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ΝΙΚΟΛΑΟΥ Ε ΚΟΥΝΔΟΥΡΑΚΗ

Οι επιδράσεις διαφορετικής προπονητικής επιβάρυνσης στην παραγωγή των επινεφριδικών και γοναδικών ορμονών του φύλου

The effects of different seasonal training programs in the production of the adrenal and the gonadal sex hormones

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Ιούλιος 2014

Ευχαριστίες

Θα ήθελα να ευχαριστήσω ιδιαίτερα τον κ. Ανδρέα Ν. Μαργιωρή, όχι μόνο για την επιλογή του θέματος και την εκπόνηση της διδακτορικής διατριβής αλλά και για την ηθική του συμπαράσταση όλη αυτήν την περίοδο, την κατανόηση του, και την αμέριστη βοήθεια που μου παρείχε οποτεδήποτε την χρειάστηκα.

Επίσης θα ήθελα να ευχαριστήσω τον κ. Ηλία Καστανά για την βοήθεια του τόσο στην διόρθωση του τελικού κειμένου, αλλά και στην βασικότατη βοήθεια που μου παρείχε για την πρώτη επιστημονική δημοσίευση.

Τον Χρήστο Τσατσάνη για την βοήθεια που μου παρείχε σε όποιο ζήτημα τον χρειάστηκα

Ιδιαίτερη μνεία βεβαίως αξίζει στην Βενυχάκη Μαρία, για την ουσιαστική και πολύτιμη βοήθεια που μου παρείχε οποτεδήποτε την χρειάστηκα .

Την Μαλλιαράκη Νίκι για την βοήθεια που μου παρείχε από την πρώτη ημέρα της έναρξης της διδακτορικής μου διατριβής

Την υποψήφια διδάκτορα κ. Ειρήνη Σπυριδάκη για την πολύτιμη βοήθεια της

*Και βεβαίως όλα τα μέλη της επταμελούς μου επιτροπής(*Ανδρέας Ν Μαργιωρής, Ηλίας Καστανάς, Χρήστος Τσατσάνης, Αχιλλέας Γραβάνης, Μαρία Βενυχάκη, Μαρία-Ελένη Καμπά, Καλλιόπη Αλπαντάκη*) για την για την βοήθειά τους και τις διορθώσεις του τελικού κειμένου.*

Αφιερωμένο στον Πατέρα μου Μανώλη, στην Μητέρα μου Ελένη, στον Αδερφό μου Βαγγέλη, στην Αδερφή μου Άννα, στην γυναίκα μου Βιβή και βεβαίως στα παιδιά μου Μανώλη και Ελένη

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Abbreviations

TT	Total testosterone
FT	Free Testosterone
3a Diol G	3 androstendiol glucuronade (3a-Diol-G),

DHEAS	Dehydroepiandrosterone-sulfate
E2	Estradiol
FSH	Follicle-stimulating hormone
LH	Luteinizing hormone
PRL	Prolactin
TC	Total cholesterol
HDL	High density lipoprotein
LDL	Low density lipoprotein
apo-AI	apolipoprotein AI
apo-B100	Apolipoprotein B100
Lp(a)	Lipoprotein a
Hct	Hematocrit
RBC	Red blood cells
ESR	Erythrocyte Sedimentation Rate
OC	Osteocalcin
b-ALP	bone alkaline phosphatase
CTX	C-terminal telopeptide
CICP	Propeptide of Collagen Type-I
VO2max	Maximal oxygen consumption

SJ	Squat jump
CMJ	Countermovement jump
HRmax	Maximal heart rate
RPE	Rate of perceived exertion
1RM	1 repetition maximum
LT	Lactate threshold
SPS	Soccer specific strength
GSC	General strength conditioning
BSG	Big Sided Games
SSG	Small Sided Games

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The effects of different seasonal training programs in the production of the adrenal and the gonadal sex hormones.

Abstract

Aim: The primary aim of the study was to examine the effects of three different seasonal soccer training programs in regard to strength training volume and load on sex steroids levels and exercise performance parameters in professional soccer players. Furthermore we examined any possible relationships between sex steroids and exercise performance responses. Our secondary aim was to examine the effects of the soccer detraining transition period just prior to the beginning of theabove seasonal intervention period on sex steroids and performance parameters. In addition, we evaluated throughout the study the responses of bone turnover markers, lipidemic profile, red blood cells, hematocrit and erythrocyte sedimentation rate. We also examined the effects of the detraining period on vitamin D levels, and its correlation with exercise performance parameters.

Design: Sixty-seven soccer players, members of three different professional teams (Team-A, n=23, height=79.1±1.3; Team-B, n=22, 182±2.3; Team-C, n=22, 181±1.4) participated in the study. Players were tested in four different occasions. The first testing was performed immediately after the end of the previous soccer season (1) followed by a second (2), third (3), and fourth (4) experimental testing after a six week detraining period, just prior the beginning of the pre-season period for the forthcoming soccer season, at the middle and immediately after the end of the soccer season (period 1). The period between testing 1 and 2 was characterized as the detraining transition period. During this period for the first two weeks of players abstained from any physical activity. The following four weeks, players performed low-intensity aerobic running three times per week. The periods between testing two and the end of the study was considered as the intervention period (period 2). The seasonal training programs during this period were expressed as high (Team-A), moderate (Team-B), and low (Team-C) strength-training stress according the strength training volume employed by each team. During the study, in each experimental period, blood samples were analyzed for total-testosterone, free-testosterone, and the metabolic product of activate testosterone 3 androstendiol glucuronade (3a-Diol-G), dehydroepiandrosterone-sulfate (DHEAS), Δ4-androstenedione, estradiol (E2), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin (PRL).

Furthermore, the concentrations of total cholesterol (TC) high density lipoprotein (HDL), low density lipoprotein (LDL), apolipoprotein AI (apo-AI), apolipoprotein B100 (apo-B100), Lp(a), red blood cell count (RBC), hematocrit (Hct) and ERS were evaluated. Bone metabolism markers i.e. of C-terminal telopeptide (CTx), osteocalcin (OC), bone alkaline phosphatase (b-ALP), and C-terminal Propeptide of Collagen Type-I (CICP) were measured only at experimental sessions (1), (2), and (4). Furthermore, only in the detraining period vitamin D was measured. In addition, players were tested for maximal oxygen consumption (VO2max), squad-jump (SJ), countermovement-jump (CMJ), 10m and 20m sprint performance prior at the beginning of the pre-season period, at the middle (mid-point), and at the end of the competition period (end-point). Also anthropometric characteristics were measured (i.e. height, body weight [BW], body fat [BF], body mass index [BMI], waste to hip ratio[WHR]).

Results Analysis of our results revealed the following findings: (a) *Detraining Period:*No significant differences were observed for none of sex steroids, lipidemic profile paremers, RBC, Hct, and ERS at the end of the detraining period ($p>0.05$). In contrast, in all teams exercise performance indices and bone formation markers decreased significantly (all $p<0.000$), while significant increases were evident for CTX, vitamin D levels increased (all $p<0.000$) and BW, BF, WHR, BMI (all $p<0.001$) and vitamin levels ($p<0.000$) in all experimental teams. Vitamin D correlated significantly with all exercise performance indices (all $p<0.000$). No correlations were evident between sex steroids and androgens. (b) *Intervention period:* All performance parameters increased significantly until the midlle of the championsip in all teams ($p<0.001$). However, performance was further increased only in Team-A for jumping and sprinting ability between end-point vs mid-point ($p<0.001$). An effect of the training program of Team-A on TT levels was evident exhibiting significant differences between at all point-measurements (2/3:$p=0.024$, 2/4:$p<0.001$, 3/4:$p=0.008$), while a marginally significant effect ($p=0.051$) was detected within Team-B and a non-significant effect in Team-C. Similar results were obtained for 3a-Diol-G in Team-A ($p=0.001$) where significant differences were found between end-point to both baseline ($p=0.001$) and mid-point ($p=0.038$).Despite these alterations no significant correlations were evident between these changes in the specific times points and exercise performance parameters. No differences were detectable for none

of the rest sex steroids, parameters of the lipidemic profile, RBC, Hct, and ESR ($p>0.05$). In regard to bone metabolism, we observed significant increases in all bone formation markers (all $p<0.000$) and a decrease in the bone resorption marker (CTX, $p<0.000$) in team A at the end of the study compared to baseline. Similarly, weak significant increases in OC ($P=0.038$), b-alp ($p=0.032$), and CICP ($p=0.045$), and a reduction in CTX ($p=0.039$) were observed in team B at the end of the study compared to baseline, whereas no alterations were evident in team C. No significant correlations were evident between sex steroids and exercise performance indices, bone metabolism markers, blood lipids RBC, Hct, and ESR.

Conclusions. Our main finding is that the volume of strength training combined with soccer training caused an elevation of circulating TT and 3a-Diol-G levels in parallel to the induction of performance capacity, bone metabolism, and optimal body composition status. It is our opinion that the elevation of endogenous androgens as a result of the volume of strength training indicates that the only method to improve athletic performance is hard training. There are no substitutes or shortcuts. If the organism needs more androgens it will produce them endogenously. Furthermore, our findings indicate that a short term detraining period in soccer does not seem to affect sex steroid levels, but induce a rapid loss in optimal exercise performance capacity, body composition and bone metabolism adaptations to exercise. In regard, to lipidemic profile, RBC, Hct, and ERS our findings indicate that in chronically trained professional soccer players variation in training volume does not affect their levels. Finally, the soccer detraining period beneficially affects vitamin D as a result of both higher exposure to the sun but also reduced strength training stress. Notably, irrespective the level of performance this secosteroid is related to both aerobic and neuromuscular exercise capacity in soccer players.

Keywords: Sex Steroids, Androgen metabolite, Exercise performance, Vitamin D, Bone metabolism

Περίληψη

Σκοπός: Πρωτεύων σκοπός της παρούσας μελέτης ήταννα εξετάσει την επίδραση τριών διαφορετικών προγραμμάτων κατά την διάρκεια μιας ποδοσφαιρικής χρονιάς όσον αφορά τον όγκο και το φορτίο της προπόνησης δύναμης στα γοναδικά και επινεφριδικά στεροειδή του φύλου και σε παραμέτρους της αθλητικής απόδοσης σε επαγγελματίες ποδοσφαιριστές. Επιπλέον, εξετάσαμε τις πιθανές σχέσεις μεταξύ των στεροειδών του φύλου και των μετρούμενων παραμέτρων της αθλητικής απόδοσης. Ως δευτερεύων σκοπός ήταν να εξετάσουμε τις επιδράσεις την μεταβατικής περιόδου μειωμένης προπονητικής επιβάρυνσης, που έλαβε χώρα πριν την έναρξη της ποδοσφαιρικής αθλητικής χρονιάς (κύριος στόχος της έρευνας), στα γοναδικά και επινεφριδικά στεροειδή του φύλου και στις παραμέτρους της αθλητικής απόδοσης αλλά και τις πιθανές συσχετίσεις μεταξύ τους. Επιπλέον εξετάσαμε τις επιδράσεις και των δυο περιόδων σε επιλεγμένους δείκτες οστικού μεταβολισμού, στο λιπαιδιμικό προφίλ, στον αριθμό ερυθρών αιμοσφαιρίων και στην ταχύτητα καθίζησης. Τέλος εξετάσαμε την επίδραση της μεταβατικής περιόδου στα επίπεδα της βιταμίνης D, και τις πιθανές συσχετίσεις αυτής με τις παραμέτρους της αθλητικής απόδοσης.

Μεθοδολογία: Εξήντα επτά (67) ποδοσφαιριστές, μέλη τριών ποδοσφαιρικών ομάδων (Ομάδα Α, n=23, ύψος=79.1±1.3; Ομάδα Β, n=22, ύψος=182±2.3; και Ομάδα Γ, n=22,ύψος=181±1.4) πήραν μέρος στην μελέτη. Οι παίκτες αξιολογήθηκαν στις μετρούμενες παραμέτρους σε τέσσερις διαφορετικές περιόδους. Η πρώτη εξέταση έλαβε χώρα αμέσως μετά το τέλος της προηγούμενης ποδοσφαιρικής περιόδου, η δεύτερη αμέσως πριν την έναρξη της επόμενης ποδοσφαιρικής περιόδου, και η τρίτη και τέταρτη στο μέσο και στο τέλος της επόμενης ποδοσφαιρικής περιόδου. Σε όλους του ποδοσφαιριστές μετρήθηκαν τα ανθρωπομετρικά τους χαρακτηριστικά σε όλες τις περιόδους που περιλάμβαναν ύψος (μετρήθηκε μόνο στην πρώτη αξιολόγηση), σωματικό βάρος (BW), ποσοστό σωματικού λίπους (%BF), δείκτης μάζας σώματος (BMI), και σχέση μέσης-ισχίων (WHR). Η πειραματική περίοδος που αποτελούνταν από τις μετρήσεις 1 και 2 χαρακτηρίστηκε ως μεταβατική περίοδος μειωμένης προπόνησης (περίοδος 1). Η περίοδος που περιλάμβανε το διάστημα μεταξύ της δεύτερης αξιολόγησης και της τέταρτης χαρακτηρίστηκε ως παρεμβατική περίοδος. (περίοδος 2). Τα προπονητικά προγράμματα κατά την περίοδο 2 χαρακτηρίστηκαν ως υψηλής (Ομάδα Α), μεσαίας (Ομάδα Β), και χαμηλής (Ομάδα Γ) προπονητικής επιβάρυνσης προπονητικών

μονάδων δύναμης. Κατά την διάρκεια της μελέτης, σε κάθε μια από τις τέσσερις (4) πειραματικές περιόδους αξιολόγησης τα δείγματα αίματος που συλλέχτηκαν εξετάστηκαν για τις συγκεντρώσεις των κάτωθι: ολική τεστοστερόνη (TT), ελεύθερη τεστοστερόνη (FT), γλυκουρονίδιο της 3α-ανδροστενεδιόλης (3aDiolG), Θεϊκή δεύδροεπιανδροστερόνη (DHEAS), Δ4-ανδροστενδιόνη, οιστραδιόλη (E2), ωοθυλακιοτρόπος ορμόνη (FSH), η ωχρινοτρόπος ορμόνη (LH), και η προλακτίνη (PRL). Επίσης σε όλες τις πειραματικές περιόδους (1), (2), (3), και (4) μετρήθηκαν μια σειρά από παραμέτρους όπως τα ακόλουθα λιπιδια του αίματος ολική χοληστερόλη (TC), υψηλής πυκνότητας λιποπρωτεΐνη (HDL), χαμηλής πυκνότητας λιποπρωτεΐνη (LDL), απολιποπρωτεΐνη AI (apo-AI), απολιποπρωτεΐνη B100 (apo-B100), λιποπρωτεΐνη-a [Lp(a)],οαριθμός ερυθρών αιμοσφαιρίων (RBC), αιματοκρίτης (Hct) και ταχύτητα καθίζησης (ESR). Στις πειραματικές περιόδους μέτρησης (1), (2),και (4) εξετάσθηκαν οι δείκτες οστικού μεταβολισμού οστεοκαλσίνη (OC), οστικό κλάσμα αλκαλικής φωσφατάσης b-ALP, προπεπτίδιο του προκολλαγόνου I (CICP) και C-τελοπεπτίδιο του τύπου I του κολλαγόνου (CTX). Μόνο στις δυο πρώτες περιόδους μετρήθηκε η συγκέντρωση της βιταμίνης D στον ορό του αίματος. Επιπλέον μετρηθήκαν η μέγιστη πρόσληψη οξυγόνου (VO2max), το άλμα από ημικάθισμα (SJ), το αντιθετικό άλμα (CMJ), και η ταχύτητα 10 και 20 μέτρων.

Αποτελέσματα: Η ανάλυση των δεδομένων μας έδειξε τα κάτωθι ευρήματα: *(α) <u>Μεταβατική Περίοδος:</u>* Καμία στατιστικά σημαντική διαφορά δεν παρατηρήθηκε για κανένα από τα εξεταζόμενα στεροειδή του φύλου ($p>0.05$), τις παραμέτρους λιπαιδιμικού προφίλ ($p>0.05$) και οστικού μεταβολισμού ($p>0.05$), τον αριθμό ερυθρών αιμοσφαιρίων ($p>0.05$), του αιματοκρίτη, και της ταχύτητας καθίζησης σε καμία από τις εξεταζόμενος ομάδες ($p>0.05$). Τουναντίον, παρατηρήθηκε σε όλες τις ομάδες στατιστικώς σημαντική μείωση στις τιμές των OC ($p<0.000$), b-ALP ($p<0.000$), CICP ($p<0.000$), VO2max ($p<0.000$), SJ ($p<0.000$), και CMJ ($p<0.000$), και ομοίως στατιστικά σημαντική αύξηση στο χρόνο 10μ ($p<0.000$), 20μ ($p<0.000$), BW($p<0.000$), BF($p<0.000$), WHR($p<0.000$), BMI ($p<0.000$) και βιταμίνη D ($p<0.000$). Καμία σημαντική συσχέτιση δεν βρέθηκε μεταξύ ανδρογόνων και απόδοσης. *<u>Παρεμβατική Περίοδος:</u>* Παρατηρήθηκαν σημαντικές βελτιώσεις σε όλες τις παραμέτρους της αθλητικής απόδοσης μέχρι το μέσω της αγωνιστικής περιόδου σε σχέση με την μέτρηση στην αρχή της προετοιμασίας σε όλες τις ομάδες, και στο

ίδιο βαθμό (p<0.000):VO2max (p<0.001), SJ (P<0.001), 10μ (P<0.001), και 20μ (P<0.001). Μόνο στην ομάδα Α υπήρξε περαιτέρω βελτίωση των SJ (P<0.001), 10μ (P<0.001), και 20μ (P<0.001) στο τέλος του πρωταθλήματος σε σχέση με το μέσω της προετοιμασίας. Στις άλλες δυο ομάδες τα επίπεδα παρέμειναν σταθερά στις τιμές του μέσου του πρωταθλήματος. Παρατηρήσαμε μια επίδραση του προγράμματος της ομάδας Α στην ΤΤ μεταξύ όλων των πειραματικών περιόδων μέτρησης (αρχή προετοιμασίας/μέσο πρωταθλήματος p=0.024, αρχή προετοιμασίας/μέσο πρωταθλήματος, μέσο πρωταθλήματος/τέλος πρωταθλήματος p=0.008), ενώ μια οριακά σημαντική διαφορά της ποδοσφαιρικής σεζόν παρατηρήθηκε για την ομάδα Β (p=0.051) και καμία για την ομάδα Γ. Όμοια αποτελέσματα παρατηρήθηκαν για την 3a-Diol-G στην Ομάδα Α (p=0.001) όπου στατιστικά σημαντικές διαφορές παρατηρήθηκαν μεταξύ του τέλους της αγωνιστικής περιόδου τόσο με την αρχή της προετοιμασίας (p=0.001), όσο με το μέσω του πρωταθλήματος (p=0.038). Καμία άλλη σημαντική διαφορά δεν παρατηρήθηκε για τα υπόλοιπα στεροειδή του φύλου σε καμία από τις τρεις ομάδες. Καμία σημαντική συσχέτιση δεν παρατηρήθηκε μεταξύ των ανδρογόνων και της απόδοσης στις συγκεκριμένες χρονικές περιόδους, παρά τις μεταβολές που παρατηρήθηκαν και στα δυο. Όσον αφορά τον οστικό μεταβολισμό παρατηρήσαμε στατιστικά σημαντικές αυξήσεις σε όλους τους δείκτες οστικού αναβολισμού (p<0.000) στην Ομάδα Α και σημαντική μείωση στον δείκτη οστικής απορρόφησης (CTX; p<0.000) στο τέλος του πρωταθλήματος σε σχέση με τα επίπεδα στην αρχή της προετοιμασίας. Επίσης, παρατηρήσαμε ασθενής σημαντικές αυξήσεις στους δείκτες οστικού αναβολισμού (OC; p =0.038, b-alp;p=0.032, και CICP;p=0.045), στην Ομάδα Β και ασθενής σημαντική μείωση στον δείκτη οστικής απορρόφησης (CTX; p=0.039) στο τέλος του πρωταθλήματος σε σχέση με τα επίπεδα στην αρχή της προετοιμασίας, ενώ καμία μεταβολή δεν παρατηρήθηκε στους δείκτες οστικού μεταβολισμού στην Ομάδα Γ. Τέλος, μερικές ανακόλουθες συσχετίσεις, αμφιβόλου σημαντικής αξίας, σε απομονωμένες πειραματικές περιόδους μέτρησης στις τρεις πειραματικές ομάδες παρατηρήθηκαν μεταξύ των στεροειδών του φύλου και παραμέτρων αθλητικής απόδοσης, δεικτών οστικού μεταβολισμού, και λιπιδαιμικού προφίλ και των ρεολογικών συστατικών του αίματος που μετρήθηκαν.

Συμπεράσματα. Το κύριο μας εύρημα ήταν ότι ο όγκος της προπόνησης δύναμης σε συνδυασμό με προπόνηση ποδοσφαίρου είχαν ως αποτέλεσμα την αύξηση των κυκλοφορούντων επιπέδων της ΤΤ και του 3a-Diol-G στον ορό του αίματος σε

συνδυασμό με βελτίωση της αθλητικής απόδοσης, του οστικού μεταβολισμού και της σωματικής σύστασης του σώματος. Κατά την γνώμη μας η ενδογενής αύξηση της συγκέντρωσης των ανδρογόνων ως αποτέλεσμα του όγκου προπόνησης δύναμης υποδεικνύουν ότι η μόνη μέθοδος για να βελτιώσουμε την αθλητική απόδοση είναι η σκληρή προπόνηση. Δεν υπάρχουν υποκατάστατα ή συντομεύσεις. Εάν ο οργανισμός χρειάζεται περισσότερα ανδρογόνα θα τα παράγει ενδογενώς. Επίσης, τα ευρήματα μας υποδηλώνουν ότι περίοδοι μειωμένης προπόνησης για μικρή χρονική περίοδο δεν φαίνεται να έχουν καμία επίδραση στα στεροειδή του φύλου, αλλά προκαλούν ταχεία απώλεια των προπονητικών προσαρμογών όσον αφορά τις παραμέτρους αθλητικής απόδοσης όσο και τον οστικό μεταβολισμό. Σε σχέση με τις παραμέτρους του λιπαιδιμικού προφίλ και των RBC, Hct, και ΤΚΕ τα ευρήματα μας καταδεικνύουν ότι σε χρόνια προπονημένους επαγγελματίες ποδοσφαιριστές διακυμάνσεις στον όγκο της προπόνησης δεν επηρεάζουν τα επίπεδα τους. Επίσης παρατηρήσαμε ότι η μεταβατική περίοδος μειωμένης προπονητικής επιβάρυνσης είχε ευεργετικές επιδράσεις στα επίπεδα της βιταμίνης D, και ότι αυτή η αύξηση πιθανόν ήταν συνδυασμός αυξημένης έκθεσης σε υπεριώδη ακτινοβολία, και μείωση του προπονητικού στρες. Τέλος, ανεξάρτητα από το επίπεδο της απόδοσης, η βιταμίνη D φαίνεται να παίζει ένα σημαντικό ρόλο τόσο στην αερόβια ικανότητα όσο και στην νευρομυϊκή απόδοση.

Λέξεις Κλειδιά: Στεροειδή του φύλου, μεταβολίκό προϊόν των ανδρογόνων, αθλητική απόδοση, Βιταμίνη D, οστικός μεταβολισμός

A. General Information

1. Introduction

The last decades a lot of interest has been focused on endogenous gonadal and adrenal sex hormones in males. The adrenal and gonadal sex hormones, apart from their key effects on the development of the primary and secondary sex characteristics, play a major role on the mechanisms and adaptation to training stress, affecting the cardio - respiratory function , muscle mass, and bone metabolism (Valimaki et al. 2004; Griggs et al. 1989; Hartgens and Kuipers , 2004). Furthermore, some evidence exists suggesting that a biological mechanisms extending from the sex hormones influences physical activity in humans (Kraemer, 2004; Raastad et al. 1989; Rames et al. 2004).

In athletes, the ability to perform efficiently during exercise performance depends primarily on a good level of the musculoskeletal system, optimum body composition and the capacity of the body to recover from the excess training stress, during training and competition. A number of published evidence indicate that changes in adrenal and gonadal hormones level result in exercise -related increase in muscle mass and strength , reduced body fat and faster recovery from the stress of training (Tremplay et al. 2004; Myhal and Lamb 2000; Dohi et al. 2003; Tiidus, 2003). Furthermore, although some controversy exits, evidence suggest that there is a positive effects of sex steroids (i.e primarily testosterone and estradiol) on blood rheology and optimum lipid profile which may affect performance by a mediated effects on enhanced oxygen (O_2) transport to the exercising muscles (increased red blood cells count, reduced hematocrit, reduced blood viscosity, more elastic blood vessels) (Kenny et al. 2002; Handa et al. 1997; Shephard 1999).

The short term effects of exercise in the production of adrenal and gonadal hormones has been extensively investigated on (Tiidus, 2003; Raastad et al. 2000; Tremblay et

al. 2004). Rather scarce and often conflicting data exist however, on long-term effects of exercise on levels of sex hormones (Hoffman et al. 2005; Hakkinen et al. 1987, 1988, Fry et al. 1994; Lucia et al. 1996, 2001) and most of are confined to investigating testosterone. Changes in adrenal and gonadal hormones seem to depend on the duration of the training program, the mode of exercise, the training status of the subjects, and the intensity, duration and volume of the training program (Tremblay et al. 2004; Slewinska-Lisowska and Majda, 2002) alongside the performance of the athlete.

2. Sex Steroids.

Sex Steroids are discrete chemical substances that are synthesized and discreted by the gonads and the adrenal cortex in males. The wide variety of their action is accomplished by acting on receptors (androgen and/or estrogen receptors) on or in target cells, which respond by altering their biological activities into a specific fashion. The interaction of sex steroids with the receptors may affect the activity of cells, tissues and, organs (Wierman, 2007). This is due to the activation of enzyme systems, alteration in membrane permeability, initiation of muscle contraction and relaxation and stimulation of protein synthesis.

2.1. Gonadal and Adrenal Sex Steroid Physiology

The biochemistry of sex hormones is well understood and is summarized in Figure 1. Sex hormones primarily consist of androgens and estrogens. Both are primarily derived via dehydroepiandrosterone, androstenedione and androstenediol, which are formed from cholesterol upon stimulation by adrenocorticotropic hormone. While the primary sex hormones differ between males (testosterone) and females (estrogen) as do the primary sites of synthesis (testes for male; ovaries for female), quantities of testosterone and estrogen occur in both sexes. Of the most important androgens are

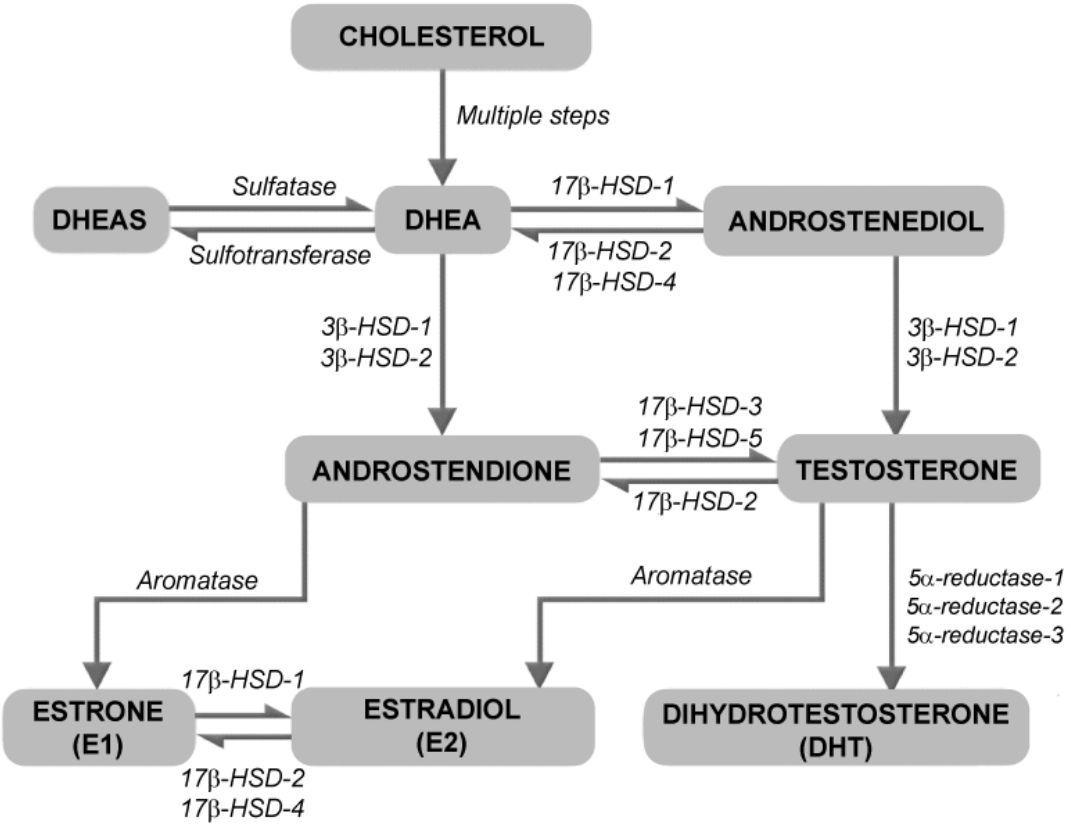


Figure 1. Sex Steroid Physiology

Δ4-androstenedione, DHEA and its sulfate DHEAS, testosterone, which may occur in protein-bound and active free form, as well as their metabolites (in the context of this thesis, 3a Diol G, a metabolite of testosterone). The most important estrogen in males is estradiol (E2).

2.2 Androgens

The main and most active androgen is testosterone. The majority of circulating testosterone derivesfrom its production within the Leydig cells in the testes. Small amounts are also produced by the adrenal gland. Regulation of testicular production occurs via a negative feedback loop system, involving the anterior pituitary hypothalamus and the gonads (hypothalamic pituitary gonadal – HPG axis). Testicular function is controlled by both luteinizing hormone (LH) and follicle stimulating hormone (FSH). A bisphasic on testicular androgen synthesis effect is also played by prolactin (PRL). More specifically, in the hypothalamic-pituitary-gonadal axis, gonadotropin-releasing hormone is secreted, in an episodic fashion, from the hypothalamus to activate the production of gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) from the anterior (Kraemer

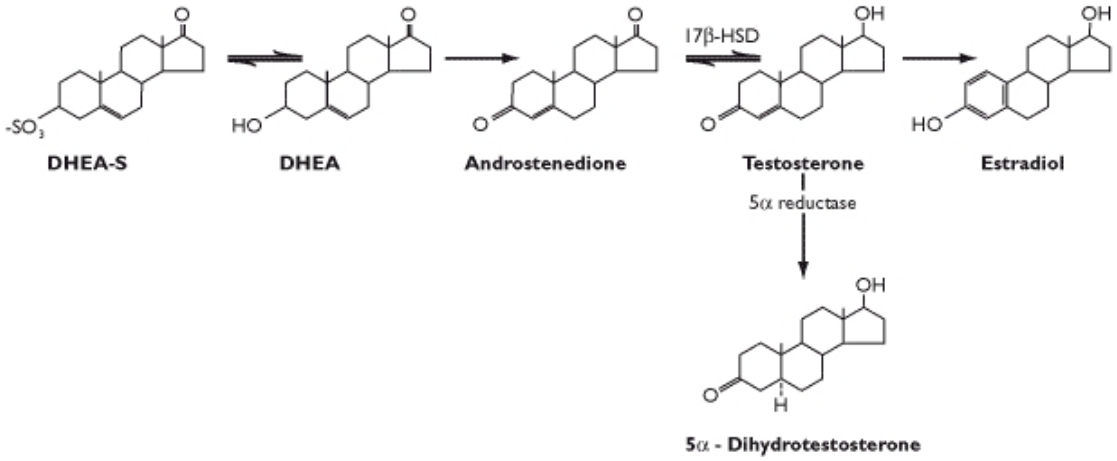


Figure 2. Peripheral metabolism of andrenal androgens

and Rogol, 2006; Borer, 2003). LH and FSH are released in a pulsatile manner to act at the gonad to control both gametogenesis (spermatogenesis or ogenesis) as well as steroidogenesis. The major sex hormones testosterone is secreted in response to gonadotropins and, in turn, feedback at the level of the hypothalamus and pituitary to control normal reproductive function.

Testosterone secreted into the circulation is mostly reversibly bound to plasma proteins, primarily to sex hormone binding globulin (SHBG) and albumin. In the circulation, total testosterone is composed of 0.5% to 3.0% free testosterone unbound to plasma proteins, 30% to 44% SHBG-bound testosterone, and 54% to 68% albumin-bound testosterone (Melmed and Williams, 2011).Clinically, biologic actions of testosterone, like those of other steroid hormones, are thought to conform to the free hormone hypothesis; that is, the biologic activity of testosterone is mediated only by its free (unbound) concentration or the concentration that is easily dissociable from plasma proteins in circulation (Melmed and Williams, 2011). Testosterone is tightly bound to SHBG with such high affinity (1.6×10^{-9} mol/L) that it is not easily dissociable and available to target tissues for biologic action. In contrast, testosterone is loosely bound to albumin, with a binding affinity (1.0×10^{-4} mol/L) that is several orders of magnitude less than that of SHBG binding. Therefore, albumin-bound testosterone is more easily dissociable and available to target tissues for action. Together, free and albumin-bound testosterone are referred to as bioavailable testosterone, because these fractions are available to diffuse into target tissues, bind to androgen receptors (AR), and affect gene transcription, resulting in specific androgen actions. Testosterone is also an intermediate substance in the formation of

estrogen in both males and females. Through an aromatization process, using the aromatase enzyme complex, testosterone is converted to 17b-estradiol; this conversion is not reversible. In males, while some testosterone is converted to estrogen, the majority of testosterone is converted into dihydrotestosterone (DHT), which cannot be aromatized into estrogen.

2.3 Adrenal Androgens

A subset of androgens are synthesized and secreted by the adrenal cortex. More specifically, the adrenal sex steroids that show an adrogenic behavior are Dehydroepiandrosterone (DHEA), its sulfate DHEAS and Δ4-androstenedione. The androgens of the adrenal cortex can formate testosterone (figure 2). The endogenous synthesis of these androgens is stimulated by the adrenocorticotropic hormone (ACTH).

2.3.1 DHEA and DHEAS

Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) are metabolic intermediates in the production of potent androgens (Dillon 2005, Traish et al. 2011). Dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAS) are weak

Androgen Metabolism

- Androstenedione, DHEA and its sulfate DHEAS are metabolized to the more potent testosterone by 17β-hydroxysteroid dehydrogenase via androstenedione.
- In some target tissues, testosterone is 5α-hydroxylated to the more potent 5α-dihydrotestosterone or estradiol.
- Most androgens are conjugated prior to excretion in the urine. Testosterone and androstenedione are cleared three times more rapidly in men than women.
- DHEAS can be excreted unchanged.
- Androstenedione may be metabolized to androsterone or etiocholanolone and, thence, 17β reduced, conjugated and excreted.
- 5α-dihydrotestosterone is reversibly inactivated by 3α-reduction and a smaller amount is converted to 5α-androstanedione.

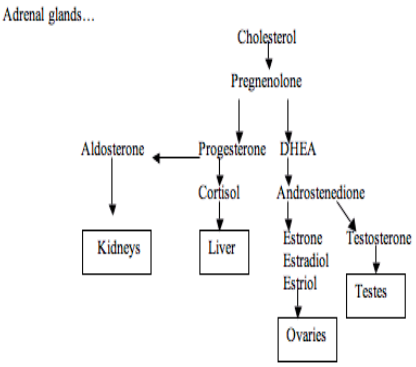
- the androgens of the adrenax cortex are (DHEA), DHEA-S and Δ4-androstenedione.

- These androgens can converted in peripheral tissues to testosterone but laso dihydrotestosterone, and both are aromatized to estrogens

- The endogenous synthesis of these androgens is stimulated by the adrenocorticotropic hormone (ACTH).

- DHEA in the circulation is by 90% by the adrenal cortex. Δ4-androstenedione is produced by 50% from the adrena cortex and 50% by the gonads

Adrenal Cortex Androgens



androgens produced primarily by the adrenal gland**.** Approximately,90% of the DHEA and DHEAS levels comes from the adrenal cortex. Formation of DHEA is stimulated by ACTH, producing a significant diurnal variation in serum concentrations. DHEA is rapidly converted to DHEAS by an enzyme present in the adrenals, liver and small intestine. DHEAS is present at concentrations greater than 200 times that of DHEA and has a longer half-life, which largely removes the diurnal variation. In males, the amount released from the adrenal glands and converted to testosterone is physiologically insignificant, compared to the amount secreted by the testes. In plasma, where the major portion of these hormones is present in the sulfate form, it is possible that DHEAS serves as a reservoir for DHEA (Leowattana, 2001). Neither form has significant androgenic activity, they have much lower affinity to the androgen receptor than testosterone, and they are precursors to about 50% of androgens in men. In regard to DHEAS is has been found to be the most abundant circulating steroid hormone in humans (William 2005). where it functions predominantly as a metabolic intermediate in the biosynthesis of

the androgen and estrogen sex steroids (Mo, 2006). More specifically, DHEAS can be converted in peripheral tissues not only to testosterone but also to androstenedione, which may be are aromatized to estrogens, and todihydrotestosterone. Notably, no receptor has been found for either of these steroids.

2.3.2 Δ4-androstenedione

The circulating Δ4-androstenedione levels are produced by the adrenal cortex (50%) and by the gonads (50%). As with DHEAS, Δ4-αndrostenedione is generally termed as a 'weak androgen' and has a much lower affinity for the androgen receptor than testosterone. Δ4-androstenedione is the common precursor of male and female sex hormones (Devlin, 2010). Some androstenedione is also secreted into the plasma, and may be converted in peripheral tissues to testosterone and estrogens. Δ4-androstenedione can be synthesized in one of two ways. The primary pathway involves conversion of 17-hydroxypregnenolone to dehydroepiandrosterone by way of 17,20-lyase, with subsequent conversion of dehydroepiandrosterone to androstenedione via the enzyme 3-β-hydroxysteroid dehydrogenase. (Devlin, 2010) The secondary pathway involves conversion of 17-hydroxyprogesterone, most often a precursor to cortisol, to androstenedione directly by way of 17,20-lyase. Thus, 17,20-lyase is required for the synthesis of androstenedione, whether immediately or one step removed. (Devlin, 2010) The production of adrenal androstenedione is governed by ACTH, whereas production of gonadal androstenedione is under control by gonadotropins.

2.4 Androgens Metabolite

An end by product of testosterone metabolism is androstanediol glucuronide (3a diol G). As seen in Figure 3, testosterone is converted to dihydrotestosterone (DHT) which

in turn is gives 3a diol G (Hawkins et al. 2008), a process governed by specific enzymes (Figure 3). This metabolite has a strong androgenic activity estimated at 75% of the bioactivity of testosterone (Doffman and Shipley, 1956). A significant amount of 3a diol G also derives from DHEAS and androstenedione.

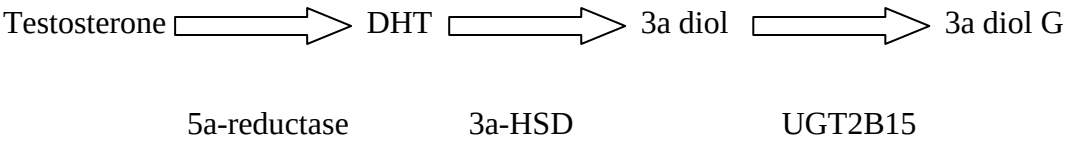


Figure 3. 3a Diol G production

2.5 Estrogen

In males, estrogens derive from circulating androgens. The most important estrogen is estradiol. Most of its formation comes from extraglandular aromatization of circulating androgens, mainly testosterone. Aromatase catalyzes the conversion of testosterone to estradiol as well as the conversion of the weaker androgen, Δ^4-

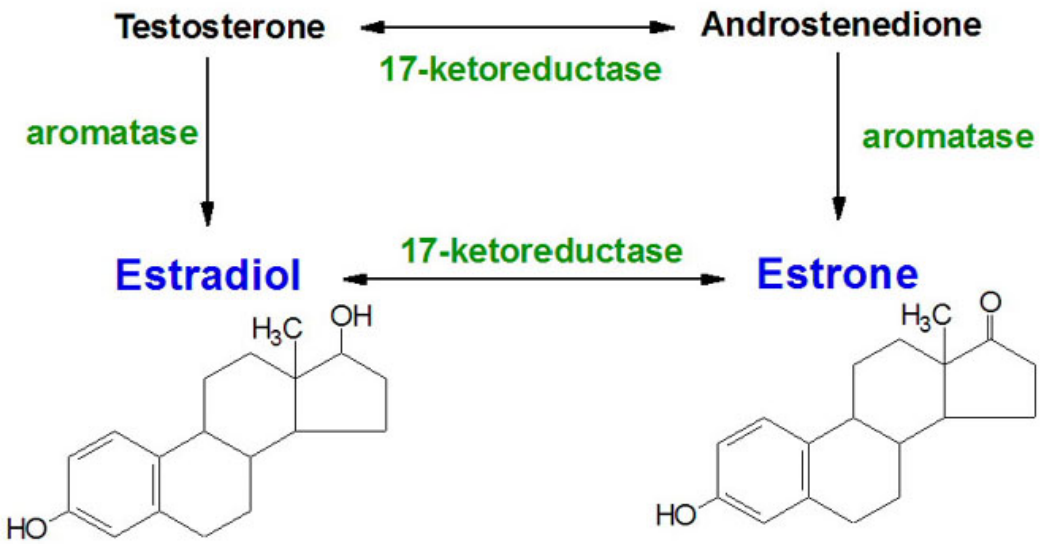


Figure 4. Estradiol Production

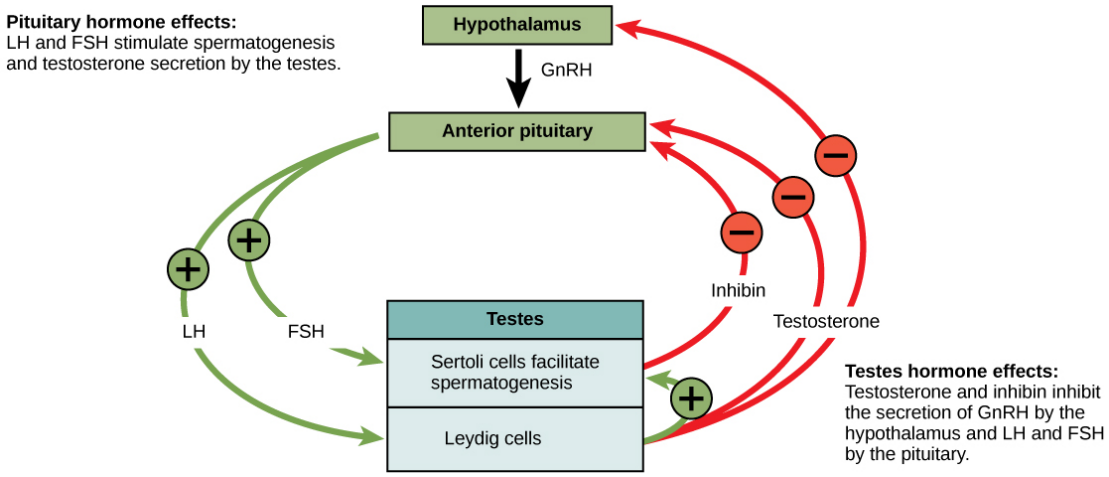


Figure 5. Stimulation of testosterone secretion by LH and FSH in males

androstenedione, to the weaker estrogen, estrone (which is then converted to estradiol by various isoforms of the enzyme, 17β-HSD). Evidence also suggest that DHEA and DHEAS they can be converted to physiological estrogens in peripheral tissues. A smaller part of E2 is derived from direct secretion by the testes (Hess, 2003). Since estradiol derivesmainly from the aromatization of testosterone, and in males LH has been reported to be the regulator of testosterone, changes in LH levels could also be related to estradiol concentration. In addition, elevated estradiol levels can stimulate the HPG axis to increase LH concentration. Furthermore, endogenous estradiol secretion has been suggested to play a role in to the regulation of both serum PRL and FSH concentration in males (Borer, 2003). Prolactin however, a hormone synthesized in the adenohypohyseal lactotrophs, has no known target organ or defined role in male reproduction. Yet, expression of prolactin receptors inthe choroid plexus and hypothalamus, presupposes a latent role for this hormone in the regulation of male fertility (Grattan, 2001; Mangurian et al. 1992). Furthermore, it has been well demonstrated that PRL is also related to stress response. Luger et al. (1987) suggested that PRL responses to absolute workloads were inversely proportional to the degree of training, so no response could be registered in highly trained subjects, as the soccer players in our study, during the insufficient stimulus of the 6-week detraining period.

Finally it should be mentioned that prolactin, which on the evolutionary scale is also related to stress response, has been found to be stimulated by interleukin-6 (IL-6) (Mastorakos et al. 2005).

3. Physiology of exercise

Exposure to exercise training results to variety of adaptations on all the physiological system of the body. These adaptations vary, according to the nature of the training stimulus. Competitive athletes that participate in different sports present different sports specific body adaptations, which improve their performance.

Sports are mainly characterized as endurance (aerobic or anaerobic), and strength and power events. These differences are due to the specific mode of exercise that is performed during these events, the metabolic pathways, which are primarily responsible for the energy provision during these events, and the type of training individuals have to undergo. Each type of these events induces specific kinds of physiological adaptations. The majority of the physiological adaptations are a result of aerobic endurance and strength and power events. However the physiological system of the body responds also to anaerobic endurance training.

3.1 Physiological Adaptations of Aerobic (Endurance) exercise training

3.1.1 Definition and Characteristics:

An athletic event is characterized as an endurance event when it involves continuous physical activity (training) over a longer period of time and requires a physical capability, which is defined as endurance. Endurance in other words can be defined as the maximum duration for which an exercise can be sustained. Traditional endurance training increases the ability to perform low load, high repetition exercise. Endurance activities can be classified into short (<4 min), medium (4-15 min), and

long duration (>15 min). During this kind of activities energy provision comes from the aerobic pathways and, at low exercise intensities mainly derives from fat oxidation. However as the intensity of endurance exercise increases so does the amount of energy supplied from the aerobic oxidation of carbohydrates.

3.1.2. Physiological and Biochemical Adaptations.

Endurance training, and thus endurance activities, induce physiological and biochemical adaptations in almost every system in the body, especially within the skeletal muscle and the cardiorespiratory system. This kind of training facilitates aerobic processes and has a marginal effect on muscular strength and anaerobic power. There is a number of biochemical adaptations in response to aerobic endurance training. The myoglobin level of the muscle has been found to significantly increase as a result of endurance training (Mole et al. 1971) and that this increase is only present in the muscles involved in the aerobic training program. Training also has been found to improve the capacity of the skeletal muscle to completely break down glycogen in the presence of CO_2, ATP and H_2O. This is mainly accomplished by specific subcellullar adaptations, involving changesin the size, number and membrane surface area of mitochondria (Costill et al. 1976). In addition, an increase in the concentration and the activity if the enzymes involved in the Krebs cycle and the electron transport system is detected (Benzi et al. 1975), as well as a shift towards a preferential fatty acid oxidation. The increase in the muscle capacity for fatty acidoxidation is related to three reasons: First, endurance training leads to increased muscle triglycerides stores (Hoppeler et al. 1971). Second, the increased availability of fats to the skeletal muscle can have a positive effect on endurance performance (Mole et al. 1971) leading to a glycogen sparing effect as a result of greater fat oxidation. Thirdly, there is an increase in the enzymes that are responsible for the fat

molecules break down before entering both the Krebs Cycle and the Beta-Oxidation pathway (Benzi et al. 1975). All these adaptations lead to an increased ability to generate ATP. Finally it must be mentioned that changes in skeletal muscle secondary to endurance training induce apart from the increased oxidation of fat, improved oxidation of carbohydrates (Costill et al. 1976) leading to increased glycolytic capacity.

Apart of the aforementioned biochemical alterations there is a variety of cardiorespiratory and muscular adaptations as a result of endurance training (respiratory, circulatory, and tissue level adaptations). These changes have been evident in a different term at rest, during submaximal, and during maximal exercise. At rest there is an increased heart size among athletes compared to none athletes (Turpeinen et al. 1996). The increase is due to an increase in the size and cavity of the ventricles (endurance trained athletes and/or and increase in the thickness of the ventricular wall (non-endurance athletes). There is also a decreased heart rate at rest, increased blood volume, hemoglobin levels, capillary density, and red blood cells volume. Notably, despite the increase in red blood cells volume, hematocrit shows a decrease when compared to untrained individuals, due to an increased portion of fluid in the blood stream. This in turn reduces blood viscosity, enhancing oxygen delivery to the exercising muscles. In addition endurance exercise induces type I muscle fiber hypertrophy, and alters the size and ratio of type II muscle fibers by decreasing the cross sectional area, while increasing type IIa and decreasing Type IIb percentages (Gollnick et al. 1972). Following chronic exposure to endurance training several important changes occur during steady state submaximal exercise. There is a slight decrease in VO_2max. In addition at a given VO_2max the amount of glycogen utilization is less, leading to reduced lactate formation. Both adaptations result to an

increase in sustained submaximal work effort (Gollnick and Saltin, 1982). Furthermore there is an increase in stroke volume leading to increased O_2 transport to the exercising tissues at a given workload, a decreased heart rate during a given exercise intensity leading to modifications within the heart muscles and/or the autonomic nervous system (Winder et al. 1978) and a decrease of blood flow per kg of active muscle at a given submaximal exercise intensity (Smith and Mittchell, 1993). This can be attributed in part to the higher capillarization in the muscles. The exercising muscles compensate for the lower blood flow in the trained state by extracting more of the O2 that is available. Furthermore during submaximal exercise ventilation rates are lower (Wetter et al. 1999).

Maximal work capacity increase as a result to endurance training (maximal exercise adaptations). This improvement can be attributed to a number of physiological adaptations. There in and increase in VO_2max, the overall measure of the body to transport and to utilize O_2, increased cardiac output and stroke volume. Furthermore, endurance training affects lung function. Athletes have a higher diffusion capacity compared to non-athletes. This is due to an exercise training induction in pulmonary blood flow (increase), and the fact that maximal minute ventilation is increased by training.

3.2 Physiological adaptations of Anaerobic exercise training

3.2.1 Definition and Characteristics:

Anaerobic exercise training represents a local characteristic of the muscles that exists independent of blood and oxygen supply to that of muscle. As anaerobic activity is considered any short-duration exercise (<2 min) that is powered primarily by metabolic pathways that do not use oxygen. Such pathways produce lactic acid and

several other by-products (H, Pi, NH_4), resulting in metabolic acidosis and subsequently impairment of exercise performance.

3.2.2 Physiological Adaptations

Although the majority of the studies have examined the physiological adaptations of aerobic training, there is also evidence in regard to the response of the physiological systems of the body to anaerobic training. These adaptations include increases in peak and mean power output, during short term activities, which are translated to increased exercise performance on the performed physical activity tasks (i.e. all out exercise <1 min duration). Apart from these general fitness effects of anaerobic training, several adaptations occur within the skeletal muscle. There is evidence of an increase in ATP concentration, increased activity of the enzymes that are responsible for energy provision via both the ATP-phosphocreatine (Pcr) and anaerobic glycolysis systems. These changes demonstrate a beneficial effect of anaerobic training on muscle metabolism, leading to an enhanced and more rapid release of the immediate energy for movement. In regard to muscle fibers, evidence from muscle biopsy studies indicate that there is a hypertrophy of type II muscle fibers, is response to anaerobic training. In addition, although changes in enzymatic activity and alterations in theATP-Pcr stores seems to exist in both type I and type II muscle fibers , these changes seem to be more specific to type II muscle fibers.

3.3 Physiological adaptations in Strength and Power Events

3.3.1 Definition and Characterisitics

Strength and power events can be defined as the physical activities which mainly consist of strength/power resistance training. Strength can be defined as the maximum

ability to apply or to resist force. Power is simply the product of strength and speed, or more appropriate, power= force x velocity. This is the key component for most athletic events, especially in those that performance depends on the ability to repeatedly produce near maximal force. This is called strength endurance. Both muscular endurance and power are directly related to the strength level of each individual. Strength alone seems to be a pure component.

Traditional strength and power (resistance) training involves high load, low repetition muscular contractions. Energy provision during this kind of activities comes mainly from anaerobic pathways although aerobic metabolism has a small contribution (Knuttgen, 2003). This kind of training increases muscular strength and anaerobic power and has a slight effect on aerobic processes.

3.3.2 Physiological and biochemical adaptations

A number of biochemical adaptations have been reported to occur following strength training. It has been observed that this kind of training results to increased concentration of ATP, PCr, muscle creatine and glycogen (MacDougal et al. 1977). In addition the aerobic enzymes involved in the Krebs cycle have been found also to increase as a response of strength training (Costill et al. 1979).The same authors observed increased activities of glycolytic enzymes. In contrast, Thorstensson and co-workers (1976) found no alteration for glycolytic enzymes activity. Furthermore, little or inconsistent changes in the activity of myokinase and creatine phosphokinase, the ATP turnover enzymes, were observed (Costill et al. 1979; Thorstensson et al. 1976).

The main resistance training physiological adaptation is increased hypertrophy, as measured by increased cross sectional area especially in type II muscle fibers, reflecting an increase in muscle protein content resulting to increased fiber size and

possible fiber number (Tanaka and Swensen, 1998). Type IIa fibers display the greatest growth, followed by type IIb fibers. Type I muscle fibers exhibit the least amount of hypertrophy.

In accordance with endurance training, resistance trained subjects show a transformation of type IIb muscle to type IIa muscle fibers but in opposite-direction changes in muscle contractile properties and fiber size (Tanaka and Swensen, 1998). Also there is evidence of a decrease in the volume of mitochondria due to hypertrophy–induced dilution. Moreover, there is an increased muscle buffering capacity (Maughan et al. 1997) strength and high intensity ability. Lastly, strength and power training results to adaptations within the nervous system (Gabriel et al. 2006). It has been observed that resistance training leads to beneficially altered recruitment pattern and synchronization of motor units, and reductions in inhibitory mechanisms such as the Golgi tendon organs (Gabriel et al. 2006). The available evidence supports the hypotheses that increases in strength depends on both muscle hypetrophy and neural adaptations of the trained muscle (Gabriel et al. 2006).

4. Physiology of Soccer

Soccer is classified as a high intensity intermittent team sport.As a sport, soccer is an event of a mixed alternating aerobic (endurance) – anaerobic type, with high intensity exercises carried out at irregular intervals. It requires players to be competed in several aspects of fitness, which include aerobic and anaerobic capabilities and power and muscle strength.

4.1 Physical demands of the game

During soccer training and competitions there are incorporating periods of high intensity exercise with periods of medium or low intensity activities. Especially, throughout the competition, the aerobic loading is high and the anaerobic energy turnover is extensive in several stages of the game (Bangsbo, 1994; Ekblom, 1986; Krustrup et al. 2005).

During 90 minute soccer game players typically cover a distance of 9 to 12 Km (Mohr et al. 2005) showing the importance of endurance in this sport. As mentioned, the activities during soccer game vary in intensity and duration. According to evidence from time motion analysis there is a change in activities every 4 – 6 seconds (Bangsbo 1994; Mohr et al. 2005). In the endurance context of the game, each player performs approximately 1350, mainly short, activities (Mohr et al. 2003), with maximal speed efforts sprinting) to constitute 1-11% of the total distance covered during the game (Stolen et al. 2005). It has been reported that players perform high intensity running approximately every 70 seconds, 10 to 30 activities with maximum speed, about 10 headings, 15 tackles, 50 involvements with the ball, 30 passes (Ekblom 1986; Stolen et al. 2005) and approximately 50 turns, comprising sustained forceful contractions to maintain balance and control of the ball against defensive pressure (Stolen et al. 2005). The latter clearly states the importance of strength and power for soccer players. Interestingly, only <2% of the total distance is covered with the ball (Stolen et al. 2005).

These patterns of activities are influenced by the specific requirements of the playing positions in the team. The greatest distances in the field are covered by the middle fielders and the less distance is complete by the centre backs (Bangsbo 1994; Rienzi et al. 2000). Other investigators observed that full backs make significantly more sprints than centre-backs and middle-fielders (Mohr et al. 2003).

4.2 Metabolism in Soccer

Due to the intermittent and acyclic nature of soccer, during the game there is an interaction of all three energy systems, aerobic, alactic and lactic anaerobic. The dominant system at any time throughout the game depends on the intensity of the activity performed at that specific time.

4.2.1 Aerobic Metabolism and Performance

Soccer relies predominantly on aerobic metabolism. It is estimated that aerobic pathways provide approximately 90% of the energy cost during the game (Bansgbo, 1994). According to Astrand and Rodahl (2003) the energy derived from aerobic pathways can reach 98% with the remaining 2% generated from anaerobic processes. Players at competitive level play at an average intensity close to the lactate threshold (the highest exercise intensity where lactate production and removal is equal). A number of studies using both elite junior (Helgerud et al. 1990) and adult (White et al. 1988; Mohr et al. 1993) soccer players observed the lactate threshold corresponded to 75-85% of maximal oxygen uptake and 87-90% of maximal heart rate. According to Bangsbo (1994), the anaerobic energy production of elite players is estimated to approximately 70% of VO_2max. Since blood and muscle lactate accumulation can impair performance it could be impossible to sustain a higher average intensity over a long period of time (Stolen et al. 2005). During the game there are periods where players are performing high intensity activities and lactate and other metabolic by-products accumulation occur. These activities are interspersed with periods of low intensity activities (intermittent nature of soccer) where lactate is removed from the exercising muscles and exercise performance is not impaired (Bangsbo, 1994; Stolen et al. 2005).

4.2.2 Importance of Aerobic Endurance in Soccer

Maximal Oxygen Consumption (VO_2max), defined as the highest amount of that the body can utilize during exhaustive exercise while breathing air at sea level (Astrand and Rodahl, 2003), is regarded as the best objective measure of cardiorespiratory endurance, aerobic metabolism and power and probably the single most important factor determining success in aerobic endurance sports (Howley et al. 1995). This parameter is improved by aerobic training which is traditionally an important component of physical training in soccer (Impelizzeri et al. 2005). The importance of VO_2max in soccer has been confirmed by several investigations. According to the obtained results from these studies there is a significant correlation between the distance covered during the game (Smaros, 1980; Bangsbo, 1994), the number of sprints performed by the players (Smaros, 1980), the competitive ranking, quality of play, and VO_2max (Bangsbo and Lindquist, 1992; Wisloff et al. 1998). The players with higher VO_2max values apart from the above mentioned advantages are also subjects to some other favorable effects: Higher VO_2max is leading to an enhanced recovery from high intensity intermittent exercise, enhanced lactate clearance, enhanced phosphocreatine regeneration, lower blood and muscle lactate levels at a given submaximal work as a result of increased reliance on aerobic pathways (Hoff and Helgerud, 2004; Stolen et al., 2005). In addition improved endurance capacity increases lipid oxidation in relation to carbohydrates, and utilization of intramuscular triglycerides.

Aerobic (endurance) training can positively affect another parameter of endurance performance, the lactate threshold (LT), which has a reference point blood concentration at 4 mmol/lt. Lactate threshold marks the transition of moderate to heavy exercise. Bangsbo (1994) observed a significant correlation to lactate and the

amount of work performed. In addition any improvement of the LT at a given submaximal exercise intensity could lead to reduced production of lactate at a given exercise intensity (Bangsbo, 1994). This could lead to increased reliance on aerobic pathways, accompanied by glycogen sparing which can favorably affect performance. In other words, the latter adaptation allows players to perform exercise at higher intensities for prolonged periods, throughout the game. In addition, glycogen sparing is leading to an increase in sustained submaximal work effort (Gollnick and Salton, 1982).

4.3 Anaerobic Metabolism and Performance

Although aerobic metabolism dominates the energy supply during a soccer game, anaerobic energy provision is of crucial importance. During a soccer game the fundamental characteristics of soccer is evident: intermittent high intensity exercise. The high intensity bouts of activities during the game are depending on anaerobic energy sources. These most decisive actions during the game are covered by means of alactic and lactic anaerobic glycolysis. For an elite player, the total duration of high intensity exercise is about 7-9 minutes, including around 30 sprints of a mean duration of 2 seconds and other activities (turning, jumping, tackling) of less duration (Bangsbo 1994). During these actions energy is derived from Phosphocreatine (PCR) degradation and adenosine triphosphate (ATP) energy stores. Phosphocreatine plays an important role during a soccer match, although its net utilization is small, functioning as energy buffer providing phosphate for ATP resynthesis. In order to restore these energy sources aerobic energy is used, making a necessity for the players to spend a substantial time at intensities lower than the LT (Hoff and Helgerud, 2004).

Glycolisis in the muscle is activated and lactate is formed almost immediately at the onset of exercise (Baker et al. 2010; Bogdanis et al. 1998). The concentration of blood lactate is often been used as an indicator of anaerobic energy production in soccer. Average blood lactate concentrations of 3 – 6 mmole/lt with individual values above 12 mmole/lt have been recorded during soccer games (Krustrup et al. 2003). These data and observation from other investigations well demonstrate that anaerobic energy system is highly taxed in soccer (Ekblom, 1986; Mohr et al. 2003).

4.4.1 Importance of Anaerobic Metabolism

To perform sprints, stops, turns, jumps, tacks and duel of play, anaerobic energy release is important. Anaerobic metabolism can favorably affect soccer performance as a result from the physiological adaptation of anaerobic training. Increased neuromuscular coordination, greater anaerobic enzymes activity and capacity, and enhanced removal of lactate are reported (Deschemes and Kraemer, 2002; Stolen, 2005). Furthermore high intensity exercise can be performed more frequently throughout the game due to increased lactate clearance, and enhanced phosphocreatine regeneration (Tomlin and Wegner, 2001). All these are often crucial for the match outcome (Wragg et al. 2000).

4.4 Strength and Power Importance for Soccer

Strength and power are equally as important as endurance in soccer. Maximal strength refers to the highest force that can be performed by the neuromuscular system during a given task. In soccer, strength is associated with high-intensity functional performance. Many activities in soccer are forceful and explosive in nature. The ability of the players to develop maximal force is related to the strength of the muscles involved in the movements. Fast alterations in direction and dislocation

velocity are frequent demands in soccer matches and they are crucial for marking, dribbling and game tactics (Pacobahyba et al. 2012). These demands may be influenced by human and athletic performance, being dependent on muscle strength and power. Increases in maximal strength and power are usually leading to improvements in relative strength (Stolen et al. 2005). This could result to improved strength and power abilities and their derivatives acceleration, jumping and sprinting. Indeed, a number of studies reported a significant relationship between maximal strength (expressed as 1RM[1]) and power with acceleration and movement velocity (Hoff and Almåsbakk, 1995) and jump and sprint performance (Wisloff et al. 1998; 2004). Notably, strength increase is associated with better neural coordination, as well as increase in the area of transversal section of the muscle. Increase in muscle mass depends on protein synthesis, influenced by the endogenous responses of the many anabolic hormones, such as high testosterone (Kraemer and Rogol, 2006; Hansen et al. 1999). In addition high levels of maximal strength are suggested to prevent injuries in soccer. Thus, the acknowledgment on the muscle strength levels contributes to the prescription of rehabilitation exercises as well as to the development of athletic properties (Brown and Weir, 2001). Therefore, the fact that soccer is dominated from speed, acceleration, jumping and breaking verifies the crucial importance of strength and power for soccer players.

4.5 Nernous System

4.5.1 General information

[1]Maximal strength is defined in terms as 1 repetition maximum (1RM) in a standardized movement and Power as the ability to develop as much force as possible in the shortest period of time.

The nervous system is a stimulus processing and generating organ. This process is called sensory motor integration. Each stimulus is received by sensory receptors and transmitted along neurons to the central nervous system (CNS). The CNS in turn interprets the incoming information and determines which response is most appropriate (Deschemes and Kraemer, 2002).

Soccer players are confronted with numerous situations during the game. Before making a decision they must first recognize every simple situation. Visual information, hearing, sense of touch and sense of movement play an important role to perception. Sensory processes are responsible to provide all the information-data about players own movements and the constantly situation around him to the brain. When the brain has the information for the action the motoric processes of the nervous system become active. The brain sends signals to the muscles to execute the specific action (Gabriel et al. 2006). Thus is it clear that the nervous system, apart from the various physiological systems, is a very important facilitating factor in the context of playing soccer.

4.5.2 Nervous System Importance

At the beginning of a training period increase in strength are correlated to an increased physical activity. The central nervous system recruits motor units by sending nerve impulses to the motor neuron. Increased firing frequency results to increased potential of force development (Aagaard, 2003; Sale, 1992). An increased activation of the muscles may be a result of lower threshold recruitment and an increased firing frequency of the nerve impulses (Stolen et al. 2005). All four, maximal strength, rate of force development (Reilly and Thomas, 1976), decision

making and action recognizing are important factors in successful soccer performance because of the demands apparent of the game.

4.6 Neuromuscular System

In the development of muscular strength two mechanisms are responsible, muscular hypertrophy and neural adaptations. It is well known that muscular hypertrophy occurs as an increase in the myofibril content of the muscle fibers (Wilmore and Costill, 1994). A strong correlation exists between the cross sectional area of the muscle and maximal force development. However in certain sports, including soccer, increased hypertrophy in a great degree is not desirable because players will have to carry greater body mass throughout the game leading to higher energy expenditure. Furthermore, increased muscle mass does not necessarily leads to high velocity muscle strength (Tesch and Larson, 1982).

The contraction speed is depending on the type of muscle fibers involved and the neural adaptations. The most complex the action performed the more important the latter becomes (Aagaard, 2003; Gabriel et al. 2006). The term neural adaptations describes of a number of factors: selective activation of motor units and muscles, ballistic contractions, synchronization, increased recruitment of motor units, firing frequency, reflex potential and co-contractions of antagonists (Aagaard, 2003; Gabriel et al. 2006). Noticeably, one of the most important factors in order to improve power and strength is to increase neuromuscular coordination. In that way players will be able to coordinate other muscle groups involved in a specific action such as the muscles that stabilize the body (Rutherford and Jones, 1986; Hoff and Helgerud, 2004). Maximal force development depends on the number of active motor units. In a

maximal voluntary contraction the small oxidative fibers are recruited first (Freund, 1983) followed by the fastest glycolytic fibers.

5. Sex Steroids and Exercise

The association between sex steroids and exercise is complex and bidirectional. Indeed, it is has been reported that exercise training might affect endogenous sex steroids production and that this suggested effect depends on several factors such as age, the training status of the participants, and the mode, intensity, and volume of the employed exercise activity (Tremplay et al. 2004; Kraemer and Rogol, 2006; Borer, 2003). In addition, several studies support the hypothesis that sex steroids may also play a regulatory role in the ability to perform efficiently during exercise (Kraemer and Rogol, 2006; Borer, 2003). The following chapters will review the bidirectional association between sex steroids serum levels and exercise performance.

5.1. Androgens

5.1.1. Testosterone

Androgens have gained considerable attention because of their influence in several physiological systems in the body. The majority of the evidence exists regarding the primary male hormone, testosterone. The proposed beneficial effects are characterized direct or indirect.

5.1.1.1. Proposed Direct Effects of testosterone

Well conducted studies have revealed that testosterone affects a variety of parameters that interfere with the ability to perform efficiently during exercise. Indeed, it is widely accepted that testosterone has a direct effect on muscle growth (Bhasin et al. 1996; Cardinale and Stone, 2006). Scientific evidence suggest that the testosterone increases muscle mass by increasing muscle protein synthesis (Ferrando et al. 1998; Griggs et al. 1989). In addition, testosterone has been found to influence skeletal muscle by altering skeletal muscle morphology. More specifically, it has been

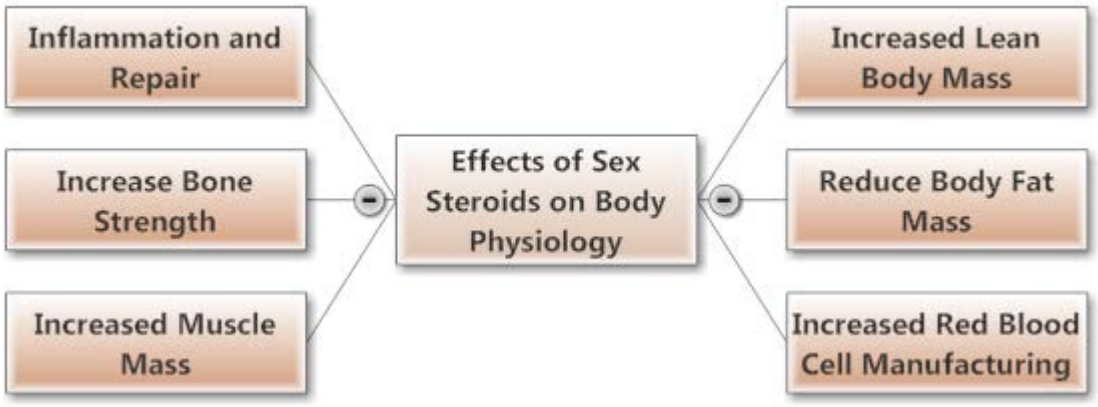


Figure 6. Proposed Effects of Sex Steroids on Body Physiology

observed that testosterone is altering the cross-sectional area of both type I and II muscle fibers. (Sinha-Hikim et al. 2002). Evidence from animal based studies indicate that testosterone may also have a strong influence on muscle skeletal muscle excitation-contraction coupling and a phenotypiation on fast fibers. Based on the aforementioned suggestions and the findings of recent studies that show a strong association between testosterone and explosive exercise performance (Bleisch et al. 1982; Cardinale and Stone, 2006), it could be suggested that testosterone might play an important role in the development of a high rate of force in muscular activity with a short duration contraction time (Bosco et al. 1996a; Bosco et al. 1996b; Ferrando et al. 1998). Taking into account the latter hypothesis and the well documented findings that there is a significant correlation of muscle mass with muscle strength and explosive type human movements (such as jumps, spring, acceleration, change of direction), which are of vital importance for most athletic events (Aagaard, & Andersen, 1998; Perez-Gomez et al. 2008) it has been suggested that testosterone may directly enhance exercise performance, especially of explosive type human movements. This is further supported by the observation that there is a significant relationship between testosterone levels, vertical jump height, and power output in professional soccer players, sprinters, and elite team athletes, suggesting that this

androgen plays a regulatory role on the development of type II muscle fibers (Bosco et al. 1996a, 1996b). Interestingly, the influence of testosterone seems to extend also to neuromuscular transmission, indicating that the effects of this androgen on athletic performance may involve alterations not only within the muscle (Leslie et al. 1991; Blanco et al. 1997). Further support to this hypothesis in coming from the evidence showing that testosterone has been found to influence the number of acetylcholine receptors at the neuromuscular function (Bleisch et al. 1982). Regarding neuromuscular coordination, a variety of studies has observed that both vertical jump and sprint performance are influenced by neuromuscular features (Missitzi et al. 2004; Pereira et al. 2008) and that optimal neuromuscular coordination is essential for these types of activities (Ecker, 1996; Dhesi et al. 2004). Furthermore, it has been documented that strength gains without accompanied by muscle hypertrophy are a neural adaptation due to an increase in the neuromuscular coordination of the muscles involved (Tudor, 1992). Therefore, the latter evidence clearly state that testosterone may play a crucial role in exercise performance by affecting the neuromuscular coordination of the muscle acting in the specific tasks.

5.1.1.2 Proposed indirect effects of testosterone

Apart from the above mentioned **direct** effects of testosterone, there also some evidence suggesting that it may influence performance by some **indirect mechanisms**. More specifically, the well documented relationship between testosterone and aggression suggest that there is an effect on muscle function and behavior mediated by alterations in its levels (Montoya et al. 2012). Based on these findings it may be speculated that since increased testosterone levels may determine high levels of aggressiveness, a facilitation of neural input during maximal explosive effort may occur. In addition it has been reported that testosterone is an important

determinant of regional fat distribution (Bhasin et al. 2003) and testosterone decline is related with fat mass increase (De Maddalena et al. 2011; Bhasin et al. 2001). The significance of this mechanism relies on the well documented finding that both neuromuscular performance capacity and aerobic endurance are inversely related with body fat percentage (Caldwell and Peters, 2009). Therefore, a possible enhanced body composition status by testosterone will be in turn translated with improved performance capacity.

Another indirect mechanism could be based on the suggested anticatabolic effect of testosterone and its interaction with the androgen receptors in the subsequent phase of recovery (Knorving et al. 2006). Testosterone appears to impact immune function, as cells of the immune system including macrophages, lymphocytes and vascular smooth muscle cells all possess androgen receptors (Fragala et al. 2011) Evidence of this interaction is apparent in a biofeedback loop where testosterone inhibits the secretion of specific cytokines, while specific cytokines appear to impair synthesis and release of testosterone (Fragala et al. 2011). Testosterone is capable to inhibit the anticatabolic effects of glucocortioids as well as reducing the suppression of muscle protein synthesis exerted by them. Depending on the amount of glucocortioid receptors occupied by testosterone there is a proportional decrease of the catabolic hormones actions (Mayer and Rosen, 1977). Therefore, reduced catabolism, positively altered protein muscle rebuilt, and enhanced recovery from exercise stress after exercise training sessions and/or competitions via increased testosterone levels, could provide an additional beneficial effect on exercise performance.

Testosterone might also provide endurance advantages though alterations in energy availability and oxygen carrying capacity of the blood. Indeed, testosterone has been reported to boost RBC production by stimulation of EPO production, synthesis and

secretion, direct stimulation of bone marrow hematopoiesis, and stimulation of iron incorporation into RBCs. Also it has been observed that androgen supplementation increase Hct and hemoglobin (Neff et al. 1981; Palacios et al. 1983). Furthermore, there is evidence indicating an androgen related left ventricular hypertrophy in athletic population (strength athletes) (McKillop et al. 1986) which is leading to increased strength of the cardiac muscle and consequently enhanced cardiac output during exercise. In regard to energy availability, an early study has reported a preservational effect of testosterone on glycogen stores during endurance exercise suggesting an enhanced lipolysis and usage of lipids during this kind of activities (Guezennec et al. 1984). Indeed, Xu et al. (1990) observed that testosterone stimulated catecholamine-induced lipolysis in a dose-dependent manner, including physiological concentrations. Enhanced lipolysis would hypothetically result to subsequently carbohydrate sparing, which is a beneficial adaptation of endurance type activities, since carbohydrate sparing ensures energy provision nearby the end of this kind of activities. Furthermore, testosterone has been found to activate glucose metabolism-related signaling pathway in skeletal muscle (Sato et al. 2008).

Lastly, testosterone may also influence muscular performance due to its observed linear relationship with Ca^{2+} metabolism. Indeed, since Calcium is an important metabolite in muscle contraction, and testosterone stimulates intracellular *calcium* release (Kadi, 2003) any alterations in Ca^{2+} release could affect exercise performance in a similar manner.

5.1.2 DHEAS and Δ4-Androstenedione

Although there are enough evidence to support that the main androgen testosterone may indeed affect exercise performance via several direct and indirect mechanism, scarce evidence exist regarding its precursors DHEAS and Δ4-Androstenedione.

In regard to DHEAS there are some evidence indicating that its levels are linearly related with muscle strength in elderly individuals. Indeed, in men aged 60-79 years, circulating DHEAS is an independent factor, correlatingwithmuscle strength and calf muscle area (Valenti et al. 2004). Furthermore, DHEA sulfate levels are associated with increased physical performance in this type of populations (O'Donnell et al. 2006). In addition, the elderly individuals with low DHEAS levels appeared to be poor responders in terms of exercise training adaptations indicating that optimal levels of DHEAS are needed in order to gain optimal exercise adaptations (Huang et al. 2006). However, all these evidence are referred to elderly individuals;no studies, to the best of our knowledge, have shown any direct effect of DHEAS on exercise performance capacity in athletes.

Another mechanism via which DHEAS could affect exercise performance isthe suggested neuroprotection effects on neurite growth, neuroneogenesis and neural survival protection against apoptosis and antagonistic effects on oxidants and glucocorticoids. DHEAS has been observed to have anti-inflammatory and immunomodulating effects, and influence catecholamine synthesis and secretion (Stárka et al. 2014). Therefore, apart from and optimal function of the nervous systems which is an essential regulatory parameters for exercise performance, DHEAS might also play a key role in muscle damage, acting as antioxidant and muscle repair, via its anticatabolic effects, which could in turn be translated with enhanced recovery after exercise training stress. In addition, DHEAS levels have

been linked with optimal body composition status (Hernandez-Morante et al. 2008) and low DHEAS (Ho et al. 2008) with increased waist-to-hip ratio. This being the case, DHEAS could affect exercise performance capacity by alterations in body weight and body fat (Ozkan et al, 2012).

Furthermore, DHEAS could also affect muscle metabolism during exercise. Hernandez-Morante et al. (2008) demonstrated for the first time in vitro that DHEAS stimulated lipolysis in 85 obese patients, preferably in subcutaneous fat in women and in visceral fat in men. Lastly, DHEAS could also affect cognitive function (Frye and Lacey, 1999). Indeed, Moffat et al. (2000) determined endogenous levels of DHEAS and related them to quantified cognitive status, which could be important parameter in several sports.

In regard to Δ4-androstenedione no evidence exists examining the association between its endogenous levels and exercise performance parameters. However a recent review paper summarized several studies that examined the effect of Δ4-androstenedione on strength training (Brown et al. 2006). The only available studies are concerning supplementation that is supposed to beneficially affect performance via several mechanisms, muscle strength, and fat mass reduction (Leder et al. 2000). At dosages of 50 mg or 100 mg per day, Δ4-androstenedione had no effect on muscle strength or size, or on body fat levels. One study used a daily dosage of 300 mg of Δ4-androstenedione combined with several other supplements, and also found no increase in strength when compared to a control group that did not take the supplements. Therefore, Δ4-androstenedione most probable could inducea beneficial effect on athletic population by an indirect effect i.e. enhancement on testosterone production of preservation of its levels (Leder et al. 2000).

5.2 Estrogen

The interest regarding estrogens response to exercise training has been emerged due to the suggestion that these sex steroids may have a beneficial effect on muscle damage, inflammation, and repair. Indeed, estrogens have suggested to have a protective role in muscle during exercise stress (Tiidus, 2003). More specifically, estrogen, and primarily estradiol, has been reported to have a significant influence on muscle membrane stability and possibly on diminishing exercise-induced muscle damage. Indeed, estradiol has been reported to decrease post-exercise membrane disruption and structural damage (Bär et al. 1988). It is well documented that certain exercise protocols that induce damage to skeletal muscle result in an efflux of several intramuscular proteins and enzymes into circulation, such as creatine kinase (CPK), lactate dehydrogenase, aspartate aminotransferase and myoglobin, (Tarnopolsky, 1999). In particular, CPK is the most commonly measured circulating marker of muscle damage (Mair et al. 1992; Nosaka et al. 1992). Under resting conditions, women have lower CPK activity in the blood as compared with males. Furthermore, human studies have observed that females have significantly attenuated muscle CPK loss and moreover lower circulating CPK levels after exercise induced muscle damage compared to males (Clarkson 1999; Stupka et al. 2000). This attenuation has often been attributed to a protective role that estradiol plays as an antioxidant and membrane stabilizer during exercise with a relatively high oxidative stress (Fragala et al. 2011). In addition, there is sufficient evidence to support the hypotheses that estrogen act as an antioxidant and membrane stabilizer in muscle (Kendall and Eston, 2002). According to a number of studies estrogen can be directly incorporated into cell membranes, in a similar way as cholesterol, and may act in a same manner in order to optimize membrane fluidity and polyunsaturated fatty acid arrangement. By

this mechanism estrogen seems to have the ability to reduce the magnitude of muscle membrane disruption after injury, by a direct interaction with membrane components (Tiidus, 2003).

Estradiol has also been shown to have an anti-inflammatory effect (Xing et al. 2004). In fact, systemic administration of estradiol has been shown to attenuate both expression of inflammatory mediators and infiltration of leukocytes following muscular injury (Xing et al. 2004). Leycocytes are playing an important role in the inflammatory responses of the muscle and on the repair mechanism after exercise induced muscle damage. Their physiological actions of neutrophills is the infiltration of muscle almost immediately after muscle damage with a main role on removal of damaged tissue. However, neutrophills have been also found to be responsible for further collateral muscle damage to healthy muscle tissue during the initial inflammatory period, since they are also responsible for cytokine production (Kendall and Eston, 2002). Based on this evidence it has been hypothesized limited leycocytes infiltration into the muscle tissue may reduced further inflammatory related damage which will thus enhance healing (Tiidus, 1999), with a consequentincreased muscle repair. However, more evidence is needed to support this hypothesis. The physiological consequences of estrogenic attenuation of postdamage muscle leukocyte infiltration have not been yet clearly established, but may theoretically inhibit or enhance muscle recovery. In conclusion, the potential for estrogen to influence muscle damage and repair may have important implications on exercise performance capacity, and these implications could be of a greater magnitude especially in top level competitions, where fast and optimum recovery from exercise stress is one of the major parameters in order to perform adequately in the forthcoming competition.

Apart for the possible effects of estrogen on muscle damage, inflammation and repair, another indirect potential mechanism that could result in beneficially altered exercise performance is the estrogen change of muscular glycogen metabolism (Kendrick et al. 1985). These data indicate that estradiol replacement spares tissue glycogen during submaximal exercise. These glycogen sparing effects have contributed to the significant improvements in exercise performance observed in a study by Kendrick et al. (1985). Furthermore, it has been suggested that estrogen control lipolysis by up-regulating alpha2A-adrenergic receptors directly in human adipose tissue through the estrogen receptor alpha (Pedersen et al. 2004). Therefore, based on these available evidence estrogens could affect exercise performance capacity, by regulating muscle metabolism.

Summing up, the available evidence indicate that alterations in sex steroids endogenous levels may affect performance via several physiological pathways. More specifically, according to the literature, the expected beneficial effects could be increased muscle mass, increased neuromuscular performance (i.e. explosive type human movements such as jumps and sprint, increased aerobic capacity due to a beneficially effect on erythropoeisis, increased aerobic and anaerobic endurance due to an effect on muscle metabolism and a decrease in body fat, increased recovery from exercise training stress due to the anti-inflammatory effects of sex steroids, and finally reduction in the possibility of injuries in the musculoskeletal system as a result of increased strength, enhance recovery from exercise and their proposed effect on bone metabolism in favor of bone formation (Banfi et al. 2010).

6. Effects of Exercise on Sex Steroid levels

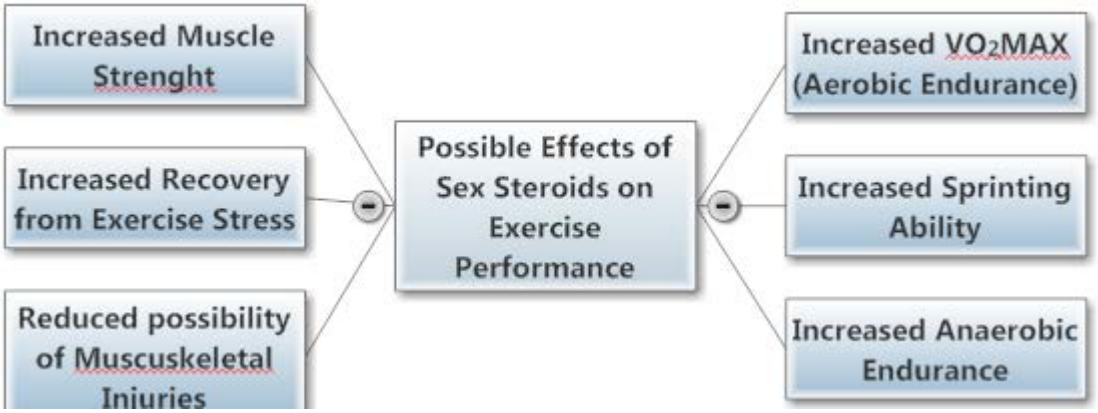


Figure 7. Suggested relationships between Sex Steroids and Exercise Performance

Despite the increased interest regarding the effects of exercise training on sex steroids the vast majority of the available studies have examined the responses of these steroids under either endurance or resistance/strength sport activities. Furthermore scarce evidence exists examining their responses in team sports, including soccer. However, since all team sports are mixed in nature combining aerobic endurance, anaerobic endurance, and resistance (strength)-power training, the available evidence regarding sex steroids response to endurance and resistance training periods, studies provide valuable data in order to understand the responses of these hormones on these sports. It should be mentioned that a number of studies tried to evaluate only the differences in adrenal and gonadal sex steroids levels between trained and/or physically active individuals versus sedentary controls.

6.1 Effects of exercise training on Androgens

6.1.1 Endurance Exercise and Androgen levels

The last decades a great number of studies have focused on the chronic effects of endurance exercise on androgen levels in males. Several researchers have examined the possible differences in androgen basal levels between endurance trained and inactive individual, and furthermore the effects of different training endurance

protocols on these sex steroids. It should be noted that the majority of the available studies has examined the main androgen testosterone.

6.1.1.1 Testosterone levels in trained versus untrained individuals

It has been suggested that endurance trained athletes might exhibit some alterations in basal testosterone levels. Indeed, the published bibliography indicate that this kind of activities compromise the hypothalamic–pituitary–gonadal axis (HPG axis) in regard to this androgen.

The relationship of endurance trained subjects compare to untrained individuals has been examined by a large number of retrospective and prospective studies: The work by Wheeler et al. (1984) has been considered the landmark study. It was observed that resting testosterone levels of endurance trained men were significantly lower in respect to untrained individuals. In accordance were the findings of Arce and co-workers (1993). Evaluation of the obtained results showed that participants on endurance exercise training had lower levels of both total and free testosterone. Furthermore this was related to subclinical modifications in semen characteristics. In addition, Hackney et al. (1988) examined the resting reproductive hormonal profiles of select athletic groups. Athletes that were exposed to chronic endurance training had lower basal testosterone levels. Interestingly, Ayers et al. (1985) although reported lower levels of total testosterone for endurance trained subjects against sedentary individuals, failed to show any difference for free testosterone levels. A recent study by Izquierdo et al. (2003) found that basal testosterone levels were significantly lower in endurance trained cyclists compared to weight lifters or sedentary controls. The authors suggested that testosterone levels are lower in sports characterized by high endurance requirement. In agreement are the findings of several studies using serial

sampling, Duclos et al. (1996) studied the long term effects of endurance exercise on the function of gonadotropic axis in marathon runners and untrained individuals for 5 consecutive days. Testosterone concentration had similar responses as in the above mentioned studies, inisolated blood samples. Similarly, another study that has used serial sampling reported decreased testosterone basal levels, giving further support to these findings (Mc Coll et al. 1989). In the above mentioned studies, the reduction in resting testosterone levels appeared to be in between 40-80% compared to untrained individuals. According to Wheeler et al (1986) and Mac Connie and associates (1986), it appears to be a training threshold of about 65 km/week above which testosterone levels are reduced. Duclos and co-workers further support the threshold hypotheses, suggesting being between 60 to 80 Km/week.

The magnitude of testosterone levels decrements have been reported to be 60-85% to that of physically inactive individuals (Ayers et al. 1988; Duclos et al. 1996; MacConnie et al. 1986). Even if it is well documented that long term exposure to endurance training leads to significantly decreased basal testosterone levels, this alterations are within the physiological levels for adult males, and above the values that are considered as subclinical levels (Vaamonde et al. 2005; De Souza and Miller. 1997).

6.1.1.2 Exersixe Training Periods and Testosterone levels

A large number of studies tried to evaluate the response of testosterone under several periods of endurance exercise training. Wheeler et al. (1991) examined how 6 months of endurance training may affect its levels, in male athletes. Over the 6 months of the training period, the subjects increased the weekly running mileage to an average of 56 Km/week;total testosterone decreased significantly. In another study Urhausen and

associates (1987) examined the behavior of serum testosterone over a 7-week period during the rowing competition season. Blood collection throughout the study revealed a continuous decrease in testosterone levels. Interestingly, a follow up regenerative week halted this reduction. The authors concluded that periods of intense physical stress leads to an increase in catabolic activities, and that regenerative phases tend to reduce this activity. Similar results in response to testosterone were reported by Lehmann et al (1993). The authors showed that that a 6-week period, consisted of 6 times/week endurance bicycle training decreased free testosterone levels. Decreased basal testosterone levels have been also reported using different endurance exercise protocols such as military training (Opstad, 1992), swimming (Bonifazi et al. 1995), running (De Souza et al. 1994) and cycling (Lucia et al. 2001). Izquierdo et al (2003) suggested that testosterone levels are lower in sports characterized by high endurance requirement. Notably, Fellman and co-workers (1985) have proposed that the lower testosterone levels in these athletes are a positive adaptation. An increased anabolic effect due to elevated testosterone levels could develop excess body muscle mass resulting to taxed O_2 delivery system during prolonged exercise which could in turn limit endurance performance.

However, the above mentioned findings are not universal. Lucia et al. (1996) examined the relationship of endurance exercise and sex hormones. Professional cyclists, recreational marathon runners and sedentary controls were recruited in this study. Measurement of resting testosterone levels throughout a competitive season observed no significant differences. Similar observations were reported by Jensen and associates (1995). The authors examined the effects of endurance training on hormonal profile over one year in 24 healthy marathon runners. Although some hormonal alterations were evident, testosterone levels were not altered significantly.

The findings of Mujika and associates (1996) give further support to the latter observations. No changes in testosterone concentration of competitive swimmers were found after 12 months of training and competition. Using an exercise training protocol of 12 weeks training/ 6d/week, Bell et al. (2000) also failed to show any effect on testosterone levels. In accordance are the findings of Fellman et al. (1985). The authors investigated effects of endurance training on the androgenic response to exercise training in males. No significant differences were evident for testosterone resting values. Notably, Daly et al. (2005) have suggested that the resting total and free testosterone values may be reduced with training but not in all individuals.

Adding to the discrepancies in the literature, a study on prepubescent boys a correlation between indices of physical performance (endurance, strength and speed) and the increase in testosterone was described over one year (Mero et al. 1990).

Taking into account the aforementioned evidence in seems that testosterone response to endurance exercise in depending on several factors. Indeed, its response seems to be dependent on the training status and the age of the participants, the specific training regimen used, and the duration of the study, as well as the level of training stimulus administered, and the volume (as per the suggestion for 60km/week), intensity and duration of training load employed. Also endurance trained males with lower testosterone may display different testosterone response that males with normal testosterone levels.

Furthermore, it also should taken under consideration the hypotheses that circulating testosterone levels, may do not really indicate the endogenous production since there is a possibility in these studies the measured levels of testosterone were also in that magnitude as a result of peripheral extrasplanchnic utilization by the tissues and/or the

target organs [this latter change may be offset by an increased uptake by peripheral target tissue (i.e. skeletal muscle)].

In general it seems that endurance exercise training might have significant effects upon the major male reproductive hormone, testosterone. A growing body of evidence suggests that rest testosterone levels are lowered in endurance-trained males. The mechanism of this decreaseis currently unclear, but may be related to dysfunction within the HPG axis. The effects of exercise to significantly reduce testosterone levels as well as disrupt the HPG axis may require months or years of endurance exercise training. Potentially, the lowered testosterone levels of the endurance-trained male could disrupt their anabolic and androgenic functions – processes (Hackney et al. 1998; Arce and DeSouza, 1993). Presently, however, there are only limited findings to indicate that any consistent disruption of the testosterone-dependent processes in the male occurs due to endurance training (Hackney et al. 1998).

6.1.1.3 DHEAS levels and endurance exercise

The results are more mixed for DHEAS, although more studies have investigated the repercussions of chronic exercise on DHEAS basal concentrations, the results appear to be contradictory. Most have shown no significant effects of endurance exercise training in healthy or pathological subjects (i.e., with coronary artery disease, rheumatoid arthritis, and type 2 diabetes) irrespective of gender, age or the type of training (Colomp et al. 2014). These studies were conducted with young and older subjects, males and females, and over periods ranging from eight to 24 weeks of training with a wide variety of endurance protocols. In agreement are the findings of a prospective study Houmard and co-workers (1994) reported that 14 weeks of moderate intensity endurance exercise in middle-aged men did not significantly alter

DHEAS concentration. Similarly, Kraemer et al. (2001) found no significant change in DHEAS levels during an athletic running season. In another study, Milani and associates (1995) examined DHEAS concentration in 96 males with coronary artery disease after they were exposed to a period of physical activity. Although exercise capacity increased 43% no significant changes were observed for DHEAS basal concentration. The authors concluded that exercise training alone has no significant impact on DHEAS, thereby strengthening the suggested role of behavioral changes in modifying this hormone. However 8 weeks of similar kinds of physicall activity in middle aged weight stable type II diabetic men resulted in a 36% increase in DHEAS basal values (Milani et al. 1995). Similar finding were reported from a study by Opstad et al (1992). The authors observed the responses of androgenic hormones during a 6 day military endurance training course with sleep and energy deficiency. Physical activity was correnspondingto 35% VO_2max. To avoid the effects of acute physical exercise on hormonal plasma levels, the cadets had only light physical activity for 1-2 hours before sampling. Analysis of the results showed that there was a decrease in androgens concentration, except from DHEAS which exhibited an increase in its resting levels. In agreement are the findings of Vaamonde et al. (2005) in physically active men with no infertility or hypothalamic pituitary problems. The exercise protocol included exhaustive endurance exercise sessions, 4 times/week, on a cycloergometer for a 2-week period. After the completion of the experimental period the authors observed that DHEAS basal levels were significantly increased.

The effects of endurance physicall activity on DHEAS concentration was addressed by Ravaglia et al. (2001) in a cross-sectional study. The participants were active and sedentary elderly men. Physical activity positively associated with DHEAS resting levels. Similarly findings were reported by Keizer and associates(1989). The authors

examined the changes in resting DHEAS levels in previously untrained males preparing for a marathon. The duration of the trainings were 18 to 20 months and was divided in three periods of 6, 5, and 7 months. The first, second, and third periods were concluded with a 15, 25, 42 km road race respectively. The competitive distance always exceeded the maximal distance covered in any previous training session. The plasma levels of DHEAS was increased in all three contests and remained elevated for subsequent 1-2 days. DHEAS increments were greater after the marathon (42 km). The authors hypothesized that training may increase the adrenal secretion of androgen. However, since blood samples were obtained immediately after the contests, it is contradictory whether these finding are suggested to the be clear indicators of the DHEAS response to long period exercise training stress and not the results of acute exercise on DHEAS levels.

Adding to the discrepancies in the literature, Gomez-Merino et al. (2005) reported a significant decrease in DHEAS and testosterone after five days of intense military training in young male soldiers. In order to explain their findings the authors suggested that this finding was most probable a result of the extreme military training stress during the study. Consitt et al. (2002) noted that, the anabolic hormones including DHEAS appeared to decline slightly with excessive endurance training. The observed decrease in the study of Gomez-Merino et al. (2005) was attributed to a response to chronic stressors. The authors hypothesized that this decrease in exercise DHEAS response may reflect excessive training, similar to the decrease in basal DHEAS concentrations shown by Flynn et al. (1997). However, in order to support the latter hypotheses further studies are necessary to examine the DHEAS exercise response to endurance exercise as a possible marker of overreaching and/or overtraining in athletes.

The question whether regular exercise alters basal DHEAS cannot be answered at this time, given the contradictory results. The discrepancies observed in the published bibliography could be related to the initial physical aptitude of the subjects or/and the short duration of the training program. It may be needed long period of training, at least one year to increase the basal values. It should be noted that in most studies 8 to 24 weeks of exercise training significantly improved physical performance capacity, and body composition (Collomp et al. 2014) despite the lack of change in DHEAS. Another hypothesis responsible for the observed discrepancies in the literature is that of high and low responders to exercise. It has been observed recently reportedthat the individuals with low DHEAS levels appeared to be poor responders in terms of exercise training adaptations whereas individuals with high DHEAS levels have been found with improved reactive time and locomotive function in response to training (Huang et al. 2006).

6.1.1.4 Δ4 – androstenedione and endurance exercise

The existing literature concerning the effects of short or long term exercise training periods on Δ4-androstenedione levels is limited. Only two studies, to the knowledge of the author, tried to give some light to the lack of evidence for the effects of chronic exercise on Δ4-androstenedione levels. Fellmann et al. (1985) failed to show any effect on Δ4-Androstenedione concentration after 40-week training program on a bicycle ergometer. These results suggest that long-term training does not enhances adrenal function in regard to Δ4-Androstenedione. Similarly, Kourkoulias et al. (2008) reported that a two week period which included a marathon race in between failed to alter Δ4-Androstenedione levels. However, these results should be taken into account with the considerable percussions when related to competitive athletes, because of the difference in age and the fact that there was no definition of the

magnitude (intensity, volume, duration) of the endurance exercise training performed by the subjects.

6.1.1.5 **3a - androstenadiol glucuronide and endurance exercise**

To date there is no study examining the effects of a long period of endurance exercise on 3a-androstenadiol glucuronide (3a Diol G) and athletic population. The only recorded relationship between 3a Diol G and long term exercise comes from a recent study by Hawkins et al. (2008). The authors examined the relationship of this metabolite and exercise during a long period of physical activity in physically inactive middle aged to older men. Subjects participated in a 12 month program of moderate to rigorous exercise training. The authors reported that exercise training did not induce any alteration on 3a-diol G levels. However in regard to competitive athletes these findings should be considered with caution. Differences that exist in training status and age between these two populations and taking into account the fact that competitive athletes participate in exercise training characterized by great intensities and volumes, could lead to different androgen responses, including 3a-diol G, to exercise training. Therefore, although this investigation gives some evidence of the behavior of 3a-diol G to physical activity, these results can not directly related to athletes participating in competitive sports.

6.1.2 Strength training and sex steroids

6.1.2.1 Resistance trained vs Sedentary Individuals

The effects of resistance training seem to differ from the outcome of endurance training not only by changes at the physiological and biochemical adaptations to training, but also to hormonal responses (Kraemer and Dogol, 2005). Kraemer and

associates (1999) examined the effects of heavy resistance exercise on hormonal response patterns in young vs older men. One of the findings of the study was that resistance trained subjects seems to have higher basal testosterone levels compared to sedentary controls. In agreement are the findings of Hakkinen and co-workers (1998). It was reported that resistance trained subjects have higher testosterone levels compared to physically inactive individuals. However, contradictory findings were observed by a cross-sectional study (Arce et al. 1993). The authors examined the effects of two forms of trainings (endurance and resistance) on hormonal profile. Compared with sedentary controls, not only endurance-trained but also resistance-trained athletes presented with significantly lower levels of total and free testosterone. The authors concluded that both endurance and resistance training modify the male reproductive hormone profile in a similar manner. Further contradiction comes from a study on elite sprinters (Grandys et al. 2011). In this athletic population usually, training designed to increase muscular strength, speed, and power which has been suggested to increase gonadal hormone concentrations (Grandys et al. 2011). Furthermore, it is confirmed by scientific evidence that athletes with higher explosive strength and better sprint running performance have a higher basal level of testosterone (Bosco et al. 1996; Cardinale and Stone, 2006.). However, the highly trained track and field sprinters showed no differences in the basal TT, basal FT and bioavailable testosterone concentrations, compared with untrained, physically active men (Grandys et al. 2011) .The results of this study were unexpected. Indeed, the

Table 1. Trained individuals compared to sedentary individuals and Sex Steroids Levels

Study	Physical activity characteristics	No of subjects/Characteristics	Sex Steroids	Observations	Comments
Wheeler et al. 1984	Endurance athletes (Running)	31 men running at least 64 km each week and 18 sedentary controls	TT,FT,LH,FSH,PRL	TT↓ than sedentary PRL↓ than sedentary, No difference FT, LH, FSH	The lowered TT and PRL levels parallel the changes reported in women runners.
Arce et al. 1992	Endurance training (running) and resistance training (weight lifting)	28 healthy male volunteers, (10 endurance-trained runners, 8 resistance-trained weight-lifters, and 10 sedentary controls).	TT,FT,LH,FSH,PRL, E2	Both endurance trained and strength trained had ↓TT and↓FT values vs controls. No differences were observed for LH,FSH,PRL, and E2	Only endurance training, in the form of running, is associated with subclinical modifications in semen characteristics.
Hackney et al. 1998	endurance trained (ET) and sedentary (SED) men	ET men (n = 53) who had been involved with chronic endurance exercise training for > or = 5 years. SED men (n = 35) were selected of comparable ages and the fact that they had done no formal exercise training	TT,FT,LH,,PRL	ET men had lowered basal T and fT levels vs controls, no differences were found for LH,PRL	this suppression may be related to an alteration in the hypothalamic-pituitary-testicular regulatory axis since the LH of the ET was not elevated
Ayers et al. 1985	Endurance athletes (marathon runners)	20 male marathon athletes	TT, FT	TT was significantly decreased in 14 of 20 subjects, FT was within the normal range in the majority	From these data the authors concluded that vigorous endurance training may be associated with significantly decreased TT values
Wheeler et al. 1986	Endurance running Sedentary individuals	31 high mileage, 18 low mileage runners and 18 non-running controls.	TT, FT, PRL, LH, FSH	TT, FT, and PRL levels were significantly lower in high mileage runners than controls. LH and FSH	

Table 1 continued

				were not significantly different.	
MacConnie et al. 1986	Marathon runners	six highly trained male marathon runners compared with healthy controls		The mean (+/- SEM) frequency of spontaneous LH pulses was diminished in the runners, as compared with healthy controls TT levels were similar in the two groups	
De Souza et al. 1994	Running	11 high mileage runners (HR) (9 moderate mileage runners (MR) and 10 sedentary controls (SC) of similar age	TT, FT, LH, FSH, PRL	TT and FT were significantly lower in HR vs MR and SC. No differences were found in LH, FSH, and PRL among the three groups	
Ravaglia et al. 2001	Cycling	Twenty four middle-aged (57.4+/-4.7 years) and 24 elderly (68.3+/-2.6 years) physically active men who in the past 10 years had been regularly bicycling during leisure time were compared with 24 middle-aged (57.9+/-4.0 years) and 24 elderly (67.2+/-1.7 years) sedentary men.	DHEAS, FT	physically active men had on average higher DHEAS than sedentary men. No differences were observed for TT	in aging men, regular moderate p hysical activity is associated with higher levels of DHEAS levels
Arce et al. 1993	endurance training (running) and resistance training (weight lifting)	10 endurance-trained runners, 8 resistance-trained weight-lifters, and 10 sedentary controls	TT, FT, E2,LH,FSH, PRL	Compared with sedentary controls, endurance-trained and resistance-trained athletes presented with significantly lower levels of TT and FT. No differences were observed for E2,LH,FSH, PRL .	
Grandys et al. 2011	high and top-class	16 sprinters and 15untrained men	TT, FT	No differences were observed for TT	

Table 1 continued

	track and field sprinters untrained men			and FT levels between the groups. TT levels were significantly higher at a period of the annual training period. of low-intensity training than during heavy sprint specific training period.	
Cangemi et al. 2010	Endurance trained and sedentary individuals	24 men who had been practicing caloric restriction (CR) 24 age- and body fat-matched endurance runners (EX), and 24 age-matched sedentary controls eating Western diets	TT, DHEAS, E2	TT was significantly lower in the CR group vs CX and SD groups DHEAS no difference between groups E2 was significantly lower in the CR and EX groups than in the WD group	
Maimoun et al. 2003	Cycling, Triathlon, Swimming	cyclists (CY; n = 11), triathletes (TR; n = 14) and swimmers (SW; n = 13) and compared with less active controls (n = 10).	TT, LH	TT was lower in CY and TR, No alteration was evident for LH	
Cooper et al. 1998	Endurance training	15 Masters runners and 15 minimally exercising men (MEM) aged 60-70 years	TT, FT, E2	TT ↑ in runners No differences for FT, E2	

Abbreviations:TT=total-testosterone; FT=free-testosterone; DHEAS=dehydroepiandrosterone-sulfat; E2=estradio; LH=luteinizing hormone; FSH=follicle-stimulating hormone, PRL=prolactin;↑=significant increase, -=no alteration;↓=significant decrease

high-level performance of these athletes may be partly explained by higher androgen concentrations, because testosterone exerts a strong anabolic effect on muscle tissue (Bhasin et al. 2001) and has a potent stimulatory effect on muscle hypertrophy (Sinha-Hikim et al. 2002). Accordingly, this notion constitutes a primary reason for the widespread anabolic–androgenic steroids abuse in athletes (Grandys et al. 2011).

These observed discrepancies could be attributed to the different training status of the participants, the employed training regime and possible alterations in volume and intensity prior to the measurements that are parameters to affect testosterone response to exercise and the training regimes used (Tremplay et al. 2004). Furthermore, these different findings could be related to the training phase of the annual training program that is to the exercise loads performed by the athletes in the training

6.1.2.2. Periods of resistance exercise training and testosterone

The available evidence regarding testosterone response to resistance training provides different findings. Studies have evaluated young individuals and have demonstrated an increase in androgenlevels at rest after strength training (Cadore et al. 2008). Nevertheless, other studies, including investigations involving middle-aged and elderly individuals, have found no differences in circulatory testosterone levels at rest after a period of training (Cadore et al. 2008). However, the literature contains very little information about possible endocrine adaptations to training in recreationally long-term strength-trained men (Cadore et al 2008).

Notably, the studies that have examined the relationship between the gonadal and adrenal androgens and resistance exercise androgens have focused on testosterone. However, the results do reveal great discrepancies. The hormonal responses to resistance training were evaluated in 11 college men who completed 12 weeks of high

volume resistance training by McCall et al. (1999). The authors observed that resting testosterone concentration was not affected by the experimental period. In another investigation, Ahtiainen and associates (2003) examined the hormonal adaptations of strength trained subjects and non-strength trained subjects during a 21 week strength training period. The strength training model used in this study also failed to reveal any significant alteration in basal testosterone levels. Similar findings were reported by Alen and colleagues (1988). The authors examined the effects of a 24 week of progressive strength training on hormonal profile. During the course of the training period no systematic change was found in serum total testosterone levels although there was a decreasing tendency in free testosterone levels. In a recent study, Sarah and associates (2006) examined the effects of an eight-week (3d/week) unilateral resistance exercise on hormonal status. The results showed that although the chosen training program induced local muscle hypertrophy to the exercising limb, this occurred in the absence of any changes in testosterone levels. Further confirmation to these findings derivesfrom several other studies that also failed to observe any alteration in total (Kraemer et al. 1995; Craig et al. 1989) but also free testosterone levels (Willoughby et al. 2014), after long or short periods of resistance training. It should be mentioned that in the study by Greg et al. (1989) after the completion of the 12 weeks of resistance training basal testosterone levels decreased although this decrease was not statistically significant.

In contrast, a number of studies reported increased testosterone levels as a response to resistance training. Izquierdo and associates (2003) examined the differential effects of 11-week strength training program leading to either repetition failure or to non-repetition failure on hormonal profile. The authors found the not to failure training program, which was reported to be a potential beneficial stimulus for improving

Table 2. Resistance training and Sex Steroids

Study	Physical activity characteristics	No of subjects/Characteristics	Training	Sex Steroids	Results
Grandys et al. 2011	high and top-class track and field sprinters untrained men	16 sprinters and 15untrained men	Habitual training	TT, FT	No differences were observed for TT and FT levels between the groups. TT levels were significantly higher at a period of the annual training period. of low-intensity training than during heavy sprint specific training period.
McCall et al. 1999	Resistance training	11 college men	12 weeks (33 sessions) of high volume resistance training	TT	TT-
Ahtiainen et al. 2003	Strength/resistance training	8 male strength athletes (SA) and 8 non-strength athletes (NA)	21-week strength-training period	TT, FT	TT-, FT-
Alen et al. 1988	strength training	21 strength-trained male subjects	24 weeks of progressive strength training and during a subsequent detraining period of 12 weeks.	TT, FT	TT-,FT- (in regard to FT there was a decreasing tendency towards the end of the study)
Craig et al. 1989	resistance strength training	9 elderly and 6 young males	12 weeks of weight training	TT	TT-
Willoughby et al. 2014	resistance training	Twenty apparently resistance-trained males	28 days of heavy resistance training, 4 times/wk	TT,FT, E2, LH, PRL	TT-,FT-, E2-, LH-, PRL-
Häkkinen et al. 1988	Weight lifting/resistance training	9 elite weight lifters	2 years of training	TT, LH, FSH	TT↑, LH↑, FSH↑

Table 2 continued

Busso et al. 1990	Resistance training	6 elite weight-lifters	1-year training period, 5d/week		TT levels were significantly elevated after 15 weeks, and despite the observed decline in its levels towards the end of the study they still remained significantly higher compared to baseline. The reductions in TT levels after week 15 were linearly correlated with fatigue
Häkkinen et al. 2000	resistance training	10 middle-aged men, 11 middle-aged women, 11 elderly men, and in 10 elderly women	6 months of heavy resistance training combined with explosive exercises	TT, FT, DHEAS	No changes in TT, FT, DHEAS
Hakkinen et al. 2002	Resistance training	11 elderly women and 10 elderly men	strength/power training twice a week for 24 weeks	TT, FT, DHEAS	No changes in TT, FT, DHEAS
Riechman et al. 2004	Resistance training	62 young men and 58 young women	10-wk resistance training program	DHEAS	DHEAS↑
Häkkinen et al. 1997	Resistance training	11 male elite weight lifters	1 year follow up study. Hormone concentrations were measured during seven test occasions. In addition, the same measurements were repeated three times during a 6-week period preceding the primary competition, which took place about 5 months after beginning of the follow-up	TT,LH	The primary findings were observed during the 6-week period from which the first 2 weeks of stressful training was associated with significant decreases in TT and increased LH levels
Willoughby and Leutholtz, 2013	Resistance training	20 Resistance-trained males	resistance trained 4 times/wk for 28 days	TT, FT, E2, LH	No changes in TT, FT, E2, and LH levels

Abbreviations:TT=total-testosterone; FT=free-testosterone; DHEAS=dehydroepiandrosterone-sulfat; E2=estradio; LH=luteinizing hormone; FSH=follicle-stimulating hormone, PRL=prolactin;↑=significant increase, -=no alteration;↓=significant decrease

strength and power, resulted to elevated basal testosterone levels. Similar observations come from a study by Staron et al. (1994). An eight-week progressive resistance training program (2days/week) managed to significantly increase basal testosterone concentration in male subjects. In agreement are the findings of Hakkinen et al. (1988). Long term neuromuscular and hormonal adaptations were studied in elite weight lifters for a 2-year period. It was reported that at the end of the study there was a significant increase in endogenous testosterone levels. The authors suggested that neuromuscular and hormonal adaptations to prolonged resistance training may influence the pituitary and possible the hypothalamic function, resulting to enhanced testosterone production. Further support comes from another long term study (Busso et al. 1990). The authors studied a system model providing an estimation of fatigue and fitness levels during a 1-year period in elite weight lifters, which were training 5 times/week. Serum testosterone levels were significantly elevated after 15 weeks, and despite the observed decline in its levels towards the end of the study they still remained significantly higher compared to baseline. Further affirmation comes from another study from the same laboratory (Kraemer et al. 1999). It was observed that a much shorter period of resistance training in untrained individuals, compared to the latter evidence, 8 and 10 weeks, manage to provide sufficient stimulus to enhance endogenous TT production. The authors also observed that significant positive alterations in basal testosterone levels were evident in the early phase of the employed strength training regimes. However, these finding should be taken under consideration since the observed increases in testosterone levels could be a result of the training status of the subjects. Indeed, it has be suggested that sedentary or non-resistance trained individuals are more sensitive to the initial bouts of resistance training in the early phase of strength training periods compared to well trained resistance athletes

(Hakkinen et al. 1988, Busso et al. 1990) and the endocrine mechanisms in these populations are more responsive to the workout stimulus.

In contrast to the aforementioned evidence, some studies failed to report any increases on TT levels after strength/resistance training (Adlercreutz et al. 1986, Kuopassalmi et al. 1980, Kuoppasalmi K and Adlercreutz, 1985). However, in these studies the reduction in TT levels were associated with the physiological stress of training, and specifically to fatigue, since an relationship has been associated between the variations of fatigue and testosterone (Busso et al. 1990). In addition, lower total and free testosterone levels after a training season of wrestlers have been reported (Kraemer and Rogol, 2006). However, the authors suggested that the observed reductions were associated with their practices to lose weight. During the period of this study, within the season period, dietary and fluid restriction was performed for further weight loss. Therefore, it was suggested that these reductions were not a result of training, but an effect of the excessive stress and the dietary modifications that has been indeed suggested to negatively affect testosterone levels (Fry and Kraemer 1997)

According to the aforementioned studies it seems that resistance exercise has the ability positively affect endogenous testosterone production, based on the training regime used, the training status of the subjects and the examined time-intervals. Furthermore, since resistance training has been suggested to increase muscle stereidogenesis (Vingren et al. 1985), the evidence that failed to observe an effect of resistance-strength training on testosterone levels may be a result of increased extrasplanchnic utilization and an increased uptake by peripheral target tissue (i.e. skeletal muscle) during this period by the target organs, and not an unaffected HPG axis. In addition the level of training stimulus administered and the volume of training load employed could also play an important regulatory role in testosterone response to

resistance training (Tremplay et al. 2004). Finally, there are many experimental and procedural constraints that may complicate the interpretation of hormonal findings such as blood sampling method, diurnal variations in hormone concentrations, hormone detection methodology, and research protocol (Hackney, 1998).

6.1.2.3 Testosterone Precursors and Metabolites and resistance exercise.

To date, little evidence is available regarding the effects of resistance training on testosterone precursors and metabolites. More specifically there are limited evidence regarding the response of DHEAS to resistance training periods, whereas no evidence exist for Δ4-Androstenedione and the metabolite of androgens 3a Diol G.

In regard to DHEAS the interest of the available literature, that has employed strength training programs, has mainly focused on middle-aged and elderly individuals, in an effort to find ways to counteract the documented dramatic decline of this androgens with age (Orentreich et al. 1984). In a similar fashion to the responses of DHEAS to endurance exercise, great controversy exists in the available evidence regarding the effects of resistance training on its levels. The study by Hakkinen et al. (2000) was one of the first to address the effects of a resistance training program in DHEAS, among other hormones, in middle-aged and elderly males. The authors reported that six months of heavy resistance training, combined with explosive activities failed to induce systematic alterations in basal DHEAS levels. This finding was further confirmed by a latter study from the same laboratory (Hakkinen et al. 2002). The authors reported a 24-week resistance/power training program in elderly males failed to significantly alter DHEAS levels, at least in a systematic way.

However, Aizawa et al. (2003) reported a significant increase in DHEAS after an eight-week resistance training program in young, previously sedentary females. In

contrast, Riechman et al. (2004) reported a modest decrease in resting DHEAS after a ten-week resistance exercise program.

Based on the available evidence we could not reach to a final conclusion regarding the response of DHEAS to resistance training, and especially in young healthy male athletes. It seems that, as testosterone, this androgens response to resistance training is affected by several factors including the training status of the subjects, the specific training regime used and both the age and the sex of the participants.

6. 2 Effects of Exercise Training on Estrogens levels.

6.2.1 Estrogens and endurance exercise

6.2.1.1 Estradiol (E2) levels in endurance trained versus untrained individuals

Estradiol (E2) is the most active form of the estrogens. There is very little information available on the response of E2 to endurance exercise in males. Melnikov and Vikulov (2004) performed a study with the participation of 14 male athletes who trained for endurance and a control group of 10 males who did not participate in sports. On average, basal estradiol concentration was significantly lower on athletes compared to controls. In agreement are the findings of Mendoza et al (1991) who observed decreased E2 levels to subjects exposed to 3 months of endurance exercise (30 minutes, 3d/week) compared to sedentary controls. It must be mentioned that the participants of the study were individuals with a recent history of myocardial infraction. Similarly, Ayers and co-workers (1985) observed low basal levels of E2 in endurance trained males compared to physical inactive controls.

On the contrary, Hawkins et al. (2008) reported that there was no difference between physically active individuals (12 months training, 6days/week) and controls (less than

Table 3. Endurance training and Sex Steroid levels

Study	Physical activity characteristics	No of subjects/Characteristics	Training	Sex Steroids	Observations	Comments
Izquierdo et al. 2003	Endurance Running, cycling, sedentary controls	1 middle-aged (46 year old [M46]) and 11 older (64 year old [M64]) men	16 week of endurance training	TT,FT	After 16 weeks no changes observed for TT. FT ↓ in both groups after 16weeks	The relationships found in this study between various indices of cycling testing and serum hormone concentrations after strength training suggest that maximal incremental cycling might be used as an additional test to detect anabolic-catabolic responses to prolonged strength training in middle-aged and older men.
Duclos et al. 1996	Marathon runners Sedentary males	4 marathon and 4 sedentary men,	5 days successively under different combinations of two factors: duration and intensity of running tests.	TT, FT, LH	Marathon runners had lower TT and LH values compared to sedentary controls	
Vaamonde et al. 2006	Physically active males	Sixteen healthy adult male volunteers were divided into experimental (8) and control (8) groups	Endurance trainin on cycloergometer for 2 weeks. The experimental group exercised four times a week;	TT, LH, FSH	TT↓, LH↓, FSH↓	These data confirm previous findings of physiological reduction in serum testosterone and PRL levels and suggest that the testosterone decrease is not related to changes in LH pulsatile release, weight, or increased serum cortisol levels.
Wheeler et al. 1991	Sedentary males	15 previous sedentary males	6 months of endurance training	TT,LH, FSH, PRL	TT↓ PRL↓, LH-, FSH-	
Urhausen et al. 1997	Rowers	9 rowers (six men of the regional	7 consecutive weeks of the	TT	observation period: TT↓	

Table 3 continued

		and three women of the national top class)	competition period (observation period), and a regenerative week		regenerative week: halted the decrease in TT levels	
Lehmann et al. 1993	Recreationally athletes	6 recreational athletes.	6 weeks of training on 6 days a week was examined. Endurance training on a bicycle ergometer for 31-33 min was performed on 4 days each week at 90-96% (weeks 1-3) and 89-92% (weeks 4-6) of the 4 mmol lactate thresholds determined on day 0 and day 21, respectively, with interval training of 3-5 x 3-5 min in addition on 2 days a week at 117-127% and 115-110%, respectively	FT, FSH, LH	FT↓ FSH↑ LH↓	
Bonifazi et al. 1995	Swimmers	8 top-level male endurance swimmers	The swimmers participated in three test sessions which occurred 6, 12 and 24 weeks after the beginning of the season.	TT, FT	The values of T increased after the exercise but returned to their initial concentrations during the recovery period. The values of fT increased after the exercise in the first and third sessions.	
Fellmann et al. 1985	Physically active males	6 healthy subjects	40-week training program on a bicycle ergometer [three 60-min sessions per week at 80%-85% of maximal oxygen uptake (VO2 max)] Before training and at the 10th, 20th, 30th, and 40th weeks of the training program, plasma testosterone, cortisol, and androstenedione concentrations were	TT, Δ4-androstenedione	TT-, Δ4-androstenedione-	

Table 3 continued

			measured			
Jensen et al. 1985	Marathon	24 healthy male marathon runners, 25 to 54 years of age.	Prospective longitudinal study over 1 year. The intensity of training increased significantly in the first 5 months of the study	T, LH, FSH, PRL, E2,	5 first months:PRL↑,TT-,LH-,FSH-,E2- End of study: No differences compared to the frst 5 months	
Lucia et al. 1996	Endurance	Professional cyclists [n = 12], elite triathletes (n = 9), recreational marathon runners (n = 10), and sedentary subjects (control group; n = 9)	Measurements were performed three times during the sports season (training period: winter; competition period: spring; resting period: fall):	TT, FT,LH, FSH	TT-, FT-, LH-, FSH-	
Mujika et al. 1996	Swimming	highly trained swimmers (n = 8)	12 weeks of intense training and 4 weeks of tapering off (taper) on plasma hormone concentrations and competition performance were investigated in a group of	TT,LH	TT-, LH- during intense training and taper	
Bell et al. 2000	Physically active university students	45 male and female subjects were randomly assigned to one of four groups; strength training only (S), endurance training only (E), concurrent strength and endurance training (SE), or a control group (C). Groups S and E trained 3 days a week and the SE group trained 6 days a week for 12 weeks.	volunteers were randomly assigned separately by sex into one of four groups: strength training only (S, 7 men, 4 women), endurance training only (E, 7 men, 4 women), concurrent strength and endurance training (SE, 8 men, 5 women), and a control	TT	TT-	

Table 3 continued

			group (C, 5 men, 5 women).			
Houmard et al. 2004	Endurance	13 males	Subjects were examined before and after 14 weeks of endurance-oriented physical training (3-4 days/week, 30-45 min/day).	TT, DHEAS	TT-, DHEAS-	
Milani et al. 1995	Cardiac rehabilitation activities in patients	96 patients with coronary artery disease,	Measurements were performed at baseline and after 12 weeks of cardiac rehabilitation and exercise training.	DHEAS	DHEAS-	
Ravaglia et al. 2001	Cycling	Twenty four middle-aged (57.4+/-4.7 years) and 24 elderly (68.3+/-2.6 years) physically active men compared with 24 middle-aged (57.9+/-4.0 years) and 24 elderly (67.2+/-1.7 years) sedentary men.	Regular exercsice for 10 years	DHEAS, FT	physically active men had on average higher DHEAS than sedentary men. No differences were observed for TT	in aging men, regular moderate physical activity is associated with higher levels of DHEAS levels
Keizer et al. 1989	Endurance	25 males and 11 females .	Athletes were monitored for an 18- to 20-month training period during which the training distance was gradually increased. The training period was divided into three periods of 6, 5, and 7 months, respectively. The first, second, and third period were concluded with a 15-, 25-, and 42-km road race, respectively	TT, DHEAS	plasma testosterone concentration was increased in males during the course of the training period. The plasma levels of this hormone remained elevated both in males and females for 1-2 days after the contests.	
Gomez-Merino et al. 2005	Military training	21 cadets	3 weeks of physical conditioning followed by a 5-day combat course with	TT, DHEAS, PRL	TT↓, DHEAS↑, PRL↑	

Table 3 continued

			energy restriction, sleep deprivation and psychological stress			
Flynn et al. 1997	Endurance Running-Cycling	11 well-trained distance runners	Participants completed two randomly assigned 10 day periods of increased training volume (200% normal training). Each increased training regimen was preceded by two weeks of reduced training (80% normal training). The increased training regimens consisted of either running only (RT) at 200% of normal training distance or running (100% normal training) and cycling (kcal = 100% normal training: CT)	TT, FT,DHEAS, LH	FT was significantly) reduced on day 5 and day 11 of RT and CT and TT was lower on day 5 however no significant treatment or interaction effects were observed for total testosterone or free testosterone. DHEAS was also significantly lower across time, LH did not change	
Kourkoulias et al. 2008	Endurance exercise - Marathon	11 non-elite marathon runners participating in the classic Athens Marathon of 2004. The group comprised ten middle-aged men and one 69-year-old man, all well-trained (running:62 km/week, active for 19.5 years)	Two-week period of endurance training including a marathon race between weeks 1 and 2	TT, FT,DHEAS, Δ4-adrostenedione, LH, FSH, PRL	TT-, FT-,DHEAS-, Δ4-adrostenedione-, LH-, FSH-, PRL-	
Hawkins et al. 2008	Endurance training	102 sedentary men, ages 40-75 yr	12 months of exercise intervention or a control group (no change in activity. The combined facility- and home-based exercise program consisted of moderate/vigorous-intensity aerobic activity for 60 min.d(-	TT, FT. 3a Diol G, E2	No changes in TT, FT. 3a Diol G, E2 levels	

Table 3 continued

			1), 6 d.wk(-1)			
Kraemer et al. 1995	Physically acrtive individuals	8younger [30-yr-old (30Y)] and 9 older [62-yr-old (62Y)]	10-wk periodized strength-power training program (nonlinear,multiset, multiexercise 3 times per week for 10 wk). The daily workouts were alternated by varying the resistance (intensity) and the volume (sets × repetitions × load) over the week.	TT, FT	Higher TT concentrations were observed at 6 and 10 wk in the 30Y than in the 60Y men 30Y men, resting serum concentrations for FT were elevated at 10 wk compared with 0 wk. For the 62Y men, FT remained unchanged throughout the training program	These data indicate that older men do respond with an enhanced hormonal profile in the early phase of a resistance training program, but the response is different from that of younger men.
Remes et al. 1979	Endurance training	39 army recruits	6-months' physical training	TT,Δ4-androstenedione, LH	TT↑, LH↑, No changes in Δ4-androstenedione	
Hall et al. 1999	Endurance training	8 male runners and e8 age-matched sedentary control subjects	The training regimen for the runners consisted of 2 weeks at normal training (NT), 2 weeks at 143% of NT (IT1), 2 weeks at 186% of NT (IT2), and 2 weeks at 50% of NT (RT).	TT, LH, FSH	No changes in TT, LH, FSH	
Kyröläinen et al. 2008	-	7 Healthy healthy male soldiers	20-day military field exercise . During the first 7 days (Phase I, (very heavy); Phase II, 6 days (easy) and PIII The last week, days 14–20 (Heavy)	TT, FT, LH, FSH	Phase I TT↓, FT↓, LH↓, FSH –; Phase II: FT returned to pre-exercise levels; Phase III:TT ncreased compared to baseline levels	

Abbreviations:TT=total-testosterone; FT=free-testosterone; DHEAS=dehydroepiandrosterone-sulfat; E2=estradio; LH=luteinizing hormone; FSH=follicle-stimulating hormone, PRL=prolactin;↑=significant increase, -=no alteration;↓=significant decrease

90 min training per week). A possible explanation for this discrepancy could be that control group was not totally inactive, a parameter that could have indeed affected the findings of this study. However, Arce et al. (1993) also failed to find any difference between 10 endurance-trained runners and 10 sedentary controls.

According to the limited aforementioned data, it remains unclear whether endurance exercise does affect E2 levels. However, it can be speculated that the different study designs, the training status of the subjects (Tremplay et al. 2004) and the time point of the athletic season that the measurement was performed (Cadore et al. 2008) could have interfered with the findings of the study . Also uncontrolled and/or variability on dietary intake could have also added to the observed discrepancies (Cangemi et al. 2010).

6.2.1.2 Luteinizing hormone (LH) levels in endurance trained versus untrained individuals

Luteinizing Hormone (LH) is the primary regulator of testosterone secretion from the Leydig cells. The LH response to the individuals exposed to endurance training compared to sedentary individuals, shows considerable variability. MacConnie et al. (1986) investigated the integrity of the hypothalamic-pituitary-gonadal axis in 6 highly trained marathon runners (mean 125-200Km/week). The authors reported that LH levels of the athletes were significantly lower compared to healthy untrained controls. Similar observations come from a study by Wheeler et al. (1984). Blood samples were obtained from 31 men who were running at least 64Km/week and 18 sedentary individuals. The obtained results showed significantly lower LH levels for the runners. Similarly Duclos and associates (1996) reported that basal blood levels of

LH were inappropriate lower in endurance trained subjects compared to physically inactive individuals.

On the contrary, Maimoun et al. (2003) reported different findings. The authors compared the basal levels of reproductive hormones in three groups of three different endurance athletic events. The subjects recruited in this study were cyclists, triathletes, swimmers and less active individuals used as control group. No differences in response to LH levels were evident between any of the four experimental groups. Similarly, Arce et al. (1993) reported no significant difference for resting LH levels in endurance trained subjects and sedentary individuals. These findings indicate that the basal levels of LH showed no significant difference between trained and untrained individuals. Unchanged LH resting levels of endurance athletes and endurance trained aged males were also reported by other studies (Arce et al. 1993; Tissandier et al. 2001).

Adding to the controversy Hackney et al. (1988) presented evidence showing that long term exposure to endurance exercise significantly increases LH levels. The same authors performed another investigation and gave further confirmation to the elevated LH levels as a response to periods of endurance exercise training (Hackney et al. 1990). However the authors concluded that this finding was most probably a result of a testosterone decline after participation to chronic excess endurance training stress.

It should be noted that the findings of altered LH levels at rest in endurance trained men with low testosterone have been labeled by some researchers as a dysfunction of the hypothalamic-pituitary-testicular (HPT) regulatory axis within these individuals (Hackney, 1998). It is characterized as a dysfunction because it represents an inability in the HPT axis to appropriately regulate hormonal levels. Interesting, these

variability in the LH responses to endurance exercise have been reported in several studies of both the retrospective and prospective type (Hackney, 1998). In general LH levels in endurance trained versus untrained individuals vary, and further research in needed to determine the reason for these discrepancies.

6.2.1.3 Follicle-stimulating hormone (FSH) levels in endurance trained versus untrained individuals

Follicle stimulating hormone (FSH) as LH plays an important role in the control of the testicular function. A number of studies tried to evaluate the possible effects of long term endurance exercise training on FSH basal concentration. Wheeler et al (1984) were the first to examine whether exposure to endurance training (running) of minimum 64 Km/week could affect hormonal profile, including FSH levels. Analysis of the results revealed that the obtained values of runners did not significantly differ from the one of the sedentary individuals. Further confirmation to these findings comes from a large number of studies using male runners or physically active aged men (De Soouza et al. 1994; Arce et al. 1993; Tissandier et al. 2001) suggesting that individuals who are exposed to regular endurance physical activity does not meet any significant alterations in FSH basal levels. Therefore, the available scientific evidence indicate that FSH does not seem to be affected by endurance exercise.

6.2.1.4 Prolactin (PRL) levels in endurance trained versus untrained individuals

Prolactin (PRL) is a hormone which permits the inference of the mechanisms involved in the regulation of testosterone production. Only a few studies have examined the possible difference in PRL levels in endurance trained and untrained individuals. Wheeler et al. (1994) investigated whether endurance exercise in men produced basal hormonal alterations compared to sedentary individuals. The authors

observed that PRL levels were lower in endurance runners compared to controls. Similar observations come from a study by Hackney and colleagues (1990). Endurance trained subjects had lowered PRL levels compared to untrained individuals. In addition the authors suggested that endurance trained males with lower testosterone also display other reproductive hormonal abnormalities with the most frequently reported changes involve decreased resting levels of prolactin even though decreased testosterone levels exist. Further confirmation to these findings come from several studies showing that endurance trained individuals had lowered PRL levels compared to untrained individuals (Ayers et al. 1988; Hackney et al. 1988; Duclos et al. 1996). However, conflicting results come from a study of De Souza and co-workers (1994). The authors studied 19 mileage runners and sedentary controls in order to check whether endurance exercise does alter resting PRL concentration. It was concluded that endurance exercise does not seem to have any effect on PRL levels.

Although the majority of the studies show that endurance training lowers PRL levels the observed discrepancy could be related with the different training regime used, and the period that the measurement was performed, since PRL is suggested to be affected by alterations in exercise induced training stress (Urhausen et al. 1995).

6.2.2 Estrogen response to exercise training

6.2.2.1 Endurance Training Programs and Estradiol levels

Vaamonde and associates (2005) examined the effects of a 2 week exhaustive endurance exercise program on the profile of sex hormones. Sixteen healthy non-professional athletes participated in this study. The authors concluded that 2 weeks of exhaustive endurance exercise stress has no effect on basal E2 levels. In another

study, Arce et al. (1993) tried to evaluate the effects of two different forms of training on the hormonal status of athletes. The period of endurance training (running) didn't manage to alter E2 concentration. In agreement are the findings of Jensen and co-workers (1995). In this prospective and longitudinal study, 1 year of training for marathon running did not significantly affect resting values of E2. Similar results were obtained by the findings of two studies which tried to investigate the effects of endurance physical activity on 40 to 75 years males engaged in 12 month program with 60 min/day, 6 days/week of exercise (Hawkins et al. 2008) and healthy men 60 to 70 years old participating regular endurance exercise for at least 2,5 hours per week (Cooper et al. 1998). Interestingly, only one study to our knowledge has reported increased E2 levels as a response of 6-months of endurance training in male rowers. (Vinogradova et al. 1992).

This discrepancy in the literature could be a result of the different mode of exercise used (rowers vs runners) and the fact that rowing also implements resistance training activities apart from endurance training. Furthermore, parameters such as diet, blood sampling method, diurnal variations in hormone concentrations, hormone detection methodology, and research protocol could have partly accounted for these findings. In general, based on the aforementioned evidence we could suggest that endurance training does not affect basal E2 levels. However, due to the limited existing evidence further research is needed to determine whether endurance training affects of not E2 endogenous production.

6.2.2.2. Endurance training programs and Luteinizing Hormone

Inconsistent findings exist for the response of LH to endurance exercise training programs. Vaamonde et al. (2005) observed that 2 weeks of exhaustive endurance

exercise resulted to decreased resting LH levels. The participants in this study were physically active individuals with minimum 3 hours / week training time. Similar observations came from Oprstad (1992). In contrast, different findings were reported from a one-year prospective longitudinal study (Jensen et al. 1995). The authors reported that marathon training does not significantly alter LH resting levels. Similarly, Lucia et al. (1996) reported that the physical stress of 1 year of training of professional cyclists, elite triathletes and recreational marathon runners had no effect on LH concentration. In accordance are the findings of a number of studies that have examined the effects of 12 months intense swimming training (Mujika et al. 1996), 6weeks- 6 days/week training on recreational endurance athletes (Lehmann et al. 1993), 3 weeks of cycling competition in a highly competitive level (Lucia et al. 2007) and 6 months of endurance training on male subjects (Wheeler et al. 1991) on LH levels. In all these studies, despite the different exercise training protocols used, the authors failed to observe any alteration in LH basal levels.

Notably, increased basal levels of LH as a response to endurance trainings programs have been also reported (Remes et al. 1979). The authors demonstrated that endurance military physical activity performed for a 6 month period resulted to increased resting concentrations of LH.

The reasons for the observed inconsistencies have not yet been determined. However, differences in testosterone levels among the participants in these studies, since the LH response is suggesting to be related with TT behavior (Hackney et al. 1998), and methological issues such as blood sampling method, hormone detection methodology, and research protocol could be related for these findings. Furthermore, according to Hall et al. (1999) since pulsatile release and amplitude characteristics of LH were not

determined in these studies the observed findings could not define the LH responses to endurance training.

6.2.2.3 Endurance training programs and Follicle Stimulating Hormone

Although it seems that there is no difference on FSH levels between trained and untrained individuals the existing bibliography examining the effects of endurance training periods on FSH concentration are inconsistent.

Wheeler et al. (1991) showed that 6 months of endurance training has no effect on resting FSH levels. Similarly Lucia and co-workers (2007) reported that 3 weeks of cycling in a competitive level did not manage to alter FSH concentration The same authors examined the effects of 1 season of training on the FSH basal levels in endurance athletes participating in three different sports (cycling, triathletes, and marathon). Likewise, no significant difference was evident for FSH levels in none of the three sports examined in this study (Lucia et al. 1996). Further support comes from a study by Lehmann et al. (1993). The authors demonstrated that 6 months of endurance training did not show any significant changes for FSH concentration.

On the contrary, Vaamonde and co-workers (2005) reported that 2 week of exhaustive endurance exercise resulted to decreased FSH basal levels in physically active males. Similarly, 1 year of training for marathon decreased significantly FSH concentration (Jensen et al. 1995). Follicle Stimulating Hormone resting levels were also found to decrease following 5 days of intensive military training (Opstad, 1992).

These discrepancies could be attributed to the different training regimes used, the training status of the participants, and the mode of the performed training activity. In addition, changes in FSH could be related with alterations in TT levels which have

been reported to stimulate FSH secretion (Bhasin et al. 1994) and furthermore with the pulsatile release and amplitude characteristics of FSH (Hall et al. 1999)

6.2.2.4 Endurance training programs and Prolactin levels

Conflicting results have been reported regarding the PRL response to endurance training. Wheeler et al. (1991) reported that 6 months of endurance training in previously sedentary males resulted to reduced resting PRL levels. In addition Gomes-Merino and associates (2003) observed decreased basal levels of PRL in physically active subjects after 5 days of intense military training. Further confirmation came from a study from Vaamonde et al. (2005). Two weeks of exhaustive endurance exercise resulted to decreased PRL levels.

Contradictory data were observed by other laboratories. Lehmann and associates (1993) examined the influence of 6 weeks endurance training program (6 days/week) on recreational athletes. Endurance cycling training was performed 4 days per week, with two sessions consisted of intense interval training. Prolactin levels remained unaltered despite the long term exercise stress. Further contradiction comes from the results of another study by Jensen et al. (1995). The objective was to examine the effects of marathon training on hormonal profile of male endurance athletes. After 5 months of endurance training a significant rise in PRL basal levels were reported. According to the authors this increase could be attributed to the fact that during this period the training intensity was significantly increased.

In general, PRL response to exercise reveals large inter-individual differences. One of the reasons for the existing discrepancies is the observation that it depends partly on the intracellular glucose availability (Galdo, 1983; Keizer et al. 1987). The exercise-induced rise in PRL only seems to be significant in better-trained individuals (Bullen

et al. 1984) or at higher exercise intensities (>90% VO_2max in untrained people). As a result of training, the release of PRL is reported to be enhanced or starts at lower exercise intensities, (Keizer et al. 1987} but could not be confined in all studies (Snyder et al. 1993). The exercise response may be associated with anaerobic lactic contribution, which would be interesting in view of the hypothesis of anaerobic lactic exercise for being one important trigger mechanism for inducing overtraining syndrome (Urhausen et al. 1995). Furthermore, PRL as cortisol, has been found to respond as a stress hormone (Kramer and Rogol, 2006, Urhausen et al. 1995). Accordingly, differences in the training volume and intensity, and the training status of the participants executing the specific training regimes could add to these contradictions.

6.2.3 Estrogen and Resistance training

Little research has been conducted for the effects of resistance training on estrogens. The majority of the studies have examined the LH response to resistance training apparently due to the suggestion that this gonadotropin play a significant role as a regulator of testosterone secretion.

6.2.3.1 Resistance exercise and Luteinizing Hormone

Hakkinen et al. (1985) examined the effects of a 24-week progressive resistance training program on the hormonal profile of 21 males. The employed training regime failed to induce any changes in LH serum levels at the end of the study compared to baseline. Similarly, a two week training period, an 8-week period (Wilkinson et al. 2006) and 28 days of resistance training 4 times/wk (Willoughby et al. 2014) in elite weight lifters, healthy young male subjects, and resistance trained athletes respectively failed to find any effects of the resistance training programs on LH level.

In contrast, Busso et al. (1992) showed that a six week training period in elite weight lifters resulted to increased LH levels. Interestingly, this finding was concomitant with a decrease in TT levels, suggesting that the reduction in serum levels was not mediated by an impairment of the hypothalamic pituitary function. The results of an early study is adding to the controversy reporting increased LH levels in after a period of 11-weeks on elite weight lifters (Hakkinen et al. 1987).

In general it can be suggested that resistance training does not seem to affect the pituitary of the hypothalamus in regard to the LH response. The observed controversies could be attributed to the pulsatile release and amplitude characteristics of LH (Hall et al. 1999), the basal levels of testosterone of the participants, since, according to some authors, LH and testosterone levels are affected by this main androgen levels. Furthermore, the training status of the participants could also play a role since untrained or less trained individuals have been shownto have a higher response in regard to testosterone, which could in turn be translated to alteration in LH (Hakkinen et al. 1985, 1987; Raastad et al. 2001). It should be mentioned, regarding the latter suggestion, that concomitant increases in testosterone and LH levels during long period of resistance training, or uncorrelated changes of these two hormones has been reported (Hakkinen et al. 1985, 1987; Raastad et al. 2001). Lastly, the blood sampling method, the hormone detection methodology, and the research protocol could also add to the inconsistent findings.

6.2.3.2 Other Estrogens and resistance exercise

To the best of our knowledge only one early study has examined the effects of resistance exercise training on all three, E2, FSH, and PRL serum levels. Hakkinen and associates (1985) studied the effects of a 24-week resistance training program on

E2, FSH, and PRL levels. No alteration was observed in none of the examined estrogens. The authors suggested that this kind of training has no lasting endocrinological effects at pituitary level. The same authors, in another study reported that prolonged resistance training in elite weight lifters failed to alter resting FSH levels (Hakkinen et al. 1988). Furthermore, two recent studies coming from the same laboratory also found that resistance training had no effects on the serum levels of estradiol, and prolactin. (Willoughby et al. 2014; Willoughby and Leutholtz, 2013). Based on the available evidence it seems that resistance training fails to affect the endogenous levels of these sex steroids. However, due to the restricted bibliography further research is needed to determine whether resistance training does not affect E2, FSH, and PRL levels. Especially for PRL, since it has been suggested to be a stress hormone, future studies should examine its response to different strength training stress (Urhausen et al. 1995, Kraemer and Rogol, 2006).

7. Team Sports and Sex Steroids

There have been a number of studies that has examined the endocrine profile in athletes participating in team sports, including soccer. Not all studies agree regarding the effects of team-sport training on sex steroids endogenous levels. However, the fact that these sports implement different activities, such as aerobic and anaerobic endurance and resistance –power trainings in different proportions, could give justification to these discrepancies.

In a recent study Coutts et al. (2007) examined the effects of six weeks of normal training on testosterone levels in 18 semi-professional rugby players. The authors reported that the experimental period resulted to significant reductions in testosterone concentration. Similarly, decreased levels of TT and DHEAS has been reported by a

study on basketball players (Slowiska-Lisowska et al. 2006). In agreement are the findings of Fillaire et al. (2001). The authors observed that the levels of salivary testosterone in 17 French national players were significantly reduced during a period of poor performance during the competitive season. However, it is unknown whether this finding should be attributed to training stress, it could be as well result of psychological stress, a parameter that is widely accepted that affects TT levels (Schultheiss and Rohde, 2002; Stanton and Schultheiss, 2009) as the authors suggested.

Contradictory evidence was reported by Celani and Grandi (1989). The authors examined the effects of 3 months of regular soccer training on the pituitary-testicular axis on non-professional soccer players. Basal levels of testosterone, LH, FSH, and PRL were measured. The endocrine evaluation was performed before the beginning of the seasonal training (after a 30 days rest period), and repeated on 2 consecutive days at the end of a 3 months regular training program. The 3-month soccer training period failed to induce any alterations in the measured sex steroid levels. In agreement are the findings of Hoffman et al. (2005). The authors examined the hormonal profile during an intercollegiate soccer season. Testosterone concentration remained at baseline levels throughout the competitive season. These observations suggest that intercollegiate soccer season does not affect testosterone production. Notably, in this study, starters were found with an average of 11,8% greater resting concentrations than non-starters throughout the season. Although this was not statistically significant result, it is interesting in the light of the observation that elevated testosterone levels are negatively correlated with fatigue (Salvador et al. 2003), as one should expect starters to be in a higher fatigue state than non-starters. However, it could also argued that this marginal increase was a result of the higher training stress. Evidence from

other athletic population indicate that TT is positively affected by strenuous exercise of high volume (Hakkinen et al. 1988).

Recent evidence report that periods of soccer training do increase endogenous total testosterone levels. Kraemer et al. (2004) examined the hormonal responses of twenty-five male collegiate soccer players throughout a season in an 11-week period. Players were divided in two groups, starters and non-starters. The authors showed there was significant increase in both groups at the end of the study, despite the observed variations in its levels during in-season. Notably, the levels of TT were low, but within the normal range, at the beginning of the study. In agreement are the observations of Gorostagia et al. (2004). The authors wanted to determine the effects of explosive strength and soccer training in young men, 8 experimental and 11 control players. Analysis of their results revealed that a progressive strength/resistance training program including full squad, vertical jump, and sprinting in conjunction with soccer training significantly increased TT levels, but on FT. The findings off the latter study indicate that the combination of regular soccer training with resistance activities might beneficially affect endogenous TT production. Further confirmation to the aforementioned studies is coming from a recent study (Pacobahyba et al. 2012). The authors reported that non-linear periodization program and non-periodized program, both performed for 12 weeks (3 times per week) in professional soccer players resulted to increased TT levels. Interestingly, the non-linear training in soccer players was more effective than the non-periodized training in promoting increase in serum testosterone levels since it resulted to more pronounced increases in TT levels.

Based on the aforementioned data it seems that great contradictory exist in the responses of sex steroids in team sports. In general, the mechanism for these changes in the aforementioned studies in unclear. It could be a result of interactive

modifications of the hypothalamic-pituitary axis (Duclos et al. 1988) or dependent upon modifications of hormonal uptake by the target organs, or even alterations in their catabolism (Bonofazi et al. 2000; Hoogeveen and Zonderland, 1996). Furthermore, the mode of exercise, and the type of the employed training regimes might also play a regulatory role (Tremplay et al. 2004). In addition, these discrepancies could have been a result based on the fact that in each team sport (even teams of the same sport) there are differences in the employed training regimes .In particular, training programs do vary in the means of the proportion of endurance and resistance/strength activities used. This variability could induce different effects on HPG axis, and therefore the different observations in the aforementioned studies could be attributed to the degree of which each training regime, endurance or resistance, is used.

8. Intervention Studies

It is generally accepted that the two parameters that are of the majors regulators of sex steroids, and mainly testosterone response to exercise, are training intensity and training volume (Tremplay et al. 2004; Grandys et al. 2011). The following short reviews of the literature are providing the available evidence regarding these two factors, showing that there are several discrepancies in the literature.

8. 1 Training Intensity and Sex Steroids

To date most of the existing research suggests that the intensity of the physical activity is one of the main determinants of the magnitude of the exercise elicited hormonal responses. The majority of the published reports have examined the relationship of acute exercise and exercise intensity endogenous gonadal and adrenal

Table 4. Team Sports and sex steroids levels

Team Sports						
Coutts et al. 2007	Rugby	7 semi-professional rugby players	6 weeks of progressive overload training with limited recovery periods. A short 7-day stepwise reduction taper immediately followed the overload period.	TT	TT↓	
Slowinska-Lisowska et al. 2006	Basketball	12 professional basketball players	three mezocycles I increased training load II decreased training load III increasing the training load	TT, DHEAS	Mezocycle I: TT↓,DHEAS ↓ Mezocycles II :TT ↑, DHEAS – Mezocycle III: TT↓,DHEAS ↓	Increased training load resulted to decreased TT and DHEAS circulating levels
Filaire et al. 2001	Soccer	17 male soccer players	The initial measures were performed 1 day following the start of season training (T1). They were then performed before and after a high-intensity training programme (T2 and T3, respectively) and 16 weeks after T3 (T4)	TT, FT	TT↓ at T3 andT4 No changes for FT	
Grandi and Celani, 1990	Soccer	7 professional soccer players (PSP)	Players were examined after a 30 days rest period and 14-15 h from the end of both a customary training session and a strenuous football match, performed at the end of a 3 months regular training program.	TT, LH,FSH,PRL	No changes in TT, LH,FSH, and PRL levels	

Table 4 continued

Celani and Grandi, 1989	Soccer	10 trained non-professional soccer players	Testing was performed before the beginning of the seasonal training (after a 30 days rest period), and repeated on 2 consecutive days at the end of a 3 months regular training program, 14-15 h from the end of both a customary 3 h training session and a 90 min strenuous soccer match	TT, LH,FSH,PRL	No changes in TT, LH,FSH, and PRL levels	
Gorostagia et al. 2004	Soccer	8 experimental (S) and 11 control (C) regional soccer players	11-week in season training, The strength training program in the S group consisted of two training sessions each week . The subjects of the S group also performed some light strengthening exercises (with loads of 40–60% of one maximum repetition)	TT,FT	TT↑ IN THE S group only No changes for FT in both groups	
Kraemer et al. 2004	Soccer	25 male collegiate soccer players	11 week period in-season, with variations in volume	TT	TT↑ at the end of the study	
Pacobahyba et al. 2012	Soccer	24 U20 soccer players	non-linear periodization program (G1) and non-periodized program (G2), both for 12 weeks, 3 times /week	TT	TT↑ at the end of the study in both G1, G2 compare to baseline. G1 vs G2 resulted to higher increase in TT	The non-linear training in soccer players was more effective than the non-periodized training in promoting increase in TT

Abbreviations:TT=total-testosterone; FT=free-testosterone; DHEAS=dehydroepiandrosterone-sulfat; E2=estradio; LH=luteinizing hormone; FSH=follicle-stimulating hormone, PRL=prolactin;↑=significant increase, -=no alteration;↓=significant decrease

sex hormones (Raastad et al. 2000;Copeland and Tremblay, 2004; Tremblay et al. 2004). Only a few authors examined the effects of variations in intensity in several training regimes on sex steroids using short or long duration training programs.

In a recent study Slowinska-Lisowska and associates (2006) studied the changes in the serum concentrations of testosterone and DHEAS in during three training cycles in basketball players for a period of 65 days. The authors reported that increases in exercise intensity in the first and third mesocycle resulted to significant reductions in total testosterone and DHEAS levels. In contrast, after the second experimental period, reduced training load resulted to increased total testosterone levels, while DHEAS levels remained unaltered to this alteration in the exercise training stress. The authors concluded that in the specific athletic population training stress, as expressed by different training intensities, affects androgens concentration. Similar observations were reported by Kyrolainen and co-workers (2008). The purpose of this study was to test the hypotheses that the magnitude of the hormonal concentration alterations during a 20 day military field exercise with constant energy intake is influenced by changes in energy deficit induced by varying exercise intensity. The experimental period was divided in three stages, a very demanding phase, an easy phase, and a somewhat demanding phase. Total testosterone, free testosterone, and LH levels were reduced during the first phase although FSH levels remained unaltered. The alterations in total and free testosterone and LH were attenuated by the second less intensive phase. Interestingly the concentrations of both testosterone and FSH tended to increase above the pre-exercise training levels by the last part of the 20-day training period. It should be mentioned that according to Wheeler et al. (1986) and Macconie et al. (1996) the decrease of plasma concentration of total and free testosterone is proportional to the training intensity of endurance athletes. In addition, a decrease in serum testosterone concentration was observed during the 4-week period of intensive training (Busso et al. 1990) and this depression was not primarily mediated by an impairment of the hypothalamic- pituitary system.

Table 5. Intervention studies and Sex Steroids

Authors	Sport	No of subjects	Type of training	Sex Steroids	Effect
Slowinska-Lisowska et al. 2006	Basketball	12 professional basketball players	three mezocycles I increased training load II decreased training load III increasing the training load	TT, DHEAS	Mezocycle I: TT↓,DHEAS ↓ Mezocycles II :TT ↑, DHEAS – Mezocycle III: TT↓,DHEAS ↓
Häkkinen et al. 1991	Swimming, weight lifting	9 elite swimmers, elite weight lifters	1 year of training: Endurance training: 8 sessions per week, with seasonal variation from 6 to 12 sessions. Weight lifting training: average seven times a week	TT, FT	during the most intensive and during prolonged training periods TT-, FT-
Häkkinen et al. 1991	powerlifters and bodybuilders	9 strength trained athletes	two separate 3-week intensive strength training periods. The overall amount of training in the periods was maintained at the same level. In both cases the training in the first 2 weeks was very intensive: this was followed by a 3rd week when the overall amount of training was greatly decreased. The two training periods differed only in that training period I included one daily session, while during the first 2 weeks of period II the same amount of training was divided between two daily sessions		FT , LH
Mujika et al. 2000	well-trained male middle-distance runners	8	either a moderate volume taper (MVT, N = 4) or a low volume taper	TT	TT↑
Moore and Fry, 2007	Nine skill position players from a National Collegiate Athletic Association (NCAA) Division I-A football team		15-week off-season training program for American football. Following 4 weeks of weight training (phase I), subjects performed weight training concurrently with high-volume conditioning drills (phase II). Phase III consisted of 15 spring football practice sessions executed over a 30-day period.	TT	Phase I: TT – Phase II: TT↓ Phase III: TT return to baseline values
Flynn et al. 1997	11well-trained distance runners		Randomly assigned 10 day periods of increased training volume (200% normal training). Each increased training regimen was preceded by two weeks of reduced	TT, FT, DHEAS, LH	FT was significantly ($p < 0.05$) reduced on day 5 and day 11 of RT

Table 5 continued

			training (80% normal training). The increased training regimens consisted of either running only (RT) at 200% of normal training distance or running (100% normal training) and cycling (kcal = 100% normal training: CT)		and CT total testosterone was lower on day 5 than day 0 luteinizing hormone were not significantly altered during RT or CT
Hug et al. 2003	11 male elite cyclists		training high-living low'; 'training low-living low'		Living low training high: no differences in FT TT. Living low training low the 6-week training period, there was a significant increase in FT (P<0.05; up to 122.1±7.8%) and TT (P<0.05; up to 120.3±6.3%).

Abbreviations:TT=total-testosterone; FT=free-testosterone; DHEAS=dehydroepiandrosterone-sulfat; E2=estradio; LH=luteinizing hormone; FSH=follicle-stimulating hormone, PRL=prolactin;↑=significant increase, -=no alteration;↓=significant decrease

The observation of a depression in serum androgens was in agreement with previous findings (Kuoppasalmi and Adlercreutz 1985; Adlercreutz et al. 1986). A decrease in serum testosterone concentration has already been observed after a short period of intensive and extensive training over several days (Hakkinen et al. 1988) without a significant change in performance. This decrease in the serum testosterone concentration during a short training period of a few days could serve as an index of the physiological stress of training. In the study by Busso et al (1990) there was also evident a reduction in LH levels. Incomplete recovery from preceding training could have reduced the LH release during intensive training. This interpretation would better match the results of Barron et al. (1985) who have observed a dysfunction of the hypothalamic-pituitary system in overtrained athletes. A 4-week period of rest allowed them to recover normal hypothalamic-pituitary function.

In another study performed by Hakkinen and Pakarinen (1991) the authors investigated the training induced adaptations in the endocrine system in 9 male strength athletes during two separate 3-week intense strength training program. In both cases the training in the first two weeks was very intense, followed by a week of reduced training. The overall amount of training was maintained in the same level during both periods in order to avoid variations in their result due to different training volume. The two training regimes differed only in that the first period included 1 session per day while during the second period in the first two weeks the same amount of training was divided in 2 sessions per day. During phase 1 only slight and non significant increase occurred in hormonal levels. In contrast, the two first weeks of intense training in the second phase reduced free testosterone levels accompanied by significant increased levels on LH. No alteration was evident for total testosterone levels, indicating that the employed training regime does not affect its levels.

In a 1-year prospective study, Jensen and associates (1995) examined the effects of marathon training on hormonal profiles of male athletes. During the first 5 months of the study there was a significant increase of the intensity of training. This was accompanied by increased PRL levels. In contrast, hormonal evaluation showed the plasma concentration of testosterone, LH, FSH, and E2 were not affected by the increased exercise intensity. This increase in PRL levels was attributed to the high stress induced by the training protocol, since this hormones is categorized as a stress hormone (Kraemer and Rogol, 2006; Urhausen et al. 1995).

Hakkinen et al. (1989) studied over an entire year the effects of prolonged training in elite endurance trained and strength trained athletes. Hormonal determination was performed at about 4 month's interval during the course of the year. No changes were observed for both total and free testosterone levels. This findings in the two groups of elite athletes, who differed greatly with regard to the type of physiological loading, demonstrated that the overall hormonal responses both during the most intensive periods were rather similar and the infrequent small changes remained well within the normal physiological range.

Similar observations were reported by Ahtiainen and colleagues (2005). The authors studied the long term (6 months) hormonal adaptations to hypertrophic strength training in 13 recreationally strength trained men. The exercise protocol was divided in two 3 month phases and was similar in regard to the total volume of work but differed with regard to intensity and rest period between the sets. No significant differences were found in serum total testosterone and free testosterone during the six month strength training period suggesting that the intensity of exercise in combination with the rest periods did not have an influence on the magnitude of hormonal responses to long term training. However, since another parameter apart from

intensity (rest periods) was involved these results cannot be clearly attributed to exercise intensity.

The results of other investigation add to the contradiction for the effects of intensity on sex steroids basal levels. Duclos and associates (1996) examined the testosterone and LH responses in 4 marathon and 4 sedentary males during 5 days using different combinations of two factors, intensity and duration. Light to moderate exercise did not modify testosterone and LH levels in both groups. In contrast, intense exercise increased testosterone concentration and lowered LH levels in both groups. However the small sample of the participants in this study could have resulted to the specific findings. In regard to LH, during very intensive training phases, a reduced pulsatile LH secretion and response was found in marathon runners and athletes, after the administration of gonadotropin-releasing hormone (GnRH). (Hackney, 1990; MacConnie et al. 1986). However, the influence of an insufficient energy intake on the pulsatile LH release should also be considered. This would suggest an impaired hypothalamic-pituitary mechanism. In another study on 800m runners Myjika et al. (2000) examined the effects of a 6-day training period at different intensities. Total testosterone was inversely correlated with low intensity continuous training distance whilst a positive correlation was evident with the high intensity interval training distance. Similar finding were reported by Myjika et al. (2002) on 800 meter runners. The athletes who tapered for 6 days showed increased total testosterone values after the taper, attributed to the elimination of low intensity continuous training from the main part of the training session during the taper. The mechanism for the increased total testosterone levels post taper is thought to relate to the preceding period of intense training bringing about a positive influence on androgenic anabolic activity during the subsequent taper characterized by reduced levels of physiological stress.

In conclusion, these findings indicate that training intensity may affect sex steroids concentration, however the possible effects depend on several parameters such as the mode of exercise used, the training status of the participants, and the rest periods between training activities (Tremplay et al. 2004). Furthermore, there is a possibility that energy intake could also play a role in the responses of these hormones to alterations in training intensity.

8.2 Training Volume and Sex Steroids

A number of studies have shown that the magnitude of exercise training volume has a direct effect on the hormonal status in male athletes. The majority of the studies have mainly focused on the responses of testosterone and the gonadotropins LH and FSH to alterations in training volume.

De Souza and co-workers (1994) suggested that exercise volume is of critical importance for significant alterations in male sex hormones. In their study it was demonstrated that a high volume of endurance exercise resulted to significantly decreased levels of both total and free testosterone levels. However no alterations were observed for LH, FSH and, PRL levels accounted to training volume. In addition, the authors reported the existence of a certain training volume threshold (100km/wk) that can results to significant alterations in circulating hormones.

In another investigation Flynn et al. (1997) examined the changes in blood hormones levels elicited by increases in training volume in 11 well trained distance runners. Subjects had to follow two increased either a 10 day training regimen consisting on 200% of their normal-habitual running or running at 100% normal training and cycling at 100% of normal training During each regimen subjects had to run for 10 consecutive days at a distance equivalent to 100% of normal trainings. Free

testosterone and DHEAS levels were significantly decreased on days 5 and 11 in both groups. This response was most probably an effect of the excessive training stress.

Specifically for DHEAS it has been suggested that although it seems to be unaffected by exercise training, excessive training stress may do so (Collomp et al. 2014). No changes were observed for LH concentration.

The purpose of a recent study by Moore and Fry (2007) was to determine the performance and hormonal responses to a 15 week off-season training program for American football. The experimental period was divided in three phases, a 4-week weight training period (phase I), followed by a period of weight training concurrently with high volume conditioning drills (phase II), and a latter 30-day phase (III) of reduced volume. Testosterone levels decreased significantly during the second phase. In addition this period was accompanied by evident reductions for power values. This decrease in TT at the second phase could have been a result of the increased training stress which showed a tendency to compromise the pitutirary-gonadal axis in regard to testosterone production. The reduced stress period of the third phase managed to return basal testosterone values at pre-exercise training basal levels. Further confirmation for the alterations in testosterone and LH levels in response to increased endurance training volume came by Wheeler and associates (1991). Testosterone level decreased whereas LH and FSH concentrations remained unaltered. In addition the authors observed decreased prolactin levels indicating that there was an increase in training stress which resulted to these hormonal responses. These findings are in agreement with other studies where gonadal hormones were monitored in the different training groups and during different training phases (Hakkinen et al. 1985; Hug et al. 2003). In each of these papers, the importance of the applied training loads on the testosterone changes was emphasized. The heavy training phases with high volume of

training led to a decrease in testosterone concentration, whereas reduced training loads were accompanied by its increase and return to the initial, pre-training level. Moreover, it has been recently demonstrated that moderate-intensity and low-volume endurance training program increases total and free testosterone concentrations in young, healthy men (Grandys et al. 2011).

In 1987, Hakkinen and collaborators examined the effect of a six week prolonged resistance training period on testosterone levels (on elite weight lifters). The study was divided in three parts, one 2 weeks high volume training period, one 2 weeks normal volume training period and finally 2 weeks of reduced training volume. The first two weeks resulted to decreased testosterone levels. The changes during the other two stages were modest indicating the importance of exercise training volume on testosterone levels. Interestingly, serum LH concentration was significantly reduced as a result of the first two weeks with high training volume showing a positive correlation to the volume of resistance training. In accordance were the findings from the same group (Hakkinen et al. 1988) suggesting that the observed decrease in androgens concentration was associated with the physiological stress of training. Another study from the same laboratory (Hakkinen et al. 1989) observed in a 1-year study on elite endurance trained and strength trained athletes that a significant decrease in serum testosterone levels occurred in the strength trained group during the period which was characterized by the highest overall amount of training. Similarly, Busso et al. (1992) showed examined the effects of a 6 week weight training program. The authors observed that the first 4 weeks of intensive training resulted to decreased serum testosterone levels, whereas the next 2 week of reduced training remained unaltered. Serum LH concentrations were significantly increased during the first 4 weeks of intense exercise. Further support to these suggestions comes from the

findings of other studies showing that increased volume of aerobic and strength exercise training have resulted in decreased concentrations of several hormones, including testosterone, resting levels (Di Luigi et al. 2002; Maestu et al. 2005; Fry et al. 1998).

However, a number of investigations failed to observe any effect training volume alterations on androgen levels. Hakkinen et al. (1989) examined the effects of a 1-year period of training on serum total and free testosterone levels in elite endurance trained (swimmers) and strength trained (weight lifters) athletes. These two groups had a correspondingly long background of training, but differed greatly with regarded to the type of training examined. The primary findings demonstrated that during the period of most intensive training in preparing for the major competitions of the year, the endocrine responses were minor and very similar between the groups in spite of the totally different type of training. Similarly, no alteration in testosterone basal levels were reported by two studies from the same laboratory (Mäestu et al. 2005; Jürimäe et al. 2006). The authors showed that testosterone profile remained unaffected by increased training volume in elite rowers after a 6 months period. Further support comes from the observation of other studies (Mackinnan et al. 2000).

It has been reported that significant increase in training volume failed to affect testosterone basal levels. Lucia et al. (1996) examined the reproductive function of three groups of endurance athletes of three different training levels over a sports season. For each group hormonal evaluation was done 3 times during the season, pre-competition, competition and rest period. The pre-competition period had significantly higher training volume compared to the other phases. However no significant differences were observed for any of the groups for total testosterone, free testosterone, LH, and FSH levels throughout the study. Furthermore, the authors

observed that the levels of circulating total testosterone were significantly higher in the cyclists compared to the other subjects. Noticeably, the cyclists were the subjects exposed to the highest training demands.

Although the above mentioned evidence indicate that sex steroids levels either remain constant or are decreased as a result of alteration in training volume, other studies have observed an increased androgenic effect as a response to exercise. Antiainen et al. (2003) reported significant higher free and total testosterone concentrations during 7 weeks strength exercise of high training volume. However, reductions were observed in a subsequent 7 weeks period when volume was reduced and intensity increase. In a recent study, Purge and associates (2005) examined the hormonal adaptations during a 24-week preparatory period of eleven elite male rowers. Before the preparatory period, the mean values of training volume was 90min/day. Compared to the initial values, there was a consistent significant increase to training volume until the 20th week (up to 167m/day). At the end of the study the mean training volume was reduced to 116min/day. Basal testosterone concentrations were significantly elevated after weeks 4, 8 and 20, indicating a high anabolic profile of the subjects following hard endurance training sessions. Testosterone levels was significantly related to mean weekly training volume suggesting that testosterone is sensitive to changes in training volume. In the end of the 24-week period testosterone were not significant different compared with pre-training, Although basal testosterone levels remained within the normal range, circulating values were considered high during the experimental period indicating a positive anabolic adaptation (Kraemer et al. 2004) to endurance training, as did the improved aerobic capacity. In another study Fry et al. 1994 studied nine elite male weightlifters. The athletes participated in a one week intensive strength training volume (overreaching) period during which they doubled

their usual volume of training. After one year of chronic weight lifting the subjects repeated the same protocol again (doubled training volume). Although after the first part of overreaching testosterone levels actually decreased, a year later testosterone levels were increased suggesting that the overreaching stimulus appears to decrease any possible detrimental effects of stressful training on the endocrine system. These findings indicate that the mode of exercise and the employed training volume play a role in testosterone response. In other words it seems that severe alterations in strength training volume and/or long term strength training periods may lead to beneficially altered testosterone levels. Further support is coming from studies on soccer players. A recent intervention study on soccer players demonstrated that the combination of strength training with soccer training (2 and/or 3 sessions per week versus baseline) significantly increases the endogenous production of testosterone. Similarly, Gorostagia and associates (2004) observed that 2 strength training per week versus 1 do beneficially affect TT levels but no FT. These two studies in the specific population suggest that alterations in the weekly training volume can induce alterations in the gonadal-pituirary axis regarding the main androgens testosterone. Interestingly, in regard to FT, Hakkinen et al. (1989) demonstrated that in elite strength trained athletes the increase in its levels occurred concomitantly with the decrease in the amount of training during the last training period of their study indicating an inverse relationship of this bioavailable form of testosterone with exercise volume. On the other hand, in the endurance-trained group the amount of training decreased, but statistically significant changes were not observed in serum-free testosterone, during the latter two training periods. The latter findings clearly suggest that the mode of exercise is of primary importance for the response of FT to

exercise training. It should be mentioned that alterations in training volume also may affect PRL levels.

In general, these discrepancies in the literature could be partly attributed to the fact that training has been expressed in a variety of ways which make it difficult to district to what degree these training volumes differ. In general chronic research shows that higher-volume resistance programs tend to elicit the greatest hormonal responses. However it seems that modifications of strength training volume, have the ability to alter testosterone levels, however, it remains unknown which parameters also contribute to this increase, apart from the employed volume of training, i.e. training status of the participants, sex, age, mode of exercise, excessive stress (overreaching, etc.) Furthermore, the available evidence indicate that resistance exercise protocols that stress large muscle mass (multi-joint exercises), are high in volume, and moderate to high intensity, tend to produce the greatest hormonal elevations for optimal muscular fitness benefits (Kraemer and Ratamess, 2005)

9. Detraining and Sex Steroids

Two types of detraining can be described: short-term detraining, with a period of less than 4 weeks, and long-term detraining (period longer than 4 weeks). Few studies have examined the detraining effects on the neuroendocrine system. Even less evidence exists for the relationship between detraining and the male reproductive system. The suggestion that sex hormones are related with physical activity and various physiological systems in the body (Borer, 2003) raised, apart from training periods, the question regarding their responses response to detraining periods. Generally the majority of the available evidence shows no significant differences in testosterone, luteinizing hormone, and follicle-stimulating hormone, after

detraining (Häkkinen et al.1985; Kraemer et al. 2002) More specifically, in regard to TT and FT,a four-week detraining period in strength trained individuals(Izquierdo et al. 2007), did not revealed any significant changes in TT and FT, after both training cessation and tapering. Similarly, Kraemer and associates (2002) showed that 6 weeks of detraining in recreationally trained men did not affect TT concentration. On the contrary, two weeks of inactivity on strength trained individuals resulted in significantly increased TT levels (Hortobágyi et al. 1993). The authors suggested that this could indicate an enhanced stimulus for tissue remodeling. Notably, Kraemer and associates (2002), based on their observation of an insignificant increase in TT levels after the first 2 weeks in their study, gave a possible explanation of this finding. The authors suggested that the first 2–3 weeks of detraining, after stressful training periods, could possibly result in increased anabolic hormones concentration, and that this increase was attenuated after the following 3–4 weeks of detraining.

In regard to LH and FSH, the observations of a study on resistance trained males (Häkkinen et al. 1985). Reported that 12 weeks of detraining did not affect these two gonadotropins resting levels. Similarly, Hall and associates (1999) reported that LH and FSH were not altered by a short detraining period in endurance athletes. Therefore, according to the aforementioned published reports we could propose that detraining does not affect the hypothalamus or the pituitary gland regarding these two hormones.

In the published bibliography no evidence exists for E2, Δ4-androstenedione, and PRL and their responses after a detraining period. In regard to DHEAS, only one study to our best knowledge has examined its responses to detraining. Wang et al. (2006) noted a significant decrease in DHEA-S after two months of detraining in

highly trained young male badminton players. This findings could be supported buy the observation that physical activity was positively associated with DHEAS in a cross-sectional study of active and sedentary middle-aged and elderly men (Ravaglia et al. 2001). However, since there is only one study to support the decrease in DHEAS levels (Wang et al. 2006) to detraining and the individuals in the latter study (Ravaglia et al. 2001) were not young competitive athletes indicate that further research in needed to further support this hypotheses, including the examination also of testosterone levels.

10. Detraining and Exercise performance

Sports periodization typically incorporates a transition – off-season period of reduced stress, in order to allow physical and mental recovery after the end of the competition season (García-Pallarés et al. 2010). This phase of reduction or complete training cessation has been defined as detraining (Mujika and Padilla, 2000). This detraining period can partial or complete reverse the adaptations of training, resulting in compromised exercise performance (Mujika and Padilla, 2000). The magnitude of the alterations in performance capacity is dependent on several factors, such as the selected recovery strategy, the duration of the detraining phase, and the initial fitness level of the participants season (García-Pallarés et al. 2010).

Evidence from various athletic populations indicate that 3 to 6 weeks of detraining affects negatively aerobic capacity (Coyle et al. 1984), strength (Izquierdo et al. 2007), neuromuscular performance (Izquierdo et al. 2007), and body composition (Hoshikawa et al. 2004). In contrast, some studies on recreational athletes and untrained individuals failed to support these findings in regard to aerobic capacity and muscle strength. It was observed that training cessation or insufficient

training stimulus for a period of 2 to 6 weeks did not result in decrements in these two parameters (Izquierdo et al. 2007; Mujika and Padilla, 2000), .. These discrepancies in the literature were attributed to the different initial training fitness levels of the participants. It has been suggested that the higher the training state of the participants, the greater the rate of decline in both VO_2max and strength adaptations (Izquierdo et al. 2007).

This negative effect of detraining on exercise performance adaptations is a result of complex physiological mechanisms. It has been reported that the decline in aerobic capacity is related to reductions in blood volume, stroke volume, cardiac output, ventilator functions, and cardiac dimension due to insufficient training stimulus or training cessation (Mujika and Padilla, 2000). Similarly, the observed detraining related decrease in muscle strength and strength related performance appears to be a result of decreases in muscle fiber size, mostly due to reduced type II muscle fiber area, mitochondrial ATP production, and enzymatic activities (Mujika and Padilla, 2000).

11. Physiology of bone metabolism

Bone, which is a metabolically active tissue, undergoes continuous remodelling, involving bone resorption and formation. In health, these two processes are tightly coupled through intricate mechanisms. Bone mass depends on the balance between resorption and formation. The cells responsible for resorption and formation are osteoclasts and osteoblasts, respectively. Numerous factors, systemic and local, regulate the function of these cells types. Bone remodeling takes place on the surface of bone and in a typical remodeling cycle, resorption takes 7–10 days while formation takes 2–3 months (Maimoun and Sultan, 2011; Banfi et al. 2010).

11.1Biochemical markers of bone formation

Osteoblasts synthesize and secrete a number of proteins which can be measured in serum as markers of their activity and, therefore, of bone formation. The most commonly used markers of bone formation are alkaline phosphatase (b-alp) and osteocalcin (OC). Furthermore, an important bone formation marker is C-Terminal Propetide of Type-I-Collagen (CICP).

11.1.1 Alkaline phosphatase (b-ALP)

Bone alkaline phosphatase (b-ALP) is the bone-specific isoform of alkaline phosphatase. A glycoprotein that is found on the surface of osteoblasts, b-ALP reflects the biosynthetic activity of these bone-forming cells. b-ALP has been shown to be a sensitive and reliable indicator of bone metabolism (Kress, 1998). The bone-derived isoenzyme of alkaline phosphatase (b-ALP) reflects a specific measure of the cellular activity of osteoblasts (Maimoun and Sultan, 2011). It is thought to participate in the mineralization process and thus represents bone matrix maturation (Garnero and Delmas, 1993).

11.1.2 Osteocalcin (OC)

Osteocalcin is a noncollagenous protein of 49 amino acids and predominantly is a unique product of synthesized by osteoblasts and odontoblasts and is incorporated into the extracellular matrix of bone. There is abundant evidence for the utility of OC as a bone formation marker (Banfi et al. 2010). It is the most abundant noncollagenous protein in bone.Most of the OC that is produced is incorporated into the bone matrix where it is found to hydroxyapatite. A small fraction of the neosynthesized OC is released into the circulation and can thus be used as an index of mineralization (Price et al. 1981). The proportion of OC incorporated into the matrix varies from 90% in the young to 70% in adults.

11.1.3 C-Terminal Propetide of Type-I-Collagen (CICP)

Type 1 Collagen is a primary organic constituent of bone. The release of these peptides into the circulation as the result of the cleavage of C-Terminal Propetide of Type-I-Collagen (CICP) provides a stoichiometric representation of the production of collagen. Type I collagen levels have been linked to bone growth and formation. Levels of CICP are indicative of collagen production in vivo.

11.1.4 CTX

Carboxy-terminal collagen crosslinks, and known by the acronym CTX is a C-terminal telopeptide composed of an octapeptide of the C terminus of the a1 type I collagen. CTX is liberated during the degradation of type I collagen (Banfi et al. 2010) and therefore reflects various enzymatic mechanisms of type I collagen degradation (Maimoun and Sultan, 2011). Recent studies have shown that CTX reflects better than other markers bone resorption (Walts, 1999). CTX fragments are also released into the circulation, as a result of the osteoclast mediated degradation of type 1 collagen during the bone resorption process. These fragments are highly bone specific because osteoclasts are not active in the degradation of other type 1 collagen-containing tissues (Maimoun and Sultan, 2011; Banfi et al. 2010).

11.2 Effects of Exercise on bone metabolism

The contribution of physical activity on optimal bone mineral density (BMD) is well established (Banfi et al. 2010). More specifically, BMD is beneficially affected by exercise training whereas subsequent training cessation and/or reduction tend to counteract this effect. However, BMD, as measured by dual-energy X-ray absorptiometry (DEXA), responds slowly and cannot reflect short term effects of exercise or the lack of it on bone physiology. On the other hand, markers of bone turnover (resorption and formation) respond immediately to changes of bone turnover

and thus they can be used as dynamic measurement of bone status (Maimoun and Sultan, 2011). Indeed, studies on several types of athletic and non-athletic populations have shown that bone metabolism markers are very sensitive to changes in physical activity levels (Banfi et al. 2010, 2012; Maimoun and Sultan, 2011). Based on these bone markers, it has been shown that bone turnover is altered in favor of bone formation following training stress (Banfi et al. 2010; Maimoun and Sultan, 2011). The available evidence suggests that exercise could be followed by a variety of changes in bone turnover. Studies have reported normal or slightly increased bone formation markers and decreased bone resorption markers as a result of aerobic exercise (Eliakim et al. 1997; Woitge et al. 1998; Fujimura et al. 1997), increased bone formation markers and bone resorption markers with anaerobic activity (Woitge et al. 1998), no increase in bone formation markers with low-grade exercise (Dalsky et al. 1988), decreased bone bone formation markers and bone resorption markers with anaerobic activities (Brahm et al. 1997), increased bone formation after 20 weeks of resistance exercise, (Hu et al. 2011), and increased bone resorption markers but unaltered bone formation markers with aerobic activity (Welsch et al. 1997). Furthermore, a volume-extended heavy training period in elite rowers resulted to increased bone formation compared to the relative rest values (Jurimae et al. 2006).

These discrepancies in the aforementioned studies could be a result of several factors that have been reported to affect the bone turnover response to exercise. Indeed, different responses have been reported when comparing short of long terms of training (Karlsson et al 2003a). Additionally, it has been suggested that bone response to mechanical loading depends on the mode of exercise (Brahm et al. 1997; Malm et al. 1993) and variations in the intensity, the volume of the performed physical activity.

Lastly, other factors that might affect bone metabolism markers response to exercise include the age, and the sex of the participants (Maimoun and Sultan, 2011).

11.2.1 Detraining and Bone Metabolism

The majority of the available studies have examined the effects of training on bone metabolism. On the contrary, little evidence exists regarding the effect of short term detraining on bone markers (resorption and formation) and even less in elite competitive athletes (Maimoun and Sultan, 2011). The available data provide contradictory findings. More specifically, Satorio et al. (2006) found no significant variations in bone markers between the competition and rest periods in athletes. Similarly other studies reported insignificant minor variations in bone turnover markers in male triathletes, rowers, power athletes, and controls (Maimoun and Sultan, 2011) as a consequence of alterations (i.e. increases or reductions) in training stress. This lack of response on the aforementioned studies was mainly attributed to the high biological variability of the measured bone markers. On the contrary, two studies on soccer players reported that training reduction during the off-season soccer transition period resulted in decreased bone resorption and increased bone formation (Karlsson et al. 2003a; Weiler et al. 2012) indicating the reversibility of exercise training on the skeletal system. Further support to the latter suggestions is coming from studies on several athletic populations. The literature infers that athletes who reduce the duration of activity seem to reach a new level of serum remodeling markers of bone turnover week to months after changed activity level (Karlsson et al. 2003).

Table 6. Exercise training and Bone Metabolism

Study	Sport	No of Subjects	Type of training/Training protocol	Bone formation markers	Bone resorption markers
Eliakim et al. 1997	Sedentary	38 (20 trained – 18 controls, 44 y)	Endurance type exercise for 5 wk(2h/day-5d/wk). Blood collection before and after training	b-ALP↑ OC↑ PICP ↑ in trained subjects	No changes for Pyr, CTX, NTX
Woitge et al. 1998	Sedentary	34: aerobic training (n=10; 25.3 ± 2.6 y, anaerobic training (n = 10; 23.5 ± 2.9 y), controls (n = 12; 25.3 ± 2.7)	8 wk of training for 3 groups; aerobic: endurance running (60-85 VO2max); anaerobic: sprint session and weightlifting; controls. Blood collection before at wk 4 and wk 8	b-alp↓ at wk4 in aerobic group, ↑ at wk 8 vs wk 4 at aerobic and anaerobic group, ↓ wk 4 (aerobic vs controls) OC ↓ wk 4 (aerobic), ↑wk 8 vs wk 4 (aerobic and anaerobic group), ↑ wk 4 and wk 8 at anaerobic vs controls.	Pyr ↓ at wk4 vs baseline and controls in aeobic and anaerobic groups, ↑ at wk 8 vs wk 4 (anaerobic group) and vs controls. Dpd ↓ wk 4 aerobic group vs baseline and controls, ↑ wk 8vs in anaerobic group vs baseline
Maimoun et al. 2004	Triathlon (national level)	7 (19.2 y)	Blood collection at the beginning and the end of 32 wk later during competition period	No changes for OC, b-ALP ↓	No changes for CTX
Shibata et al. 2003	Sedentary	28	Blood collection at the beginning of training (daily walking for 17 subjects, dayly walking and jumping for 11 subjects) and 1 year later	b-ALP↑ in both groups, b-ALP ↑ in walking and jumping vs walking alone. No changes for OC	No changes for CTX
Jurimae et al. 2006	Rowing (international level)	12 (20.8 ± 3.0 y)	Blood collection at the beginning of training and 6 months later during completion period	OC↑	
Karlsson et al 2003	Soccer	12 (22.8 ± 1.2 y),	Blood collection just before the end of soccer	PICP ↓ at 2-4 wk of resting period t-ALP ↓ at 3 and 4	

Table 6 continued

		27 controls (23.4 ± 1.1 y)	season, and weekly for 4 wk of resting period and after 10 d of restart training	wk of resting period and restart training; ICTP ↑ at 4 wk of resting period, no changes in OC	
Sartorio et al. 2006	Regionally or nationally ranked athletes	25 males (22 ± 4 y), 22 women (22 ± 5 y)	Blood collection at the start and after 2, 4, and 6 months 2-3 h/d for 4-5×/wk of habitual training)	No changes in ICTP	
Ryan et al. 1994	Trained individuals	36:20 trained (61 ± 1 y), 16 controls: 59 ± 2 y	Blood collection training and after 16 wk of training (16 wk strength training, 3×/wk)	No changes in OC, b-ALP	TRAP ↑ in trained and CO
Fujimura et al. 1997	young adult Oriental males	15; Training group (n = 8; 26.4 ± 1.2 y), control (n = 7; 24.6 ± 1.0 y)	Blood collection at Before and after 1, 2, 3, and 4 months of training ;Weight training program: 45 min/d; 3×/wk during 4 mo	OC (+26.3%) and B-ALP (+30%) ↑ in trained; PICP (−18%) ↓ in CO	No change in Dpd

Abbreviations:OC=osteocalcin; t-ALP= total alkaline phosphatase; b-ALP=bone alkaline phosphatase; PICP=Procollagen I carboxyterminal propeptide; ICTP=pyridinoline cross-linked carboxy-terminal telopeptide of type I collagen; PDP=Deoxypyridinoline; NTX= N-terminal telopeptide; Pyr= pyridinoline crosslinks; CTX= C-terminal telopeptide;↑=significant increase, -=no alteration;↓=significant decrease

11.3 Bone Metabolism and Sex Steroids

Apart from exercise training, scientific evidence suggest that sex steroids play an important regulatory role on bone health. In particular is has been reported that estrogens, and mainly estradiol, may be important in the regulation of bone resortion whereas both estradiol (E2) and androgen may play an important role in the maintenance of bone formation markers in the elderly and healthy male population (Ackerman et al. 2012; Sinnesael et al. 2011). Further support for the dominant role of sex steroids on bone metabolism comes from the findings of a study in 92 males between the ages of 20 and 44 (Leber et al. 2003). The authors reported that both gonadal androgens and estrogens are independent mediators of bone resorption in adult men. However, these findings are not universal. Aromatase inhibition in the elderly has been found to increase testosterone and reduce estrogen levels but hardly to affect bone metabolism markers (Falahati-Nini et al. 2000). In addition, studies on adolescent and collegiate athletes and on chronically trained middle aged men failed to observe any association between bone metabolism markers and total testosterone (TT), free testosterone (FT), follicle-stimulating hormone (FSH) and luteinizing hormone (LH). The latter evidence question the association between sex steroids and bone turnover markers, especially in young healthy trained individuals (Ackerman et al. 2012 ; Sinnesael et al. 2011; Falahati-Nini et al. 2000).

12. Vitamin D

12.1 Vitamin D physiology

Since its discovery in the early 19s Vitamin D understanding changed from a calcicotropic vitamin to a more complex factor with a role in multiple physiological systems in the body, including cell function and differientation (Hollick, 2002).

Vitamin D is a secosteroid produced in the skin under the influence of ultraviolet radiation converting 7-dehydrocholesterol to pre-vitamin D3. In the dermis pre-vitamin D3 is converted to vitamin D3 (cholecalsiferol) before its subsequent conversion to 25-hydroxy vitamin D (25(OH)D) in the liver. In the kidneys hydroxylation of 25-hydroxy vitamin D takes place to calcitriol (1,25-dihydroxy vitamin D (1,25(OH)2D)), its biologically active form. Dietary intake also provides small quantities of vitamin D2 (ergocalciferol) which follows the same hydroxylation pathway. After hydroxylation vitamin D is transported in the blood bound to vitamin D binding protein (Hamilton, 2010) and/or albumin. Vitamin D is well recognized for its role in calcium (in conjuction with parathyroid hormone (PHT)) and phosphorus homeostasis (Cashman, 2007). Vitamin D acts as a regulator of bone mineral homeostasis controlling serum levels of calcium and phosphate to be sufficient for the normal mineralization of type I collagen matrix in the skeleton (Haussler et al. 1997).

Like all steroid hormones calcitriol acts as a molecular switch to signal genetic transcription. More than 1000 human genes are direct targets of calcitriol (Taavera-Mendoza and White, 2007). Among the different vitamin D receptors (VDR) that have been observed, one has been found to be at the nucleus, acting as a classical nuclear receptor and another one at the membrane (Norman, 1998). A VDR activation-role has been well demonstrated in cell function and tissue development mainly in bone and muscle and is a lesser degree to other tissues such as parathyroid cells, liver, and chondrocytes (Montero-Odasso and Duque, 2005).

12.2 Vitamin D and exercise performance

Muscle weakness is also associated with vitamin D deficiency in elderly individuals (Clerup et al, 2000; Ward et al. 2009). Higher plasma concentrations of vitamin D are

associated with muscle strength, physical activity and ability to climb stairs, and lower concentrations among elderly people (Mowe and Bohmer, 1999; Dhesi et al. 2003) due to muscle atrophy. In addition, biopsy studies have revealed that vitamin D deficiency has no effect on type I muscle fibers, but results to a reduced proportion and diameter of type II muscle fibers (Ziambaras et al. 1997), and reversely affect neuromuscular coordination (Dhesi et al. 2004). Indeed, in a number of studies, muscle biopsies on vitamin D-deficient subjects before and after vitamin D treatment revealed atrophy of type II muscle fibers prior to treatment and significant improvement after treatment (Young et al. 1981). In addition, several cross-sectional studies have reported associations between vitamin D and various parameters of neuromuscular performance (Bischoff-Ferrari et al. 2004). Muscle strength appears to be influenced by the presence of vitamin D receptors (VDR) in the muscle cell. A role of VDR activation in muscle cell function and tissue development has been reported. In elderly individuals serum levels of vitamin reduce significantly which in turn may lead to reduction in VDR expression (Bischoff-Ferrari et al. 2004; Lee et al. 2003) activation and therefore reduction in their function, affecting muscle strength. Furthermore vitamin D levels are important for the release of calcium into the cytosol. Calcium is a critical modulator of skeletal muscle function and any perturbation to calcium handling may impact on both its contractile and relaxation properties (Berchtold et al. 2000). Therefore vitamin D levels may affect muscle function by increasing or not the calcium pool. In addition vitamin D affects the activation of protein kinase C (CPK) which affects protein synthesis in the muscle cell (Selles and Boland, 1991).

Evidence from observational studies has shown an association between vitamin D status and physical performance. The majority of the studies concerning vitamin D levels have been performed in elderly population showing a positive relationship

between vitamin D levels strength and physical fitness. Cross-sectional studies show that serum 25-hydroxyvitamin D (25OHD) levels are associated with quadriceps strength. Mowe and associates (1999) showed that low levels of vitamin D concentration in older adults were significantly related to reduced muscular function. In an analysis of men and women age 60 and over who participated in the cross-sectional NHANES III survey, individuals with higher serum 25(OH)D levels were able to walk faster (8-foot walk test) and to get out of a chair faster (sit-to-stand test) than subjects with lower levels (Bischoff-Ferrari et al. 2004). Similar results were observed by Stein et al. (1999) in an Australlian nursing home and hostel. In addition vitamin D deficiency has been reported to affect predominantly the weight-bearing antigravity muscles of the lower limb which are necessary for walking and postural balance (Glerup et al. 2000). Further confirmation for the correlation of vitamin D and muscle strength comes from a number of studies where subjects under vitamin D supplementation showed increased muscle strength (Glerup et al. 2000). A recent study by Ward et al. (2009) using as samples post-menarchal adolescent girls, and not elderly individuals, revealed a positive relationship between serum vitamin D levels and jump height, jump velocity, and power.

Limited evidence is available for the potential relationship between vitamin D serum levels and athletic performance. Early studies (Allen and Gurreton, 1945; Gorkin et al. 1938) suggested that both cardiovascular fitness and muscle endurance and speed were enhanced after explosion to ultraviolet radiation. In 1944 German investigators showed that irradiated students showed a 13% improvement in performance on a bike ergometer under ultraviolet radiation, whereas the performance of the control subjects was unchanged (Lehmann and Mueller, 1944). American researchers found that even a single dose of ultraviolet irradiation tended to improve the strength, speed, and

endurance of college women (Cheatum 1968; Canell et al. 2009). Furthermore a consisted literature indicates that physical and athletic performance is seasonal, it peaks when vitamin D levels peaks and declines as they decline (Cannell et al. 2009).

13.1 Lipid Profile and Exercise

The term 'lipid profile' describes the varying levels of lipids in the blood. The most commonly reported ones in the studies that examine the effects of exercise on lipid profile are total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol. Furthermore, apolipoprotein A (apo AI) which is the major protein component of HDL in plasma, apolipoprotein B (occurs in the plasma in 2 main isoforms, ApoB48 and ApoB100) which is its primary apolipoprotein component and is absolutely required for LDL formation in plasma, and Lipoprotein(a) [Lp(a)] which consists of an LDL-like particle and the specific apolipoprotein(a) that is covalently bound to the apo-B of the LDL like particle, have nowadays taken under consideration when examining the effects exercise on lipid profile.

There is substantial, consistent and strong evidence that physical activity is a deterrent for developing many forms of cardiovascular disease (Fletcher et al. 1992). Among its many benefits, habitual physical activity is thought to reduce cardiovascular disease risk, at least in part, by its favorable influence on circulating blood lipids and lipoproteins (Durstine et al. 2000; 1994). It has been suggested that exercise has the largest impact on so-called atherogenic dyslipidemia, characterized by low high-density lipoprotein cholesterol (HDL), and high total cholesterol (TC), and density lipoprotein cholesterol (LDL) particles (Trejo-Gutierrez and Fletcher, 2007). Comparisons between sedentary and physically active individuals and professional

athletes has been used to establish whether there is a positive influence of physical activity on blood lipid profile.

13.1.1 Physically Active versus Inactive individuals

13.1.1.1 Total Cholesterol (TC) and Low Density Lipoprotein choresterol (LDL)

In regard to LDL and TC, there is limited evidence to suggest that physically active individuals and athletes exhibit lower levels of TC and LDL compared to sedentary individuals. Some studies have reported lower plasma TC and LDL levels in males and females participating in endurance and resistance sport activities, professional skiers, and professional cyclists suggesting a dramatic influence of exercise on LDL and TC (Durstine et al. 2001; Mann et al. 2014). However when these findings were adjusted to potentially lipid-altered characteristics such as body-weight, body fat, caloric intake and dietary intake these differences in the majority of the studies were diminished or were even no longer significant (Kokkinos et al. 1995; Lakka and Salonen, 1992; Durstine et al. 2001; Mann et al. 2014). Notably, in the studies that have observed reductions in LDL levels between runners and sedentary individuals, the reduction in this lipoprotein was inversely related to the distance covered (Durstine, 1994). The majority of the evidence failed to observe any significant differences TC and LDL concentration between sedentary and exercise trained individuals. It has been shown that males participating in endurance and resistance events, power or speed related sports, cross-country skiers and recreational and master athletes did not show any difference compared to inactive individuals in TC and LDL (Buyukyazi, 2005; Fett et al. 2009; Durstine et al. 2001; Mann et al. 2014). These findings are supported by epidemiological evidence that did not show any association between physical activity and TC and LDL levels in normo and hyperlipiademic groups (Gordon et al. 1983, 1984).

Table 7. Exercise training and Blood Lipids

Study	No of subjects	Training protocol	Measure	Effect
Nybo et al. 2012	26	Aerobic exercise (prolonged) 12 weeks 150 min/week 65 % VO2max	TC,HDL,LDL	HDL↑, No changes in TC,LDL
LeMura et al. 2000	48	Aerobic exercise 16 weeks 3 sessions/week 70–75 % HRmax (weeks 1–8), 85 % HRmax (weeks 8–16) 30 min (weeks 1–8), 45 min (weeks 8–16)	TC,HDL,LDL	HDL↑, No changes in TC,LDL
Kraus et al. 2002	111	Aerobic exercise (jogging) 24 weeks 65–80 % VO2peak Jogging Calorific equivalent of 20 miles/week	TC,HDL,LDL	HDL↑, LDL↓ No changes in TC,
O'Donovan et al. (2005)	64	Aerobic exercise (moderate intensity) 24 weeks 3 sessions/week 60 % VO2max 400 kcal/session Aerobic exercise (high intensity) 24 weeks, 3 sessions/week 80 % VO2max , 400 kcal/session	TC,HDL,LDL	TC↓, LDL↓ No changes in HDL
Fett et al. (2009)	50	Resistance training, circuit training, 8 weeks, 3 sessions/week (weeks 1–4), 4 sessions/week (weeks 4–8) 60 min	TC,HDL,LDL	TC↓, no changes HSL, LDL
Shaw et al. (2009)	28	Aerobic and resistance training, 16 weeks 3 sessions/week, Aerobic: 60 % HRmax, resistance: 60 % 1 RM 45 min	LDL	LDL↓
Manning et al. 1991	16 obese Females	Resistance training; 12wk; f = 3/wk; I = 60-70% 1RM; D = 3 sets, 6-8 reps	TC, HDL, LDL, apo B100	No changes in TC, HDL, LDL, apo B100
Despres et al. 1990	5 males	Cycle ergometry; 100d; f = 6/wk; I = low; D = 2 × 53 min sessions to expend 1000 kcal/d	TC,HDL,LDL	TC↓, HDL↑, LDL↓
Williford et al. 1996	12 (participating in an exercise conditioning program) and 5 (controls participating in a team sports program)	15-week exercise training program 5 d.wk-1 for 15 weeks (weight training 2 d.wk-1 and aerobic training 3 d.wk-1)	HDL, LDL	No changes in HDL, LDL, no difference compared to controls
Kishali et al. 2005	20 male athletes (national team wrestlers), 44 male students.	Habitual training	TC, HDL, LDL,apo AI, apo B100, Lp(a)	No difference in TC, HDL, LDL,apo AI, apo B100, Lp(a) among 4 groups
Holme et al. 2007	198 sedentary males and 21 females : control (n ¼ 43), exercise	1 year of exercise, average 3 sessiions/week, consisting of one hour endurance training (aerobics, circuit training and fast walking/jogging). Workouts were offered at 60–80% of maximal heart rate	HDL, LDL, apo AI, apo B	No changes in HDL, LDL, apo AI, apo B in none of the intervention groups

Table 7 continued

	alone (n ¼ 54), diet alone (n ¼ 55) and combined dietary and exercise intervention (n ¼ 67)			
Mankowitz et al. 1992	8 male runners	male runners who ran 30-40 miles/week were studied in the trained state and after 14-22 days of detraining.	TC, HDL, LDL, Lp(a)	HDL ↓, no changes for TC, LDL, and Lp(a)
Fripp et al. 1987	14 old male adolescents	9-week resistive exercise program	HDL, LDL	HDL ↑, LDL ↓
Vatani et al. 2011	30 healthy males	Intervention:[MI: moderate intensity (45-55% 1RM), HI: high intensity(80-90% 1RM)] and one Control (no intervention) groups. Subjects in MI and HI intervention groups underwent 3 supervised resistance-training sessions per week for six weeks.	ApoB, ApoA, LDL, HDL	HI group : HDL↑, TC↓, LDL ↓ No changes in apo B, apo A
Honkola et al. 1997	38 males (type 2 diabetic subjects) 18 participated in the training program, 20 served as controls	circuit-type resistance training 5-month individualized progressive resistance training programme (moderate intensity, high volume) twice a week	TC, LDL	TC↓, LDL ↓
Shaw et al. 2009	38 male volunteers: non-exercising control group (NE) (n = 12), an aerobic training (AT) group (n = 12), or a combination training (CT) group that used both aerobic and resistance training (n = 13)	Exercise training groups: 3 times a week training/16 weeks. AT subjects: exercise for 45 minutes at 60% of their individual age-predicted maximum heart rates using treadmills, rowers, steppers and cycle ergometers. Intensity was increased by 5% every four weeks (65, 70 and then 75% of their individual age-predicted maximum heart rate) by increasing treadmill, rower, stepper and/or cycle ergometer speed and/or resistance (grade or tension).	LDL	AT programme: LDL↓ CT programme: LDL↓

Abbreviations: TC=total cholesterol; LDL=low density lipoprotein; HDL=high density lipoprotein; apo A=apolipoprotein A; apo B=apolipoprotein B; Lp(a)=lipoprotein a;↑=significant increase, -=no alteration;↓=significant decrease

13.1.1.2 High Density Lipoprotein Cholesterol (HDL)

On the contrary exercise seems to have a more profound effect on HDL. Most of the published reports suggest that HDL is higher in physically active individuals and athletes compared to sedentary controls, although these findings are not universal (Ruiz et al. 2004). The available literature indicate that individuals who have physically demanding jobs and/or engaged in endurance exercise activities have higher HDL levels compared to their less active counterparts or inactive individuals (Frey et al. 1990; Williford et al. 1996; Williams, 1993). Similarly, highly trained skiers and cyclists, professional and recreational athletes (Buyukyazi, 2005), and

moderately trained students (Kishali et al. 2005) have been observed to have much higher HDL levels compared to sedentary individuals. Notably, as with LDL, in the studies that have found beneficially altered HDL the observed differences were associated with the distance covered (Mann et al. 2014).

13.1.1.3 Apolipoproteins

Compared to TC, LDL, and HDL less evidence exist in regard to the apolipoproteins apo-A and apo-B. A recent study in middle aged males showed that apo AI levels showed a favorable association with leisure time physical activity, even at moderate levels (eg, walking, cycling, gardening, bowling), while apo-B levels ratios showed an association with only vigorous leisure time physical activity (Behre et al. 2010). Similarly, an earlier cross-sectional study in 113 non-smoking men aged 30 to 45 years showed that exercise is associated with lower apo B concentrations (O'Donovan et al. 2005). In addition, apo-AI levels and apo-B100 levels were shown to be higher and lower in endurance-trained male athletes, swimmers and volleyball players compared with aged-matched men enjoying an active life style and moderately trained subjects compared to sedentary controls (Kishali et al. 2005; Ruiz et al. 2014; Olchawa et al. 2004). Furthermore, a randomized longitudinal intervention study of 219 healthy men aged 40 to 49 years, characterized by overweight and physical inactivity, showed that apo B levels as well as the apo B/apo AI ratio correlated inversely with changes in fitness during 1 year of follow-up. Interestingly, Despres et al. (1990), reported reductions in apo B in conjunction with reductions in LDL levels suggesting that these two indices change in a same pattern (Holme et al. 2007).

However, not all studies managed to show a beneficial effect of exercise on these apolipoproteins. Endurance-training studies failed showed an increase in apo AI concentrations in sedentary males even after 1 year under endurance exercise (Holme

et al. 2007; Durstine and Haskell, 1994; Durstine et al. 2001) and between high levels athletes, recreationally athletes, and sedentary controls (Buyukyazi 2005). This percentage is higher in studies examining apo B that have failed to show any difference between active and inactive individuals (Durstine and Haskell, 1994; Durstine et al. 2001). Similarly, no difference was observed in apo B and its subtraction apo B100 as a response to resistance exercise (Manning et al. 1991). Although the reason for the differences in these studies is not clearly apparent, it could be related with changes in body fat, body weight, dietary intake, and other lifestyle characteristics (Durstine et al. 2001, 1994).

13.1.1.4 Lipoprotein a (Lp[a])

Despite the limited data, Lp(a) does not seem to be beneficially affected by exercise. Indeed, early observational and longitudinal exercise studies failed to observe an association between exercise training and Lp(a) (Durstine 1994) indicating that exercise has no effects on Lp(a) levels (Lobo et al. 1992). In additions Mankowitz et al. (1992) reported no effect of Lp(a) after 14 or more days of detraining. Confirmations to these observations come from the findings of Ruiz et al. (2014). The authors reported that Lp(a) levels were not different between inactive individual, swimmers, and volleyball players. In agreement are the findings of recent studies that did not observe any difference in Lp(a) levels in elite, recreationally, and collegiate athletes compared to sedentary individuals. (Buyukyazi, 2005; Kishali et al. 2005).

Since Lp(a) is very similar in structure and composition with LDL and consists of an LDL-like particle one should expect that exercise-induced changes in LDL levels could also influence its values . However, present information indicate that Lp(a) is a heritable trait (Lamon-Fava et al. 1989) and is not linked metabolically with LDL

(Dahlen et al. 1986). Therefore, physical interventions programs that alter plasma LDL levels most probable would not affect on Lp(a) concentration.

Notably, under specific training induced condition exercise might alter Lp(a) levels. A study examining the long term effect of exercise in sedentary individuals reported that Lp(a) levels rose almost twofold over a period of 9 months (Clavel et al. 1997).Similarly, other studies have reported higher Lp(a) levels in soccer players compared to swimmers and volleyball players (Ruiz et al. 2014) and in adult long distance runners compared to non-athletic controls (Hubinger et al. 1996). According to these findings athletes are presented with significantly higher Lp(a) levels compared with controls. It should be hypothesized that this median Lp(a) concentration in these athletes could have been be due to greater physical demands which cause muscular or systemic stress reaction. The latter suggestion might be responsible for these observed discrepancies since this condition may be associated with raised Lp(a) values, as Lp(a) has been shown to share characteristics of inflammatory parameters. Therefore the above mentioned findings indicate that exercise may affect Lp(a) concentration under extremely stressful situations that can cause inflammatory responses.

13. 2 Intervention studies

Several interventional studies have tried to evaluate the effects of different exercise protocols on lipid profile. The authors of these studies have examined mainly the effects of aerobic and resistance exercise on blood lipids under various intensity and volume regimes, that elicit different caloric demands.

Significant changes in TC and LDL from training interventions are not usually reported with few exceptions, that has reported 4 to 7% improvements by regular exercise in TC

and LDL in both sexes (Durstine et al. 2001, Mann et al. 2014). Therefore, there is little evidence to support a training threshold (volume or intensity threshold) above which those two parameters are beneficially altered. The available evidence indicate that in untrained individuals, differences (i.e increases) in the volume of the endurance/aerobic training programs are the most effective in producing a healthiest profile in TC and LDL. This is supported by the findings that the total distance covered is inversely correlated with the reductions in LDL, indicating the importance of training volume. (Dunn et al. 1997). Similarly, high intensity/high volume exercise have been found to decrease TC and LDL levels versus high intensity low volume and moderate intensity low volume (O´Donovan et al. 2005). It should be mentioned when changes are observed in TC and LDL these are associated with training programs in which participants extended more than 1200 Kcal/week (Durstine et al. 2001, Mann et al. 2014). However, these observations describe training intensities that cannot be sustained for a long period by general population since it can lead to increase rate of injury and drop out (Durstine et al. 2001). In contrast, well trained individuals do not seem to respond even in extremely increases in training volume, probably due to an already beneficial adaptation of long term strenuous exercise on TC and LDL. Although several factors have been proposed to influence TC and LDL changes in these studies such as their baseline levels, changes in body weight and body fat, and exercise intensity, it does not seem according to the majority of the literature to be determinants for any exercise induced changes in LDL and TC.

In regard to exercise intensity, is has been proposed that increased intensity of the aerobic exercise has more consistent impact upon LDL than moderate levels of physical activity, but does not seem to affect significantly TC. In general, regarding LDL, although its levels are not-consistently changed during aerobic exercise, there

exists a relationship that appears to be dose-dependent with both the amount and the intensity of exercise (Kraus et al. 2002). Less information exists supporting resistance training as a modifier of plasma lipids compared to endurance exercise. In particular, resistance exercise training seems to be less effective in lowering TC and LDL level, especially in males. In contrast to the majority of the studies (Kokkinos et al. 1991; Lemura et al. 2000; Boyden et al. 1993) evidence from other laboratories have observed decreases in their levels (Trejo-Gutierrez and Fletcher, 2007), however these studies had employed a small sample size and furthermore the majority of those have been limited in females. The explanation for these suggestions could be related to the relatively few calories that expended during resistance exercise versus endurance activities.

The importance of the caloric expenditure during training interventions programs is supported by recent studies which have shown that changes in TC and LDL should not be expected after low volume exercise interventions of either resistance or aerobic activities (Durstine et al. 2001; Mann et al. 2014). Apart from energy expenditure it has been suggested that circuit resistance training does beneficially affect TC and LDL levels (Honkola et al. 1997; Fett et al. 2009). The latter findings are adding to the speculation that the volume of movement might be as important as the amount of weights lifted. Lastly, the evidence from some studies indicate that reductions in fat mass and increases in lean body muscle after resistance training are associated with decreased TC and LDL levels although this findings are not universal (Mann et al. 2014).

In regard to HDL, it has been suggested that it is the component of the lipid profile that is most likely to improve with changes in physical activity through training interventions, even with moderate levels of training. It has been observed that training

volume of aerobic or resistance activities, opposed to training intensity (Nydo et al. 2010) play the major role in beneficial improvements in HDL, especially when they accomplish an energy expenditure limit of 12000 to 22000 Kcal/week. This is further supported by the findings of Kraus et al. (2002). The authors reported that the group assigned to high-amount, high-intensity exercise (equivalent of jogging 20 miles per week at 65% to 80% of maximal oxygen consumption during 8 months) had the only significant increase in HDL (3.8 mg/dL) compared to the control group. These findings support the reported notion that along with greater exercise training volumes the alterations in HDL are greater (Durstine et al. 2001). The above mentioned energy expenditure is translated in the aerobic events to a distance covered of 24-37 km/week. (LeMura et al. 2000). The more effective factor, increasing HDL levelswas the volume and intensity of the performed exercise training (Kraus et al. 2002). In accordance are the observations that aerobic exercise of high training intensity (80% of VO_2max) versus moderate intensity (60% VO2max) resulted to significantly greater HDL levels (Durstine et al. 2001; Mann et al. 2014). These data suggest that in relation to aerobic exercise, both total energy expenditure and intensity are regulatory factors for alterations in HDL levels.

According to the findings observed in the intervention studies that have employed resistance exercise activities, discrepancies exist regarding the HDL response. With the volume controlled, acute changes in HDL levels were observed in low and moderate training intensities (50% of 1RM, and 75% of 1RM respectively) compared to maximal intensity (110% of 1RM). Vatani et al. (2011) observed that only the high intensity training program (80-90% of 1RM) compared to the moderate intensity program (45-55%) resulted to significantly increased HDL levels, a surprising finding since previous studies have shown that increased level of this lipid fraction is likely to

increase even in low levels of physical activitiy (Kesaniemi et al. 2001). However, this finding further supports the hypothesis that training intensity when equalizing the training load facilitates an additional benefit on HDL profile (Mann et al. 2014). In regard to the resistance training volume it has been consistently shown that the volume of movement has a greater impact on lipid profile including HDL (Mann et al. 2014) than increased intensity. Notably the majority of these studies have been performed in inactive or recreationally physically active individuals but not professional athletes.

Some interventions studies tried to evaluate the effectiveness of the combination of aerobic and resistance exercise training on lipid profile, since both of these type of activities are employed in the training regimes of many individual and team sports. However, the available evidence coming from studies that have employed various modes, frequencies, intensities and volumes of exercise show contradictory findings (Tambalis et al. 2009). Interestingly, evidence from the studies that observed beneficially affected lipid profile after the combination of aerobic and resistance activities showed that no difference were evident between the resistance /aerobic group versus the aerobic group although they both beneficially affected lipid profile (Shaw et al. 2009). The authors concluded that the combination of these two modes of exercise do not result to an additional beneficial effect on lipid profile.

In regard apo AI, apo B100, and Lp(a) to the best of our knowledge no evidence exist in intervention studies. However, some conclusions can be made by examining physically active individuals or athletes participating in different sports, where the mode of the performed activities during training and competitions vary considerable. No significant differences were observed in to apo A, apo B, and Lp(a) levels between wrestlers and physically active collegiate students (Kishali et al. 2005). Similarly top level athletes under high intensity and higher volume training showed no different

values in to apo AI, apo B, and Lp(a) compared to recreationally athletes (Buyukyazi, 2005). In contrast, Berg et al. (1986) reported that athletes participating in power activities (weight lifters, hammer throwers) had lower apo AI compared to aerobic trained athletes (endurance athletes cyclists, and cross county skiers), and higher apo B levels compared to both aerobic and anaerobic athletes (decathletes, sprinters wrestlers). In a recent study Ruiz et al. (2014) observed higher Lp(a) levels in soccer players compared to swimmers and volley ball players, higher apo AI values in swimmers compared to both soccer and volley ball players, and lower apo B100 levels compared to both soccer and volleyball players. These findings indicate that different sports that employ various modes of training activities may affect differentially these three lipid profile parameters. However, more detailed intervention studies are needed to drive to more conclusive suggestions.

1.3.3 Sex Steroids and Lipids Profile

Although to the best of our knowledge no studies has examined the association between sex steroids and lipid profile in athletes, evidence from studies on non-athetic population suggest that these hormones are related with alterations in blood lipids.

In regard to estrogen, it has been demonstrated that in men with coronary artery disease (CAD) blood level of estradiol correlated positively with blood level of TC and LDL (Wranicz et al. 2005). A positive, significant correlation was found between blood concentrations of estradiol and TC and LDL (Denti et al. 2000). In addition blood level of estradiol positively correlated either with TC or LDL in different populations of men with CAD. (Khaw and Barrett-Connor 1991; Kiel et al. 1989). In contrast, in men with inherited mutation of gene encoding aromatase, an enzyme necessary for estrogen biosynthesis, i.e. in estrogen deficiency, increased levels of LDL and decreased HDL were found (Morishima et al. 1995). In another study,

Shono et al. (1996) which reported that E2 level were negatively related to LDL. In addition, Lipoprotein (a) is probably also reduced by the action of estrogens since it has reported that estrogen is known to lower Lp(a) (Samsioe, 1994) This finding suggest that the higher levels E2 within physiological ranges in healthy men may partially help to maintain a desirable profile of the plasma lipid.

In regard to androgens the majority of cross-sectional studies have found a positive correlation of endogenous testosterone with HDL and a negative correlation with total cholesterol, and LDL (Malkinet et al. 2003). Low TT might have adverse effects on the lipid profile and thus represent a risk factor for hypercholesterolemia, hypertriglyceridemia, high LDL, and low HDL, suggesting the importance of maintaining an appropriate TT level in men (Zhang et al. 2014). In addition, several studies on patients with coronary artery disease have shown that higher testosterone levels are associated with higher HDL concentrations (Wickramatilake et al. 2013; Denti et al. 2000; Wranicz et al. 2005). Furthermore, a significant linear correlation has been observed between FT and LDL, although other authors failed to support any correlation (Denti et al. 2000; Wranicz et al. 2005). However, these findings are not universal since epidemiological data suggest that testosterone levels are negatively associated with total cholesterol and LDL-C, while in men testosterone levels appear to have a complicated and controversial relationship with HDL-C levels and cardiovascular risk (Kraemer and Rogol, 2006).

There is a more consistent relationship with testosterone and other lipid fractions. Most studies on elderly or hypogonadal populations report reductions in total cholesterol, LDL and apo B, although this findings are not universal (Durstine et al. 2001; Mann et al. 2014). Testosterone treatment has also been shown to reduce Lp(a)

level (Zmunda, 2003). Furthermore, it has been observed that increasing testosterone level results to decreased ApoB/A1 ratio (Ohlsson et al. 2011).

In regard to androgen precursors, it has been reported that higher DHEAS concentrations were associated with better lipid profile. Notably, DHEA has been found to be positively associated in men only with HDL (Vaidya et al. 2008). Further support to the proposed beneficial effect of DHEAS on lipid profile comes from another study which has suggested DHEAS to be as one of the independent predictors of HDL with a positive relation, and of cholesterol/HDL ratio, with a negative relation (Shono et al. 1996).

14. Red Blood Cells, Hematocrit, and Exercise

14. 1 Effects of Red blood cells and Haematocrit on exercise

Exercise seems to affect blood rheology. Several studies have observed that the fluidity of blood can be improved by regular physical exercise. This phenomenon may have important aspects for sports medicine, atherosclerosis research, and anti-ischaemic therapy. Hematocrit (Hct) and red blood cells (RBC) are two haemorheological parameters that show a bidirectional relationship with exercise performance (El-Sayed et al. 2005; Mairbaurl, 2013).

Hematocrit is defined as the ratio of the volume of red blood cells to the total volume of blood. Actually, it represents the direct measurement of red cells blood volume (El-Sayed et al. 2005). Changes in red blood cell count may affect hematocrit which in turn could result to alterations in blood and plasma volume in a linear manner (El-Sayed et al. 2005; Mairbaurl, 2013). This would lead to altered blood viscosity. In this way hematocrit may affect exercise capacity due to its relationship with blood viscosity. An increase in plasma volume, mediated by Hct changes, normally results in enhanced exercise performance due to reduced blood viscosity, thereby optimized

microcirculation and improved oxygen delivery capacity to the working muscle (Schumacher et al. 2000). According to these suggestions Hct not only affects the amount of oxygen transported per volume of blood but also affects the rheological properties of the blood.

Red blood cells play also an important role in the ability to perform efficiently during exercise. The main function of red blood cells in exercise is the transport of O_2 from the lungs to the tissues and the delivery of metabolically produced CO_2 to the lungs for expiration (Mairbäurl, 2013). Furthermore, Despite O_2 transported blood cells fulfill a variety of other functions, all of which also may improve exercise performance. Likely the most important one is the contribution of red blood cells in buffering changes in blood pH by transport of CO_2 and by binding of H+ to hemoglobin (Mairbäurl, 2013). Red blood cells also take up metabolites such as lactate that is released from skeletal muscle cells during high intensity exercise. Uptake into red blood cells decreases the plasma concentration of these metabolites that may compromise exercise performance (Maughan et al. 1997). Finally, red blood cells seem to be able to decrease peripheral vascular resistance by releasing the vasodilator NO and by releasing ATP which stimulates endothelial NO formation causing arteriolar vasodilation and augments local blood flow (Mairbäurl, 2013).

14.2 Exercise Effects on Blood Rheology

The aforementioned evidence clearly state the importance of both Hct and red blood cell concentration on exercise performance. On the other hand exercise can influence these haematological variables. Several authors have reported that athletes display several rheological changes that differ than those of healthy sedentary individuals. Indeed, decreased levels of Hct and RBC have been reported in athletes compared with physically inactive controls (El-Sayed et al. 2005; Mairbaurl, 2013; Shumacher

et al. 2002). Regarding Hct, it has been observed that blood volume and plasma volume increased rapidly after exercise training sessions, whereas red blood cells volume did not change for several days, before it began to increase indicating that Hct values were decreased for several days (El-Sayed et al. 2005; Mairbaurl, 2013). Similarly, Hct has been found to be reduced after 4, 8 days of prolonged exercise and a mountain bike competition respectively (Mounier et al. 2003; Wirnitzer and Faulhaber, 2007). In regard to RBC count, its values have been found to be lower in athletes compared to sedentary individuals (Convertino, 1991). However, these findings are not universal and it has been documented that these changes are not mainly an effect of physical activity in general, but a result of the specific type of exercise, and mainly aerobic endurance in nature (Guglielmini et al. 1989; Spodaryk et al. 1993). Indeed, this suggestion seems to be the case in endurance events. Endurance training can lead to what has been termed sports anaemia. This is a result of a dilutional pseudoanaemia secondary to plasma volume. It is considered as anaemia in that the haemoglobin concentration is lower than the usually defined limits of normal. For a long time it has been explained with increased RBC destruction during exercise (Mairbaurl, 2013). However, most well conducted studies have demonstrated that the red cell mass is actually unchanged or even expanded, not decreased as it is in most true anaemias (Maimun et al. 2005; Mairbaurl, 2013). The observations of a literature review indicate that the effects of endurance training on red blood cell include actually increases in their number (Szygula, 1990). Evidence suggest that mainly endurance training may increase the rate of RBC production, concomitantly with accelerated turnover, resulting in a preserve of a new steady-state population of younger RBCs. Although under normal conditions, red blood cells (RBCs) have a lifespan of about 120 days, the rate of aging may increase to 70 days

during intensive aerobic training. This may lead to hypoxia in working muscle during single episodes of exercise and possibly an increased rate of RBC destruction with long term exercise, which facilitates a higher RBC turnover. As described in endurance runners, there is an increased incident of haemolysis as a mechanical compression of mainly old RCB in capillaries (Schobersberger et al. 1990) which could act as a stimulus for erythropoiesis. This mechanism is leading to a higher proportion of young RBC into the circulation. This physiological pathway may enhance athletic performance because younger (newly formed) RBC are more deformable and are able to deliver oxygen to the exercising muscles more efficiently than older cells (Smith, 1995). This may be partly due to the higher concentration of 2,3-diphosphoglycerate in younger RBCs which decreases the affinity of hemoglobin for oxygen at the partial pressures found in the contracting muscle, and therefore facilitating oxygen uncoupling in the periphery (Smith, 1995).

Notably, these phenomena has been only described as effects of endurance sport such as long distance running, cycling and swimming (Schobersberger et al. 1990). In addition it should be mentioned that providing RBC destruction during exercise does not exceed the rate of RBC production, no detrimental effect to athletic performance should occur.

Less evidence exists for strength training or mixed sports. Schumacher et al. (2001) examined the hematological indices in various athletes. The authors observed that within the different athletic population higher values of RBC and Hct were found in strength trained individuals compared to endurance athletes and participants in sports with mixed activities. Mixed trained athletes showed a tendency for lower values compared to strength trained individuals. The findings of this study could be related with the widely accepted suggestion that endurance/aerobic exercise induces plasma

volume expansions (El-Sayed et al. 2005), therefore the lack of similar activities in strength trained individuals compared to mixed sports failed to lead to similar rheological changes. Hu et al. (2008) examined the effects of 20 weeks of strength training on physically inactive men. Unlike sports anemia induced by endurance training, this training period resulted to increased Hct and RBC count. Similarly, a 7-week period of moderate to intense exercise resulted to increased RBC count but not in Hct (Kilgore et al. 2002; Schobersberger et al. 1990). Bodeuf et al. (2009) after 6 months of resistance training, failed to show any difference in plasma hematocrit and red blood cell count, in older adults. These data suggest that in contrast to endurance type athletic training, resistance training do not induce at least pronounced sports anemia. Therefore the mode of exercise produces different affects in blood rheology regarding both RDC count and Hct. Furthermore, it could be hypothesized that a mediated TT increase due to strength training would may in turn increased RBC production. Since plain strength training, which could increase TT levels (Kraemer and Rogol, 2006), does not elicit sports anemia, this speculative increase in RBC would be in turn translated to increased Hct.

14.3 Intervention Studies

No intervention study has examined the effects on long periods of training on Hct and RBC count. In general moderate endurance exercise resulted to pseudoanemia (i.e. reduced Hct and RBC count) which with ongoing training was accompanied by indices of hemolysis and shifts towards younger RBC in the circulation (Maimoun et al. 2005; Mairbaurl, 2013). Nevertheless these adaptations are evident only with specific duration of endurance training, and are connected with increased muscle mass. Notably, athletes that participate in endurance training for leisure (3h/week) display lower Hct and RBC values compared to groups of endurance training

competitive athletes (Schumacher et al. 2002). In this study endurance trained athletes with increasing amount of weekly training show an increased RBC and Hct levels which could indicate an increased erythropoeisis.

14.4 Detraining and Blood Rheology

All the above mentioned studies have examined periods of training on blood rheology indices. However, very scarce evidence exist regarding the detraining effects on RBC and Hct in athletic population. To the best of our knowledge only two studies have evaluated the response of RBC and Hct to short period of detraining (Mujika et al. 2004; Koury et al. 2005). No differences were observed in neither Hct nor RBC count indicating that during short term detraining the effects of exercise in these two hematological indices are retained.

14. 5 Red Blood Cells and Sex Steroids

The available literature indicates that androgens have the ability to affect erythropoeisis. One of the effects of androgens, and mainly testosterone, is to increase the production of erythropoietin. Another is to increase the responsiveness of immature bone marrow cells to the effects of erythropoietin. Simplistically, testosterone increases the output and effectiveness of erythropoietin, which in turn stimulates the production and regulation of red blood cells (Shahani et al. 2009) and thus possible Hct. Furthermore, it has been observed that reduced androgens levels are related with reductions in red blood cells count and similar alterations also in Hct (Fonseca et al. 1998). The aforementioned evidence indicate that testosterone plays a key role in erythropoiesis and that low testosterone are related with a deterioration in the ability of to produce optimal red blood cells levels. This decreased cell production may cause a significant drop in exercise performance by affecting the oxygen delivery capacity to the exercising muscles during exercise.

15. Erythrocyte Sedimentation Rate (ESR)

Erythrocyte Sedimentation Rate (ESR) is a nonspecific measure of inflammation that is commonly used as a medical “in vitro” screening test. In particular, it reflects mainly the increase in production of a certain protein produced in response to necrosis or inflammation. These changes occur in many acute and chronic infections, tumors and degenerative disease. There are several factors which will affect the results of this test such as (a) an abnormal increase in some plasma proteins may prevent the RBCs from sedimenting, causing a falsely decreased ESR. In contrast, some other abnormal plasma proteins cause the RBCs to clump quickly and fall at an abnormally increased rate, resulting in a falsely increased ESR and (b) Red Blood Cell factors: Anemia causes a falsely increased ESR because the change in the RBC to plasma ration favors rouleaux formation, causing the RBCs to sedimentquickly. Rouleaux is a condition in which the RBCs clump together like stacks of coins. Microcytes (abnormally small RBCs) will descent more slowly than macrocytes (abnormally large RBCs). RBCs with an abnormal or irregular shape, such as sickle cells (sickle shaped RBCs) or spherocytes (round RBCs, do not have biconcave shape), hinder rouleaux formation and cause a falsely decreased ESR.

In regard to athletes extremely limited evidence exist regarding the response and the relationship of ESR and exercise. In a recent study ESR was assessed in 44 athletes, with the main presenting symptom of fatigue between 1997 and 2004; only one had a clinically significant elevated value of 35 mm h−1, this athlete was diagnosed with recurrent sinusitis. ESR in this study, was one of the tests to yield clinically relevant abnormal results (Toit et al. 2007). In another study Hsieh et al. (2014) tried to determine possible relationships between aerobic capacity, pulmonary function, and disease-related variables in patients with ankylosing spondylitis (AS). Neither aerobic

capacity nor vital capacity correlated with ESR (Hsieh et al. 2014). Interestingly, Elosua et al. (2005) reported that compared to sedentary men, men practicing light physical activity had significantly lower ESR. Furthermore, it was reported that, compared to low physical performance, intermediate and high physical performance were inversely associated with ESR. In other words these authors, using data obtained in an epidemiological study performed in a population-based sample, demonstrated that physical activity and performance are associated with lower ESR in humans.

These findings support public health recommendations suggesting that a signicant benefit could be already achieved with light to moderate physical activity (Elosua et al. 2005). The above mentioned data suggest that reduced inflammation is associated with increased exercise. However, prospective studies will be required in order to verify these findings and to establish the effects of strenuous exercise training that professional athletes undergo on ESR, and furthermore the possible effects of different modes intensities and volume of exercise in ESR.

15.1 Erythrocyte Sedimentation Rate and Sex Steroids

Studies on patients with several parthological situations have observed a relationship between ESR and sex steroids. More specifically, positive correlations were noted between testosterone and ESR in patients (Brennan et al. 2003). In another study although there was no association of ESR with testosterone, there was evident a significant correlation with LH and FSH (Leal et al. 2006). In addition, the findings of another study reported that an increase in ESR was related to a decrease in TT, FT, and E2 (Mikkola et al. 1999).

B. Specific Information

1. Soccer, Sex Steroids and Exercise performance

In regard to soccer fairly limited number of studies have examined the relationship between soccer training and sex steroids. The majority of the available evidence has mainly focus on testosterone. Soccer is a high intensity intermittent exercise that combines aerobic and anaerobic activities and places considerable demands on the neuromuscular and hormonal systems (Bangsbo, 1994). Although the main metabolic functions in soccer are sustained by aerobic metabolism (Filaire et al. 2001)the ability of the neuromuscular system to produce maximal strength and power and their derivatives (sprints, jumps, acceleration) appear to be of crucial importance (Bangsbo, 1994). As a consequence of the demands placed on soccer players, the current conditioning programs involve the development of specific physical capacities such as endurance, speed, strength and power. However, the maintenance or improvement in performance is not solely depended on sufficient conditioning. It is now believed that several other factors influence exercise performance among which is the circulating sex steroids (Tiidus, 2004; Cardinale and Stone, 2006; Kraemer et al. 1995),

The association between soccer and sex steroids levels is complex and still controversial. Thus, periods of high intensity training in soccer reduce androgen basal concentration(Filaire et al. 2001) whereas its levels remain unchanged (Grandi and Celani, 1990),or increase(Kraemer et al. 2004) as a response to regular soccer training. Even less evidence exist for interventional studies combining soccer training and strength sessions. The existing literature suggest that the combination of soccer training with linear or non-linear strength sessions result in increased total testosterone (TT) basal levels and neuromuscular (Pacobahyba et al. 2012, Gorostagia et al. 2004), but does not affect free testosterone (FT) and endurance capacity (Gorostagia et al. 2004).

Notably, no evidence at all exists regarding the effects of seasonal alterations in training volume and load on both the sex steroid levels and ergometrics. Furthermore, to the best of our knowledge, to date there is no evidence examining the responses of several parameters that has been reported to be affected by sex steroid such as bone metabolism markers, lipidemic profile and specific blood rheological indices in soccer players under different seasonal training regimes.

2. Aim of the study

The main aim of the present work was to examine the effects of three different seasonal soccer training programs on serum sex steroid levels and on exercise performance capacity in professional soccer players. More specifically, we examined the effects of different soccer training regimes on the fluctuation of adrenal and gonadal sex steroid levels and on performance capacity over time (pre-season period until the end of the championship). The training programs differed among the three teams, i.e. high volume training program in Team A, moderate in Team B, and low in Team C. The difference involved (a) volume and load of general (GSC) and (b) soccer-specific strength training (SPS) (a common training method in today's soccer training). The effects were measured by maximal oxygen consumption (VO2max) and neuromuscular performance parameters, i.e. squat jump (SJ), countermovement jump (CMJ), and sprint performance. To assess the effect of the three training programs on circulating endogenous sex steroids, we measured total-testosterone (TT), free-testosterone (FT), dehydroepiandrosterone-sulfate, (DHEAS), Δ4-androstenedione, 3a-androsten-diol-glucuronade (3a-Diol-G), estradiol (E2), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin (PRL). In addition, we examined the association between changes in performance parameters to changes of levels of endogenous androgens. Furthermore, we examined the effects of these three different

training regime on bone metabolism markers (OC, CTX, b-ALP, CICP), lipidemin profile [TC, HDL, LDL, apo AI, apo B100, Lp(a)], selected blood rheological indices (i.e. RBC, Hct), erythrocyte sedimentation rate, body weight, body fat, waist to hip ratio, and body mass index and any possible relationships between these parameters with sex steroids levels throughout the study.

The secondary aim was to examine the effects of the six week off season detraining period, prior to the beginning of the pre-season period for the forthcoming soccer season, in all the above mentioned parameters i.e. sex steroids, exercise performance, lipidemic profile, RBC, Hct, ERS, body weight, body fat, waist to hip ratio, and body mass index. Furthermore, we examined the association between changes in sex steroids levels to possible alterations in exercise performance, bone turnover, blood lipids, ERS and the selected blood rheological properties. In addition, during this six week detraining period we examined the responses of vitamin D levels to reduced training, and any possible relationship of this secosteroid with exercise performance indicesAccording to the study design our research protocol aimed to provide an answer to the following scientific questions:

(a) Intervention Period

1. *To examine whether the different training regimes in strength training volume and load over an entire soccer season could affect sex steroids basal levels and ergometrics (i.e. exercise performance indices).*
2. *To examine whether any observed changes in the examined performance parameters and sex steroids response to the different seasonal training regimes were associated.*
3. *Whether our three different seasonal training regimes elicited different effects on specific bone metabolism markers, blood lipids, selected blood rheological*

indices, and ERS and if any observed alterations were related with the examined sex steroids levels.

4. *To examine the alterations induced by three different seasonal programs on body weight, percent body fat, waist to hip ratio, and body mass index*

(b) Detraining Period

5. *To examine whether the off-season short-term detraining soccer period, which is characterized by a massive reduction in training stress has any effect on sex steroids levels, ergometrics, and on the examined metabolism markers, blood lipids, selected blood rheological indices, and ERS.*
6. *To examine whether any possible alterations in exercise performance parameters were related with sex steroid levels during the detraining period.*
7. *To examine the alterations induced by the soccer detraining period on body weight, percent body fat, waist to hip ratio, and body mass index*
8. *To examine the effects of the soccer detraining period on vitamin D levels, and any possible association between its levels and ergometrics.*

Sex Steroid Measurements

The adrenal and gonadal sex steroids that were measured in our study are as follow:

- Total testosterone,
- Free Testosterone,
- 3alpha diolG,
- Δ 4-androstenedione,
- DHEA-S,
- Estradiol
- FSH,
- LH
- PRL.

3. Materials and Methods

Participants

Sixty seven professional soccer players, members of two Greek Superleague teams (Team A (mean±SD); n=23, age=25,5±5,3, height=1,82±0,11, Team B; n=22, age=24,7±4,9, height=1,81±0,07) and one Greek Football League 2 team (Team C; n=22, age=23,8±4,1, height=1,81±0,053) participated in this study (table 8). All the participants were professional soccer players from more than 5 years. Players received verbal explanation for the study and a written consent was obtained. The study was conducted according to the declaration of Helsinki and was approved by the ethics committee of the University of Crete. Allparticipants have signed an informed concent before their participation in the study

Table 8. Anthropometric Characteristics of the three experimental teams

	Team A	Team B	Team C
Age (years)	25,5±5,3	24,7±4,9	23,8±4,1
Height (m)	1,82±0,11	1,81±0,07	1,81±0,05

Exclusion Criteria

The exclusion criteria were as follow: a) any medical or endocrine disorder that could affect the ability of the players to participate in the study and/or affect endogenous hormonal

Ergometrics

The soccer exercise performance that were measured in our study are as follow:

a) Aerobic Capacity
- Maximal OxygenConsumption

b) Jumping Ability (Field test for maximal strength evaluation)
- Squat Jump (SJ)
- Countermovement jump (CMJ)

c) Speed
- 10 meters sprint,
- 20 m spint

production; b) suspicion of the use of exogenous agents; c) players that their contract was ending before the end of the conclusion of the study; d) the use of vitamin D supplements; and e) players that failed to follow the programmed experimental sessions, or were absent from more than 15 days from normal training.

As a result of the third criterion (exclusion criterion c) seven players were excluded from the study (four from team A and three from team B). As a result of the fourth criterion (exclusion criterion d) 2 players were excluded from the study. Therefore form the initial sample size of seventy seven players (76), finally sixty seven (67) players participated in the study.

Table 9. Weekly Training Program during the Competition Period

Team A	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Morning			moderate volume high intensity strength circuit training + moderate intensity specific soccer strength training	specific soccer strength training + low intensity technical tactical training		speed training + moderate intensity game in the half filed (10') + freecicks, corner cicks	reaction speed training 20'-25'
Afternoon	starters recovery training non starters friendly game vs U20 or SSG training	Day off	speed, agility coordination training + SSG	moderate intensity technical tactical conditioning (BSG)	low to moderate intensity technical tactical training (BSG)		Game
Team B							
Morning	-	-	high intensity strength training + low to moderate intensity technical training		-	-	-
Afternoon	starters recovery training non starters friendly game vs U20 or SSG training	Day off	speed, agility coordination training + SSG	soccer specific strength training + moderate intensity technical tactical conditioning (BSG)	low to moderate intensity technical tactical training (BSG)	speed training + moderate intensity game in the half filed (10') + freecicks, corner cicks	Game
Team C							
Morning	-	-	-	-	-	-	-
Afternoon	starters recovery training non starters friendly game or SSG training	Day off	speed, agility coordination training + SSG	moderate intensity technical tactical conditioning (BSG)+ Soccer specific strength training **or** general strength training (every second week)	low to moderate intensity technical tactical training (BSG)	speed training + moderate intensity game in the half filed (10') + freecicks, corner cicks	Game

Table 10. General Strength Training Characteristic in the thee experimental teams

Team	Strength Training Type	Intensity	Strength Training Exercises
Team A	Circuit Strength Training in the Field, 10 stations, 4 sets 10 reps in free weight exercises, 4' rest between sets	Moderate Intensity (70-80% of 1RM)	Core Strength exercises (2 exercises) + Free weight exercises: lunge, squats, steps up on bench with external weight, pullover, arm curls (biceps), triceps, bench press
Team B	4 sets, 5-6 reps, explosive action high execution speed	High Intensity GSC (90% 1RM)	Leg extension, hamstring curl, chest press, calf raise, pullover, arm curls (biceps), triceps
Team C	4 sets, 5-6 reps, explosive action high execution speed, (alternating with SPS training every second strength training session).	High Intensity GSC (90% 1RM) or SPS	Leg extension, hamstring curl, chest press, calf raise, pullover, arm curls (biceps), triceps

GSC; General strength conditioning, SPS; soccer-specific strength

3.1 Study Design

The study was performed over a period of 12 months, and was divided in two periods. The first period i.e. detraining period (period 1) had a 6-week duration which had specific aims (see Period 1 [detraining] sub-section) in order to support the integrity of the forthcoming second intervention period (period 2) which was starting at the beginning of the pre-season period for the next competitive season until the end of the championship (approximately10 ½ months).

Period 1 (Detraining period)

The duration of the first period was six weeks. The first testing was performed at the end of at the previous competitive soccer season, while the second testing was performed after a six-week transition off-season period. Notably, all three experimental teams followed a similar, but not identical, weekly training plan (similar training intensity, volume, and load) the last 4 months of the previous competition season consisting of 5 training/week including all aspects of physical conditioning and a competition (soccer match)**.** Furthermore, they were instructed to follow an identical off-season training regime. The experimental testings that were performed during this detraining period had a variety of aims in order the selection of the participants to support the accuracy of the main aim of this project,i.e. the seasonal intervention soccer season. The aims during this period were as follows: a) selection of the

participants, i.e. to examine primarily which players would continue to participate in the study since our participants were professional soccer players and (their contracts were ending just prior to the beginning of the intervention period of our study (i.e. just before the baseline experimental testing at the beginning of the pre-season period) b) controlling the training regime used by all three teams during the off-season period in order to avoid variations in both ergometric and sex steroids values prior to the beginning of period that could long term or short term interfere with our findings, c) an effort to control the dietary intake during off-season just prior to the beginning of the intervention period, since sex steroids levels have been reported to be affected by dietary manipulations, d) to examine whether this period have resulted in any difference in sex steroids levels which could have most probable been an effect of using anabolic agents throughout the previous competition period, and thus to exclude these players from the study, and finally due to these two performed measurements, an experimental aim which was e) to examine the effects of the detraining off-season transition period primary on sex steroids and ergometrics, and moreover in all the other examined parameters in our study including vitamin D.

During the detraining period, all players were tested in two different occasions. The first experimental testing took place immediately after the end of the competition period in the middle of May (pre). The second experimental testing was performed at the beginning of July (post), prior to the beginning of the preparation period for the forthcoming season. Each experimental testing consisted of two days of consecutive measurements. The first day of each experimental session anthropometric characteristics were measured at 08:30 am. From 09:00 to 10:30 am, venous blood samples were obtained to determine the concentration of the different analytes, after squat jump (SJ), countermovement jump (CMJ), 10 m, and 20 m sprint performance. The second day of each experimental session our participants were tested for the determination of maximal oxygen consumption (VO2max). The measurements for the determination of VO2max values started at 09:30 am. All hormonal and exercise performance measurements, during the two experimental sessions, were performed at the same time of the

day and players were tested in the same order to avoid any circadian variation on the measured variables.

Detraining Period training program

During the first two weeks of this recuperation period, participants were informed to avoid any kind of physical activity. After this two-week period, they were instructed to perform low intensity (50%–60% of VO2max) aerobic running of 20 to 30 minute total duration (30, 20, 2×15, 3×10, 2×10) three times a week, divided by one day of rest.

Period 2 (Intervention period)

The second, and main part, of our study was performed over a period of 10 ½ months (42 weeks), from the beginning of the pre-season period until the end of the championship. Players were tested in three different occasions throughout the study. As baseline testing was considered the one performed prior to the beginning of the preparation period at July. The second experimental (mid-point) session was performed in the mid-season point at January immediately after the end of the first half of the competition period, and the latter testing (end-point) was placed the week immediately after the end of the competition period at May. Each experimental session included two days of consecutive testing. Prior to each testing period players were under no exercise stress for at least two days to avoid any fatigue effects. During the first day at 08:30 am of each experimental period anthropometric characteristics were measured. Afterwards, from 09:00 to 10:30 am venus blood samples were obtained for the determination of the concentration of the measured hormones, bone turnover markers, lipidemic profile, RBC, Hct, and ERS. At the afternoon of the same day (17:00 pm) players were tested for squad jump (SJ), countermovement jump (CMJ), 10 and 20 m sprint performance. The second days of each experimental session players were tested for the determination of maximal oxygen consumption (VO_2max) starting from the morning at 09:30 am. All measurements were performed during all three experimental sessions in the same time of day and players were tested in the same order to avoid any circadian variation on the

measured variables. Only in the first experimental session, players were tested for the determination of 1 repetition maximum (1RM) in the specific strength exercises (free weight exercises or machine weight exercises) that were implemented in strength training programs. This testing was performed the morning of the next day of the VO2max measurements staring at 10:00 am. Verbal encouragement was given to all subjects during all testing procedures, ensuring the maximal effort throughout all testing procedures

Training

Playing Schedule

During the whole experimental period players of teams A, B, and C participated in 33, 32, and 33 official (competition and cup) games respectively.

Training Program

The training program in the three experimental teams was set by the team coaches with the cooperation of the researchers. During both pre-season and competition periods (Table 9) players participated in approximately 75-95 minute mean duration training sessions , focusing on all aspects of physical conditioning, and technical-tactical elements of the game (Tables 10,12).

Categorizing Training Sessions

Players participated in low, moderate, and high intensity conditioning with (soccer drills) or without the ball (aerobic continuous, intermittent running, fartleck training, speed and agility drills). In order to analyze and evaluate the weekly training load specific sub-components of each session were categorized according to the conditioning target of each training as follow: Technical-Tactical training (TT) when targeting to the technical- tactical elements of the players and Technical-Tactical Conditioning (TTC) when targeting to the technical- tactical elements of the players7 ,both performed in conjunction with soccer drills such as small sided games (SSG), and big sided games (BSG) aiming to improve physical conditioning, speed

and agility conditioning(SAC) when the target was speed and agility performance, endurance conditioning (EC) when running was performed (without the ball), general strength conditioning (GSC) when the aim was general strength conditioning, and soccer specific strength training (SPS) when players performed soccer specific strength exercises. The strength sessions employed were categorized as follow according to the recommendations by Baker (1996): General strength exercises when the aim is to increase the maximal strength of the selected muscle groups, special strength (SC) when the aim is to train for power once strength levels have increased, and specific strength when the employed exercises provide a training stimulus that is very similar to the specific skills needed during an actual competition (i.e. sprints, jumps, shots, changes of direction etc.)

Differences in Training Programs among Teams

The three training schemes that were used by teams A, B, and C were organized in such way to mainly differ in the number of the GSC and SPS during both the preparation and the in-season period (Table 10). Team A had the higher weekly number, and frequency of strength based session (Table 12). The second weekly higher values in these two parameters were observed in team B (Table 12). Lastly, the training scheme employed by team C had the lowest values in volume and frequency of strength sessions per week (Table 12). In that way the three different seasonal training plans in our study were expressed as follow: High strength training stress, Moderate strength training stress, and Low strength training stress for teams A, B, and C respectively.

Preparation Period

During the pre-season period players of teams A, B, and C participated in 74, 63, and 52 training sessions respectively in a period of 7 weeks. Participants of teams A and B participated in the same number and nature (training volume, load, and intensity) of training sessions including TT (15 sessions) , TTC (+SSG,BSG; 20 sessions), SAC (4 sessions), EC (9 sessions) whereas players of team C had similar number of TTC (+SSG,BSG; 20 sessions)

and SAC (4 sessions), and slightly lower training sessions in TT (11), and EC (6 sessions). The main difference accounted in the number of SPS and GSC (team A; GSC=11, SPS=15, team B; GSC=6, SPS=9, Team C: BSC=4, SPS=7) in the three experimental teams, with the training plan of team A showing the highest number of both SPS and GSC whereas the training plan of team C the lowest. The seventh week of the pre-season period all teams followed their in-season weekly training plan (Table 12). During this phase all friendly games were recorded as TTC.

In-season Period

In-season weekly training plan was kept constant throughout the competition period in each team (Table 9). The weekly training program was mainly different in the number and nature of the SPS and GSC sessions performed by each team (Tables 9, 10). The training plan that was employed by team A had one high intensity-moderate volume GSC and 2 SPS session per week. In regard to team B, its players participated in one high intensity-low volume general strength training and one SPS every week. Players of team C had the lowest number of strength training sessions in our study, only one per week. The weekly training plan in this team was organized in such way that the first week a GSC was performed alternated by a SPS sessions every second week throughout the in-season period. Apart from the different weekly number of the SPS sessions in each team, an extra training session was performed by the players of team A at the morning of the match day, consisted of warm up, reaction speed (6-8 short sprints) and cool down. During the competition phase teams A, B and C had 3, 2, and 1 cup matches respectively at midweek (Wednesdays). During these cup-weeks training plan was different including 2 recovery sessions, 2 low intensity-low volume TT sessions, and 1 SAC session in all three experimental teams.

Training Load, Volume, and Intensity

Training load of each session was assessed with the use of the 10 point RPE scale modified by Foster et al (1995). Training load was calculated by multiplying PRE score by the duration

of each session in minutes. The RPE of each session was collected about 30 minutes after the each session to ensure that the perceived effort was referred to the whole training, rather the most recent exercise intensity. All participants were familiarized with the PRE scale at least twice, during the last soccer season. In order control the training load and to avoid any overtraining effect that would negatively affect both the adrenal-gonadal axis and exercise performance (Urhausen et al. 1995) daily training monotony (average daily training load/standard deviation of the daily training load calculated over a week) was measured. In regard to training volume, it was expressed during the study as total number of training sessions and training time in minutes (min).

Heart Rate maximal (HR max) assessment was used for the determination of the training zones (<50-60% of HR max, 60-70% of HR max, 70-80% of HR max, 80-90%, of HR max, 90-100% of HR max). Heart Rate was periodically measured throughout the experimental period one week per month in each team (Polar Team2 Pro,Polar Electro,Oy, Finland), to verify the aimed exercise training intensities that were set by the coaches and the researchers for every training session.

Soccer Specific Strength (SPS)

All three experimental teams employed SPS sessions throughout the study (Table 9), but in a different weekly frequency. Soccer specific strength has been defined as the ability of a player to use muscle strength and power effectively and consistently throughout soccer season (Bangsbo, 1994). These sessions included various forms of activities such as skipping over cones, jumping on one or two legs, jumping over hurdles or obstacles, shooting, heading, acceleration, repeated sprints, eccentric movements such as deceleration and stopping, changes of direction etc. (Manolopoulos et al. 2006) combined with maximal intensity soccer activities such as crossing, shooting and heading (Mujika et al. 2009). Notably, regarding the employed SPS sessions in the in-season weekly training program by team A, different activities were used in each session. Due to the long duration of the experimental period the

coaches did not use identical drills throughout the study in the performed SPS sessions, but employed a large variety of exercises. However, all performed drills in each SPS were similar in nature, duration, and volume. During every SPS the number of plyometric exercises (jumps) was kept constant to 45 jumps per sessions for each individual. Every SPS was of 35-40 minutes duration and was performed in conjunction with other training- conditioning activities (Table 9).

General Strength Conditioning (GSC)

General strength conditioning was used by all teams (table 9). Teams B and C employed the same high intensity low load GSC regime, whereas team A players followed a high intensity, moderate volume strength training regime in conjunction with two core strength exercises performed in a circuit manner (Table 10).

Laboratory Measurements

Anthropometry

Anthropometric characteristics of the subjects are shown in Table 12. Prior the beginning of the first experimental day in each three testing sessions, height was measured using a stadiometer (Charder HM210D, Charder Electronics CO, LTD, Taiwan) to the nearest 0,1 cm and weight was obtained using an electronic weight scale (Seca Alpha 770, Seca Vogel, Hamburg, Germany) to the nearest 0,1 kgr. Body fat percentage was assessed by skinfold thickness measurement (Lange Skinfold Caliper, Cambridge Scientific Instruments, Cambridge, UK) using the 4 spot formula by Jackson and Pollock (1978). All measurements were made by the same investigator.

Neuromuscular Performance Measurements

The jumping ability of the soccer players (SJ, CMJ) was assessed with a jumping mat (Powertimer, Newtest Ltd., Oulu, Finland). For each of two jump types three tests were carried out and the best result was used for the analysis. During both SJ and CMJ the arms

were kept in the iliac crest to minimize their contribution. Subjects performed SJ starting from a standing position bending the knees to 90^0, stopping for three seconds, and then jumping as high as possible. CMJs were performed starting from a standing position after which players were instructed to jump as high as possible after a fast preparatory downward eccentric movement. Sprint times were measured with infrared photoelectric cells (Powertimer, Newtest Ltd., Oulu, Finland). At each sprint type two consecutive measurements were performed, from a standing position. Players commenced the sprints when ready from a standing start 0,5 behind the start. Three sprints were executed with 120 seconds rests in between, to avoid any fatigue effects, and the best value was recorded. The best time of two trials of each distance was used for the analysis. Before each of the experimental sessions a low intensity 15 min standardized warm up was performed.

Maximal Oxygen Consumption (VO2max) Measurement

The second experimental day players were tested for the determination of VO2max and Heart Rate max on a motorized treadmill. HR max was recorded from the highest 5-s average of the HR recorded with a portable HR monitor (Polar, s610, Polar, Electro, Kempel) Warm up consisted of a six minute run at 8kmh. speed was set at 10km-h and held constant for 3 minutes. Thereafter speed was increased by 2km/h every 3 minutes until 16 Km/h and then speed was increased every 2 minutes until voluntary exhaustion. Achievement of VO2max was considered as the attainment of at least 2 of the following criteria: (a) a plateau in VO2 despite increasing speeds, (b) a respiratory exchange ratio above 1.10, or (c) a heart rate (HR) within 5% of age-predicted maximal HR (220-age). Expired gases were analyzed using a breath-by-breath, automated gas-analysis system (VMAX29, Sensormedics, Yorba Linda, CA). Before each test, flow and volume were calibrated using a 3-L capacity syringe (Sensormedics, Yorba Linda, CA). Gas analyzers were calibrated using 2 tanks of oxygen (O2) and carbon dioxide (CO2) of known concentrations (Sensormedics, Yorba Linda, CA).

One Repetition Maximum Test

The determination of 1RM was carried through the procedures suggested by Kraemer et al (1995c). Players were tested in all free weight exercises or machine weight exercises (Cybex International, Inc. USA) that were employed in the study. Subjects were always kept under surveillance of the first author and the coaches to ensure proper exercise technique and safety.

Blood Collection and Analysis

On each test day, venous blood samples were obtained after a period of a ten-minute rest in a lying position. Blood samples were collected in tubes, containing a clot activator and serum gel separator and were centrifuged at 3000 rpm for 10 minutes to separate serum. Serum samples were then stored at −70°C till analysis. All samples were tested in duplicate. Total testosterone (ng/ml), LH (IU/L), FSH (mIU/L), E2 (pg/Ml), and PRL (μg/L) concentrations were measured using AIA 21 fully automated immunoassay analyzer (TOSOH-Eurogenetics Tokyo, Japan). The sensitivity of the assays for TT, E2, FSH, LH, and PRL were 7 ng/ml, 25 pg/ml, 1,0 mlU/ml, 0,2 mlU/ml, and 1,05 respectively. The intra and inter coefficient of variation were 3,1–5,2% and 2,48–5,99% for TT, 2,6–6,1% and 3,8–9,1% for E2, 1,5–2,6% and 4,3–5,6% for FSH, 1,8–2,5% and 2,1–2,7% for LH, and 1,8–2,1% and 2,7–2,9% for PRL.

Free testosterone (pg/ml), 3a-Diol G (ng/ml), Δ4-androstenedione (ng/mL), and DHEAS (μg/mL) concentrations were measured using enzyme-linked immunoabsorbent assays (Alpco Diagnostics, Windham, NH). All procedures were carried out according to the instructions of the manufacturer. The sensitivity of the assays for FT, Δ4-androstenedione, DHEAS, and 3a Diol G were 0,17 pg/ml, 0,04 ng/ml, 0,005 μg/ml, and 0,1 ng/ml respectively. The intra and inter coefficient of variation were 4,7–17% and 5,3–12,4% for FT, 4,9–5,8% and 7,7–9,7% for Δ4-androstenedione, 7,5–11,5% and 4,2–15,3% for DHEAS, and 6,0–7,8% and 6,5–10,8% for 3a Diol G. All samples were analyzed in duplicate and in the same assays.

Bone Metabolism Markers

The standard commercial assays that were used in order to measure the examined bone formation and bone resorption markers are shown in table 11.

Table 11. Bone metabolism measurements

Bone Metabolism Marker	Method of measurement	Assay
Osteocalcin:Serum intact bone gla protein (OC)	competitive immunoassay which uses a monoclonal mouse antibody that recognizes only intact (de novo) human osteocalcin	NovoCalcin®, Metra Biosystems, USA
Serum bone-specific alkaline phosphatase (S-B-ALP)	EIA which uses a murine monoclonal anti-B-SAP that has selective, high affinity for the bone alkaline phosphatase isoform, low cross-reactivity to the liver form of alkaline phosphatase and negligible binding of intestinal isoenzyme	Alkphase-B™, Metra Biosystems
Type I collagen C-terminal propeptide (PICP)	enzyme-linked immunoassay (ELISA) using the CICP assay	METRA® CICP EIA Kit, Quidel
Urinary C-terminal cross-linking telopeptide of type I collagen (U-CTX-I)	competitive EIA, which uses an antibody recognizing the (8AA) sequence in the C-terminal telopeptide region of the α1 chain of collagen I, where the aspartate 1211 residue (D) is β-isomerized	CrossLapsEIA, CIS Biointernational, France

Measurements of Lipid Profile

Serum concentrations of TC, HDL-C, and triglycerides (TG) were measured by use of an automated chemistry analyzer (Olympus AU-600) with reagents from the same manufacturer. LDL-C was calculated according to the Friedewald formula. (Friedewald et al. 1972.). Serum apolipoprotein A-I (apo AI), apolipoprotein B100 (apo B100), and Lp(a) were measured by rate nephelometry (lipoprotein [a] [LPA] test, apolipoprotein A-I [APA] test, and apolipoprotein B100 [APB] test, Beckman Instruments Inc, Galway, Ireland). Serum thyrotropin (reference range: 0.15-3.20 mIU/mL), FT4 (reference range: 9-27 pmol/L), FT3 (reference range 3-8.5 pmol/L) were measured by Vitros Immunodiagnostic products (Ortho-Clinical Diagnostics, Amersham, UK).

Hematocrit, Red Blood Cells count and Erythrocyte Sedimentation Rate (ERS)

Whole blood was processed using a Cell-Dyn 3700 automated Hematology Analyzer (Abbott Laboratories, Chicago, Illinois, USA) and Hct RBC and ERS levels, were measured according to the manufacturer's instructions.

Vitamin D measurement

Venus blood samples were obtained following a ten-minute period of rest in a lying position, after an overnight fast. Vitamin D levels were assessed using DiaSorin 25 hydroxy vitamin D (DiaSorin Inc. S.p.A, Italy), repeatability CV= 3-6 % and reproducibility CV=6-11%, according to the laboratory standard operating procedures.Calibrator 1 and 2 (human serum) concentrations are referenced to standard preparations containing highly purified 25(OH) vitamin D. According to the manufacturer the correlation of the immunoenzymatic assay with LC-MS/MS is described by the equation: concentration = 5,6+0,83*LC-MS/MS (R=0,93). The intra assay coefficient of variation ranges between 3-6%. In our study vitamin D levels below 10ng/ml were considered as severe deficiency, levels between 10- 20ng/ml as deficiency and levels between 20 - 30ng/ml as insufficiency.

Delimitations of the study

Before each experimental session players were informed to avoid consuming any supplement that could promote performance at least 2 days prior the testing, to keep their hydration status, and to avoid any caffeine or alcohol beverages at least 3 hours prior each testing. Nutritional guidelines were given to all layers in order to ensure a >60% carbohydrate dietary intake during the study. All players had been familiarized with the testing protocol, as they have been previously tested on several occasions during the last soccer season with the same testing procedures. This study was a part of a larger research project examining adaptations of many hormonal, physiological, and biochemical parameters to the different training schemes employed by our three experimental groups.

3.2 Statistical Methods

Our statistical analyses was performed differentially at the two experimental periods i.e. Detraining period (period 9) and Intervention period (period 10), since at period 1 we examined the differences between two time-points, while at the intervention period, which was and the main aim of our study measurements were performed at three different time points, at the beginning of the pre-season, at the middle, and at the end of the soccer competition period.

Detraining Period (Period 1)

Statistical analysis was performed using software program SPSS 19.0. Standard statistical methods were used for the determination of means and standard deviations (±SD). The changes between the experimental periods in the measured parameters within the groups were analyzed by the paired samples t-test for normally distributed variables and Mann-Whitney test for non-normally distributed variables. Pearson's (for normally distributed variables) and Spearman's (for non-normally distributed variables) correlation coefficients were used to assess the linear relationship between quantative variables. The differences between the groups at baseline, in all hormonal and performance parameters, and body composition parameters were analyzed with the General Linear Model (GLM) analysis of variance, aiming to examine whether both teams had similar values in sex steroids and exercise performance variables at the starting point. The level of significance was set at $p<0.05$.

Intervention Period (Period 2)

Differences at baseline between the three groups in all hormonal, performance, bone metabolism, blood lipids, rheological indices and ERS, body composition parameters, training load, and training volume were examined in the context of univariate ANOVAs. When equal variance was assumed, Bonferroni adjustments were employed, while in cases where assumption of homogeneity of variance was violated, the Welch test and DunnetT3 adjustment were used. Pearson’s (for normally distributed variables) and Spearman’s (for non-normally distributed variables) correlation coefficients were used to assess the linear

relationship between variables. Evaluation of the effects on all measured variables in question (performance parameters: VO2max, SJ, CMJ, 10m, 20m; body composition parameters: body weight, %fat body composition, BMI, WHR; sex steroids: total testosterone, free testosterone, 3a Diol G, Δ4-androstenedione, DHEAS, E2, FSH, LH, PRL), lipidemic profile parameters, and blood rheological properties (of each training regime over time was pursued through a series of two-way 3x3 mixed ANOVA with season time (baseline, mid-point, end-point) as the within subjects variable and training regime (Team A representing high training load, Team B moderate and Team C low training load) as the between-subjects variable. In order to examine the differences in bone metabolism markers the paired samples t-test for normally distributed variables and Mann-Whitney test for non-normally distributed variables since only measurements at two times points wre performed, at the baseline and at the end of the study. Pearson's (for normally distributed variables) and Spearman's (for non-normally distributed variables) correlation coefficients were used to assess the linear relationship between quantative variables. Significance was set at $p<0.05$. Power analyses indicated that for the effect size of interactions observed in the study, estimated power for detecting significant simple effects ranged between 0.89 and 0.96 at alpha=0.05. Statistical analysis was performed using the software program SPSS 19 and power analysis was conducted with GPower 3.1.

Detraining Period (Period 1)

3.3 Results

Differences between the Groups at Baseline

No significant differences were observed between the three experimental groups at the beginning of the study for VO2max ($F = 1.769$, $p = 0.179$), SJ ($F = 1.008$, $p = 0.371$), CMJ ($F = 1.849$, $p = 0.166$), 10 m ($F = 1.102$, $p = 0.339$), 20 m ($F = 0.660$, $p = 0.520$), body weight ($F = 0.016$, $p = 0.89$), and body fat percentage ($F = 0.003$, $p = 0.95$). Similarly, no significant differences were observed for any of the TT ($F=1.411$, $p = 0.249$), 3a Diol G ($F = 0.626$, $p = 0.538$), FT ($F = 2.74$, $p = 0.10$), DHEAS ($F = 0.208$, $p = 0.813$), Δ4-androstenedione ($F =$

1.466, p = 0.191), E2 (F = 1.085, p = 0.344), FSH (F = 1.525, p = 0.225), LH (F = 0.849, p = 0.432), and PRL (F = 1.193, p = 0.310) at the beginning of the study between the three experimental groups. In regard to FT no difference was evident at at the beginning of the study for teams A and B (F = 2.741, p = 0.105) andbetweens teams B and C F = 0.603, p = 0.442). In contrast, a significant difference was observed between teams A and C in regard to FT F = 6,347, p = 0.016).

Body Weight, Body Fat Percentage, WHR, and BMI

Body weight increased significantly in teams A (77,60±5,88 vs 79,13±6,16; p<0.001), B (77,89±8,75 vs 79,49±8,95; p<0.001) and C (78,76±5,14 vs 79,69±5,48; p<0.001) at the end of the study compared to the beginning of the study. Similarly, a significant increase in body fat percentage was observed in both teams A (9,2±3,33 vs 11,01±4,11; p<0.001), B (9,43±3,55 vs 10,40±4,08; p=0.001), and C (8,65±1,95 vs 9,71±2,48; p<0.001) at the end of the six-week detraining period compared to pre-values. In addition, analysis of our data showed significant increases in both BMI and WHR in teams A (2.340±1.235 vs 2.386±1.358; p<0.001; 0.845±0.026, 0.856±0.027, p<0.001 respectively), B (2.358±1.720, 2.407±1.790, p<0.001; 0.843±0.029, 0.855±0.031, p<0.001 respectively), and C (2.388±0.786, 2.417±0.868, p<0.001; 0.830±0.019, 0.845±0.019, p=0.001 respectively) at the end of the study compared to their values at the beginning of the study.

Sex Steroids

Sex steroid values at the beginning and after the six-week detraining period are presented in table 13. No significant differences were observed for any of the measured hormones in team A: TT (p = 0.14), FT (p = 0.32), Δ4-androstenedione (p = 0.28), DHEAS (p = 0.13), 3a Diol G (p = 0.40), E2 (p = 0.09), FSH (p = 0.11), LH (p = 0.44), and PRL (p = 0.72), team B: TT (p = 0.73), FT (p = 0.90), Δ4-androstenedione (p = 0.95), DHEAS (p = 0.052), 3a Diol G (p = 0.50), E2 (p = 0.36), FSH (p = 0.88), LH (p = 0.067), and PRL (p = 0.69), and team C: TT

Table 12. Mean weekly values (SD) of Training volume, Training Load, and Monotony during the whole experimental period

	Pre Season			1st Half of the competition period			2st Half of the competition period		
	Team A	Team B	Team C	Team A	Team B	Team C	Team A	Team B	Team C
Volume									
Number of training sessions	74	63	52	138	104	86	142	106	88
Session mean duration (min)	90,88 (4,27)	94,39 (6,60)	85,62 (5,35)	81,75 (16,79)	80,33 (11,75)	73,6 (15,85)	80,78 (15,45)	80,11 (12,34)	74,1 (15,67)
Mean weekly volume (min)	966,69*† (35,29)	871,41*# (16,84)	653,33*†# (12,79)	653,12*† (4,33)	481,76*# (4,40)	368,41*†# (4,32)	653,13*† (15,12)	481,41*# (8,84)	368,41*†# (9,66)
Training Load									
Mean weekly RPE	3641,57† (387,79)	3614,02# (572,30)	2720,43†# (151,44)	2871,73*† (57,20)	2254,31*# (20,71)	2218,42*†# (15,67)	2832,25*† (76,61)	2258,28*# (11,94)	2167,04*†# (7,29)
Monotony	1,97 (0,027)	1,77 (0,094)	1,88 (0,14)	1,178 (0,019)	1,199 (0,015)	1,084 (0,024)	1,170 (0,017)	1,195 (0,016)	1,087 (0,014)
Number of matches	-	-	-	19	18	18	15	15	16
Mean matches RPE	-*	-	-	640,6 (4,32)	637,3 (5,12)	638,2 (487)	640,2 (4,51)	636,1 (4,36)	637,8 (4,98)

* team A vs. team B, † team A vs. team C, # team B vs. team C within the same season training measurement at $p < 0.05$ (equal variance assumed: Bonferroni adjusted or equal variance not assumed: DunnetT3 adjusted)

(p = 0.74), FT (p = 0.92), Δ4-androstenedione (p = 0.33), DHEAS (p = 0.53), 3a Diol G (p = 0.97), E2 (p = 0.57), FSH (p = 0.75), LH (p = 0.51), and PRL (p = 0.40) at the end of the six-week detraining period compared to their pre-detraining resting values.

Exercise Performance

The mean values ± SD of VO2max (ml/kg/min) decreased significantly at the end of the study compared to their pre-detraining values in team A (p<0.001), team B (p<0.001), and team C (p<0.001). Similarly, in teams A, B, and C there was observed a significant decline in SJ (all p<0.001), and CMJ (all p<0.001) values (cm) in the same period. All three teams showed significant increases in 10 m (all p<0.001), and 20 m (all p<0.001) sprint times (sec) at the end of the study compared to pre-detraining values.

Bone metabolism markers

The changes in the measured bone metabolism markers and the examined sex steroids are presented in table 14. Our findings revealed that the six-week detraining period resulted

Table 13. Mean values (SD) of Sex Steroids concentration during the detraining period

Hormones	**Team A**		**Team B**		**Team C**	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
3a Diol G (ng/ml)	8,88 (3,01)	8,43 (2,54)	8,40 (3,08)	8,69 (2,87)	9,66 (4,89)	9,86 (5,76)
TT (ng/dl)	656,97(136,5)	604,98(141,9)	679,89(109,7)	686,81(124,3)	611,26 (161,73)	622,34 (125,93)
FT (pg/ml)	12,50 (4,86)	13,81 (6,13)	13,11(14,79)	13,9(18,30)	9,17 (3,93)	9,25 (3,38)
Δ4 (ng/mL)	1,78(0,46)	1,86 (0,39)	2,07 (0,93)	2,077 (1,26)	2,29 (1,26	1,91 (0,72)
DHEAS (µg/mL)	1,99(0,63)	2,35 (1,07)	2,08 (1,38)	2,89 (1,18)	1,87 (1,16)	1,53 (0,51)
E2 (pg/mL)	23,69 (11,99)	19,62 (12,82)	28,70 (16,04)	25,38 (11,06)	26,01 (13,97)	
FSH (mIU/L)	8,29 (7,46)	7,41 (5,56)	5,77 (4,08)	5,71 (3,96)	6,06 (3,36)	6,00 (3,87)
LH (IU/L)	4,97 (1,95)	4,69 (1,67)	4,38 (1,92)	4,95 91,71)	4,99 (1,990	4,63 (1,45)
PRL (µg/L)	12,80 (7,70)	12,25 (6,71)	9,96 (4,74)	10,24 (5,00)	11,16 (5,68)	9,69 (3,31)

Pre=prior to the beginning of the detraining period; post=after the end of the detraining period

in significant reductions in all measured bone formation markers (OC; p<0.001,b-ALP; p<0.001, and CICP; p<0.001) at the end of the study compared to thei pre-detraining values in all three experimental teams. The examined bone resorption markers CTX exhibited a significant reduction at the end of the study compared to their pre-detraining values in all three experimental teams of the same statistical magnitude (p<0.001).

Blood lipids, Hct, RBC and ESR.

No significant differences were observed at pre to post values for TC (p>0.05), HDL-C (p>0.05), LDL-C (p>0.05), apo AI (p>0.05), apo B100 (p>0.05) and Lp(a) (>0.05)(Table 15). Similarly no significant differences were observed at the end of the study compared to the pre-detraining values for RBC (p>0.05), Hct (p=0.05), and ESR (p=0.05).

Vitamin D

The values of Vit D and exercise performance parameters at pre and after the six-week transition period are presented in table 16. The analysis was performed used the whole sample size (n=67) and not individually by each team. Vitamin D levels increased significantly

Table 14. Bone metabolism mean values (SD) during the detraining period in the three experimental teams

	Pre			Post		
	Team A	Team B	Team C	Team A	Team B	Team C
Bone Formation						
OC	23,52 (11,46)	12,09 (4,36)	12,68 (8,38)	22,73 ** (11,31)	11,15** (4,25)	12,25 ** (8,26)
b-ALP	41,13 (20,06)	20,97 (4,38)	23,72 (10,05)	39,71** (18,99)	19,84 ** (4,29)	22,31** (10,23)
CICP	143,26 (47,08)	109,45 (34,74)	121,68 (42,26)	134,34 ** (47,6)	102,04** (35,27)	114,63** (41,33)
Bone Resorption						
CTX	1,04 (0,33)	0,81 (0,24)	0,71 (0,023)	1,13** (0,33)	0,94 ** (0,30)	0,79** (0,24)

Pre: measurement prior to the beginning of the off-season transition period; Post: measurement at the end of the off-season transition period.* significant difference at the level of significance p<0.05** , significant difference at the level of significance p<0.001.

Table 15. Blood Lipids, RBC, Hct, and ESR mean values (SD) during the detraining period in the three experimental teams

	Pre			Post		
	Team A	Team B	Team C	Team A	Team B	Team C
Blood Lipids						
TC	172,13 (26,38)	166,00 (22,60)	164,63 (39,6)	172,65 (28,03)	165,5 (23,15)	171,31 (23,11)
HDL	50,13 (12,53)	52,22 (8,84)	49,45 (7,48)	51,73 (9,14)	51,77 (9,29)	49,36 (7,96)
LDL	102,47 (27,29)	90,00 (20,87)	93,27 (21,39)	102,34 (27,59)	85,72 (23,76)	95,68 (22,42)
apo AI	166,65 (26,87)	164,82 (23,89)	166,86 (25,95)	166,95 (27,05)	165,52 (23,04)	167,54 (25,18)
apo B100	83,54 (28,77)	73,47 (25,49)	80,13 (31,12)	83,52 (28,46)	73,36 (25,39)	80,18 (31,05)
Lp(a)	23,41 (24,59)	24.73 (22,11)	26,97 (25,86)	23,45 (24,60)	23,37 (21,37)	25,37 (25,18)
Blood Rheology						
Hcr	43,89 (2,14)	43,43 (1,89)	42,72 (1,66)	43,85 (2,40)	43,46 (1,95)	42,75 (1,70)
RBC	4,90 (0,37)	4,73 (0.59)	4,79 (0,31)	4,91 (0,37)	4,68 (0,64)	4,83 (0,34)
ESR	3,13 (1,57)	3,22 (1,54)	3,04 (1,25)	3,00 (1,53)	3,00 (1,41)	3,40 (1,22)

Pre: measurement prior to the beginning of the off-season transition period; Post: measurement at the end of the off-season transition period.* significant difference at the level of significance $p<0.05$** , significant difference at the level of significance $p<0.001$.

($p<0.001$) during the off season period compared to pre-detraining in all teams (table 16).

Correlation between vitamin D levels and exercise performance parameters

The correlations between vitamin D levels and exercise performance parameters, during the beginning and the end of the 6-week transition period, are presented in table 17. Analysis of our results revealed a significant positive correlation between vitamin D levels and SJ, CM, and VO2max values at the beginning and at the end of the experimental period. A significant

negative correlation was observed between vitamin D levels and 10m, and 20m sprint times at the beginning and at the end of the study.

Correlations between Sex Steroids and Exercise Performance Parameters

Correlations between sex steroids and performance parameters, during the beginning and the end of the 6-week detraining period, are presented in table 18 (team A), table 19 (team B) and table 20 (team C). Analysis of our results did not reveal any significant differences ($p>0.05$) between 3a Diol G, TT, FT, Δ4-androstenedione, DHEAS, E2, FSH and PRL in none of our experimental teams A, B, and C, during both experimental sessions. In regard to LH there were observed significant correlations with VO2max at both the first ($p = 0.013$) and the second ($p = 0.010$) experimental sessions in team A. Significant correlations were evident in team B, regarding LH levels with both 10 m, and 20 m values at the first ($p = 0.022$ and $p = 0.004$ respectively) and the second ($p = 0.028$ and $p = 0.006$ respectively) experimental sessions. No other correlations were evident in none of the three experimental teams regarding LH.

Table 16. Vitamin D and Performance values (SD) during detraining (n=67)

Measurements	**Pre**	**Post**
Vitamin D (ng/ml)	34,41 (7,08)	47,24** (13,50)
SJ (cm)	39,50 (3,87)	37,10** (3,59)
CMJ (cm)	40,91 (4,57)	38,62** (4,00)
VO2max (ml/kg/min)	59,44 (3,07)	58,89** (3,45)
10m (sec)	1,74 (074)	1,79** (0,08)
20m (sec)	3,02 (0,06)	3,07** (0,07)
Vitamin D (ng/ml)	34,41 (7,08)	47,24** (13,50)
SJ (cm)	39,50 (3,87)	37,10** (3,59)

Pre: measurement prior to the beginning of the off-season transition period; Post: measurement at the end of the off-season transition period.* significant difference at the level of significance $p<0.05$** , significant difference at the level of significance $p<0.01$.

Intervention Period (Period 2)

Differences between the Groups at Baseline

No significant differences were observed between the three experimental groups at the baseline measurement for VO2max (F = 1.769, p = 0.179), SJ (F = 1.008, p = 0.371), CMJ (F = 1.849, p = 0.166), 10 m (F = 1.102, p = 0.339), 20 m (F = 0.660, p = 0.520), body weight (F = 0.016, p = 0.89), and body fat percentage (F = 0.003, p = 0.95). Similarly, no significant differences were observed for any of the TT (F = 2.409, p = 0.098), 3a Diol G (F = 0.823, p = 0.444), FT (F = 2.74, p = 0.10), DHEAS (F = 0.208, p = 0.813), Δ4-androstenedione (F = 0.377, p = 0.688), E2 (F = 1.456, p = 0.241), FSH (F = 0.903, p = 0.411), LH (F = 0.246, p = 0.783), and PRL (F = 1.505, p = 0.230) at baseline between the three experimental groups.In regard to FT no difference was evident at baseline for teams A and B (F 3.975, p = 0.0.053) and betweens teams B and C (F = 0.515, p = 0.477). . In contrast a significant difference was observed between teams A and C in regard to FT F = 9.397, p = 0.004)). In regard to DHEAS no differences were observed between teams A and B (F = 2.624, p = 0.113). In contrast a significant difference was observed in regard to DHEAS between teams A and C (F = 10.417, p = 0.002) and teams B and C (F = 24,419, p = 0.000). Finally, no significant differences were observed at baseline between teams B and C (F=0,448, p=0.507). In contrast, there was a

Table 17. Correlations (correlation coefficients and p-values) between Vitamin D levels and exercise performance parameters

Exercise Performance	Pre Vitamin D (ng/ml)	Post Vitamin D (ng/ml)
SJ (cm)	0.731 (p<0.001)	0.597 (p<0.001)
CMJ (cm)	0.740 (p<0.001)	0.476 (p<0.001)
VO2max (ml/kg/min)	0.436 (p<0.001)	0.394 (p=0.006)
10m (sec)	-0.649 (p<0.001)	-0.410 (p<0.001)
20m (sec)	-0.673 (p<0.001)	-0.426 (p<0.001)

Pre: measurement prior to the beginning of the off-season transition period; Post: measurement at the end of the off-season transition period.

Table 18. Correlations (p-values) between circulating sex steroids and exercise performance parameters in team A during detraining

Team A	VO_2max (ml/kg/min)		SJ (cm)		CMJ (cm)		10m (sec)		20m (sec)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
3a Diol G (ng/ml)	0.394	0.368	0.873	0.355	0.833	0.601	0.177	0.203	0.510	0.177
TT (ng/dl)	0.513	0.794	0.682	0.151	0.660	0.122	0.851	0.717	0.564	0.800
FT (pg/ml)	0.788	0.487	0.741	0.833	0.927	0.451	0.932	0.613	0.540	0.714
Δ4 (ng/mL)	0.381	0.699	0.505	0.571	0.312	0.605	0.79	0.362	0.305	0.791
DHEAS (μg/mL)	0.714	0.374	0.611	0.222	0.820	0.202	0.799	0.809	0.666	0.275
E2 (pg/mL)	0.782	0.615	0.405	0.727	0.465	0.975	0.072	0.321	0.308	0.580
FSH (mIU/L)	0.240	0.090	0.866	0.843	0.531	0.723	0.417	0.324	0.711	0.422
LH (IU/L)	0.013*	0.010*	0.687	0.543	0.702	0.225	0.767	0.161	0.621	0.064
PRL (μg/L)	0.330	0.276	0.634	0.591	0.528	0.417	0.680	0.185	0.660	0.247

* siginifcant diference at the level of significance p<0.05

Table 19. Correlations (p-values) between circulating sex steroids and exercise performance parameters in team B during detraining

Team B	VO_2max (ml/kg/min)		SJ (cm)		CMJ (cm)		10m (sec)		20m (sec)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
3a Diol G (ng/ml)	0.719	0.075	0.521	0.268	0.196	0.400	0.163	0.327	0.188	0.699
TT (ng/dl)	0.911	0.136	0.34	0.718	0.692	0.480	0.728	0.848	0.216	0.863
FT (pg/ml)	0.304	0.926	0.65	0.976	0.383	0.879	0.760	0.366	0.602	0.948
Δ4 (ng/mL)	0.052	0.052	0.212	0.930	0.765	0.625	0.769	0.464	0.468	0.592
DHEAS (μg/mL)	0.480	0.109	0.263	0.143	0.659	0.678	0.960	0.985	0.952	0.967
E2 (pg/mL)	0.481	0.564	0.811	0.651	0.645	0.929	0.436	0.496	0.406	0.518
FSH (mIU/L)	0.555	0.907	0.312	0.713	0.995	0.165	0.252	0.077	0.228	0.072
LH (IU/L)	0.613	0.352	0.266	0.177	0.082	0.051	0.022*	0.028*	0.004**	0.001**
PRL (μg/L)	0.290	0.561	0.204	0.546	0.238	0.804	0.688	0.95	0.629	0.710

* siginifcant diference at the level of significance p<0.05, ** siginifcant diference at the level of significance p<0.01

Table 20. Correlations (p-values) between circulating sex steroids and exercise performance parameters in team C during detraining

Team B	VO_2max **(ml/kg/min)**		**SJ (cm)**		**CMJ (cm)**		**10m (sec)**		**20m (sec)**	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
3a Diol G (ng/ml)	0.740	0.411	0.128	0.249	0.865	0.366	0.744	0.942	0.258	0.436
TT (ng/dl)	786	0.735	0.096	0.227	0.866	0.151	0.701	0.177	0.121	0.487
FT (pg/ml)	155	0.142	0,360	0.863	0.479	0.983	0.992	0.771	0.976	0.766
Δ4 (ng/mL)	0,313	0,340	0,265	0,118	0.515	0,205	0,280	0,732	0,046*	0,913
DHEAS (μg/mL)	0.842	0,664	0.467	0.735	0.711	0.992	0.656	0.932	0.237	0.378
E2 (pg/mL)	0.950	0.368	0.992	0.740	0.852	0.366	0.743	0.236	0.832	0.815
FSH (mIU/L)	0.828	0.101	0.070	0.554	0.169	0.652	0.022*	0.052	0.217	0.204
LH (IU/L)	0.458	0.215	0.350	0.237	0.959	0.493	0.858	0.702	0.499	0.703
PRL (μg/L)	0.285	0.650	0.427	0.803	0.939	0.405	0.194	0.478	0.449	0.626

* siginifcant diference at the level of significance p<0.05, ** siginifcant diference at the level of significance p<0.01

significant difference at baseline between teams A and B (F=5.051, p=0.030) and between teams A and C (p=7.921, p=0.07).

Volume and Training Load (RPE)

In order to confirm the intentional differentiation between the three training regimens, one-way ANOVAs were used to test volume and training load (as expressed by RPE) among the three teams within the same training period. As expected (Table 12), teams significantly differed both in volume (pre-season p=0.001, 1st half of competition period p<0.001, 2nd half of competition period p0.001) and RPE (pre-season p<0.001; 1st half of competition period F: 2,48=1848,63 p<0.001; 2nd half of competition period p 0.001).

Table 21. Comparisons of means (SD) of performance, body composition parameters, and androgen levels by the training regime over time

	Team A			**Team B**			**Team C**		
	Pre	Mid	post	pre	Mid	Post	pre	Mid	post
VO_2max (ml/kgr/min)	***57,67 [a, b]***	***60,72 [a]***	***60,94 [b]***	***58,30 [a, b]***	***60,59 [a]***	***60,66 [b]***	***56,36 [a, b]***	***58,61 [a]***	***58,69 [b]***
	(2,54)	***(2,89)***	***(2,67)***	***(3,88)***	***(3,95)***	***(4,12)***	***(2,52)***	***(3,12)***	***(3,20)***
Neuromascular performance									
SJ (cm)	***37,30 [a, b]***	***40,17 [a, c]***	***41,70 [b, c]***	***38,18 [a, b]***	***41,27 [a]***	***41,55 [b]***	***37,55 [a, b]***	***39,77 [a]***	***40,00 [b]***
	(3,08)	***(3,43)***	***(3,51)***	***(3,03)***	***(3,44)***	***(3,66)***	***(3,51)***	***(3,62)***	***(3,46)***
CMJ (cm)	***39,13 [a, b]***	***41,96 [a, c]***	***43,65 [b, c]***	***40,09 [a, b]***	***43,18 [a]***	***43,09 [b]***	***38,86 [a, b]***	***40,73 [a]***	***40,91 [b]***
	(3,27)	***(4,10)***	***(4,58)***	***(2,79)***	***(4,12)***	***(4,10)***	***(3,93)***	***(3,93)***	***(3,84)***
Speed									
10m Sprint (cm)	***1,79 [a, b]***	***1,75 [a, c]***	***1,73 [b, c]***	***1,78 [a, b]***	***1,73 [a]***	***1,73 [b]***	***1,79 [a, b]***	***1,76 [a]***	***1,76 [b]***
	(0,06)	***(0,06)***	***(0,06)***	***(0,72)***	***(0,72)***	***(0,69)***	***(0,08)***	***(0,09)***	***(0,09)***
20m Sprint (cm)	***3,06 [a, b]***	***3,03 [a, c]***	***3,02 [b, c]***	***3,06 [a, b]***	***3,01 [a]***	***3,01 [b]***	***3,07 [a, b]***	***3,05 [a]***	***3,04 [b]***
	(0,06)	***(0,05)***	***(0,06)***	***(0,06)***	***(0,06)***	***(0,07)***	***(0,07)***	***(0,07)***	***(0,06)***
Androgen levels									
TT	***604.98[a,b]***	***681.66[a,c]***	***777.04[b,c]***	686.81	685.63	750.64	622.34	620.91	655.78
	(141.92)	***(153.41)***	***(151.52)***	(124.32)	(136.84)	(191.47)	(125.93)	(160.79)	(219.47)
FT	13.70	14,69	15.58	10.28	9.58	11.72	9.26	9.26	10.07
	(5.80)	(6.68)	(6.16)	(5.80)	(4.48)	(7.34)	(3.39)	(5.51)	(3.69)
3aDiol G	***8.43[b]***	***9.20[c]***	***10.42[b,c]***	8.70	8.29	8.45	9.87	9.84	9.70
	(2.55)	***(2.92)***	***(2.67)***	(2.87)	(2.68)	(3.13)	(5.76)	(4.66)	(4.92)
Δ4-androstenedione	1,86	1,95	1,99	2,07	2,13	2,04	1,91	1,84	2,23
	(0,46)	(0,69)	(0,61)	(1,26)	(0,69)	(0,45)	(0,60)	(0,74)	(0,88)
DHEAS	2,35	2,56	2,41	2,80	2,58	2,45	1,53	1,75	1,94
	(1,07)	(0,87)	(0,68)	(1,21)	(0,84)	(0,91)	(0,51)	(0,73)	(0,99)

Intervention Period: Pre=baseline measurement; mid=mid-season measurement; post=post-season measurement; VO_2max=Maximal Oxygen Consumption; SJ=Squad jump; CMJ=countermovement jump; BW= body weight; BF%= body fat percent; a: pre vs. mid, b: pre vs. post, c: mid vs. post within the same team at $p < 0.05$ (Bonferroni adjusted).

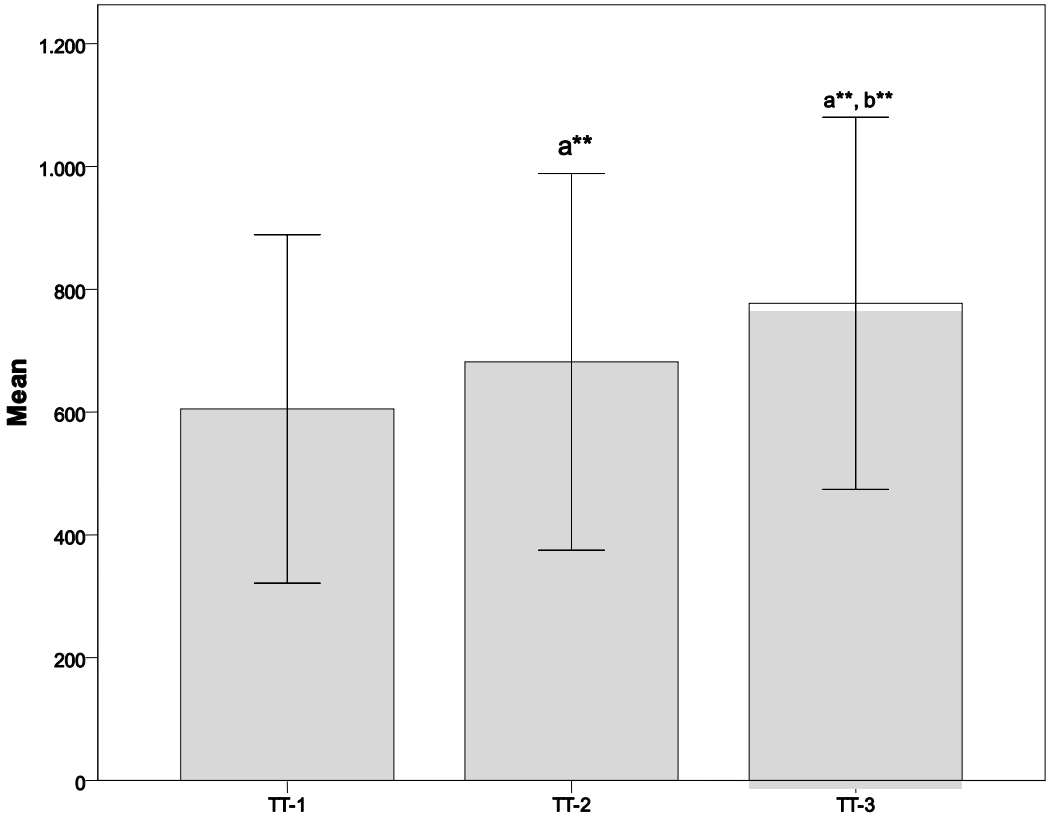


Figure 8. Total testosterone (TT) during the three experimental sessions of the intervention period in team A. TT1=baseline; TT2=midlle of championship;TT3=end of championship, a= statistical different from baseline; b=statistical different from middle of the championship, *p<0.05;p<0.01.**

Performance

Two-way mixed ANOVAs showed a significant interaction between the time points of evaluation i.e. beginning of season (early July), mid-point (mid January) and end-point (mid-May) and the training regime used. As expected there was a considerable improvement over time in all performance parameters within each experimental team (Table 21). *Neuromuscular Performance*, assayed by SJ and CMJ, showed a significant increase ($p<0.001$) at the mid- and end-point measurements compared to baseline and a significant decrease ($p<0.001$) of sprint times (10 and 20 m) for all three teams, in ad hoc Bonferroni adjusted comparisons test of simple effects. In

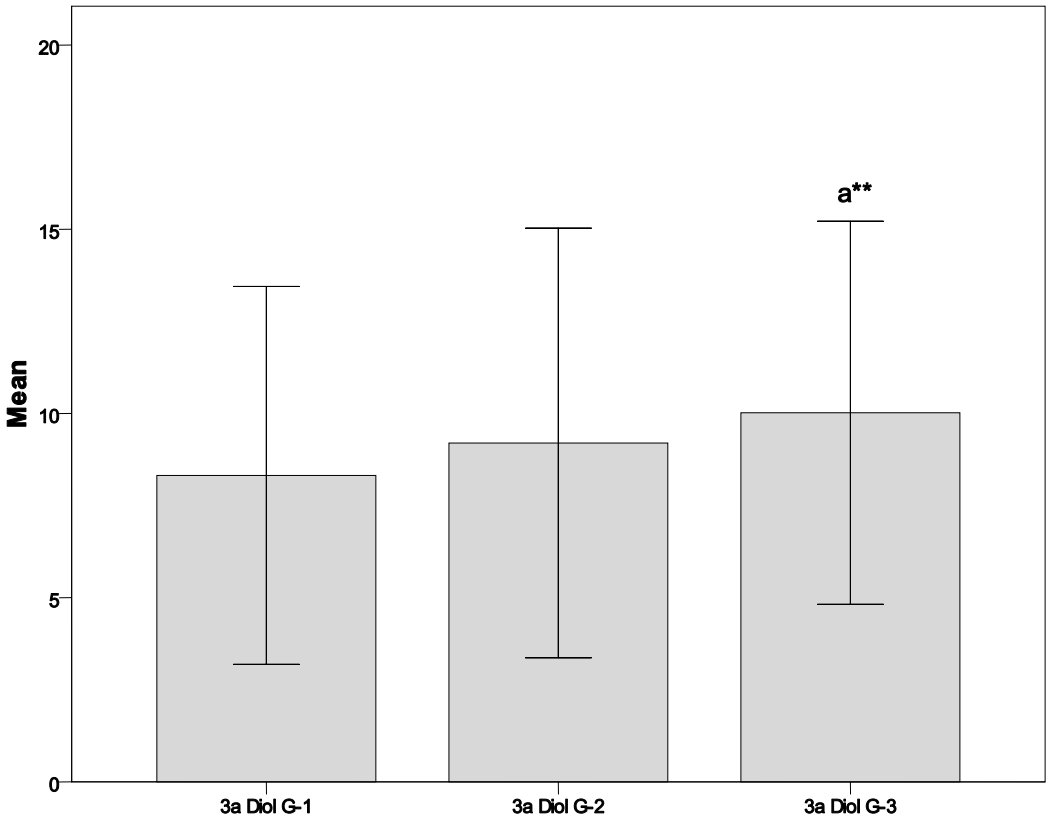


Figure 9. 3a Diol G during the three experimental sessions of the intervention period in team A. TT1=baseline; TT2=midlle of championship;TT3=end of championship, a= statistical different from baseline; b=statistical different from middle of the championship, *p<0.05;p<0.01.**

Team A, the comparison between mid- to end-point further revealed a significant increase in SJ ($p<0.001$), CMJ ($p<0.001$), and a significant decrease in 10m ($p<0.001$) and 20m ($p<0.001$) sprint times at the end of the study. No significant differences were revealed by the mid- to end-point comparison for any of the measured neuromuscular performance parameters in Team B (SJ $p=0.383$, CMJ $p=1.00$, 10m $p=0.126$, and 20m $p=1.00$) and Team C (SJ $p=0.609$, CMJ $p=1.00$, 10m $p=1.00$, and 20m $p=1.00$).*Maximal Oxygen Consumption:* The comparison between baseline to mid-point and end-point showed that there were significant increases ($p<0.001$) across teams in VO_2max. No significant differences were revealed for end- to mid-point comparisons for VO_2max in Team A ($p=0.057$), Team B ($p=1.000$), and Team C ($p=1.000$) (Table 13). *Body Composition*: The BW and BF%, BMI, and WHR

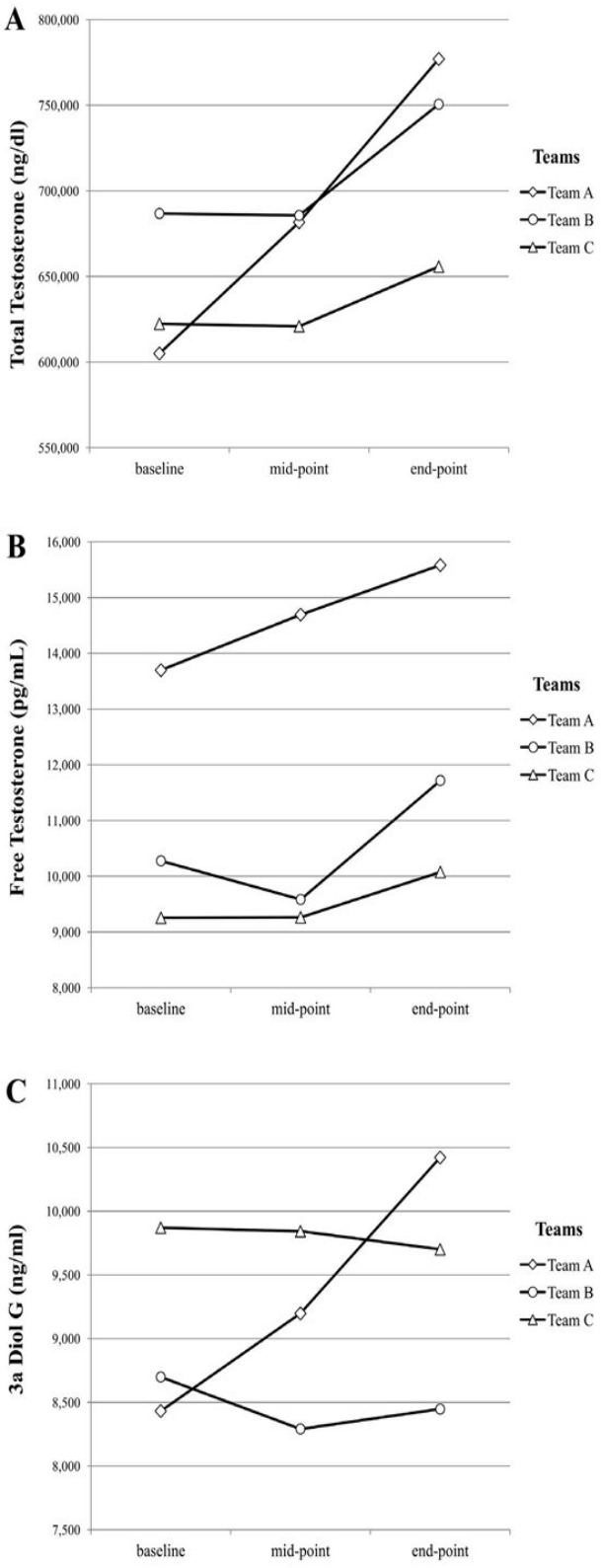


Figure 10.

Cell mean interactions by training regimen (high strength training stress=Team A; moderate strength training stress = Team B, low strength training stress=Team C) over time for Total Testosterone (A), Free Testosterone (B), and 3a Diol G (C). Significant differences were observed only in Team A between baseline to mid and end-point for both total testosterone (TT) and 3a Diol G and at the mid to end-point comparison in TT.

Table 22. Comparisons of means (SD) of estrogens and body composition parameters by the training regime over time

	Team A			**Team B**			**Team C**		
	Pre	Mid	post	pre	Mid	Post	Pre	mid	Post
Estrogen									
E2	19,6	19,9	25,8	25,38	27,94	27,08	22,33	27,33	31,12
	(12,82)	(9,6)	(16,42)	(11,06)	(15,17)	(15,74)	(9,76)	(14,13)	(13,48)
FSH	7,41	8,61	8,67	5,71	6,05	6,11	6,00	6,45	6,74
	(5,56)	(7,76)	(8,60)	(3,96)	(4,33)	(4,09)	(3,87)	(3,60)	(3,67)
LH	4,69	5,04	5,13	4,95	4,98	4,69	4,63	5,28	5,35
	(1,67)	(2,16)	(1,95)	(1,71)	(1,88)	(1,48)	(1,45)	(3,41)	(2,21)
PRL	12,25	12,02	13,14	10,24	13,24	12,62	9,69	10,39	11,24
	(6,71)	(5,83)	(7,30)	(5,00)	(7,04)	(4,62)	(3,31)	(4,93)	(7,26)
Body composition									
BW	***79,14*** ^{a,b}	***78,10*** ^{a}	***77,60*** ^{b}	***79,49*** ^{a,b}	***78,10*** ^{a,c}	***77,50*** ^{b,c}	***79,69*** ^{a}	***78,98*** ^{a}	79,14
	(6,16)	***(5,82)***	***(5,76)***	***(8,96)***	***(8,65)***	***(8,07)***	***(5,48)***	***(5,41)***	(5,48)
BF%	***11,02*** ^{a,b}	***9,19*** ^{a,c}	***6,76*** ^{b,c}	***10,41*** ^{a,b}	***8,67*** ^{a}	***8,97*** ^{b}	***9,72*** ^{a}	***8,87*** ^{a}	8,98
	(4,11)	***(3,9)***	***(1,35)***	***(4,08)***	***(3,21)***	***(2,93)***	***(2,48)***	***(2,21)***	(2,02)
BMI	23,86	23,55	23,40	24,07	23,65	23,54	24,17	23,97	24,01
	(1,35)	(1,18)	(1,15)	(1,79)	(1,74)	(1,57)	(0,86)	(0,85)	(0,69)
WHR	0,85	0,85	0,84	0,85	0,84	0,83	0,84	0,83	0,82
	(0,02)	(0,02)	(0,02)	(0,03)	(0,33)	(0,03)	(0,01)	(0,02)	(0,02)

Inervention Period: Pre=baseline measurement; mid=mid-season measurement; post=post-season measurement; E2=estradiol; FSH=follicle-stimulating hormone; LH=luteinizing hormone; PRL=prolactin; BW= body weight; BF%= body fat percentage; BMI=body mass index; WHR: waist to hip ratio; a: pre vs. mid, b: pre vs. post, c: mid vs. post within the same team at $p < 0.05$ (Bonferroni adjusted)

all assessments throughout the study are also presented in Table 22. For Team A (high strength training stress) and team B (moderate strength training stress), ad hoc comparisons between simple effects showed significant decreases in BW ($p<0.001$) between baseline to mid- and end-point measurements, but regarding BF% only Team A exhibited further loss of body fat between mid- to end-point ($p<0.001$) in contrast to Team B ($p=0.782$). For Team C, weight loss and body fat reduction were found significant only at mid-point to baseline ($p=0.008$ and $p=0.025$, respectively). The WHR and BMI followed the same pattern of alterations in all teams.

Sex Steroids

Our findings regarding sex steroids are shown in tables 21, 22 and figures 8,9,10. Two-way mixed ANOVA revealed that the main effect of training regime (teams) on TT was not significant ($p=0.117$), but a significant main effect of time on TT was found ($p<0.001$). The two factors had a significant interaction ($p=0.018$), depicted in Figure 8 (cell means interaction). Simple effect analysis of season within each level of training regime revealed a significant difference within Team A ($p<0.001$), a marginal difference within Team B ($p=0.051$), and a non-significant difference within Team C ($p=0.427$). Post hoc comparisons (Bonferroni adjusted) in Team A showed significant differences between all season comparisons (baseline/mid-point: $p=0.024$, baseline/end-point: $p<0.001$, mid/end-point: $p=0.008$). Mean values of TT by each training regime over time are shown in Table 21. The main effect of training regimens on FT was significant ($p=0.001$), while a marginal finding of season was found ($p=0.044$). There was no significant interaction between the two factors for FT (Figure 10). In contrast, 3a Diol G exhibited a significant dependence on the type of raining program ($p<0.001$) but not on the time periods ($p=0.211$). The interaction

Table 23. Bone metabolism mean values (SD) at the two time points at the intervention period in the three experimental teams

	Baseline			End of the Study		
	Team A	Team B	Team C	Team A	Team B	Team C
Bone Formation						
OC	22,73 (11,31)	11,15 (4,25)	12,25 (8,26)	24,10 *** (11,48)	11,35* (4,12)	12,58 (8,29)
b-ALP	39,71 (18,99)	19,84 (4,29)	22,31 (10,23)	43,04 *** (20,46)	20,20* (4,13)	22,52 (10,17)
CICP	134,34 (47,6)	102,04 (35,27)	114,63 (41,33)	146,12*** (47,46)	103,09* (34,75)	116,45 (41,98)
Bone Resorption						
CTX	1,13 (0,33)	0,94 (0,30)	0,79 (0,24)	1,00 *** (0,32)	0,89* (0,26)	0,78 (0,23)

Baseline: measurement prior to the beginning of the pre-season period; Post: measurement at the end of the off-season transition period.* significant difference at the level of significance p<0.05** , significant difference at the level of significance p<0.01; ***p<0.001.

between factors was significant (p=0.024; Figure 10), with simple effects showing that only in the high training load team (Team A) was the effect of time significant (p=0.001). Within this team, post hoc test comparisons (Bonferroni adjusted) revealed a significant difference between baseline- and mid-point to end-point values (p=0.001 and p=0.038, respectively). No differences were observed for none of Δ4-androstenedione, DHEAS, E2, LH, FSH, and PRL levels throughout the study in none of the three experimental teams (p>0.05)

Bone Metabolism

Analysis of our data revealed that in team A there was a significant increase in OC (p<0.001), b-alp (p<0.00), and CICP (p<0.00), and a decrease in CTX (p<0.00) at the end of the study compared to baseline (Table 23). In team B there was observed a weak significant increase in OC (P=0.038), b-alp (p=0.032), and CICP (p=0.045), and a weak significant decrease in CTX (p=0.039) at the end of the study compared to

Table 24. Comparisons of means (SD) of bone metabolism and lipidemic profile parameters during the intervention period

		Team A			**Team B**			**Team C**		
		Pre	Mid	Post	pre	mid	post	pre	mid	Post
Lipidemic Profile										
	TC	172,65 (28,03)	172,47 (27,52)	172,39 (26,80)	165,5 (23,15)	167,04 (20,93)	168,00 (21,79)	171,31 (23,11)	170,13 (23,32)	171,95 (23,06)
	HDL	51,73 (9,14)	51,95 (8,91)	52,56 (8,95)	51,77 (9,29)	52,72 (9,73)	53,04 (9,82)	49,36 (7,96)	50,31 (8,16)	51,45 (8,83)
	LDL	102,34 (27,59)	102,82 (26,78)	102,52 (26,51)	85,72 (23,76)	90,40 (20,04	91,45 (20,52)	95,68 (22,42)	89,68 (27,99)	92,63 (21,91)
	apo AI	166,95 (27,05)	167,04 (26,61)	167,30 (26,8)	165,52 (23,04)	165,13 (23,75)	164,56 (24,30)	167,54 (25,18)	167,27 (25,770	163,36 (36,88)
	apo B100	83,52 (28,46)	83,45 (29,04)	84,04 (28,70)	73,36 (25,39)	77,84 (38,44)	73,32 (25,02)	80,18 (31,05)	80,31 (31,28)	80,74 (30,65)
	Lp(a)	23,45 (24,60)	23,41 (24,54)	23,40 (24,59)	23,37 (21,37)	22,68 (21,62)	22,00 (21,39)	25,37 (25,18)	23,69 (24,38)	22,06 (24,54)
Blood Rheology										
	Hct	43,85 (2,40)	43,86 (1,98)	44,06 (1,94)	43,46 (1,95)	43,39 (2,00)	43,45 (1,76)	42,75 (1,70)	42,73 (1,68)	42,70 (1,56)
	RBC	4,91 (0,37)	4,93 (0,36)	4,94 (0,35)	4,68 (0,64)	4,85 (0,32)	4,84 (0,34)	4,83 (0,34)	4,81 (0,31)	4,81 (0.31
ESR		3,00 (1,53)	3,08 (1,41)	3,00 (1,47)	3,00 (1,41)	3,04 (1,49)	2,90 (1,30)	3,40 (1,22)	3,27 (1,45)	3,36 (1,39)

Intervention Period: Pre=baseline measurement; mid=mid-season measurement; post=post-season measurement; TC= total cholesterol; HDL=high density lipoprotein; LDL=low density lipoprotein; apo AI=apolipoprotein A; apo B100= apolilipoprotein B100,; Lp(a)=lipoprotein a; Hct=hematocrit; RBC=red blood cells; ESR=erythrocyte sedimentation rate; a: pre vs. mid, b: pre vs. post, c: mid vs. post within the same team at $p < 0.05$ (Bonferroni adjusted)

baseline. In team C we failed to observe any significant difference between baseline values compared to the end of the study in OC (p>0.05), b-alp (p>0.05), CICP (p>0.05), and CTX (>0.05).

Lipidemic Profile

No significant differences were observed in none of TC (p>0.05), HDL-C (p>0.05), LDL-C (p>0.05), apo AI (p>0.05), apoB100 (p>0.05), and Lp(a) (p>0.05) in the mid-point measurement compared to baseline (Table 24). Similarly, no significant differences were observed in TC (p>0.05), HDL-C (p>0.05), LDL-C (p>0.05), apo AI (p>0.05), apoB100 (p>0.05), and Lp(a) (p>0.05) at the end of the study compared to both baseline and mid-point measurements (Table 24).

Blood Lipids, RBC, Hct, ESR

No statistically significant alterations were observed for none of TC (p>0.05), LDL (p>0.05), HDL (p>0.05), apo AI (p>0.05), apo B100 (p>0.05), Lp(a) (p>0.05) in none experimental team throughout the study. Similarly, no significant differences were observed for none of Hct (p>0.05), RBC (p>0.05), and ESR (p>0.05) in none of the three experimental teams throughout the study (Table 24).

Correlations *between sex steroids and exercise performance parameters*

A weak significant correlation was evident between TT and both SJ (p=.025) and CMJ (p=0.042) only at the mid-point measurement in team A. Despite the increase in both parameters at the end-point no significant correlations were observed in between these parameters (Table 25). In addition, no similar findings were observed in the other two experimental teams. In addition, no other significant correlation was observed throughout the study in Teams A in regard to androgens. In regard to estrogens significant correlations were evident in LH with VO2max at baseline and

mid-point (p=0.01) and (p=0.004) respectively, FSH with VO_2max (p=0.033) at end-poin, PRL with 20m (p=0.029) at mid-poind, and E2 with CMJ (p=0.48), 10m (p=0.46) and 20m (p=0.007) at the end of the study. No other correlations were evident throughout the study between estrogens and performance parameters.

In team B there no significant observations were observed between androgens and exercise performance indices apart from a weak correlation of 3a Diol G with VO_2max (p=0.012) and DHEAS (p=0.037) at the mid-point but not at any other time point in all three experimental teams. Furthermore a weak correlation was evident between Δ4-abdrostenedione with VO_2max (p=0.033) in team C at mid-point, but not in any other time point for none of our experimental teams. In regard to estrogens we observed a significant correlation between LH and SJ (p=0.001), CMJ (p=0.001), 10M (P=0.011) and 20m (p=0.001) at baseline, LH with SJ (p=0.031), CMJ (p=0.032), 10M (P=0.005) and 20m (p=0.007) at mid-point. However no other correlation was observed throughout the study between these parameters. In addition we failed to observe any other correlation with estrogens and exercise performance parameters in the three time-points.

Analysis of our results in team C failed to observe any correlation of androgens and exercise performance parameters, apart from a weak correlation of Δ4-androstenedione with VO_2max (p=0.033) at baseline. Similarly, no significant correlations were observed in regard to estrogens, apart from FSH and SJ (p=0.034) at baseline, E2 with both SJ (P=0.042) and 20m (p=0.022) at the end-point measurement, and LH with SJ (p=0.043) at the mid of the study.

Correlations between Sex Steroids and Bone metabolism markers.

The correlations of sex steroids and bone metabolism markers are presented in table 28. Evaluation of our data revealed a statistically significant correlation between LH and OC (p=0.018) at the end of the study in team A but no at baseline (p>0.05). No other correlations were observed between sex steroids and bone turnover markers in none of the teams A, B, and C nor at baseline neither at the end of the study (all p>0.05). In team B we observed significant correlations between DHEAS and CICP (p=0.036) and PRL and OC (p=0.045) at baseline, but not at the end of the study (p>0.05). No other correlations were observed between sex steroids and bone turnover markers in none of teams A, B, and C nor at baseline neither at the end of the study (all p>0.05).

Analysis of our data in team C failed to observe any significant correlation between sex steroid and bone turnover markers apart from a significant correlation between FSH and OC (p=0.000) only at baseline.

Correlations between sex steroids and blood lipids, RBC, Hct and ERS

In team A our data revealed a significant correlation between 3a Diol G and Lp(a) (p=0.005) and DHEAS with HDL (p=0.006) at baseline however we failed to observe any significant associations between these sex steroids with Lp(a) and HDL neither at the mid-point measurement (p>0.05) nor at the ed of the study (Table 29). At the mid-point measurement we observed a significant correlation between DHEAS and TC (p=0.030), but not at baseline and at the end of the study. In addition no correlations were observed between n sex steroids levels and TC, HDL, LDL, Lp(a), apo AI, and apo B100 throughout the study (p>0.05).

In team B at baseline we observed significant correlation between TT and Lp(a) (p=0.041), FT and Lp(a) (p=0.003), Δ4-androstenedione and LDL (p=0.001), FSH and Lp(a) p=0.012, an PRL with apo AI (p=0.001) and Hct (p=0.038) but these were

Table 25. Correlations (p values) between sex steroids and performance parameters in team A throughout the intervention period

	3a Diol G	TT	FT	Δ4	DHEAS	E2	FSH	LH	PRL
Team C									
Baseline									
VO2max	0.510	0.794	0.437	0.699	0.374	0.615	0.090	0.010**	0.276
SJ	0.587	0.151	0.858	0.571	0.222	0.727	0.843	0.543	0.591
CMJ	0.948	0.122	0.540	0.605	0.202	0.975	0.723	0.225	0.417
10m	0.814	0.717	0.480	0.362	0.809	0.321	0.324	0.161	0.185
20m	0.553	0.800	0.424	0.791	0.275	0.580	0.422	0.064	0.247
Mid-point									
VO2max	0.366	0.570	0.219	0.127	0.342	0.570	0.124	0.004**	0.814
SJ	0.235	0.055	0.636	0.236	0.777	0.549	0.142	0.697	0.389
CMJ	0.282	0.052	0.817	0.297	0.540	0.599	0.651	0.489	0.402
10m	0.652	0.989	0.985	0.320	0.122	0.450	0.746	0.996	0.354
20m	0.659	0.877	0.937	0.102	0.834	0.202	0.320	0.579	0.029*
End-point									
VO2max	0.568	0.541	0.230	0.084	0.212	0.939	0.033*	0.107	0.708
SJ	0.373	0.077	0.853	0.939	0.505	0.064	0.891	0.583	0.459
CMJ	0.283	0.101	0.580	0.905	0.790	0.048*	0.865	0.331	0.331
10m	0.617	0.818	0.969	0.643	0.748	0.046*	0.506	0.409	0.644
20m	0.959	0.580	0.764	0.260	0.588	0.007**	0.703	0.254	0.502

*p<0.05; ** p<0.01

Table 26. Correlations (p values) between sex steroids and performance parameters in team B throughout the intervention period

	3a Diol G	TT	FT	Δ4	DHEAS	E2	FSH	LH	PRL
Team C									
Baseline									
VO2max	0.619	0.136	0.608	0.056	0.109	0.564	0.907	0.482	0.407
SJ	0.252	0.718	0.950	0.930	0.143	0.651	0.713	0.001**	0.480
CMJ	0.623	0.480	0.478	0.625	0.678	0.929	0.165	0.001**	0.931
10m	0.250	0.863	0.426	0.464	0.985	0.496	0.077	0.011**	0.654
20m	0.374	0.848	0.180	0.592	0.967	0.518	0.072	0.001**	0.441
Mid-point									
VO2max	0.012*	0.247	0.895	0.774	0.037*	0.122	0.897	0.449	0.209
SJ	0.995	0.075	0.765	0.373	0.157	0.153	0.929	0.035*	0.430
CMJ	0.626	0.118	0.480	0.463	0.585	0.249	0.544	0.032*	0.350
10m	0.089	0.198	0.804	0.914	0.902	0.166	0.051	0.005**	0.317
20m	0.528	0.147	0.976	0.762	0.911	0.323	0.154	0.007**	0.151
End-point									
VO2max	0.348	0.311	0.998	0.457	0.072	0.585	0.595	0.857	0.157
SJ	0.928	0.484	0.256	0.562	0.644	0.733	0.475	0.920	0.422
CMJ	0.797	0.839	0.611	0.428	0.764	0.603	0.547	0.907	0.122
10m	0.129	0.305	0.879	0.313	0.240	0.522	0.060	0.065	0.335
20m	0.142	0.630	0.857	0.276	0.293	0.618	0.052	0.257	0.304

*p<0.05; ** p<0.01

Table 27. Correlations (p values) between sex steroids and performance parameters in team C throughout the intervention period

	3a Diol G	TT	FT	Δ4	DHEAS	E2	FSH	LH	PRL
Team C									
Baseline									
VO2max	0.411	0.735	0.142	0.340	0.98	0.530	0.156	0.215	0.650
SJ	0.249	0.227	0.863	0.118	0.735	0.555	0.034*	0.237	0.803
CMJ	0.366	0.151	0.983	0.205	0.992	0.701	0.126	0.493	0.405
10m	0.942	0.177	0.771	0.970	0.932	0.953	0.868	0.702	0.478
20m	0.436	0.487	0.766	0.980	0.378	0.764	0.932	0.703	0.626
Mid-point									
VO2max	0.842	0.541	0.811	0.033*	0.549	0.606	0.164	0.737	0.202
SJ	0.868	0.746	0.241	0.251	0.262	0.805	0.469	0.043*	0.693
CMJ	0.816	0.981	0.390	0.752	0.746	0.897	0.524	0.108	0.853
10m	0.147	0.138	0.807	0.780	0.341	0.096	0.137	0.858	0.861
20m	0.158	0.100	0.735	0.586	0.307	0.333	0.345	0.181	0.692
End-point									
VO2max	0.380	0.363	0.022	0.249	0.811	0.599	0.101	0.404	0.428
SJ	0.896	0.760	0.542	0.958	0.999	0.042*	0.554	0.307	0.641
CMJ	0.931	0.951	0.748	0.323	0.868	0.450	0.052	0.631	0.662
10m	0.382	0.066	0.482	0.292	0.780	0.135	0.052	0.247	0.802
20m	0.49	0.143	0.674	0.554	0.760	0.011*	0.204	0.327	0.637

*p<0.05; ** p<0.01

Table 28. Correlation (p values) sex steroids and bone metabolism markers during the intervention period

Team A	**3a Diol G**	**TT**	**FT**	**Δ4**	**DHEAS**	**E2**	**FSH**	**LH**	**PRL**
OC pre	0.503	0.489	0.175	0.395	0.667	0.250	0.779	0,504	0.278
OC post	0.904	0.721	0.238	0.397	0.536	0.080	0.764	0.322	0.249
b-ALP Pre	0.325	0.545	0.788	0.423	0.164	0.158	0.791	0.739	0.061
b-ALP Post	0.757	0.702	0.961	0.233	0.428	0.456	0.837	0.741	0.199
CICP Pre	0.216	0.535	0.907	0.257	0.337	0.668	0.616	0.519	0.084
CICP post	0.668	0.668	0.581	0.966	0.848	0.847	0.419	0.696	0.747
CTX pre	0.641	0.971	0.169	0.252	0.308	0.237	0.438	0.616	0.901
CTX porstr	0.430	0.070	0.164	0.380	0.813	0.244	0.347	0.018*	0.289
Team B									
OC pre	0.985	0.938	0.952	0.468	0.908	0.565	0.996	0.183	0.045*
OC post	0.676	0.239	0.239	0.231	0.822	0.081	0.492	0.203	0.793
b-ALP Pre	0.360	0.851	0.313	0.723	0.543	0.605	0.260	0.945	0.558
b-ALP Post	0.603	0.700	0.928	0.608	0.171	0.433	0.582	0.691	0.049*
CICP Pre	0.853	0.629	0.558	0.315	0.036*	0.582	0.572	0.568	0.280
CICP post	0.161	0.321	0.217	0.447	0.878	0.146	0.360	0.571	0.383
CTX pre	0.112	0.483	0.526	0.275	0.405	0.487	0.879	0.065	0.942
CTX post	0.823	0.701	0.383	0.565	0.429	0.795	0.795	0.0446	0.569
Team C									
OC pre	0.728	0.707	0.628	0.419	0.373	0.795	0.000**	0.106	0.486
OC post	0.818	0.352	0.793	0.992	0.772	0.356	0.051	0.517	0.517
b-ALP pre	0.680	0.891	0.304	0.502	0.938	0.642	0.105	0.732	0.459
b-ALP post	0.306	0.202	0.350	0.797	0.863	0.901	0.622	0.421	0.057
CICP pre	0.131	0.312	0.245	0.772	0.430	0.410	0.625	0.816	0.181
CICP post	0.227	0.622	0.446	0.365	0.913	0.903	0.470	0.711	0.930
CTX pre	0.814	0.178	0.751	0.968	0.992	0.115	0.674	0.470	0.551
CTX porst	0.181	0.557	0.092	0.705	0.918	0.777	0.245	0.338	0.787

Pre=just prior to the begging of the pre-season period; Post=at the end of the completion period; *p<0.05;p<0.01**

Table 29. Correlation (p values) sex steroids and blood lipids markers during the intervention period

Team A	**3a Diol G**	**TT**	**FT**	**Δ4**	**DHEAS**	**E2**	**FSH**	**LH**	**PRL**
Baseline									
TC	0.353	0.116	0.769	0.170	0.528	0.659	0.616	0.741	0.353
HDL	0.200	0.113	0.363	0.126	0.006*	0.886	0.375	0.132	0.178
LDL	0.745	0.879	0.754	0.707	0.754	0.702	0.975	0.909	0.902
apo AI	0.645	0.547	0.352	0.573	0,485	0.870	0.727	0.171	0.370
apo B100	0.133	0.847	0.272	0.502	0.139	0.713	0.344	0.324	0.481
Lp(a)	0.066	0.442	0.832	0.323	0.349	0.188	0.826	0.064	0.921
Mid-Point									
TC	0.204	0.302	0.363	0.471	0.030*	0.356	0.697	0.811	0.680
HDL	0.071	0.345	0.403	0.938	0.124	0.911	0.448	0.679	0.584
LDL	0.856	0.884	0.355	0.649	0.240	0.645	0.270	0.099	0.700
apo AI	0.399	0.716	0.577	0.286	0.843	0.153	0.580	0.298	0.918
apo B100	0.732	0.529	0.629	0.579	0.0061	0.482	0.601	0.274	0.054
Lp(a)	0.060	0.828	0.667	0.119	0.838	0.305	0.594	0.594	0.429
End Point									
TC	0.353	0.450	0.478	0.447	0.349	0.468	0.860	0.276	0.484
HDL	0.409	0.270	0.304	0.966	0.504	0.506	0.152	0.753	0.394
LDL	0.982	0.730	0.948	0.572	0.513	0.898	0.906	0.523	0.257
apo AI	0.810	0.370	0.795	0.796	0.842	0.973	0.325	0.746	0.685
apo B100	0.608	0.902	0.415	0.952	0.060	0.176	0.440	0.173	0.809
Lp(a)	0.005*	0.658	0.652	0.283	0.884	0.3.44	0.814	0.671	0.821

Table 29 continued

Team B	3a Diol G	TT	FT	Δ4	DHEAS	E2	FSH	LH	PRL
Baseline									
TC	0.651	0.379	0.079	0.937	0.861	0.625	0.342	0.879	0.127
HDL	0.190	0.565	0.344	0.001**	0.094	0.067	0.472	0.0.067	0.893
LDL	0.345	0.138	0.834	0.126	0.422	0.512	0.248	0.512	0.444
apo AI	0.356	0.337	0.448	0.099	0.507	0.991	0.645	0.612	0.001**
apo B100	0.782	0.601	0.824	0.277	0.180	0.971	0.446	0.806	0.329
Lp(a)	0.610	0.041*	0.003*	0.308	0.706	0.121	0.012*	0.661	0.772
Mid-Point									
TC	0.890	0.347	0.001**	0.575	0.077	0.429	0.420	0.715	0.547
HDL	0.876	0.626	0.711	0.924	0.901	0.907	0.530	0.247	0.606
LDL	0.014*	0.899	0.586	0.299	0.829	0.180	0.932	0.126	0.280
apo AI	0.715	0.934	0.764	0.768	0.470	0.607	0.657	0.296	0.250
apo B100	0.665	0.536	0.151	0.044*	0.777	0.120	0.456	0.554	0.487
Lp(a)	0.917	0.604	0.700	0.764	0.709	0.632	0.454	0.938	0.573
End Point									
TC	0.415	0.777	0.196	0.895	0.975	0.064	0.083	0.401	0.947
HDL	0.819	0.765	0.681	0.286	0.591	0.983	0.628	0.060	0.739
LDL	0.876	0.798	0.295	0.976	0.450	0.813	0.717	0.131	0.134
apo AI	0.972	0.014*	0.304	0.636	0.293	0.227	0.374	0.827	0.063
apo B100	0.288	0.177	0.481	0.938	0.748	0.780	0.908	0.633	0.246
Lp(a)	0.845	0.023*	0.492	0.118	0.826	0.023*	0.112	0.946	0.119

Tadle 29 continued

Team C	3a Diol G	TT	FT	Δ4	DHEAS	E2	FSH	LH	PRL
Baseline									
TC	0.795	0.191	0.027*	0.576	0.787	0.174	0.114	0.834	0.398
HDL	0.677	0.750	0.350	0.256	0.964	0.534	0.892	0.992	0.675
LDL	0.685	0.848	0.076	0.218	0.795	0.816	0.724	0.528	0.703
apo AI	0.673	0.552	0.934	0.161	0.384	0.934	0.196	0.358	0.060
apo B100	0.998	0.342	0.775	0.425	0.631	0.669	0.623	0.359	0.807
Lp(a)	0.950	0.751	0.879	0.428	0.379	0.326	0.207	0.785	0.476
Mid-Point									
TC	0.560	0.145	0.980	0.478	0.716	0.634	0.198	0.432	0.210
HDL	0.608	0.532	0.914	0.448	0.167	0.929	0.512	0.705	0.762
LDL	0.508	0.990	0.708	0.824	0.395	0.948	0.516	0.566	0.464
apo AI	0.853	0.697	0.2532	0.353	0.531	0.984	0.784	0.817	0.932
apo B100	0.343	0.248	0.506	0.291	0.712	0.429	0.735	0.212	0.535
Lp(a)	0.205	0.654	0.589	0.742	0.296	0.890	0.879	0.974	0.506
End Point									
TC	0.411	0.020*	0.576	0.723	0.708	0.914	0.212	0.669	0.015*
HDL	0.377	0.525	0.367	0.630	0.608	0.577	0.478	0.982	0.469
LDL	0.350	0.123	0.046*	0.527	0.090	0.539	0.913	0.704	0.380
apo AI	0.846	0.486	0.348	0.654	0.850	0.550	0.931	0.303	0.678
apo B100	0.751	0.199	0.879	0.653	0.481	0.450	0.627	0.839	0.454
Lp(a)	0.832	0.636	0.960	0.712	0.553	0.413	0.961	0.432	0.685

Pre=just prior to the begging of the pre-season period; Post=at the end of the completion period; *p<0.05;p<0.01**

Table 30. Correlation (p values) between sex steroids and Hct, RBC, and ESR during the intervention period

Team A	**3a Diol G**	**TT**	**FT**	**Δ4**	**DHEAS**	**E2**	**FSH**	**LH**	**PRL**
Hct pre	0.620	0.995	0.595	0.754	0.838	0.779	0.616	0.192	0.90
Hct mid	0.356	0.331	0.792	0.471	0.501	0.356	0.0.697	0.636	0.845
Hct post	0.824	0.806	0.819	0.397	0.422	0.468	0.860	0.948	0.798
RBC pre	0.362	0.261	0.323	0.235	0.444	0.327	0.661	0.670	0.99
RBC mid	0.372	0.906	0.119	0.272	0.88	0.230	0.782	0.429	0.461
RBC post	0.661	0.535	0.283	0.686	0.733	0.068	0.759	0.222	0.792
ESR pre	0.071	0.609	0.209	0.850	0.029*	0.080	0.891	0.653	0.375
ESR mid	0.034*	0.647	0.210	0.459	0.663	0.556	0.728	0.926	0.766
ESR post	0.266	0.194	0.108	0.244	0.304	0.674	0.860	0.635	0.837
Team B									
Hct pre	0.084	0.426	0.739	0.909	0.231	0.832	0.205	0.847	0.038*
Hct mid	0.370	0.916	0.709	0.922	0.694	0.762	0.143	0.073	0.739
Hct post	0.250	0.707	0.940	0.440	0.283	0.085	0.054	0.402	0.774
RBC pre	0.742	0.485	0.678	0.590	0.956	0.145	0.787	0.490	0.946
RBC mid	0.443	0.327	0.419	0.653	0.796	0.904	0.672	0.642	0.473
RBC post	0.568	0.860	0.171	0.246	0.976	0.736	0.728	0.691	0.778
ESR pre	0.923	0.254	0.107	0.951	0.990	0.860	0.213	0.544	0.260
ESR mid	0.156	0.693	0.195	0.798	0.135	0.853	0.764	0.583	0.013*
ESR post	0.266	0.011*	0.743	0.225	0.883	0.515	0.123	0.484	0,078
Team C									
Hct pre	0.589	0.445	0.233	0.377	0.792	0.896	0.356	0.170	0.098
Hct mid	0.352	0.855	0.684	0.434	0.131	0.928	0.193	0.028*	0.876
Hct post	0.189	0.770	0.962	0.053	0.605	0.248	0.968	0.322	0.783
RBC pre	0.610	0.099	0.219	0.924	0.585	0.601	0.207	0.953	0.476
RBC mid	0.550	0.365	0.710	0.774	0.611	0.963	0.879	0.789	0.181
RBC post	0.385	0.362	0.420	0.485	0.947	0.157	0.901	0.772	0.638
ESR pre	0.178	0.218	0.378	0.947	0.759	0.460	0.764	0.472	0.460
ESR mid	0.325	0.068	0.786	0.378	0.228	0.763	0.719	0.726	0.669
ESR post	0.827	0.154	0.302	0.097	0.295	0.244	0.688	0.751	0.919

*$p<0.05$

not observed at the second (all p>0.05) and third experimental periods (all p=0.005), although, apart from TT levels at the end of the study compared to mid-point, all sex steroids and blood lipids did not show significant changes in their values. At the second experimental session the only significant correlations were observed between 3a Diol G and LDL (p=0.014), FT and TC (p=0.001) and Δ4-androstenedione while at the end of the study we observed that TT was correlated with both apo AI (p=0.014) and Lp(a) (p=0.023) and that E2 was correlated with Lp(a) (p=0.023).

In team C we observed at baseline a weak significant correlation between FT and TC (p=0.027), but not at the mid (p>0.05) and end point measurement (p>0.05), a weak correlation between LH and Hct (p=0.028) at mid-point, but not at baseline (p>0.05) and at the end-point measurements (p>0.05), and lastly only at the end of the study between PRL and TC (p=0.015), TT and TC (p=0.02) FT with LDL (p=0.046).

In regard to Hct, RBC, and ESR, we observed in Team A weak correlations between 3a Diol G and ESR (p=0.034) at the middle of the season, and between DHEAS and ESR (p=0.029) at the beginning of the pre-season period. No other correlations were evident for none of the examined parameters throughout the study in this team (Table 30). In Team B we observed correlations between PRL and both Hct (p=0.038) and ESR (p=0.013) only in the middle of the season, and between TT and ESR (p=0.011) at the end of the study. No other correlations were evident for none of the examined parameters throughout the study in this team (Table 30). Finally in Team C we observed only a weak correlation between LH and ESR at the middle of the season. No other correlations were evident for none of the examined parameters throughout the study in this team (Table 30).

4. Discussion

The main aim of this study was to examine the effects of three different seasonal training programs regarding strength volume and load on hormonal and performance parameters in soccer players. Our main finding is that the combination of soccer training with general and soccer-specific strength training can induce an elevation of TT and 3a Diol G. These changes appear to be mainly related to the volume of strength training since they both responded in proportion to volume, i.e. the higher the volume of strength training (Team A), the higher the resulting alterations. These changes in androgen levels do not correlate with any of the examined performance parameters. The only borderline correlation observed was between 3a Diol G to VO2max at the mid-point measurement in Team B. No significant differences were observed for DHEAS, Δ4-abdostenedione, estradiol, LH, FSH, and prolactin in any time point throughout the intervention soccer period. These findings indicate that alterations in the volume and load of the strength training stress do not seem to affect the endogenous production of the latter sex steroids in professional soccer players. Regarding exercise performance and body composition variables, all training programs managed to induce beneficial alterations in these parameters until mid-season, which was retained until the end of the season. However, only the high strength training stress of Team A resulted in further significant changes in neuromuscular performance (SJ, CMJ, 10m 20m), body fat percentage, and body weight in the second half of the study. Regarding bone metabolism our findings indicate that the higher the strength training stress the higher the adaptations in favor of bone formation. In addition, our findings indicate the alterations in training load and volume do not affect the lipidemic profile and RBC, Hct and ERS in professional soccer players.

Lastly, our finding show that the off-season detraining period in our study failed to affect sex steroids levels, lipid profile, Hct, RBC, and ESR, however it had detrimental effects on exercise performance, bone metabolism adaptations, and optimal body composition status.

4.1 Sex Steroids

4.1.1 Androgens and Detraining

Analysis of our data revealed that the six week off-season detraining soccer period failed to affect the circulating sex steroids levels in our three experimental teams. In

regard to TT and FT our observations are in accordance with the findings showing that detraining periods of 4 (Izquierdo et al. 2007) and 6 (Kraemer et al. 2002) weeks duration failed to affect TT and FT levels respectively. In addition the observed absence of an alteration in 3a Diol G levels during this period of reduced training stress clearly indicates that no anabolic trend was evident at the end of the six-week detraining period, since this hormone is the metabolic product of androgens and an indicator of activated androgens (Labrie et al. 1997). In regard to LH and FSH, our findings are supported by the observations of studies on resistance and endurance trained maless (Hakkinen et al. 1985; Hall et al. 1999). The authors reported that relative short detraining periods did not affect both LH and FSH levels. Therefore, according to the aforementioned published reports and our own findings, we could propose that detraining does not affect the hypothalamus or the pituitary gland regarding these two hormones.

In regard to DHEAS, only one recent study examined its behavior after 2 months of training cessation in highly trained badminton players (Wang et al. 2006). The authors observed a significant reduction in its resting levels at the end of the study. However, we were unable to confirm these observations. Indeed, we have found that the detraining period did not decrease DHEAS concentration, on the contrary, it showed a tendency to increase albeit in a non-significant manner in both experimental teams. This discrepancy could be related to the training status of the participants and the training regime used, which have been both reported to affect the hormonal responses to exercise (Tremplay et al. 2004). In the published bibliography no evidence exists for E2, Δ4-androstenedione, and PRL and their responses after a detraining period. In conclusion, our overall findings indicate the soccer off-season detraining period does not affect the circulating levels of sex steroids.

4.1.2 Androgens and Intervention Period

TT, FT, 3a Diol G

To the best of our knowledge, only two published intervention studies in soccer exist in the literature (Gorostagia et al. 2004; Pacobahyba et al. 2012). Both studies have reported comparable results to ours. More specifically, the combination of regular soccer training with two (Gorostagia et al. 2004) or three (Pacobahyba et al. 2012) strength training sessions, during an 11-week in-season and a 12-week pre-season period, respectively, managed to increase TT resting levels, whereas one session per

week did not appear to alter TT (Gorostagia et al. 2004). It should be noted here that in our teams the training scheme employed in Teams A was composed of three strength training session, in Team B two strength training session per week while Team C had only one. These similarities in the weekly strength training frequency provides supportive evidence to our own findings.

However, the TT changes in Teams A and B were evident in a different manner. The high strength stress training program employed by Team A managed to increase significantly TT resting levels at the mid-point measurement, followed by a further significant increase at the end of the study, whereas players of Team B exhibited only a tendency to increase TT resting levels, as highlighted by the mean values at the end of the study compared to baseline (Table 13). We postulate that the TT responses observed in Teams A, B, and, C were the result of the different strength training volume employed by each of the three teams. Indeed, the three types of seasonal training programs were significantly different in training volume and load throughout the study (Table 12). Team A had the highest values in these two parameters compared to the other two experimental teams, while Team B had significantly higher values compared to Team C (Table 12). The seasonal training plan was organized in such a manner as to differ mainly regarding the weekly volume/frequency of the sessions aiming to improve general and soccer-specific strength. Regarding the other aspects of physical conditioning, the training programs employed by Teams A and B were nearly identical and slightly differed compared to Team C (Table 9). Therefore, the differences observed between the weekly training volume and load were mainly the result of the different strength training stress employed by each team. Confirmation of our suggestion of a volume/load-dependant TT response comes from the observations of previously published studies. It has been reported that strength training which results in high volume (Kraemer et al. 2005), high intensity (Staron et al. 1994) and high training load (Hartman et al. 2007) can increase TT resting levels. These changes may occur in response to long (Häkkinen et al. 1988) or even short training periods.44 Therefore, we could suggest that the higher strength training stress employed by Team A was more effective in providing a sufficient stimulus for a more pronounced TT increase compared to the moderate strength training stress of Team B. Similarly, Team B's training program managed to provide sufficient stimulus to induce a detectable though non-significant elevation in TT levels (Table 13) (end of study vs just prior to pre-season values) compared to the low strength training stress of Team C that did not

appear to affect TT levels. It should be mentioned that the extra sprint session performed on the morning of each match-day in Team A was unlikely to make any significant contribution to TT response, as it has previously been reported that sprint training suppresses TT rather than elevates it (Aldercrentz et al. 1986). The elevation of TT observed in Team B at the end of the study was not significant, in contrast to what has been reported by Pacobahyba et al. (2012), who have shown that soccer training combined with two strength sessions per week can significantly elevate TT levels. This discrepancy could be due to the nature of the strength training regimes employed by our Team B (general and soccer specific strength) and the Pacobahyba et al. (2012) study in which only general strength training was used. It is generally accepted that the mode of physical activity can influence hormonal responses to exercise training.46 In regard to the unaffected androgens levels in Team C, it is well documented that one strength session per week does not provide sufficient stimulus for TT increase (Filaire et al. 2001; Gorostagia et al. 2004). Only one recent study with an in-season weekly training regime similar to that of Team C showed an elevation of TT levels at the end of the competitive season (Kraemer et al. 2004). The authors suggested that the observed increase in TT was due to the reduction of training volume towards the end of the season. However, this could not be the case in our study since not only in Team C but also in all experimental teams training volume was kept constant until the end of the season. Therefore, the absence of any periodization in training volume, and the low strength training stress employed by this team, most probably accounted for the unaffected TT level throughout the study.

Our data regarding the changes of 3a Diol G during the training period in Team A support our TT findings. The significant changes of 3a Diol G levels detected in this team followed a similar pattern to the changes in TT levels, i.e. its levels increased at mid and end of the season compared to baseline in Team A. This observation suggests that the higher strength training program employed by Team A accelerated both the production and activation rates of testosterone. However, the observed elevation of 3a Diol G, indicative of activated testosterone (Labrie et al. 1997), did not appear to have any statistically significant repercussions on ergometrics, suggesting a more subtle effect. Furthermore, it should be mentioned here that our study is the first involving professional athletes tested for the effect of training on 3a Diol G. The only other available data regarding 3a Diol G response to exercise is from a study performed in elderly and middle-aged men (Hawkins et al. 2008). The authors reported that a 12-

month training period failed to document any significant change in 3a Diol G levels, a finding which is in agreement with our results obtained from Teams B and C, i.e. from the teams with the lower volume of training. Thus, we postulate that the lower level of strength training stress does not change the rate of testosterone activation in contrast to the higher level of strength training stress used in Team A. The higher training stress in Team A may have induced activation of testosterone, the first step of which is its conversion to its biologically active compound DHT (dihydrotestosterone). This may be responsible for the alterations of the ergometrics that we have observed, since DHT has been shown to be associated with fast twitch muscle fibers, muscle strength, and body fat percentage (Yoshioka et al. 2006), parameters that directly affect exercise performance (Hartgens and Kuipers, 2004; Larew et al. 2003; Ozkan et al. 2012). Furthermore, the elevation of 3a Diol G levels indicates a positive anabolic effect, since this metabolite is a marker of the total androgen pool and an indicator of peripheral activation of androgens (Labrie et al. 1997). Overall, the importance of this finding is based on the fact that apart from an induced adaptation leading to increased endogenous TT production at the end of the study in Team A, there was a further unique adaptation, an augmented peripheral activation of testosterone.

Our results demonstrated that none of the three different training programs provided sufficient stimulus to significantly change FT levels. In agreement with our observations is a recent study in professional soccer players reporting that the combination of regular soccer training (composed of two strength training sessions per week) did not affect FT levels (Gorostagia et al. 2004). Evidence from previous studies on strength trained individuals showed that FT seems to be affected only by heavy resistance exercise of high volume (Ahtiainen et al. 2003; Kraemer et al. 1999). This hypothesis is partly validated by our findings. The higher strength training stress program in Team A showed a trend, albeit not statistically significant, of increase in FT levels at the end of the study compared to baseline. No similar trend was observable in the other two teams (lower strength volume) (Table 13, Figure 10). However, this higher strength training program failed to provide sufficient stimulus for a significant increase in FT levels. These different observations between the aforementioned studies (Ahtiainen et al. 2003; Kraemer et al. 1999) and our own findings could be a result of the difference in strength training volume between the two experimental models and the training status of the subjects, parameters that have been reported to be of paramount significance in the effect of exercise on endogenous androgen production (Trappe et al. 1997).

Δ4-Androstenedione

Analysis of our results showed that Δ4-androstenedione was not altered throughout the study in none of the three experimental teams. The only scientific data for the effects of a long training period on Δ4-androstenedione come from Fellmann and associates (1991). The authors failed to show any effect on Δ4-Androstenedione concentration after 40-week training program on a bicycle ergometer. These results suggest that long-term training does not enhances adrenal function in regard to Δ4-Androstenedione. Similarly, Kourkoulias et al. (2008) reported that a two week period which included a marathon race in between failed to alter Δ4-Androstenedione levels. However, these results should be taken into account with the considerable percussions when related to competitive athletes, because of the difference in age and the fact that there was no definition of the magnitude (intensity, volume, duration) of the endurance exercise training performed by the subjects. No other scientific evidence exist for the effects of a long training period with or without training manipulation on Δ4-androstenedione resting levels on professional or not professional athletes. Therefore, based on our findings regular soccer training, and/or seasonal variations on strength training volume and load does not seem to provide sufficient training stimulus to affect Δ4-androstenedione basal levels.

DHEAS

In our study there was no difference in DHEAS response throughout the study in none of our experimental teams. Our findings are comparable to the studies of two laboratories. It have been observed that six months of heavy resistance training, combined with explosive activities failed to induce systematic alterations in basal DHEAS levels. This finding was further confirmed by a latter study from the same laboratory (Hakkinen et al. 2002). The authors reported a 24-week resistance/power training program in elderly males failed to significantly alter DHEAS levels, at least in a systematic way. However, the available evidence in regard to DHEAS reveals a great inconsistency. Elevation, no changes, or even reductions have been observed in elderly individuals, female, and male resistance athletes (Ravaglia et al. 2001; Collomp et al. 2014; Hakkinen et al. 2002). It should be mentioned that none of existing studies have examined the seasonal changes in DHEAS levels in professional soccer players. According to Chatarde et al. (2002) the determinant of variation in DHEAS remains unclear. In the only study to our knowledge performed in teams sports, over of a 3

month period the authors observed that basketball training resulted to reduced levels when the training load was increased, whereas when the intensity of the training sessions was reduced DHEAS levels were increased (Slowiska-Liwoska et al. 2006). However, in our study the training load, apart from the first weeks of the preparation, during the whole competition season was constant in all teams. Therefore the absence of periodized alterations in load and intensity was probably the reason for the non-significant changes in Teams A, B, and C.

4.1.3 Estrogens

Evaluation of our findings revealed that the three different seasonal training regimes failed to alter E2, LH, FSH, and PRL basal levels. Our findings are comparable with the available scientific evidence in soccer (Celani and Grandi 1989; Grandi and Celani, 1990; Hoffman et al. 2005). The authors failed to find any differences in these estrogens. Therefore, our findings provide further support to the observation that E2, LH FSH, and PRL are not affected by seasonal soccer training, even if this is combined with various different strength training regimes throughout a whole soccer season.

4.2 Exercise Performance Parameters

4.2.1 Exercise Performance Parameters and Detraining period

As expected, the six-week detraining period reduced resulted to a rapid loss of aerobic and neuromuscular adaptations. Our findings are in accordance with the observations of several authors that have examined the effects of reductions in training volume in professional soccer players (Reilly and Williams, 2003; Caldwell and Peters, 2009; Izquierdo et al. 2007; Amigo et al. 1998; Mujika and Padilla, 2000). In regard to VO2max has been shown to decline in a variety of sports, even with short term detraining (less than 4 weeks), up to 14% (Coyle et al. 1984; Martin et al. 1986; Moore et al. 1987; Houmard et al. 1992). Furthermore, VO_2max has been found to decrease in highly trained athletes up to 20% after 4 weeks of detraining (Martin et al. 1986). Similarly, it have been reported that the off-season detraining period can result in a reduction in jumping and sprinting ability (Caldwell and Peters, 2009; Izquierdo et al. 2007; Amigo et al. 1998; Mujika and Padilla, 2000). It was suggested that these findings could be related with reductions in the cross-sectional area of type II muscle fibers, negatively altered anaerobic enzymatic activity, and a decline in mitochondrial ATP production as a result of the detraining period (Ross and Leveritt, 2001).

4.2.2 Exercise Performance Parameters and Intervention period

In agreement with the majority of the available evidence (Bangsbo et al. 2004; Caldwell and Peters, 2009; Gorostagia et al. 2004; Rønnestad et al. 2011) all examined performance parameters exhibited significant improvements at mid-point compared to baseline in all teams, and this effect was retained until the end of the study. However, only in Team A did we observe a further significant improvement in SJ, CMJ, 10m and 20m capacity in the second half of the study. These considerable improvements in exercise performance until mid-point and their maintenance until the end of the season indices is a result of a combined effect of the pre- and in-season conditioning in conjunction with the competitions' training stress (Caldwell et al. 2008; De Villareal et al. 2008).

The further enhancement in jumping (SJ, CMJ) and sprinting (10m, 20m) ability in Team A in the second half of the in-season period was most likely a result of the higher employed volume of both SPS and sprint sessions in this team compared to Teams B and C (Table 9). It has recently been suggested (De Villareal et al. 2008) that a moderate frequency of plyometrics (twice per week), similar to the volume used in our SPS regimes in Team A, is more effective in promoting jumping ability compared to the lower frequency (once per week) used in Teams B and C. Furthermore, a similar weekly sprint training volume (three sessions per week) as that employed by Team A (Table 9) has been found to produce beneficial adaptations in the muscle function, which is translated into an increased SJ and CMJ performance (Markovij et al. 2007). This increased jumping ability could be the reason for the observed increase in sprint performance in Team A. Indeed, it is well established that jumping ability is strongly related to sprint performance and, moreover, increases in sprint performance occur concomitantly with the largest increases in jumping ability in soccer players (Gorostagia et al. 2004; Rønnestad et al. 2011).

Another possible mechanism responsible for this increase in jumping and sprinting ability in the second half of the study in Team A could be related to the reduced BF% in this team during this period (Table 13**).** Indeed, it has been well demonstrated that both jumping ability and sprint performance improvements are significantly associated with a reduction in BF% (Davis et al. 2004; Mercer et al. 1997). Moreover, it has previously

been reported that competitive soccer players with a lower BF% invariably show better sprint performance (Caldwell and Peters, 2009).

4.3 Body Composition

4.3.1 Body Composition and Detraining Period

At the end of the 6-week detraining period, significant increases in body weight and body fat percentage were evident in both teams. In accordance are the findings of other laboratories (Ostojic, 2003; Hoshikawa et al. 2004). It was observed that the off-season soccer period resulted in increased body weight and body fat percentage in the players. Further Confirmation to our findings comes from a recent study in competitive swimmers (Ormsbee and Arciero, 2012) which observed that 35–42 days of detraining, involving light-moderate physical exercise, after a competitive swim season, resulted in significant increases in body weight and body fat percentage. The increased body fat percentage and body weight, in our study, could be attributed to the reduced training stress during the detraining period. The reduced training stimulus could have resulted in a lowering of the metabolic rate per unit of tissue mass, and effectively decreased resting metabolic rate, which could have a negative impact on body composition (Ostojic, 2003; Ormsbee and Arciero, 2012), although these finding are not universal (LaForgia et al. 1999). Furthermore, it has been observed that during detraining periods there is an increase in lipoprotein lipase (LPL) activity, which facilitates free fatty acid deposition on adipose tissue (Hardman et al. 1998).

In regard to BMI and WHR, to the best of our knowledge no evidence exist in soccer players during periods of detraining. However, the observed increases in both parameters in all experimental teams were most probable the result of the similar in manner alterations in body fat and body weight.

4.3.2 Body Composition Parameters and Intervention Period

In regard to the body fat significant reductions in the mid-point measurement, our findings are in agreement with previous studies (Bangsbo, 1994; De Villarreal et al. 2008; Morgan et al. 2005; Ostovij, 2003). It has been suggested that this decrease is a result of a combined effect from the pre-season period (Reilly,b 1996) and the in-season training stress until the first half of the season (Bangsbo, 1994; Caldwell and Peters, 2009)

The unchanged body fat percentage values in teams B and C in the end of the study are confirmed by other laboratories (Caldwell and Peters, 2009; Morgan et al. 2005) which showed that fat percentage decreases until mid-season and remains constant to the end of the competition phase. The further decrease in body fat percentage in team A is in agreement with the findings by Ostovic (2003) in professional soccer. The authors suggested that this was a result of the whole competition training stress on this parameter. In our study this further reduction in body fat in team A could be related to the higher training volume and training load in this team, since it has been suggested that high training load and volume could increase the demands of the aerobic system causing larger energy expenditure, resulting to reduced body fat (Bangsbo, 1994).

The observed significant decreases in Body weight (BW) was following the same manner as body fat percentage reductions (Table 13), its values decreased significant until mid-season in all teams, whereas a significant reduction was evident only in team A at the end of the study. This similar pattern of changes in both body composition parameters indicates that the observed changes in BW were a result of the observed alterations in body fat percentage throughout the study.

Our study, to the best of our knowledge, is the first to examine the effects of different seasonal training schemes regarding strength training volume and load on BMI and WHR in professional soccer players. Although we observed a tendency of both BMI and WHR to decrease in a similar manner as BW and BF% in our experimental teams this difference was not significant. Our findings indicate that these two body composition parameters are not affected in professional soccer players due to variations in strength training volume and load.

4.4 Relationship between Sex steroids and performance parameters

4.4.1 Detraining Period

No correlations were evident between performance parameters and circulating androgen (TT, FT, 3a Diol G, Δ4-androstenedione, and DHEAS) during both the detraining period in our study. Similarly, no correlations were evident between performance and E2, FSH, and PRL levels. It is of note that the gonadotropin LH exhibited a correlation with VO2max in team A, and with sprinting performance in team B. To our mind, the validity and physiological significance of this finding is questionable since in team A,

LH correlated with VO2max performance in both experimental sessions but not with jumping and sprinting ability, whereas in team B, LH levels correlated with sprint performance (pre and post) but not with VO2max. In addition, despite the considerable changes in all performance parameters observed at the end of the detraining period, the LH levels did not exhibit any alterations. These discrepancies indicate that these data are of minor physiological significance, and do not necessarily constitute a significant correlation between LH and VO2max, 10m, and 20m performance.

4.4.2 Intervention period

During the intervention period we failed to observe any significant correlation between androgens and performance parameters in our three experimental teams apart from a weak correlation of TT with both SJ and CMJ only at the mid-point measurement in team A, despite the significant variations that these parameters exhibited throughout the study in all teams, a weak significant correlation between VO2max and 3a Diol G levels at the end of the study in team B, and similarly a weak correlation between Δ4-androstenedione withVO2max in Team C at mid-point measurement (tables 27, 28). It should be noted that the meaning of these finding is questionable since in all the other experimental sessions these observed associations were not further supported in any team. Similarly, in regard to estrogens the observed correlations between LH, E2, FSH, and PRL and with exercise performance parameters occurred with great variability, mostly in a weak significant manner, and were not a consistent but rather in isolation at specific times-point and with no sequence in any team. These observed weak correlations could have been a result of the sample size in each experimental team.

However, we could not exclude the hypotheses that sex steroids, and especially testosterone which showed beneficial alterations in teams A and B, may have favorably affected exercise performance by several indirect mechanism. Indeed, it has been suggested that testosterone has the ability to indirectly affect exercise capacity by variety of mechanisms. Indeed, testosterone may also influence muscular performance due to its observed linear relationship with calcium metabolism. Since calcium is an important metabolite in muscle contraction, and testosterone stimulates intracellular calcium release (Kadi, 2003) any alterations in calcium release could affect exercise performance, and especially neuromuscular capacity, in a similar manner. Furthermore, testosterone is capable to inhibit the anticatabolic effects of glucocorticoids as well as reducing the suppression of muscle protein synthesis excreted by them. Depending on

the amount of glucocorticoid receptors occupied by testosterone there is a proportional decrease of the catabolic hormones actions (Mayer and Rosen, 1977) which may in turn enhance the recovery after strenuous exercise. Moreover, it has been reported that testosterone may affect performance by activating glucose metabolism-related signaling pathway in skeletal muscle (Sato et al. 2008) and thus enhance carbohydrate utilization during exercise, and moreover by stimulating catecholamine-induced lipolysis (Xu et al. 1990) in a dose-dependent manner, providing a massive availability of energy during exercise. In addition, it has been suggested that testosterone can increase cardiac muscle strength, an adaptations that could lead to enhanced cardiac output during exercise, and thus to an sufficient energy nutrients and oxygen provision to the exercising muscles (McKillop et al. 1986). Lastly, the well documented relationship between testosterone and aggression suggest that there is an effect on muscle function and behavior mediated by alterations in its levels (Montoya et al. 2012). By this mechanism it could be speculated that since increased testosterone levels may determine high levels of aggressiveness, a facilitation of neural input during maximal explosive effort may occur.

4.5 Bone Metabolism

Analysis of our results revealed that both the reduced training stress of the detraining period and the increased training volume and load of the intervention period affected bone metabolism markers. More specifically, we observed that the massive reduction in training stress during the 6-week off-season period resulted in a significant increase in bone resortpion (i.e. examined by CTX) and a decrease in all examined bone formation markers (i.e. b-ALP. OC, CICP). This observed response in favor of bone resortpion is in agreement with the only two available studies on soccer reporting that the off-season detraining period soccer has negative effects on bone metabolism and thus bone health (Karlsson et al. 2003; Weiler et al. 2012). In regard to the intervention period our findings revealed that the high (team A) and medium (team B) training volume and load have resulted in beneficially altered bone turnover, while no difference were observed in the low strength training stress team C. Our findings indicate that in our study the increase in bone anabolism and decreases in bone catabolism were in a proportional to training volume and load manner (Cadore et al. 2005). The higher training stress employed by Team A elicited the most pronounced differences. Bone turnover changes were observed in a similar manner but to a lesser degree in team B, whereas no

alterations were evident in team C. Our observations in the intervention period are in accordance with the suggestions that the osteogenic effects derived from physically activity appears to demand high training level that is characterized by a great volume and intensity. In our study, the differences in volume between our experimental teams were obvious (Table 12) and give explanations for our findings i.e. the higher the strength training stress the higher the beneficial alterations in bone metabolism. In regard to exercise intensity it should be mentioned that the subcomponents of the employed SPS training sessions, that notably were of higher volume in team A compared to the other two teams and to a lower volume in team C compared to team A, were of maximal intensity soccer related resistance/power activities. Therefore, based on the aforementioned observations and our own findings, we could suggest that the training volume and intensity was the result of the observed alterations in bone metabolism during the intervention period, and that these changes were evident in a dose response manner.

4.6 Relationship between Sex Steroids and Bone Metabolism markers`

Analysis of our results showed that there were evident only some inconsistent associations between CTX and LH at the end of the intervention period in team A, CICP with DHEAS and OC with PRL just prior to the pre-season period in team B, and between FSH and OC just prior to the pre-season period in team C that were not supported by similar association throughout the study in any team. No other correlations were evident in regard to these parameters, but also in all other sex steroids and bone turnover markers at the two time points in our study. According to these observations we could suggest that sex steroids were not a contributing factor in the alterations in bone metabolism markers in our study. Our observations are supported by the findings of other reports. Indeed, studies on adolescent and collegiate athletes and on chronically trained middle aged men failed to observe any association between bone metabolism markers and TT, FT, FSH, and LH (Ackerman et al. 2012; Sinnesael et al. 2011, Falahati-Nini et al. 2000). Similarly, it has been observed that aromatase inhidition in the elderly although managed to increase testosterone and reduce estrogen levels, no relationship was evident between these sex steroids and bone turnover markers (Falahati-Nini et al. 2000). It should be mentioned that these findings are not universal since there is evidence to suggest that sex steroids play an important regulatory role on bone health in the elderly and healthy male population (Ackerman et al. 2012, Sinnesael

et al. 2011). However, the participants in the latter studies that have reported a close association between sex steroids and bone metabolism were either elderly or inactive individuals. Therefore, based on the aforementioned reports and our own findings, (Ackerman et al. 2012; Sinnesael et al. 2011; Falahati-Nini et al. 2000) we could suggest that it is questionable whether there is a relationship between sex steroids and bone turnover markers in well trained young healthy individuals.

4.7 Lipids Profile, RBC, Hct, and ESR

Analysis of our data revealed that none of the measured parameters of the lipidemic profile i.e. TC, LDL-C, HDL-C, apo AI, apo B100, and Lp(a), and RBC, Hct, and ESR were affected by the short detraining period and the three different training regimes in any time point.

In regard to blood lipids our findings are supported from a number of studies showing that in athletic and well trained population none of TC, LDL, HDL, apo-AI ,apo-100 and Lp(a) are affected by short detraining periods (Thompson et al. 1985; Herd et al. 1998), and by increased training load and volume (Durstine et al. 2001; Mann et al. 2014). In general modifications in volume and intensity may affect TC, LDL-C, and HDL-C whereas exercise does not seem to alter apo AI, apo B100, and mainly Lp(a) (Durstine et al. 2001; Mann et al. 2014). The major explanation for our observations during the intervention period is that despite the different training regimes our players were under rigorous training sessions, with varying training loads, volumes and intensities for a long period of time (>5 years). Therefore, our participants could have already gained any possible beneficial adaptation regarding their lipidemic profile. Indeed, well trained individuals do not seem to respond even in extremely increases in training volume, probably due to an already beneficial adaptation of long term strenuous exercise (Durstine et al. 2001; Mann et al. 2014).

In regard to Hct, RBC, and ESR, to the best of our knowledge, no comparable evidence exists in the literature. Our findings suggest that in chronically trained professional soccer players short term detraining or variation in the training volume and load throughout a competitive season does not seem to affect these parameters. The explanation for this observation could be also a result of the fact that our players have already had gained the upper limit of the proposed beneficial alterations in these parameters and therefore, short reductions in training or/and seasonal alterations of

training volume and load did not provide sufficient stimulus for further alterations in their levels.

4.8 Sex steroids and Lipid profile, Hct, RBC, and ESR

Analysis of our result observed some associations is isolated time points throughout the study between sex steroids, and mostly estrogen, with blood lipids and Hct, RBC and ESR (Tables 29, 30), but this was in an inconsistent manner, and furthermore was not accompanied by similar observations within the teams in the other time points that measurements were performed.

Although, to the best of our knowledge, no studies has examined the association between sex steroids and lipid profile in professional soccer players under variations in exercise training stress, evidence from studies on non-athetic population suggest that these hormones are related with alterations in blood lipids. Androgens and estrogen have been found to be related with the lipidemic profile in the elderly and in individuals with pathological conditions, however the results reveal great inconsistencies. Indeed, the available evidence show contradictory findings in regard to both androgens (Denti et al. 2000; Bagatell et al. 1992; Barrett-Connor; Wranicz et al. 2005) and estrogens (Morishima et al 1995; Pyorala et al. 1994; Wranicz et al. 2005; Khaw et al. 1991; Kiel et al. 1989) and these effects seem to vary according to the specific kind of population that each study had employed. Due to the lack of available evidence in professional soccer players it makes difficult to evaluate our findings. Our findings clearly state that in chronically trained professional soccer players it could be suggested that lipid profile is not influenced by sex steroids, and furthermore there are no evidence in the available literature to support this observation. In addition we should mentioned that the majority of the correlations were evident between the gonadotropins and blood lipids (table 29), a fact that indicates that indeed no associations exist between these parameters since it is well documented that these hormones are characterized by a pulsatile release leading to great variations in their circulating levels.

In regard to Hct, RBC, and ESR analysis of our results failed to reveal any associations between these parameters apart from three random associations only between Hct and PRL at the first half of the season in team B, and FSH with Hct at the end of the study in team C (Table 30). These correlations were not evident in the other time-point measurements within these teams. Therefore, we could suggest that in our chronically

trained population sex steroids and these biochemical parameters including ESR are not related. This lack of an association could be a result of the chronic participation in training and competitions by our players which could have already had reach the upper limit of the suggested beneficial alterations in these parameters. This could also be the case in regard to ESR since our recorded values (Table 15) were within the near the most favorable values of the clinical beneficial range in all three experimental teams.

However, we should mention that since none of our participants was found to be with abnormal sex steroid levels below the clinical beneficial range we could not exclude the hypothesis that any possible endocrine disorder in this type of popultation could be related with deterioration of blood lipid profile, the ability of to produce optimal red blood cells levels, and negatively altered ESR.

4.9 Vitamin D

We observed that the six week detraining period resulted to increased vitamin D levels. This finding could be related with two different physiological mechanisms. (a) Scientific evidence suggest that prolonged intense training sessions or intensified training periods, similar to the ones' used repeatedly during a soccer season, suppresses athletes' immune system (Gleeson, 2007). In our study, the first experimental session was performed at the end of the nine-month competition season. This intense, long-term, training period could have resulted in a suppression of the innate immune mechanisms (Gleeson, 2007). On the contrary, the period prior to the second testing players were under minimal training stress, which could have resulted in a enhanced innate immunity. Regarding Vit D, recently published data has uncovered a linear relationship between its levels and a functional immune system (Cannell, 2007). Moreover, increased Vit D levels have been reported to boost immune mechanisms (Christakos and DeLuca, 2011). Therefore, we could hypothesize that any positive alteration in soccer players' immunity could be evident in conjunction with increased Vit D status (Zitterman, 2003). In light of the aforementioned evidence, the observed increase in Vit D levels at the end of our study could be related with the major decline in the exercise training stress during the off-season period. This suggestion could give further support to the proposed linear relationship between Vit D levels and optimal immune mechanisms.

(b) The second possible mechanism could involve the extremely importance that UVB plays on endogenous Vit D synthesis (Holick, 2002) and the observation that its

effectiveness is among other parameters, season dependent (Pfeifer et al. 2002). Indeed, the off-season period in Greek Superleague takes place at June and the beginning of July. During this period UVB reaches its peak (Galán et al. 2011), resulting in increased Vit D production. Furthermore, this off-season phase is actually a holiday period for professional soccer players. This could indicate increased time spend under sunlight, and also an increased exposure of a larger proportion of the players' body to UVB. Since both parameters are related with Vit D production, its observed increased levels at the end of our study could be partly accounted to these two factors (Holick, 2002; Hollis, 2005)

4.10 Relationship between Vitamin D and Performance

Vitamin D levels exhibited a positive linear relationship with the ergometric evaluation of muscular strength (SJ and CMJ) and speed at both experimental periods. Our observations are comparable to several studies showing that vitamin D is linearly associated with jumping ability and strength in pre-adolescent girls (Muir and Montero-Odaso, 2011) and elderly individuals (Hamilton, 2010; Geoff, 2005) and in agreement with the observation that 100m performance was enhanced after a single biodose of ultraviolet radiation in collegiate women (Cheatum, 1968). Moreover, a recent vitamin D supplementation-study on professional soccer players revealed that inadequate vitamin D concentration was detrimental to jumping and sprinting ability, whereas supplementation counteracted this effect (Close et al. 2013). Notably, regarding muscular strength, Hamilton et al. (2014) reported that vitamin D levels were not associated with lower limb isokinetic muscle function in soccer players. However, this finding was attributed to the different mode of exercise used since, as the authors suggested, vitamin D could preferentially affect muscle groups that were not evaluated in their study. It should be mentioned that both SJ and CMJ are considered to be the most accurate field tests for the determination of the strength levels of the lower limps (Marcovic et al. 2004). In order to perform SJ and CMJ the proximal muscles required are quadriceps, soleus, and gastrocnemius (Ward et al. 2009). Those muscles have been found to be predominantly affected by vitamin D deficiency (Ward et al. 2009). Furthermore, it is well established that sprint performance is linearly related with both SJ and CMJ (Ingebrigtsen and Jeffreys, 2012; Perez-Gomez et al. 2008), suggesting a direct effect of strength levels on sprinting ability. Therefore, based on the aforementioned evidence, our findings indicate a possible effect of vitamin D on

jumping ability and strength, which is in turn translated to an affected sprint performance in a similar manner.

The pathways via which vitamin D affects muscular strength (as measured by SJ and CMJ) and sprint performance are still hypothetical. However, there are several potential mechanisms conveying these effects. The ergogenic effects of vitamin D may be related to the regulation of muscle protein synthesis which could affect muscle mass, thanks to the presence of vitamin D receptors on skeletal myocytes (Bischoff-Ferrari et al. 2004; Perez-Gomez et al. 2008; Pfeifer et al. 2002). Furthermore, alterations in vitamin D levels also affect its receptors at the expression and activation levels (Bischoff-Ferrari et al. 2004; Bischoff-Ferrari, 2012) and thus affecting muscle mass (Bischoff-Ferrari et al. 2004; Lee et al. 2003), neuromuscular coordination (Dhesi et al. 2004), and the relative number and the cross-sectional area of type II muscle fibers (Young et al. 1981) Since it well documented that the major determinants of jumping and sprinting ability are muscle strength (Perez-Gomez et al 2008; Ziambaras and Dagogo-Jack, 1997), type II muscle fibers (Stone et al. 2003; Inbar et al. 1981) and neuromuscular coordination (Pereira et al. 2008), any potential effect of vitamin D on these parameters would in turn affect jumping and sprinting capacity in a similar manner.

Analysis of our data revealed a linear association between vitamin D and VO2max in both experimental sessions. This finding is supported by an early study which reported increased aerobic capacity as a result of exposure to ultraviolet radiation in collegiate students (Canell et al. 2009). Furthermore, a recent study on adolescents observed a positive relationship between vitamin D and aerobic performance on adolescents (Lämmle et al. 2013). Since VO2max is related to soccer performance, as indicated by the well-documented relationship between this parameter and the distance covered during a soccer game (Stølen et al. 2005), our findings suggest that in order to perform efficiently during a soccer game optimal vitamin D levels are needed. The observed association between vitamin D levels and VO2max could be related to its protective effect on lung function. According to recently published evidence, low vitamin D levels are associated with lower indices of lung function (Zosky et al. 2011) and increased airway reactivity (Litonju and Weiss, 2007). Since exercise performance and especially aerobic capacity (VO2max) is depended on optimal lung function (Fatemi et al. 2012), any protective and/or boosting effect of vitamin D on the function

of this organ could beneficially influence aerobic performance during exercise (Fatemi et al. 2012). Vitamin D could also influence VO2max by affecting iron metabolism and erythropoietin (Christakos et al. 2013; Li et al. 2002). According to Li and associates (2002) vitamin D deficiency results in dysregulation of innate immunity and inflammation which is affecting iron metabolism and contributes to erythropoietin resistance. It is well documented that erythropoietin is linearly associated with changes in red blood cells levels (Jones and Tunstall-Pedoe, 1989). Thus, vitamin D could influence VO2max via its effects on erythropoesis modifying the capacity of the oxygen supply to the exercising muscles and consequently affecting aerobic exercise performance (Jones and Tunstall-Pedoe, 1989).

Furthermore, we have found that this boosting effect on vit D levels was evident in parallel to a reduction in neuromuscular performance parameters. The latter finding strengthens the well-documented concept that training plays the principal role for exercise adaptation and improvements in exercise performance. However, since these changes did not affect the significant relationship between Vit D levels and neuromuscular performance, our findings indicate that this vitamin may play an important role in these parameters regardless the level of performance**.**

4.11 Conclusions

In conclusion, our findings suggest that, provided that the volume and load of the training is up to a certain level, a combination of soccer training with general strength and soccer-specific strength training can affect the circulating endogenous androgens TT and 3a Diol G but not FT, testosterone precursors i.e. Δ4-androstenedione, DHEAS, and estrogens. Indeed,

we have found that the training scheme employed by Team A resulted in an elevation of TT and in a simultaneous increase in its activation as expressed by the elevated levels of 3a Diol G. The observed effects appear to be volume-dependant, since the two teams with the lower volume did not have similar results to those of Team A. As expected, aerobic capacity in all experimental teams increased up to mid-season and thereafter plateau till the end of the competition period. Regarding neuromuscular performance, our findings indicate that despite the differences in the training schemes used, it increased in a similar manner until the first half of the season. Interestingly, the high strength training stress induced a further and significant increase till the end of the competition season. The alterations in body composition parameters and bone metabolism followed the same manner of changes i.e. were volume and load dependant.

However, circulating sex steroids levels do not appear to have at least direct effects on exercise performance parameters and bone turnover markers. It is our opinion that the elevation of endogenous androgens as a result of the volume of strength training indicates that the only method to improve athletic performance is hard training. There are no substitutes or shortcuts. If the organism needs more androgens it will produce them endogenously.

In regard to detraining, our findings demonstrate that the six-week detraining period, in our study, resulted in significant declines in aerobic, strength (as indicating by SJ and CMJ), and sprint performance adaptations. Furthermore, it had a negative effect on body composition as indicated by the observed increases in body weight and body fat percentage at the end of the study. The off-season transition period did not result in any significant differences in the measured sex steroid concentrations. Therefore, our findings suggest that sex steroids were non-contributing parameters for the reductions in exercise performance indices and the negatively altered body composition neither for the negatively altered bone turnover. The implications of our findings strongly suggest that professional soccer players should devise transition off-season periodization training programs that provide adequate mental and physical recovery after the demanding competitive season, but also allow these athletes to maintain aerobic, strength, and sprint adaptations, and retain optimal body composition status.

In regard the lipidemic profile and the rheological parameters our findings in both study periods i.e. intervention period and detraining, strongly suggest that in chronically trained professional soccer players variations in training volume and load do not affect their levels. The possible explanation is that our players have already obtained the suggested upper limit beneficial adaptations as a response to exercise in these parameters, and short detraining period (but not training cessation) and long periods of variation in strength training volume and load do not later their levels.

Lastly, in regard to vitamin D we observed that the soccer off-season detraining period results to beneficial alteration in its levels. This effect is attributed to two different mechanisms, a possible higher exposure under UVB during this period and a possible indirect effect of the reduction in training stress on the immune system, which is in turn, translated to increased vitamin D levels. Furthermore, our findings clearly suggest that vitamin D is related with both aerobic and neuromuscular indices irrespective the level of performance in professional soccer players.

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Scientific Publications

HORMONES 2014, 13(1):104-118

DOI:

Research Paper

Effect of different seasonal strength training protocols on circulating androgen levels and performance parameters in professional soccer players

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Abstract

OBJECTIVE: To examine the effects of three seasonal training programs, largely different in strength volume, on androgen levels and performance parameters in soccer players. **DESIGN:**Sixty-seven soccer players, members of three different professional teams, participated in the study. Strength intensity of the training programs were assessed as high (for Team-A, n=23), moderate (for Team-B, n=22), and low (for Team-C, n=22). Blood samples were analyzed for total-testosterone, free-testosterone, and the metabolic product of activate testosterone 3a-androstendiol glucuronade (3a-Diol-G). Players were tested for maximal oxygen consumption (VO2max), squad-jump (SJ), countermovement-jump (CMJ), 10m and 20m sprint performance prior at the beginning of the pre-season period, at the middle (mid-point), and at the end of the competition period (end-point). **RESULTS:** All performance parameters increased significantly until mid-point in all teams ($p<0.001$). However, performance was further increased only in Team-A only for jumping and sprinting ability between end-point vs mid-point ($p<0.001$). An effect of the training program of Team-A on TT levels was evident exhibiting significant differences between at all point-measurements (baseline/mid-point:$p=0.024$, baseline/end-point:$p<0.001$, mid/end-point:$p=0.008$), while a marginally significant effect ($p=0.051$) was detected within Team-B and a non-significant effect in Team-C. Similar results were obtained for 3a-Diol-G in Team-A ($p=0.001$) where significant differences were found between end-point to both baseline ($p=0.001$) and mid-point ($p=0.038$). No differences were detectable for FT. A borderline significant negative correlation was observed between 3a-Diol-G and VO2max in Team-B at mid-point. No other correlations were evident between performance and hormonal parameters. **CONCLUSION:** Our findings suggest that the volume of strength training combined with intensive soccer training caused an elevation of circulating TT and 3a-Diol-G levels in parallel to the induction of performance capacity. It is our opinion that the elevation of endogenous androgens as a result of the volume of strength training indicates that the only method to improve athletic performance is hard training. There are no substitutes or shortcuts. If the organism needs more androgens it will produce them endogenously.

Keywords

Androgen metabolite, Exercise performance, Soccer, Strength, Testosterone

INTRODUCTION

The main androgen, testosterone, affects a variety of parameters such as body composition, muscle metabolism and function, protein synthesis, and muscle mass.[18,23] These parameters are related with the ability to perform efficiently during exercise.[23,35,39] Furthermore, previous findings from different athletic populations indicate a linear relationship between maximal explosive force,[5] force production,[22] jumping and sprinting ability[8,30,32] and testosterone levels. The association between testosterone and exercise is bidirectional, i.e. testosterone affects exercise and is affected by it. Indeed, testosterone response to exercise is a result of a combination of different factors such as volume, intensity, type of exercise, and training status of the subjects.[24,46] It is generally accepted that endurance training[19] tends to decrease testosterone resting values, whereas strength exercise can increase its basal levels during periods of high volume[32] and high intensity training[44] in response to long[22] or short training periods.[44]

Soccer is a high intensity intermittent exercise that combines aerobic and anaerobic activities and places considerable demands on the neuromuscular and hormonal systems.[4] Although the main metabolic functions in soccer are sustained by aerobic metabolism,[14] the ability of the neuromuscular system to produce maximal strength and power and their derivatives (sprints, jumps, acceleration) appears to be of crucial importance.[4] As a consequence of the demands placed on soccer players, the current conditioning programs involve the development of specific physical capacities such as endurance, speed, strength, and power. However, maintenance of or improvement in performance are not solely dependent on sufficient conditioning. It is now believed that several other factors influence exercise performance, among which is circulating testosterone.[8,32]

The association between soccer and testosterone levels is complex and still controversial. Thus, periods of high intensity training in soccer reduce testosterone basal concentration,[14] whereas its levels remain unchanged[16] or increase[29] as a response to regular soccer training. Meanwhile, very few interventional studies have been conducted presenting data on the combination of soccer training and strength sessions. The existing literature suggests that the combination of soccer training with linear or non-linear strength sessions results in increased total testosterone (TT) basal levels and neuromuscular performance[17,40] but does not affect free testosterone (FT) and endurance capacity.[17]

The aim of the present work was to examine the effects of three different seasonal soccer training programs on serum levels of TT, FT, and the metabolic product of activated testosterone 3a-Androsten-diol-glucuronade (3a Diol G)[34] as well as on exercise performance. More specifically, we examined the effects of different soccer training regimes on the fluctuation of androgen levels and performance over time (pre-season period until the end of the championship). The training programs differed among the three teams, i.e. high volume training program in Team A, moderate in Team B, and low in

Team C. The difference involved (a) volume and load of general (GSC) and (b) soccer-specific strength training (SPS) (a common training method in today's soccer training). The effects were measured by maximal oxygen consumption (VO2max) and neuromuscular performance parameters, i.e. squat jump (SJ), countermovement jump (CMJ), and sprint performance. To assess the effect of the three training programs on circulating endogenous androgens, we measured total-testosterone (TT), free-testosterone (FT), and 3a-androsten-diol-glucuronade (3a-Diol-G). Finally, we examined the association between changes in performance parameters to changes of levels of endogenous androgens. Our working hypothesis was that the higher the volume, the better the performance parameters and the higher the endogenous androgen levels.

MATERIALS AND METHODS

Subjects

Sixty-seven male professional soccer players, members of two Greek Superleague teams (Team A: n=23, Team B: n=22) and one Greek Football League 2 team (Team C: n=22) participated in this study. All the participants had been professional soccer players for more than 5 years. A detailed medical history was recorded. Exclusion criteria were as follows: a) any medical or endocrine disorder that could affect their ability to participate in the study and/or affect endogenous hormonal production; b) suspicion of use of exogenous hormonal agents or of other illegal substances; and c) absence from the normal training program for more than 15 days. The individual players incorporated in the study had comparable anthropometric measures, suggesting homogeneity of the sample at the beginning of the study. Their age, weight, and height characteristics ±SD were as follows: Team A: age (years)=25.5±1.1, weight (kg)=79.1±1.3, height (cm)=182±2.3; Team B: age (years)=24.7±1, weight (kg)=79.5±1.9, height (cm)=181±1.4; Team C: age=23.8±0.9, weight (kg)=79.7±1.2, height (cm)=181±1.1. Players received verbal explanation for the study and a written consent was obtained. The study was conducted according to the declaration of Helsinki and was approved by the ethics committee of the university.

Study Design

The study was performed over a period of 10½ months (42 weeks), i.e. from the beginning of the pre-season period until the end of the championship. The players were evaluated three times: a) baseline: just before the beginning of the pre-season period (early in July); b) mid-point: in the first half of the in-season period (January); and c) end-point: at the end of the season (mid-May). Prior to each testing period, players were under no exercise stress for at least two days to avoid any fatigue effects. Each experimental session included two days of consecutive testing. In the morning of the first day, anthropometric characteristics were measured (08:30 am) and venous blood samples were obtained (from 09:00 to 10:30 am) to determine the concentration of testosterone assessed by three assays, i.e. TT, FT, and 3a Diol G. In the afternoon of the same day (17:00 pm), players were tested for neuromuscular performance assessed by two different measurements, i.e. squat and countermovement jump (SJ and CMJ, respectively) and speed performance assessed by two measurements, i.e. 10m and 20m sprint. On the morning of the second day (09:30 am), maximal oxygen consumption (VO2max) was performed. All hormonal and exercise performance measurements were performed during all three experimental sessions at the

same time of the day and players were tested in the same order to avoid any circadian variation in the measured variables. The measurement for the determination of one repetition maximum (1RM) was performed only at pre-season for the specific strength exercises used in our study. This testing took place the day after the VO2max assessment starting at 10:00 am. Before each experimental session, players were requested to avoid consuming any supplement that could promote performance at least 2 days prior to the testing to keep their hydration status as well as to avoid any caffeine or alcohol beverages at least 3 hours prior to each testing. Nutritional guidelines were given to all players in order to ensure a >60% carbohydrate dietary intake during the study. All players were familiar with the testing protocol, as they had been previously tested with the same procedures on several occasions during the last soccer season.

Training

Playing Schedule and training program

During the whole experimental period, players of Teams A, B, and C participated in 33, 32, and 33 official (competition and cup) games, respectively. The training program for the three experimental teams was designed by the team coaches with the cooperation of the researchers. During both pre-season and competition periods, players participated in approximately 75-95 minute mean duration training sessions, focusing on all aspects of physical conditioning and technical-tactical elements of the game (Table 1). *Categorizing Training Sessions*: In order to analyze and evaluate the weekly training load, specific sub-components of each session were categorized according to the conditioning target of each training session as follows: Technical-Tactical training (TT) and Technical-Tactical Conditioning (TTC) both performed in conjunction with soccer drills such as small sided games (SSG) and big sided games (BSG), speed and agility conditioning (SAC), endurance conditioning (EC) when running was performed (without the ball), general strength conditioning (GSC), and soccer-specific strength training (SPS). The strength sessions employed were categorized according to the recommendations by Baker (1996) as follows: general strength (aiming to increase maximal strength), special strength (aiming to train for power once strength levels have increased), and specific strength (aiming to train the specific skills needed during an actual competition).

Preparation Period

During the pre-season period, participants of Teams A, B, and C participated in 74, 63, and 52 training sessions, respectively, in a period of 7 weeks. Players of Teams A and B participated in the same number and nature (training volume, load, and intensity) of training sessions including TT (15 sessions), TTC (+SSG, BSG; 20 sessions), SAC (4 sessions), EC (9 sessions), whereas players of Team C had a similar number of TTC (+SSG, BSG; 20 sessions) and SAC (4 sessions), and slightly lower training sessions in TT (11) and EC (6 sessions). The main difference between Teams A, B, and C consisted in the number of SPS and GSC (Team A: GSC=11, SPS=15; Team B: GSC=6, SPS=9; Team C: BSC=4, SPS=7). During the seventh week of the pre-season period all teams followed their in-season weekly training plan (Table 1). During this phase all friendly games were recorded as TTC. *In-season Period:* The in-season weekly training plan was kept constant by each team throughout the competition period (Table 1). The weekly training program was different mainly regarding the number and the nature of the

SPS and GSC sessions performed by each team (Tables1, 2). Apart from the different weekly number of SPS sessions in each team, an extra training session was performed by the players of Team A on the morning of the match-day (Table 1). During the competition phase Teams A, B, and C had 3, 2, and 1 cup matches, respectively, at mid-week (Wednesdays). During these "cup-weeks", the training plan was different including two recovery sessions, two low intensity-low volumes TT sessions, and one SAC session in all teams.

Categorizing the three training programs

The three seasonal training programs were different regarding the volume and the load of the strength training sessions. Therefore, the seasonal training regimes were categorized as follows: high strength training stress, moderate strength training stress, and low strength training stress for Teams A, B, and C, respectively. *Training Load, Volume, and Intensity:* The training load of each session was assessed with the use of the 10-point RPE scale modified by Foster et al (1995). The training load calculation and assessment was performed according to standard procedures.[15] In order to control the training load and to avoid any overtraining effect that would negatively affect both the adrenal and gonadal axes as well as exercise performance,[47] the daily "training monotony" (average daily training load/standard deviation of the daily training load calculated over a week) was measured. In regard to training volume, it was expressed during the study as total number of training sessions and training time in minutes (min). Heart Rate maximal (HR max) assessment was performed using HR monitors (Polar Team[2] Pro, *Polar Electro*, Oy, Finland) to determine the training zones (<50-60% of HR max, 60-70% of HR max, 70-80% of HR max, 80-90% of HR max, 90-100% of HR max). *Soccer-Specific Strength (SPS):* All three experimental teams employed SPS sessions of 35-40 minutes duration throughout the study but at a different weekly frequency (Table 1). Soccer-specific strength has been defined as the ability of a player to use muscle strength and power effectively and consistently throughout the soccer season.[4] These sessions included various forms of activities such as skipping over cones, jumping on one or two legs, jumping over hurdles or obstacles, shooting, heading, acceleration, repeated sprints, eccentric movements such as deceleration and stopping, changes of direction, etc.,[36] combined with maximal intensity soccer activities such as crossing, shooting, and heading . All performed drills in each SPS were similar in nature, duration, and volume. During every SPS, the number of plyometric exercises (jumps) was kept constant at 45 jumps per session for each individual. *General Strength Conditioning (GSC):*General strength conditioning was used by all teams (Table 1). Teams B and C employed the same high intensity, low load GSC regime, whereas Team A players followed a high intensity, moderate volume strength training regime in conjunction with two core strength exercises performed in a circuit manner (Table 2).

Laboratory Measurements

Anthropometry

At each experimental session, height was measured using a stadiometer (Charder HM0D, Charder Electronics CO, LTD, Taiwan) and body weight (BW) was obtained using an electronic weight scale (Seca Alpha 770, Seca Vogel, Hamburg, Germany). Body fat percentage (BF%) was assessed by skinfold thickness measurement (Lange Skinfold Caliper, Cambridge Scientific Instruments, Cambridge, UK) using the 4 spot formula by Jackson and Pollock (1978). All measurements were made by the same investigator.

Exercise Performance

The jumping (SJ, CMJ) and sprinting ability (10m, 20m) of the soccer players were assessed with a jumping mat (Powertimer, Newtest Ltd., Oulu, Finland) and infrared photoelectric cells (Powertimer, Newtest Ltd., Oulu, Finland), respectively, according to standard procedures.[7] VO2max assessment was performed on a motorized treadmill using an automated gas-analysis system (VMAX29, Sensormedics, Yorba Linda, CA), with the use of set procedures of a standard protocol.[28] Finally, 1RM assessment was performed with the use of free weight exercises or machine weight exercises (Cybex International, Inc. USA) according to suggested procedures.[33]

Blood Collection and Analysis

On each test day, venous blood samples were obtained following a period of 10-minute rest in a lying position. Serum blood samples were collected in tubes containing a clot activator and serum gel separator and were centrifuged at 3000 rpm for 10 minutes to separate serum. Serum samples then were stored at -20^{o}C till analysis. TT levels were measured using the AIA fully automated immunoassay analyzer (TOSOH-Eurogenetics). The sensitivity of the assays for TT was 7 ng/ml and the intra- and inter-assay coefficient of variation was 3.1-5.2% and 2.48-5.99%, respectively. FT and 3a Diol G levels were measured using an enzyme-linked immune absorbent assay (Alpco Diagnostics, Windham, NH). The sensitivity of the assay for FT was 0.17 pg/ml and the intra- and inter-assay coefficient of variation were 4.7-17% and 5.3-12.4%, respectively. The sensitivity of the assay for 3a Diol G was 0.1 ng/ml and the intra- and inter- assay coefficient of variation was 6-7.8 and 6.5-10.8, respectively. All procedures were carried out according to the instructions of the manufacturer. All samples were analyzed in duplicates.

Statistical Analysis

Differences at baseline between the three groups in all hormonal, performance, body composition parameters, training load, and training volume were examined in the context of univariate ANOVAs. When equal variance was assumed, Bonferroni adjustments were employed, while in cases where assumption of homogeneity of variance was violated, the Welch test and DunnetT3 adjustment were used. Pearson's (for normally distributed variables) and Spearman's (for non-normally distributed variables) correlation coefficients were used to assess the linear relationship between variables. Evaluation of the effects on all measured variables in question (performance parameters: VO2max, SJ, CMJ, 10m, 20m; body composition parameters: body weight, %fat body composition; androgen: total testosterone, free testosterone, 3aDiol) of each training regime over time was pursued through a series of two-way 3x3 mixed ANOVA with season time (baseline, mid-point, end-point) as the within subjects variable and training regime (Team A representing high training load, Team B moderate and Team C low training load) as the between-subjects variable. Significance was set at $p<0.05$. Power analyses indicated that for the effect size of interactions observed in the study, estimated power for detecting significant simple effects ranged between 0.89 and 0.96 at alpha=0.05. Statistical analysis was performed using the software program SPSS 19 and power analysis was conducted with GPower 3.1.

RESULTS

Differences in performance variables and androgen levels between teams at baseline

No significant differences were observed at baseline between the three experimental groups in any of the measured performance (VO2max, SJ, CMJ, 10m, 20m) or body parameters (weight, fat%). Similarly, no significant differences were observed at baseline between the three experimental groups regarding circulating TT and 3a Diol G. A significant difference between teams was revealed for FT ($p=0.013$), with Team A ($M=13.70$) being different from Team C ($M=9.26$) at ad hoc test comparison ($p=0.015$; Bonferroni adjusted).

Volume and Training Load (RPE)

In order to confirm the intentional differentiation between the three training regimens, one-way ANOVAs were used to test volume and training load (as expressed by RPE) among the three teams within the same training period. As expected (Table 3), teams significantly differed both in volume (pre-season $p=0.001$, 1^{st} half of competition period $p<0.001$, 2^{nd} half of competition period p0.001) and RPE (pre-season $p<0.001$; 1^{st} half of competition period $F: 2,48=1848,63$ $p<0.001$; 2^{nd} half of competition period p 0.001).

Relationships between changes of performance parameters and circulating androgens

A weak significant negative correlation was evident between VO2max and 3a Diol G levels ($p=0.012$) at the end of the study in Team B. However, no other significant correlation was observed throughout the study in Teams A, B, and C between androgens (TT, FT, and 3a Diol G) and performance parameters. It should be mentioned that analysis of our data revealed some p values within the range $0.05<p\text{-value}<0.10$. These insignificant correlations could be a result of the sample size. These correlations were between TT and both SJ (mid-point: $p=0.055$, and end-point: $p=0.077$) and CMJ (mid-point: $p=0.053$) in Team A, between 3a Diol G and VO2max (baseline: $p=0.075$), and TT and SJ (end-point: $p=0.061$) in Team B, and FT with both SJ (baseline: $p=0.082$) and 20m (end-point: $p=0.055$).

Performance

Two-way mixed ANOVAs showed a significant interaction between the time points of evaluation i.e. beginning of season (early July), mid-point (midJanuary) and end-point (mid-May) and the training regime used. As expected there was a considerable improvement over time in all performance parameters within each experimental team (Table 4). *Neuromuscular Performance*, assayed by SJ and CMJ, showed a significant increase ($p<0.001$) at the mid- and end-point measurements compared to baseline and a significant decrease ($p<0.001$) of sprint times (10 and 20 m) for all three teams, in ad hoc Bonferroni adjusted comparisons test of simple effects. In Team A, the comparison between mid- to end-point further revealed a significant increase in SJ ($p<0.001$), CMJ ($p<0.001$), and a significant decrease in 10m ($p<0.001$) and 20m ($p<0.001$) sprint times at the end of the study. No significant differences were revealed by the mid- to end-point comparison for any of the measured neuromuscular performance parameters in Team B (SJ $p=0.383$, CMJ $p=1.00$, 10m $p=0.126$, and 20m $p=1.00$) and Team C (SJ $p=0.609$, CMJ $p=1.00$, 10m $p=1.00$, and 20m $p=1.00$).*Maximal Oxygen Consumption:* The comparison between baseline to mid-point and end-point showed that there were significant increases ($p<0.001$) across all teams in VO2max. No significant differences were revealed for end- to mid-point comparisons for VO2max in Team A ($p=0.057$), Team B ($p=1.000$), and

Team C (p=1.000) (Table 4). *Body Composition:* The BW and BF% assessments throughout the study are also presented in Table 4. For Team A (high strength training stress) and team B (moderate strength training stress), ad hoc comparisons between simple effects showed significant decreases in BW (p<0.001) between baseline to mid- and end-point measurements, but regarding BF% only Team A exhibited further loss of body fat between mid- to end-point (p<0.001) in contrast to Team B (p=0.782). For Team C, weight loss and body fat reduction were found significant only at mid-point to baseline (p=0.008 and p=0.025, respectively).

Endogenous androgen levels

Two-way mixed ANOVA revealed that the main effect of training regime (teams) on TT was not significant (p=0.117), but a significant main effect of time on TT was found (p<0.001). The two factors had a significant interaction (p=0.018), depicted in Figure 1 (cell means interaction). Simple effect analysis of season within each level of training regime revealed a significant difference within Team A (p<0.001), a marginal difference within Team B (p=0.051), and a non-significant difference within Team C (p=0.427). Post hoc comparisons (Bonferroni adjusted) in Team A showed significant differences between all season comparisons (baseline/mid-point: p=0.024, baseline/end-point: p<0.001, mid/end-point: p=0.008). Mean values of TT by each training regime over time are shown in Table 4. The main effect of training regimens on FT was significant (p=0.001), while a marginal finding of season was found (p=0.044). There was no significant interaction between the two factors for FT (Figure 1). In contrast, 3a Diol G exhibited a significant dependence on the type of training program (p<0.001) but not on the time periods (p=0.211). The interaction between factors was significant (p=0.024; Figure 1), with simple effects showing that only in the high training load team (Team A) was the effect of time significant (p=0.001). Within this team, post hoc test comparisons (Bonferroni adjusted) revealed a significant difference between baseline- and mid-point to end-point values (p=0.001 and p=0.038, respectively).

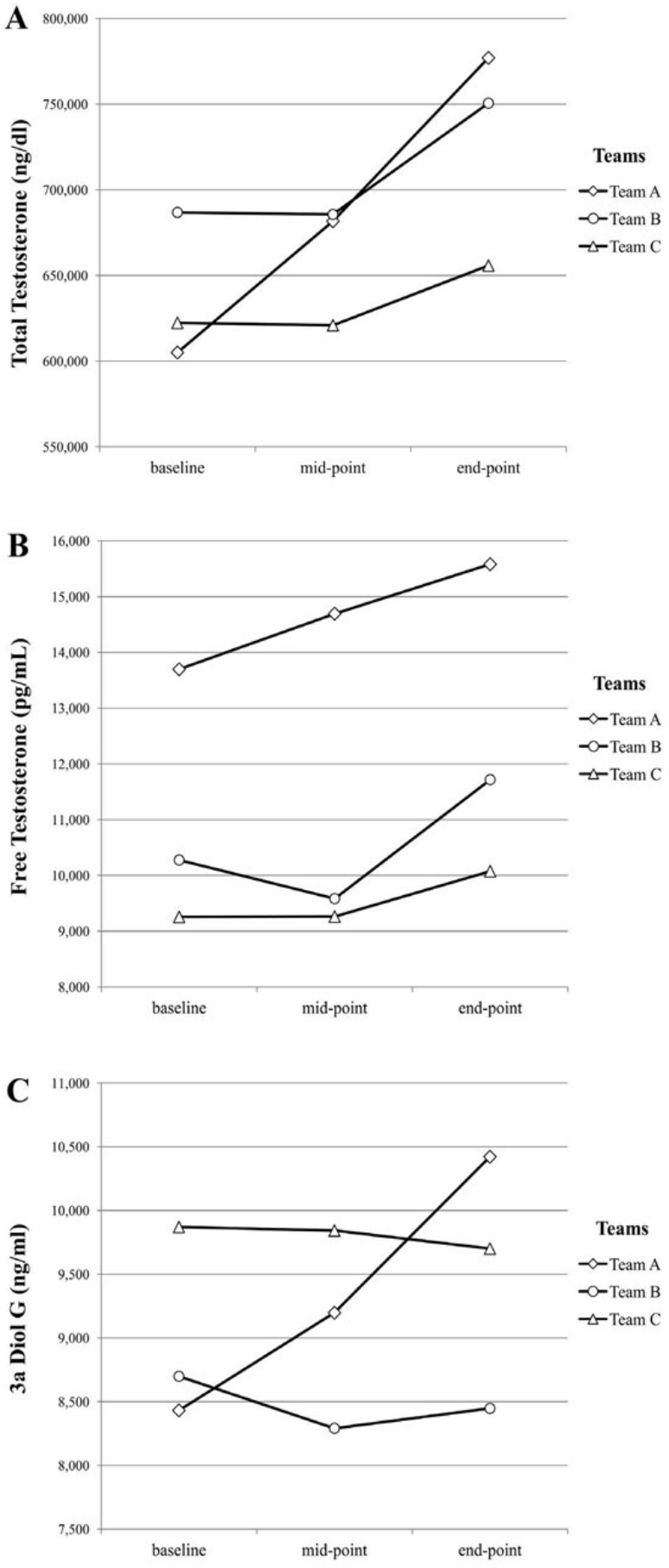

Figure 1. Cell mean interactions by training regimen (high strength training

stress=Team A; moderate strength training stress=Team B, low strength training stress=Team C) over time for Total Testosterone (A), Free Testosterone (B), and 3a Diol G (C). Significant differences were observed only in Team A between baseline to mid- and end-point for both total testosterone (TT) and 3a Diol G and at the mid- to end-point comparison in TT.

DISCUSSION

The aim of this study was to examine the effects of three different seasonal training programs regarding strength volume on hormonal and performance parameters in soccer players. Our main finding is that the combination of soccer training with general and soccer-specific strength training can induce an elevation of TT and 3a Diol G. These changes appear to be mainly related to the volume of strength training since they both responded in proportion to volume, i.e. the higher the volume of strength training (Team A), the higher the resulting alterations. These changes in androgen levels do not correlate with any of the examined performance parameters. The only borderline correlation observed was between 3a Diol G to VO2max at the mid-point measurement in Team B. Regarding exercise performance and body composition variables, all training programs managed to induce beneficial alterations in these parameters until mid-season, which was retained until the end of the season. However, only the high strength training stress of Team A resulted in further significant changes in neuromuscular performance (SJ, CMJ, 10m 20m), body fat percentage, and body weight in the second half of the study.

To the best of our knowledge, only two published intervention studies in soccer exist in the literature.[17,40] Both studies reported comparable results to ours. More specifically, the combination of regular soccer training with two[40] or three[17] strength training sessions, during an 11-week in-season and a 12-week pre-season period, respectively, managed to increase TT resting levels, whereas one session per week did not appear to alter TT.[17] It should be noted here that in our teams the training scheme employed in Teams A was composed of three strength training session, in Team B two strength training session per week while Team C had only one. These similarities in the weekly strength training frequency provides supportive evidence to our own findings.

However, the TT changes in Teams A and B were evident in a different manner. The high strength stress training program employed by Team A managed to increase significantly TT resting levels at the mid-point measurement, followed by a further significant increase at the end of the study, whereas players of Team B exhibited only a tendency to increase TT resting levels, as highlighted by the mean values at the end of the study compared to baseline (Table 4). We postulate that the TT responses observed in Teams A, B, and C were the result of the different strength training volume employed by each of the three teams. Indeed, the three types of seasonal training programs were significantly different in training volume and load throughout the study (Table 3). Team A had the highest values in these two parameters compared to the other two experimental teams, while Team B had significantly higher values compared to Team C (Table 3). The seasonal training plan was organized in such a manner as to differ mainly regarding the weekly volume/frequency of the sessions aiming to improve general and soccer-specific strength. Regarding the other aspects of physical conditioning,

the training programs employed by Teams A and B were nearly identical and slightly differed compared to Team C (Table 1). Therefore, the differences observed between the weekly training volume and load were mainly the result of the different strength training stress employed by each team. Confirmation of our suggestion of a volume/load-dependant TT response comes from the observations of previously published studies. It has been reported that strength training which results in high volume,[32] high intensity,[44] and high training load[24] can increase TT resting levels. These changes may occur in response to long[21] or even short training periods.[44]Therefore, we could suggest that the higher strength training stress employed by Team A was more effective in providing a sufficient stimulus for a more pronounced TT increase compared to the moderate strength training stress of Team B. Similarly, Team B's training program managed to provide sufficient stimulus to induce a detectable though non-significant elevation in TT levels (Table 4, Figure 1) (end of study vs baseline) compared to the low strength training stress of Team C that did not appear to affect TT levels. It should be mentioned that the extra sprint session performed on the morning of each match-day in Team A was unlikely to make any significant contribution to TT response, as it has previously been reported that sprint training suppresses TT rather than elevates it.[2] The elevation of TT observed in Team B at the end of the study was not significant, in contrast to what has been reported by Pacobahyba et al (2012), who have shown that soccer training combined with two strength sessions per week can significantly elevate TT levels. This discrepancy could be due to the nature of the strength training regimes employed by our Team B (general and soccer specific strength) and the Pacobahyba study in which only general strength training was used. It is generally accepted that the mode of physical activity can influence hormonal responses to exercise training.[46] In regard to the unaffected androgens levels in Team C, it is well documented that one strength session per week does not provide sufficient stimulus for TT increase.[14,17] Only one recent study with an in-season weekly training regime similar to that of Team C showed an elevation of TT levels at the end of the competitive season.[29] The authors suggested that the observed increase in TT was due to the reduction of training volume towards the end of the season. However, this could not be the case in our study since not only in Team C but also in all experimental teams training volume was kept constant until the end of the season. Therefore, the absence of any periodization in training volume, and the low strength training stress employed by this team, most probably accounted for the unaffected TT level throughout the study.

It should be mentioned that the intervention studies which reported significant elevations in TT levels employed only general[40] or special strength sessions.[17] In our study, we used the combination of two types of strength training, i.e. general and soccer-specific strength sessions. Since it has been well demonstrated that the combination of soccer training with only one strength training per week does not appear to be sufficient to increase the endogenous testosterone production,[14,17,29] our findings suggest that it was the weekly combination of one general with two (Team A) or one (Team B) soccer-specific strength sessions provided sufficient stimulus for the significant TT increase in Team A and it was enough for the observed marginally significant elevation of TT in Team B. Despite the lack of publications regarding the relationship between soccer-specific strength and TT levels, the sub-components of the employed soccer-specific strength

sessions have been individually studied. This kind of training is composed of various maximal intensity activities, such as repeated sprints in the manner of interval sprint training, eccentric movements (turning, changes of direction, acceleration and deceleration), plyometrics, and squat exercises with or without external weights. It has been found that interval sprint training,[13] eccentric resistance training,[49] and exercises using large muscle groups such as squats can increase TT levels.[48] In regard to plyometric training, it has recently been observed[9] that it may decrease, rather than increase, TT levels. However, the authors reported that this reduction of TT was rather the result of accelerated utilization of TT by the tissues and augmented hepatic clearance than compromised endogenous production. Based on these reports, we hypothesize that all these types of training activities which are included in the soccer-specific sessions if combined with general strength sessions could result in a sufficiently high stimulus to elevate TT levels.

The observed changes in TT, apart from the different strength exercise training stress, may also be the result of the metabolic demands elicited by the three training programs employed, each characterized by a specific training volume and load. Indeed, according to a number of published studies,[20,43,48] the threshold of TT response appears to be based more on the metabolic demand than on the training regime itself. Extensive literature supports the concept that a linear relationship exists between energy expenditure, and thus metabolic demands, with volume, frequency, and training load.[10,26,45] In our study, the TT response appears to be linearly related to each of these parameters. More specifically, the higher volume, load, and frequency of trainings increased TT resting levels in Team A, the moderate strength training stress tended to increase TT in team B, whereas the lowest strength training stress in team C did not have any effect. The observed differences in endogenous androgen responses between teams may well be the result of the different metabolic demands. This observation supports the findings of the aforementioned studies[20,43,48] as to a threshold response of TT regarding energy demands since it appears that a linear relationship exist between metabolic demands and changes of TT levels.

Our results demonstrated that none of the three different training programs provided sufficient stimulus to significantly change FT levels. In agreement with our observations is a recent study in professional soccer players reporting that the combination of regular soccer training (composed of two strength training sessions per week) did not affect FT levels.[17] Evidence from previous studies on strength trained individuals showed that FT seems to be affected only by heavy resistance exercise of high volume.[1,31] This hypothesis is partly validated by our findings. The higher strength training stress program in Team A showed a trend, albeit not statistically significant, of increase in FT levels at the end of the study compared to baseline. No similar trend was observable in the other two teams (lower strength volume) (Table 4, Figure 1). However, this higher strength training program failed to provide sufficient stimulus for a significant increase in FT levels. These different observations between the aforementioned studies[1,31] and our own findings could be a result of the difference in strength training volume between the two experimental models and the training status of the subjects, parameters that have been reported to be of paramount significance in the effect of exercise on endogenous androgen production.[46]

Our data regarding the changes of 3a Diol G during the training period in Team A support our TT findings. The significant changes of 3a Diol G levels detected in this team followed a similar pattern to the changes in TT levels, i.e. its levels increased at mid and end of the season compared to baseline in Team A. This observation suggests that the higher strength training program employed by Team A accelerated both the production and activation rates of testosterone. However, the observed elevation of 3a Diol G, indicative of activated testosterone,[34] did not appear to have any statistically significant repercussions on ergometrics, suggesting a more subtle effect. Furthermore, it should be mentioned here that our study is the first involving professional athletes tested for the effect of training on 3a Diol G. The only other available data regarding 3a Diol G response to exercise is from a study performed in elderly and middle-aged men.[25] The authors reported that a 12-month training period failed to document any significant change in 3a Diol G levels, a finding which is in agreement with our results obtained from Teams B and C, i.e. from the teams with the lower volume of training. Thus, we postulate that the lower level of strength training stress does not change the rate of testosterone activation in contrast to the higher level of strength training stress used in Team A. The higher training stress in Team A may have induced activation of testosterone, the first step of which is its conversion to its biologically active compound DHT (dihydrotestosterone). This may be responsible for the alterations of the ergometrics that we have observed, since DHT has been shown to be associated with fast twitch muscle fibers, muscle strength, and body fat percentage,[50] parameters that directly affect exercise performance.[23,35,39]Furthermore, the elevation of 3a Diol G levels indicates a positive anabolic effect, since this metabolite is a marker of the total androgen pool and an indicator of peripheral activation of androgens.[34] Overall, the importance of this finding is based on the fact that apart from an induced adaptation leading to increased endogenous TT production at the end of the study in Team A, there was a further unique adaptation, an augmented peripheral activation of testosterone.

In agreement with the majority of the available evidence,[4,7,11,17,28,38,42] all examined performance parameters exhibited significant improvements at mid-point compared to baseline in all teams, and this effect was retained until the end of the study. However, only in Team A did we observe a further significant improvement in SJ, CMJ, 10m and 20m capacity in the second half of the study. These considerable improvements in exercise performance until mid-point and their maintenance until the end of the season indices is a result of a combined effect of the pre- and in-season conditioning in conjunction with the competitions' training stress.[7,12,42] Overtraining or suboptimal aerobic loading and lack of sprint and strength sessions can counteract these effects.[7,29] However, in our study we had no evidence of any overtraining since "training monotony" values were below the suggested level of two indicatives of overtraining[2] (Table 3). In addition, aerobic loading was kept constant throughout the study, while strength and sprint sessions were included in the weekly training plan (Tables 1, 2).

The further enhancement in jumping (SJ, CMJ) and sprinting (10m, 20m) ability in Team A in the second half of the in-season period was most likely a result of the higher employed volume of both SPS and sprint sessions in this team compared to Teams B and C (Table 1). It has recently been suggested[12] that a moderate frequency of plyometrics (twice per week), similar to the volume used in our SPS regimes in Team A, is more effective in

promoting jumping ability compared to the lower frequency (once per week) used in Teams B and C. Furthermore, a similar weekly sprint training volume (three sessions per week) as that employed by Team A (Table 1) has been found to produce beneficial adaptations in the muscle function, which is translated into an increased SJ and CMJ performance.[37] This increased jumping ability could be the reason for the observed increase in sprint performance in Team A. Indeed, it is well established that jumping ability is strongly related to sprint performance[41] and, moreover, increases in sprint performance occur concomitantly with the largest increases in jumping ability in soccer players.[17,42]

Another possible mechanism responsible for this increase in jumping and sprinting ability in the second half of the study in Team A could be related to the reduced BF% in this team during this period (Table 4). Indeed, it has been well demonstrated that both jumping ability and sprint performance improvements are significantly associated with a reduction in BF%.[11,39] Moreover, it has previously been reported that competitive soccer players with a lower BF% invariably show better sprint performance.[7]

In conclusion, our findings suggest that, provided that the volume and load of the training is up to a certain level, a combination of soccer training with general strength and soccer-specific strength training can affect the circulating endogenous androgens TT and 3a Diol G but not FT. Indeed, we have found that the training scheme employed by Team A resulted in an elevation of TT and in a simultaneous increase in its activation as expressed by the elevated levels of 3a Diol G. The observed effects appear to be volume-dependant, since the two teams with the lower volume did not have similar results to those of Team A. As expected, aerobic capacity in all experimental teams increased up to mid-season and thereafter plateau till the end of the competition period. Regarding neuromuscular performance, our findings indicate that despite the differences in the training schemes used, it increased in a similar manner until the first half of the season. Interestingly, the high strength training stress induced a further and significant increase till the end of the competition season. It is our opinion that the elevation of endogenous androgens as a result of the volume of strength training indicates that the only method to improve athletic performance is hard training. There are no substitutes or shortcuts. If the organism needs more androgens it will produce them endogenously.

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Received 05-08-2013, Accepted 18-02-2014

Manuscript number: PONE-D-13-33301

Discrepancy between exercise performance, body composition, and sex steroid response after a six-week detraining period in Professional Soccer Players

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ABSTRACT

Purpose: The aim of this study was to examine the effects of a six-week off-season detraining period on exercise performance, body composition, and on circulating sex steroid levels in soccer players. **Methods:** Fifty-five professional male soccer players, members of two Greek Superleague Teams (Team A, n=23; Team B, n=22), participated in the study. The first two weeks of the detraining period the players abstained from any physical activity. The following four weeks, players performed low-intensity (50%-60% of VO_2max) aerobic running of 20 to 30 minutes duration three times per week. Exercise performance testing, anthropometry, and blood sampling were performed before and after the six-week experimental period. **Results:** Our data showed that in both teams A and B the six-week detraining period resulted in significant reductions in maximal oxygen consumption (60,31±2,52 vs 57,67±2,54; $p<0.001$, and 60,47±4,13 vs 58,30±3,88; $p<0.001$ respectively), squat-jump (39,70±3,32 vs 37,30±3,08; $p<0.001$, and 41,05±3,34 vs 38,18±3,03; $p<0.001$ respectively), and countermovement-jump (41,04±3,99 vs 39,13±3,26; $p<0.001$ and 42,82±3,60 vs 40,09±2,79; $p<0.001$ respectively), and significant increases in 10-meters sprint (1,74±0,063 vs 1,79±0,064; $p<0.001$, and 1,73±0,065 vs 1,78±0,072; $p<0.001$ respectively), 20-meters sprint (3,02±0,05 vs 3,06±0,06; $p<0.001$, and 3,01±0,066 vs 3,06±0,063; $p<0.001$ respectively), body fat percentage (Team A; $p<0.001$, Team B; $p<0.001$), and body weight (Team A; $p<0.001$, Team B; $p<0.001$). Neither team displayed any significant changes in the resting concentrations of total-testosterone, free-testosterone, dehydroepiandrosterone-sulfate, Δ4-androstenedione, estradiol, luteinizing hormone, follicle-stimulating hormone, and prolactin.

Furthermore, sex steroids levels did not correlate with exercise performance parameters. **Conclusion:** Our results suggest that the six-week detraining period resulted in a rapid loss of exercise performance adaptations and optimal body composition status, but did not affect sex steroid resting levels. The insignificant changes in sex steroid concentration indicate that these hormones were a non-contributing parameter for the observed negative effects of detraining on exercise performance and body composition.

Key words: Soccer, Detraining, Sex steroids, Exercise Performance

INTRODUCTION

Soccer periodization typically incorporates a transition – off-season period of reduced stress in order to allow physical and mental recovery after the end of the competition season [1]. This phase of reduction or complete training cessation has been defined as detraining [2]. This detraining period can partial or complete reverse the adaptations of training, resulting in compromised exercise performance [2,3]. The magnitude of the alterations in performance capacity is dependent on several factors, such as the selected recovery strategy, the duration of the detraining phase, and the initial fitness level of the participants [1].

Evidence from various athletic populations indicate that 3 to 6 weeks of detraining affects negatively aerobic capacity [4,5], strength [6,7], neuromuscular performance [7,8], and body composition [8,9]. In contrast, some studies on recreational athletes and untrained individuals failed to support these findings in regard to aerobic capacity and muscle strength. It was observed that training cessation or insufficient training stimulus for a period of 2 to 6 weeks did not result in decrements in these two parameters [2,3,7,10]. These discrepancies in the literature were attributed to the different initial training fitness levels of the participants. It has been suggested that the higher the training state of the participants, the greater the rate of decline in both VO_2max and strength adaptations [4,7].

This negative effect of detraining on exercise performance adaptations is a result of complex physiological mechanisms. It has been reported that the decline in aerobic capacity is related to reductions in blood volume, stroke volume, cardiac output, ventilator functions, and cardiac dimension due to insufficient training stimulus or training cessation [2,3]. Similarly, the observed detraining related decrease in muscle strength and strength related performance appears to be a result of decreases in

muscle fiber size, mostly due to reduced type II muscle fiber area, mitochondrial ATP production, and enzymatic activities [2,3].

Few studies have examined the detraining effects on the neuroendocrine system. Even less evidence exists for the relationship between detraining and the male reproductive system. The suggestion that sex hormones are related with physical activity and various physiological systems in the body [11], raise the question of their response to detraining periods. Generally, manipulation of the training regime results in alterations of the neuroendocrine system [7]. The majority of the available evidence shows no significant differences in testosterone, l*uteinizing hormone*, and follicle-stimulating hormone after detraining [7,12,13]. However, it has been observed that a short detraining period of a two-week duration increase total testosterone resting levels [14]. It was suggested that the first two weeks of detraining, after stressful training periods, total testosterone levels increase either significantly or insignificantly, indicating increased anabolism and this response is attenuated after the forthcoming 3 weeks of the detraining period [13].

In soccer, little interest has focused on the off-season period. The limited available data suggests that the off-season detraining period results in reductions in aerobic capacity, sprinting ability, increased body weight, and body fat percentage in both professional and semi-professional soccer players [8,9,15]. To the best knowledge of the authors, no available data exists in regard to jumping ability and hormonal responses in professional soccer players during this transition period.

Therefore, the **aim of our study** was to examine the effects of a six-week detraining period in professional Greek male soccer players on aerobic capacity, jumping ability, sprint performance, body composition, and sex steroids. More specifically, we tested soccer players for maximal oxygen consumption (VO_2max), squat jump (SJ),

countermovement jump (CMJ), 10 meters (10m), and 20 meters (20m) sprint performance. Regarding sex steroids, we evaluated the responses of the most important androgens, total testosterone (TT) and free testosterone (FT), the precursors of testosterone, Δ4-androstenedione (Δ4) and dehydroepiandrosterone sulfate (DHEAS), the metabolic product of their activated form and indicator of the androgen pool [16] 3α-Androstanediol Glucuronide (3a Diol G), and the main estrogens i.e. estradiol (E2), l*uteinizing hormone* (LH), follicle-stimulating hormone FSH, and prolactin (PRL). We hypothesized that all performance parameters and body composition variables will be negatively affected, as a result of the insufficient training stimulus over the six-week detraining period, whereas no hormonal alterations will be revealed.

MATERIAL AND METHODS

Participants

Fifty five professional male Greek football players, members of two Superleague teams (Team A, n=23; Team B, n=22) were selected and participated in this study. The mean values for age (years) ± SD and height (cm) ± SD in teams A and B were: age=25,5 ± 5,3; height= 1,82 ± 0,11 and age=24,7 ± 4,9; height=1,81 ± 0,07 respectively. The exclusion criteria were as follow: a) any medical or endocrine disorder that could affect the ability of the players to participate in the study and/or affect endogenous hormonal production; b) suspicion of the use of exogenous agents; c) players that their contract was ending before the end of the conclusion of the study. As a result of the third criterion (exclusion criterion c) seven players were excluded from the study (four from team A and three from team B).

Ethics Statement

Before testing, verbal explanation was given to each player, concerning the aim of the study and the testing procedures, and written informed consent was obtained. The study was performed in strict accordance with the ethical guidelines of the Helsinki Declaration and was approved by the Ethical Scientific Committee of the University Hospital of Heraklion, Greece.

Study Protocol

All players were tested in two different occasions. The first experimental testing took place immediately after the end of the competition period in the middle of May (pre). The second experimental testing was performed at the beginning of July (post), prior to the beginning of the preparation period for the forthcoming season. Each experimental testing consisted of two days of consecutive measurements. The first day of each experimental session anthropometric characteristics were measured at 08:30 am. From 09:00 to 10:30 am, venous blood samples were obtained to determine the concentration of the measured hormones. In the afternoon of the same day (17:00 pm) the players were tested for squat jump (SJ), countermovement jump (CMJ), 10m, and 20m sprint performance. The second day of each experimental session our participants were tested for the determination of maximal oxygen consumption (VO_2max). The measurements for the determination of VO_2max values started at 09:30 am. All hormonal and exercise performance measurements, during the two experimental sessions, were performed at the same time of the day and players were tested in the same order to avoid any circadian variation on the measured variables. Verbal encouragement was given to all participants, ensuring the maximal effort throughout all testing procedures. Before each experimental session, players were informed not to consume any supplement that could promote performance at least 2 days prior to the testing. Moreover, they were instructed to abstain from any physical

activity two days prior to each testing in order to avoid any fatigue effects. All players were familiarized with the testing protocol, as they had been previously tested on several occasions during the last soccer season with the same testing procedures. The weekly training plan, during the last 4 months of the competitive season, was similar for both teams, as it had been previously reported in Greek Superleague teams [17]. This study was a part of a larger research project examining the adaptations of many hormonal, physiological, and biochemical parameters during one year period in professional soccer players.

Nutritional Guidelines

Detailed nutritional guidelines were given to all players for the whole detraining period. In particular, our participants were instructed to consume a high carbohydrate (>60%), low fat (15-25%), and low protein (10-15%) diet [18]. All players were provided with a list of a variety of foods, which included the caloric and nutrient content (carbohydrate, fat, and protein) of each serving portion [18,19]. Moreover, each player was provided with a table which included the caloric cost of various daily and physical activities [19] (of different intensities). In addition, the resting metabolic rate of each player was assessed [20]. Based on this information, players were asked to compose a diet as per their daily energy requirements (i.e. according to the performed daily activities and exercise training regimes) in order to meet their calculated daily energy expenditure, but at the same time retaining the basic characteristic of their diet i.e. high carbohydrate, low fat, low protein. Furthermore, representative daily meals of 2000, 2500, 3000, and 3500 kcal were provided to each player. Each daily plan included the quantity of each ingredient, its caloric value, and alternative but equal in calories foods. Finally, players were asked to maintain the optimal hydration status, according to set procedures [18,21]. The aim of these

guidelines were as follow: a) to ensure that the carbohydrate content would be adequate for the training activities performed during the detraining period as well as during the two experimental sessions; b) to ensure that the production levels of endogenous sex steroids would not be altered by the nutritional parameters, and; c) to avoid weight gain and increased fat mass due to excessive energy intake and high fat percentage consumption.

Detraining Period

A six-week detraining period was set in our study. During the first two weeks of this recuperation period, participants were informed to avoid any kind of physical activity. After this two-week period, they were instructed to perform low intensity (50%-60% of VO2max) aerobic running of 20 to 30 minute total duration (30, 20, 2x15, 3x10, 2x10) three times a week, divided by one day of rest.

Anthropometric Measurements and Body Composition

Height (cm) was measured using a stadiometer (Charder HM210D, Charder Electronics CO, LTD, Taiwan) and weight (kg) was obtained using an electronic weight scale (Seca Alpha 770, Seca Vogel, Hamburg, Germany). Body fat percentage was assessed by skinfold thickness measurement (Lange Skinfold Caliper, Cambridge Scientific Instruments, Cambridge, UK) using the 4 site formula proposed by Durnin and Womersley [22].

Neuromuscular Performance

The jumping ability of the soccer players (SJ, CMJ) was assessed with a jumping mat (Powertimer, Newtest, Oy, Finland). Three tests were carried out for each jump type and the best result was used for the analysis. During both SJ (cm) and CMJ (cm), the arms were kept in the iliac crest to minimize their contribution. The players performed

SJ starting from a standing position, bending the knees to 90°, stopping for three seconds, and then jumping as high as possible. CMJs were performed starting from a standing position, and players were instructed to jump as high as possible, after a fast preparatory downward eccentric movement. Sprint times for 10m (sec) and 20m (sec) were measured with infrared photoelectric cells (Powertimer, Newtest, Oy, Finland). For each sprint type two consecutive measurements were performed from a standing position. All sprints were separated by a two-minute rest period for full recovery to avoid any fatigue effects. The best time of the two trials of each distance was used for the analysis. A standardized, low intensity, fifteen-minute warm up was performed prior to each experimental period.

Maximal Oxygen Consumption (VO_2max)

Players were tested for the determination of VO_2max (ml/kg/min) on a motorized treadmill. Warm up consisted of a six-minute run at 8 km/h. Speed was set at 10km/h and held constant for 3 minutes. Thereafter, speed was increased by 2 km/h every 3 minutes until 16m/h and then, speed was increased every 2 minutes until voluntary exhaustion. Then, the initial achievement of VO_2max was considered as the attainment of at least 2 of the following criteria: (a) a plateau in VO_2 despite increasing speeds, (b) a respiratory exchange ratio above 1.10, or (c) a maximal heart rate within ±5% of age-predicted HR maximum (220-age). Expired gases were analyzed using a breath-by-breath, automated gas-analysis system (VMAX29, Sensormedics, Yorba Linda, CA). Before each test, flow and volume were calibrated using a 3-L capacity syringe (Sensormedics, Yorba Linda, CA). Gas analyzers were calibrated using 2 tanks of oxygen (O2) and carbon dioxide (CO2) of known concentrations (Sensormedics, Yorba Linda, CA).

Blood Collection and Analysis

On each test day, venous blood samples were obtained after a period of a ten-minute rest in a lying position. Blood samples were collected in tubes, containing a clot activator and serum gel separator and were centrifuged at 3000 rpm for 10 minutes to separate serum. Serum samples were then stored at -70 ^{o}C till analysis. All samples were tested in duplicate. Total testosterone (ng/ml), LH (IU/L), FSH (mIU/L), E2 (pg/Ml), and PRL (µg/L) concentrations were measured using AIA 21 fully automated immunoassay analyzer (TOSOH-Eurogenetics Tokyo, Japan). The sensitivity of the assays for TT, E2, FSH, LH, and PRL were 7 ng/ml, 25 pg/ml, 1,0 mlU/ml, 0,2 mlU/ml, and 1,05 respectively. The intra and inter coefficient of variation were 3,1-5,2% and 2,48-5,99 % for TT, 2,6-6,1% and 3,8-9,1 % for E2, 1,5-2,6% and 4,3-5,6 % for FSH, 1,8-2,5% and 2,1-2,7 % for LH, and 1,8-2,1% and 2,7-2,9 % for PRL.

Free testosterone (pg/ml), 3a-Diol G (ng/ml), Δ4-androstenedione (ng/mL), and DHEAS (µg/mL) concentrations were measured using enzyme-linked immunoabsorbent assays (Alpco Diagnostics, Windham, NH). All procedures were carried out according to the instructions of the manufacturer. The sensitivity of the assays for FT, Δ4-androstenedione, DHEAS, and 3a Diol G were 0,17 pg/ml, 0,04 ng/ml, 0,005µg/ml, and 0,1 ng/ml respectively. The intra and inter coefficient of variation were 4,7-17% and 5,3-12,4% for FT, 4,9-5,8% and 7,7-9,7% for Δ4-androstenedione, 7,5-11,5% and 4,2-15,3% for DHEAS, and 6,0-7,8% and 6,5-10,8% for 3a Diol G. All samples were analyzed in duplicate and in the same assays.

Statistical Methods

Statistical analysis was performed using software program SPSS 17.0. Standard statistical methods were used for the determination of means and standard deviations (±SD). The changes between the experimental periods in the measured parameters within the groups were analyzed by the paired samples t-test. Pearson's (for normally distributed variables) and Spearman's (for non-normally distributed variables) correlation coefficients were used to

assess the linear relationship between quantative variables. The differences between the groups at baseline, in all hormonal, performance, and body composition parameters were analyzed with the General Linear Model (GLM) analysis of variance, aiming to examine whether both teams had similar values in all these variables at the starting point. Statistical power analysis was performed (Stata® 13 software, StataCorp LP, USA) in order to attain 80% power. Analysis was carried out at a confidence level=95% and confidence interval=13,6 [7] . Our calculations directed us to a sample size of 45 to detect any differences in changes of the measured variables between the two experimental sessions. The level of significance was set at $p<0.05$.

Results

Differences between the Groups at Baseline

No significant differences were observed between the two experimental groups at the baseline measurement for VO_2max (F=0.23, p=0.88), SJ (F=1.84, p=0.18), CMJ (F=2.44, p=0.12), 10m (F=0.12, p=0.73), 20m (F=0.15, p=0.69), body weight (F=0.016, p=0.89), and body fat percentage (F=0.003, p=0.95). Similarly, no significant differences were observed for any of the TT (F=0.38, p=0.53), 3a Diol G (F=0.28, p=0.59), FT (F=2.74, p=0.10), DHEAS (F=0.09, p=0.76), Δ4-androstenedione (F=1.83, p=0.18), E2 (F=2.18, p=0.14), FSH (F=1.95, p=0.16), LH (F=1.28, p=0.26), and PRL (F=2.19, p=0.14) at baseline between the two experimental groups.

Body Weight and Body Fat Percentage.

Changes in body composition variables are presented in table 1. Body weight increased significantly in both teams A (77,60±5,88 vs 79,13±6,16; $p<0.001$), and B (77,89±8,75 vs 79,49±8,95; $p<0.001$) at the end of the study compared to baseline. Similarly, a significant increase in body fat percentage was observed in both teams A

(9,2±3,33 vs 11,01±4,11; p<0.001) and B (9,43±3,55 vs 10,40±4,08; p<0.001) at the end of the six-week detraining period.

Hormones

Sex steroid values at baseline and after the six-week detraining period are presented in table 2. No significant differences were observed for any of the measured hormones in team A: TT (p=0.14), FT (p=0.32), Δ4-androstenedione (p=0.28), DHEAS (p=0.13), 3a Diol G (p=0.40), E2 (p=0.09), FSH (p=0.11), LH (p=0.44), and PRL (p=0.72), and team B: TT (p=0.73), FT (p=0.90), Δ4-androstenedione (p=0.95), DHEAS (p=0.052), 3a Diol G (p=0.50), E2 (p=0.36), FSH (p=0.88), LH (p=0.067), and PRL (p=0.69) at the end of the six-week detraining period compared to their baseline resting values.

Exercise Performance

The mean values ± SD of VO_2max (ml/kg/min) decreased significantly at the end of the study compared to baseline (figure 1, figure 2) in team A (60,31 ± 2,52 vs 57,67 ± 2,54; p<0.001) and team B (60,47 ± 4,13 vs 58,30 ± 3,88; p<0.001). Similarly, in teams A and B, there was observed a significant decline (figure 1, figure 2) in SJ (39,70±3,32 vs 37,30±3,08; p<0.001, and 41,05±3,34 vs 38,18±3,03; p<0.001 respectively), and CMJ (41,04±3,99 vs 39,13±3,26; p<0.001, and 42,82±3,60 vs 40,09±2,79; p<0.001 respectively) values (cm). Both teams showed significant increases in 10m (1,74±0,063 vs 1,79±0,064; p<0.001, and 1,73±0,065 vs 1,78±0,072; p<0.001 respectively), and 20m (3,02±0,05 vs 3,06±0,06; p<0.001, and 3,01±0,066 vs 3,06±0,063; p<0.001 respectively) sprint times (sec) at the end of the study compared to baseline (figure 3, figure 4).

Correlations between sex steroids and performance parameters

Correlations between sex steroids and performance parameters, during the beginning and the end of the 6-week detraining period, are presented in table 3 (team A) and table 4 (team B). Analysis of our results did not reveal any significant differences ($p>0.05$) between 3a Diol G, TT, FT, Δ4-androstenedione, DHEAS, E2, FSH and PRL in neither team A nor B, during both experimental sessions. Significant correlations were observed between LH and VO_2max at both the first ($p=0.013$) and the second ($p=0.010$) experimental sessions. Significant correlations were evident in team B, regarding LH levels with both 10m, and 20m values at the first ($p=0.022$ and $p=0.004$ respectively) and the second ($p=0.028$ and $p=0.006$ respectively) experimental sessions.

DISCUSSION

Our findings support our hypothesis. After the six-week detraining period, we observed significant reductions in aerobic and jumping capacity values, and significant increases in 10m and 20m sprint times, body weight, and body fat percentage. These changes occurred with the absence of any significant changes in the circulating sex steroids basal levels. These observations indicate that the employed detraining period, in our study, resulted in a rapid loss of aerobic and neuromuscular performance adaptations in professional soccer players.

Maximal Oxygen Consumption (VO_2max)

As expected, VO_2max was significantly reduced after the six-week detraining period in both teams A and B. In agreement are the findings by Reilly and Williams [23]. The authors reported that an eight-week detraining period in soccer resulted in decreased aerobic capacity, as indicated by the reduced VO_2max values. Similarly, a study from another laboratory observed that the off-season soccer period resulted in

reduced VO_2max performance in semi-professional soccer players [15]. In agreement are the findings from other athletic populations. Maximal oxygen consumption has been shown to decline in a variety of sports, even with short term detraining (less than 4 weeks), up to 14% [4,5,10,24]. Furthermore, VO_2max has been found to decrease in highly trained athletes up to 20 % after 4 weeks of detraining [5]. However, some studies showed that short term training cessation or drastically reduction in volume and training frequency, in a period of 4 to 5 weeks, did not affect VO_2max [1,25,26]. These discrepancies could be related with the initial training status of the participants and the training regime employed during the detraining period. Coyle and associates [4] suggested that the higher the training state in VO_2max, the greater the decline in aerobic capacity after detraining periods. Since our participants were professional soccer players with their VO_2max mean pre-values (60,31 ml/kg/min for team A, 60,47 ml/kg/min for team B) within the suggested range (55 – 70 ml/kg/min) for high level soccer players [27], this could be the case in our study. Furthermore, it has been suggested that in order to retain aerobic training adaptations during detraining periods, a high intensity aerobic training stimulus (>80% VO_2max), even once a week, should be performed [26,28]. This is further supported by the observations of a recent study in top level Kayakers [1]. The authors reported that a period of 5 weeks under reduced training stimulus, which consisted of a moderate (80% VO_2max), and not a high intensity aerobic regime, did not manage to retain VO_2max performance. Therefore, according to the aforementioned evidence, the employed low intensity (50%-60% of VO_2max) aerobic regime that was performed the last 4 weeks of the detraining period in our study could not provide sufficient stimulus to maintain aerobic capacity.

Jumping Ability

We observed a significant reduction in both CMJ and SJ after the six-week detraining period in teams A and B. These findings are in agreement with the observations of a recent study in semi-professional soccer players. The authors reported that the off-season detraining period can result in a reduction in jumping ability [15]. However, inconsistent findings have been reported by some studies that examined the detraining effects on strength trained individuals [7,13,14]. Indeed, Izquierdo and associates [7] observed that 4 weeks of complete training cessation or tapering in strength trained athletes decreased CMJ performance, whereas other studies on the same athletic population showed that a period of two [14] or six [13] weeks of training cessation did not affect vertical jump performance. These discrepancies were attributed to the different strength training status of the participants in these studies, and only the athletes with the higher strength levels [7] experienced decreases in jumping ability. In our study, the effects were rather related to the employed training regime during the off-season period than the strength status of the participants, since soccer players have been generally reported to have lower muscle strength levels compared to strength trained athletes [27]. Our players followed a low intensity aerobic regime in both teams after the 2 first weeks of training cessation. Previous evidence has demonstrated that this kind of activity does not promote jumping ability but on the contrary, it can negatively affect this kind of performance [29]. A further possible explanation for our observations comes from the suggestion that detraining can result in decreased strength after a 3-6 week period, due to a negative effect on type II muscle fibers [6,7,12]. Early research has documented that fast twitch muscle fibers are strongly correlated with maximal strength, which is linearly related with jumping ability and explosive actions such as SJ and CMJ [30]. According to these findings, the observed reductions in jumping performance, in our study, could be related to a possible negative effect of the detraining period on fast twitch muscle fibers.

Sprint Performance

The observed reductions in 10m and 20m sprint performance in both teams are in agreement with the only available studies, to our knowledge, that examined the effect of the soccer off-season period on sprinting capacity [8,31,32]. The authors observed that detraining resulted in significant reductions in 10m, 20m, and 50m sprint performance. It was suggested that these findings could be related with reductions in the cross-sectional area of type II muscle fibers, negatively altered anaerobic enzymatic activity, and a decline in mitochondrial ATP production [2,3,31]. Since sprint performance is related with muscle morphology, enzymatic activity, and ATP production [33], any possible negative changes during the six-week detraining period in any of the aforementioned parameters could be accounted for the observed reductions in sprinting capacity. Furthermore, it is well established that sprint performance depends on the ability to generate power [34] and that power is a product of strength [35], demonstrating a linear relationship between strength levels and sprinting ability. Since both SJ and CMJ are considered to be the most accurate field tests for the determination of the explosive strength level of the lower limps [36], the observed reduction in their levels, at the end of our detraining period, could further justify the decline in sprint performance.

Body Composition

At the end of the 6-week detraining period, significant increases in body weight and body fat percentage were evident in both teams. In accordance are the findings of other laboratories [8,9]. It was observed that the off-season soccer period resulted in increased body weight and body fat percentage in the players. However, in these studies, no off-season training sessions were undertaken. On the contrary, our players performed low intensity aerobic trainings during the last 4 weeks of the detraining

period. Confirmation to our findings comes from a recent study in competitive swimmers [37]. The authors reported that 35–42 days of detraining, involving light-moderate physical exercise, after a competitive swim season, resulted in significant increases in body weight and body fat percentage. The increased body fat percentage and body weight, in our study, could be attributed to the reduced training stress during the detraining period. The reduced training stimulus could have resulted in a lowering of the metabolic rate per unit of tissue mass, and effectively decreased resting metabolic rate, which could have a negative impact on body composition [8,37], although these finding are not universal [38]. Furthermore, it has been observed that during detraining periods there is an increase in lipoprotein lipase (LPL) activity, which facilitates free fatty acid deposition on adipose tissue [39]. These suggestions are supported by the unaltered circulating sex steroids levels in our study. Since changes of internal androgen and estrogen levels are affecting body composition [40], resting metabolic rate [41,42], and lipase activity [43] the observed changes in body composition should be attributed to other causes and not to changes of the sex steroid milieu. Taken together, our data suggest that the insufficient training stimulus during our detraining period could have resulted in a short-term energy surplus, leading to weight gain, characterized by an increase in fat mass, most probably, due to an increased free fatty acid deposition on adipose tissue secondary to an enhanced LPL activity [37,39].

Sex Steroids

In the present study, the detraining period did not significantly affect the circulating levels of the measured sex hormones in our experimental teams. To the best of our knowledge, this is the first study examining the hormonal responses during the off-season period in professional soccer players.

Total Testosterone, Free Testosterone, and 3a Diol G

Our results showed that neither TT nor FT and the active androgen metabolite 3a Diol G were affected by the six-week detraining period. Notably, this is the first study examining the behavior of 3a Diol G in professional athletes, which has been reported to be an excellent marker of activated androgens [16]. In regard to TT and FT, our observations are in accordance with the findings after a four-week detraining period in strength trained individuals [7]. The authors failed to find any significant changes in TT and FT after both training cessation and tapering. Similarly, Kraemer and associates [13] showed that 6 weeks of detraining in recreationally trained men did not affect TT concentration. On the contrary, two weeks of inactivity on strength trained individuals resulted in significantly increased TT levels [14]. The authors suggested that this could indicate an enhanced stimulus for tissue remodeling. We were unable to confirm this finding since, in our study, no measurement was performed at the first two weeks of the off-season period. However, Kraemer and associates [13], based on their observation of an insignificant increase in TT levels after the first 2 weeks in their study, gave a possible explanation of this finding. The authors suggested that the first 2-3 weeks of detraining, after stressful training periods, could possibly result in increased anabolic hormones concentration, and that this increase was attenuated after the following 3-4 weeks of detraining. The absence of an alteration in 3a Diol G levels, in our study, clearly indicates that no anabolic trend was evident at the end of the six-week detraining period, since this hormone is the metabolic product of androgens and an indicator of activated androgens [16]. In other words, its unchanged resting values at the end of the off-season period clearly states that there was not any enhanced muscle tissue remodeling or any other extra-splanchnic utilization of the circulating androgens.

Gonadotropins

In regard to LH and FSH, our findings are in accordance with the observations of a study on resistance trained males [12]. The authors reported that 12 weeks of detraining did not affect these two gonadotropins resting levels. Similarly, Hal and associates [44] reported that LH and FSH were not altered by a short detraining period in endurance athletes. Therefore, according to the aforementioned published reports and our own findings, we could propose that detraining does not affect the hypothalamus or the pituitary gland regarding these two hormones.

Other Hormones

In the published bibliography no evidence exists for E2, Δ4-androstenedione, and PRL and their responses after a detraining period. In regard to DHEAS, only one recent study examined its behavior after 2 months of training cessation in highly trained badminton players [45]. The authors observed a significant reduction in its resting levels at the end of the study. However, we were unable to confirm these observations. Indeed, we have found that the detraining period did not decrease DHEAS concentration, on the contrary, it showed a tendency to increase albeit in a non-significant manner in both experimental teams (Table 2). This discrepancy could be related to the training status of the participants and the training regime used, which have been both reported to affect the hormonal responses to exercise [46].

A possible explanation for the absence of an alteration, not only in regard to DHEAS but also for Δ4-androstenedione, E2, and PRL is their reported correlation with TT levels. It has been suggested that DHEAS and Δ4-androstenedione were linearly correlated with TT levels although these findings are not universal [47,48]. Similarly, studies on elderly individuals showed the E2 concentration was correlated with its

major precursor (TT) levels, in a linear manner [49]. Therefore, the insignificant changes in TT levels at the end of the study could explain the lack of an alteration in E2, Δ4-androstenedione, and DHEAS levels. In regard to PRL, it has been previously reported to have a strong reverse correlation with TT levels [50] in athletic population, indicating that the unaffected TT basal levels resulted in its unaltered resting values.

It should be stressed here that our overall findings, regarding the alterations of the examined performance parameters and the unaffected sex steroids levels, do not appear to be mediated by changes of nutritional intake since the latter was kept unchanged throughout the study. Indeed, during the six-week detraining period we paid great attention to exclude nutritional changes (including that of hydration) by retaining a constant level of a daily diet composed of high carbohydrates, low fat and, low protein consumption, and a religious matching of their energy intake to the calculated level of daily energy expenditure. It should be mentioned that both the carbohydrate content of the diet [51,52] and energy restriction [53] play regulatory role in the levels of sex steroids. In addition, reduced carbohydrate content of a diet, insufficient energy intake, and dehydration negatively influence the ability of the players to perform efficiently during aerobic and high intensity intermittent exercise activities [21,54]. Therefore, because of the aforementioned adjustments of each player's dietary intake during the detraining period, we had no measurable changes of the hormonal milieu of their body, and the observed changes in exercise performance capacity should be attributed to the reduction of training.

Finally, no correlations were evident between performance parameters and circulating androgen (TT, FT, 3a Diol G, Δ4-androstenedione, and DHEAS) during the detraining period. Similarly, no correlations were evident between performance and

E2, FSH, and PRL levels. It is of note that the gonadotropin LH exhibited a correlation with VO_2max in team A, and with sprinting performance in team B. To our mind, the validity and physiological significance of this finding is questionable since in team A, LH correlated with VO_2max performance in both experimental sessions but not with jumping and sprinting ability, whereas in team B, LH levels correlated with sprint performance (pre and post) but not with VO_2max. In addition, despite the considerable changes in all performance parameters observed at the end of the detraining period, the LH levels did not exhibit any alterations. These discrepancies indicate that these data are of minor physiological significance, and do not necessarily constitute a significant correlation between LH and VO_2max, 10m, and 20m performance.

CONCLUSION

In conclusion, our findings demonstrate that the six-week detraining period, in our study, resulted in significant declines in aerobic, strength (as indicating by SJ and CMJ), and sprint performance adaptations. Furthermore, it had a negative effect on body composition as indicated by the observed increases in body weight and body fat percentage at the end of the study. The off-season transition period did not result in any significant differences in the measured sex steroid concentrations. Moreover, apart from the observed relationship of minor physiological significance between LH with VO_2max and sprinting capacity, no other correlations were evident between the circulating sex steroids levels and the values of the performance parameters. Therefore, our findings suggest that sex steroids were non-contributing parameters for the reductions in exercise performance indices, and the negatively altered body composition. The implications of our findings strongly suggest that professional soccer players should devise transition off-season periodization training programs that

provide adequate mental and physical recovery after the demanding competitive season, but also allow these athletes to maintain aerobic, strength, and sprint adaptations, and retain optimal body composition status.

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Figure Legends

Figure 1. **Changes in aerobic and jumping capacity mean values after the detraining period in Team A.** Changes (mean values ±SD) in VO_2max (ml/kg/min), SJ (cm), and CMJ (cm) during the course of the 6 week detraining - off-season soccer period for Team A players. Pre: measurement prior to the beginning of the detraining period; Post: measurement at the end of the detraining period; SJ: squat Jump; CMJ: countermovement Jump. **Different from Pre ($p<0.001$).

Figure 2. Changes in aerobic and jumping capacity mean values after the detraining period in Team B. Changes (mean values ±SD) in VO_2max (ml/kg/min), SJ (cm), and CMJ (cm) during the course of the 6 week detraining - off-season soccer period for Team B players. Pre: measurement prior to the beginning of the detraining period; Post: measurement at the end of the detraining period; SJ: squat Jump; CMJ: countermovement Jump. ** Different from Pre ($p<0.001$).

Figure 3. Changes in sprint performance mean values after the detraining period in Team A. Changes (mean values ±SD) in 10 meters (Sprint10) and 20 meters (Sprint20) sprint performance (sec) during the course of the 6 week detraining - off-season soccer period for Team A players. Pre: measurement prior to the beginning of the detraining period; Post: measurement at the end of the detraining period. ** Different from Pre ($p<0.001$).

Figure 4. Changes in sprint performance mean values after the detraining period in Team B. Changes (mean values ±SD) in 10 meters (Sprint10) and 20 meters (Sprint20) sprint performance (sec) during the course of the 6 week detraining - off-season soccer period for Team B players. Pre: measurement prior to the beginning of

the detraining period; Post: measurement at the end of the detraining period. ** Different from Pre ($p<0.001$).

Tables

Table 1. Mean values ±SD of Body Composition measurements at baseline (Pre) and at the end of the study (Post)

	Team A		**Team B**	
	Pre	**Post**	**Pre**	**Post**
Body Weight (kg)	77,60±5,88	79,13**±6,16	77,89±8,75	79,49±8,95
Body Fat %	9,28±3,33	11,01**±4,11	9,34±3,55	10,40±4,08

** Different from Pre (p<0,001)

Table 2. Mean values ±SD of Sex Steroids concentration at baseline (Pre) and at the end of the study (Post)

Hormones	**Team A**		**Team B**	
	Pre	**Post**	**Pre**	**Post**
3a Diol G (ng/ml)	8,88±3,01	8,31±2,56	8,40±3,08	8,69±2,87
TT (ng/dl)	656,97±136,5	604,98±141,9	679,89±109,7	686,81±124,3
FT (pg/ml)	12,50±4,86	13,81±6,13	13,11±14,79	13,9±18,30
Δ4 (ng/mL)	1,78±0,46	1,86±0,39	2,07±0,93	2,077±1,26
DHEAS (µg/mL)	1,99±0,63	2,35±1,07	2,08±1,38	2,89±1,18
E2 (pg/mL)	23,69±11,99	19,62±12,82	28,70±16,04	25,38±11,06
FSH (mIU/L)	8,29±7,46	7,41±5,56	5,77±4,08	5,71±3,96
LH (IU/L)	4,97±1,95	4,69±1,67	4,38±1,92	4,95±1,71
PRL (µg/L)	12,80±7,70	12,25±6,71	9,96±4,74	10,24±5,00

Table 3. Correlations (p-values) between circulating sex steroids and exercise performance parameters in team A

Team A	VO_2max **(ml/kg/min)**		**SJ (cm)**		**CMJ (cm)**		**10m (sec)**		**20m (sec)**	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
3a Diol G (ng/ml)	0.394	0.368	0.873	0.355	0.833	0.601	0.177	0.203	0.510	0.177
TT (ng/dl)	0.513	0.794	0.682	0.151	0.660	0.122	0.851	0.717	0.564	0.800
FT (pg/ml)	0.788	0.487	0.741	0.833	0.927	0.451	0.932	0.613	0.540	0.714
Δ4 (ng/mL)	0.381	0.699	0.505	0.571	0.312	0.605	0.79	0.362	0.305	0.791
DHEAS (μg/mL)	0.714	0.374	0.611	0.222	0.820	0.202	0.799	0.809	0.666	0.275
E2 (pg/mL)	0.782	0.615	0.405	0.727	0.465	0.975	0.072	0.321	0.308	0.580
FSH (mIU/L)	0.240	0.090	0.866	0.843	0.531	0.723	0.417	0.324	0.711	0.422
LH (IU/L)	0.013*	0.010*	0.687	0.543	0.702	0.225	0.767	0.161	0.621	0.064
PRL (μg/L)	0.330	0.276	0.634	0.591	0.528	0.417	0.680	0.185	0.660	0.247

* siginifcant diference at the level of significance p<0.05

Table 4. Correlations (p-values) between circulating sex steroids and exercise performance parameters in team B

Team B	VO_2max **(ml/kg/min)**		**SJ (cm)**		**CMJ (cm)**		**10m (sec)**		**20m (sec)**	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
3a Diol G (ng/ml)	0.719	0.075	0.521	0.268	0.196	0.400	0.163	0.327	0.188	0.699
TT (ng/dl)	0.911	0.136	0.34	0.718	0.692	0.480	0.728	0.848	0.216	0.863
FT (pg/ml)	0.304	0.926	0.65	0.976	0.383	0.879	0.760	0.366	0.602	0.948
Δ4 (ng/mL)	0.052	0.052	0.212	0.930	0.765	0.625	0.769	0.464	0.468	0.592
DHEAS (μg/mL)	0.480	0.109	0.263	0.143	0.659	0.678	0.960	0.985	0.952	0.967
E2 (pg/mL)	0.481	0.564	0.811	0.651	0.645	0.929	0.436	0.496	0.406	0.518
FSH (mIU/L)	0.555	0.907	0.312	0.713	0.995	0.165	0.252	0.077	0.228	0.072
LH (IU/L)	0.613	0.352	0.266	0.177	0.082	0.051	0.022*	0.028*	0.004**	0.001**
PRL (μg/L)	0.290	0.561	0.204	0.546	0.238	0.804	0.688	0.95	0.629	0.710

* siginifcant diference at the level of significance p<0.05, ** siginifcant diference at the level of significance p<0.01

Revised manuscript PONE-D-14-13457

Vitamin D and exercise performance in professional soccer players

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Abstract

Aim. The current study had two aims. The primary purpose was to examine the association between serum vitamin D levels and the ergometric evaluation of muscle strength, aerobic capacity, and speed in professional soccer players. The secondary aim was to evaluate the effects of the soccer off-season period on serum vitamin D levels.

Methods: Sixty-seven Caucasian male soccer players (age 25.6 ± 6.2 and height 1.81 ± 0.08 m), members of two Greek Superleague Soccer teams and one Football-league championship team participated in this study. Exercise performance testing for the determination of squat jump (SJ), countermovement jump (CMJ), 10 (10m) and 20 meters (20m) sprint performance, maximal oxygen consumption (VO_2max), anthropometry, and blood sampling were performed before (pre) and after (post) the six-week off-season period.

Results: Analysis of our results showed the following: (a) a significant correlations between serum vitamin D levels and performance parameters in both pre (SJ; $P<0.001$, CMJ; $P<0.001$, VO_2max; $P<0.001$, 10m; $P<0.001$, and 20m; $P<0.001$) and post (SJ; $P<0.001$, CMJ; $P<0.001$, VO_2max; $P=0.006$, 10m; $P<0.001$, and 20m; $P<0.001$) experimental sessions. (b) Vitamin D concentration increased significantly ($P<0.001$) following the six-week off-season period compared to baseline, while at the same time all measured performance parameters decreased (SJ; $P<0.001$, CMJ; $P<0.001$, 10m; $P<0.001$, 20m; $P<0.001$, VO_2max; $P<0.001$).

Discussion: Our findings suggest that vitamin D levels are associated with the ergometric evaluation of muscle strength, as expressed by SJ and CMJ, sprinting capacity, and VO_2max in professional soccer players, irrespective the levels of performance. Furthermore, our data reaffirm the importance of UVB on serum vitamin D levels. Moreover, reductions in exercise training stress may also have beneficial effects on vitamin D levels, suggesting a possible association of its levels and the training-induced stress. Our results indicate a possibly bidirectional interaction between soccer performance indices and vitamin D levels.

***Key Words**: Vitamin D, Soccer, Exercise Performance, Exercise training stress.*

Introduction

Vitamin D is primarily synthesized endogenously following cutaneous exposure to ultraviolet B radiation (UVB) [1], [2]. Apart from its effect on calcium homeostasis and bone metabolism vitamin D exerts a host of other physiological effects on neural and muscular tissues, the immune system, and energy homeostasis, thus affecting among other parameters physical performance [3], [4], [5]. More specifically, it has been shown that vitamin D levels correlate with grip and quadriceps strength, physical fitness, and a decline of falls and bone fractures [5]. Vitamin D deficiency predominantly affects the weight-bearing antigravity muscles of the lower limbs which are necessary for walking and postural balance [5], [7]. Furthermore, vitamin D supplementation boosts muscular strength and restores balance [7].

It should be noted that the majority of the above mentioned studies have been performed in the elderly [5], [7]. Nevertheless, similar findings have been reported in younger individuals. A recent study on adolescent girls reported a positive association between serum vitamin D levels and jump height, jump velocity, and power [2]. Similarly, early studies on collegiate athletes and students have documented that cardiovascular fitness, muscle endurance, and speed are enhanced following exposure to ultraviolet radiation [8], although other authors failed to document such associations [9]. Furthermore, a consisted literature indicates that physical and athletic performance is seasonal, it peaks when Vit D levels peaks and declines as its levels decline [10], [11].

Paradoxically, a growing number of studies report a high prevalence of vitamin D insufficiency or downright deficiency even in regions with extensive sunlight in both athletic and non-athletic populations [12], [13]. The reason for this phenomenon is not entirely clear. It is mainly attributed to the limited exposure to sun, the types of clothing, and the declining ability of the skin to produce vitamin D precursors with advancing age [4]. Interestingly, a recent study reported that vitamin D levels also decline during strenuous military training [14]. This decrease was evident although training was performed outdoors in the summer and early autumn months during daylight hours and thus with adequate exposure under UVB. The

latter data may indicate a possible relationship between exercise training stress and vitamin D levels.

Soccer is a sport where aerobic capacity, muscular strength, and speed are of vital importance for most of the actions during playing. Indeed, players must perform repeated sprints, stops, jumps, and changes of direction with maximal force development, and in the shortest possible response time. Furthermore, top level players run approximately 10-12km during a soccer game, and the total distance covered is linearly related to VO_2max [15]. Vitamin D seems to be involved on this type of activities. It is well documented that its levels are related with muscle strength [5], the proportion and the diameter of type II muscle fibers [16], and neuromuscular coordination [12], which are of paramount importance for explosive type human movements, such as sprints and jumps [17], [18], [19]. Moreover, the presence of vitamin D receptors (VDR) in the muscle cell appears to influence muscle strength. The discovery of VDR within the muscle indicates that vitamin D has the potential to impact upon muscle structure and function directly and has been identified as a regulator of skeletal muscle development and action [20]. In regard to aerobic capacity the findings from recent studies provide evidence that it could be affected by vitamin D levels through an effect of this vitamin on optimal lung function and/or erythropoietin resistance [21], [22].

Our study had two aims. The main objective was to examine the potential relationship between vitamin D levels and muscle strength, as expressed by squat jump (SJ) and countermovement jump (CMJ) [23], maximal oxygen consumption (VO_2max), 10 and, 20 meters sprint performance in two different occasions, prior to the beginning and at the end of the off-season soccer period. Our second aim was to examine the vitamin D response to the reduced exercise training during the six-week off-season transition period. We hypothesized that in both experimental sessions vitamin D levels would correlate with soccer players' jumping, sprinting, and aerobic capacity, and that the off-season transition period, of reduced training stress, would favorably affect vitamin D concentration. To the best of our knowledge, no published study has examined the relationship between vitamin D levels and muscle

strength, VO_2max and speed in professional soccer players, and/or the effects off-season detraining soccer period on its levels in any kind of athletic population.

Materials and Methods

Participants

Initially, seventy seven professional players, members of three soccer teams, were evaluated for possible participation in the study. The exclusion criteria were as follow: a) any medical disorder that would affect vitamin D levels, b) players that their contract was ending before the conclusion of the study, and c) the consumption of supplements containing vitamin D. Ten (10) players failed to fulfill criteria b (8) and c (2) and were subsequently excluded from the study. Sixty seven Caucasian professional male soccer players, members of two Greek Super league teams (n=45) and one Football league team (n=22) were consecutively included in the study. The mean values of age (years) ± SD and height (m) ± SD were: age 25,6 ± 6,2 and height 1,81 ± 0,08 respectively.

Ethics Statement

Before testing verbal explanation of the aim of the study and the testing procedures were given to all participants, and written informed consent was obtained. The study was performed in strict accordance with the ethical guidelines of the Helsinki Declaration and was approved by the Ethical Scientific Committee of the University Hospital of Heraklion, Crete, Greece.

Experimental protocol

The duration of the off-season transition period was set to six-weeks, starting at the end of the competition period. During this recuperation period, participants were instructed to avoid any kind of exercise training for the first two weeks. After this two-week period, they were instructed to perform low intensity aerobic running (50%-60% of VO_2max) of 20 to 30 minute total duration (30, 20, 2x15, 3x10, 2x10) three times per week, divided by one day of

rest. This type of activity was selected by the team coaches. All players were tested at two different occasions. Each experimental testing consisted of two days of consecutive measurements. The first experimental testing took place immediately after the end of the competition period in May (pre). The second experimental testing was performed at the beginning of July (post), just prior to the beginning of the preparation period for the forthcoming season. In each experimental periods testing consisted of anthropometric measurements (i.e. height, body weight, body fat percentage), blood testing for the assessment of vitamin D levels, and ergometry tests for the measurement of SJ (cm), CMJ (cm), VO_2max (ml/kg/min), 10m, and 20m sprint performance. The first day of each experimental period anthropometric characteristics were measured at 08:30 am. From 09:00 to 10:30 am, venous blood samples were obtained in order to determine the vitamin D concentration. In the afternoon of the same day (17:00 pm) the players were tested for SJ, CMJ, 10m, and 20m sprint performance. The second day of each experimental period, starting at 09:30 am, our participants were tested for the determination of VO_2max. During the study players were instructed to avoid consuming any vitamin D supplements. Before each experimental session, players were informed not to consume any supplement that could promote performance at least 2 days prior to the testing and to avoid any caffeine or alcohol beverages. Moreover, they were instructed to abstain from any exercise training sessions two days prior to each testing in order to avoid any fatigue effects. Detailed nutritional guidelines were given to all players in order to ensure a high (>60%) carbohydrate dietary intake during the study, including a list of a variety of foods, and based on individual resting metabolic rates and the calculated daily energy expenditure as per reported activities (24). Furthermore, players were also asked to maintain their hydration status. All players were familiarized with the testing protocol, as they had been previously tested with the same procedures on several occasions during the last soccer season. The study was performed in Crete, Greece, at a latitude of 35,9° N.

Anthropometric Measurements and Body Composition

Height was measured using a stadiometer (Charder HM210D, Charder Electronics CO, LTD, Taiwan) and weight was obtained using an electronic weight scale (Seca Alpha 770, Seca Vogel, Hamburg, Germany). Body fat percentage was assessed by skinfold thickness measurement (Lange Skinfold Caliper, Cambridge Scientific Instruments, Cambridge, UK) using the 4 site formula by Durnin and Womersley [25].

Vitamin D measurement

Venus blood samples were obtained following a ten-minute period of rest in a lying position, after an overnight fast. Vitamin D levels were assessed using DiaSorin 25 hydroxy vitamin D (DiaSorin Inc. S.p.A, Italy), repeatability CV= 3-6 % and reproducibility CV=6-11%, according to the laboratory standard operating procedures. Calibrator 1 and 2 (human serum) concentrations are referenced to standard preparations containing highly purified 25(OH) vitamin D. According to the manufacturer the correlation of the immunoenzymatic assay with LC-MS/MS is described by the equation: concentration = 5,6+0,83*LC-MS/MS (R=0,93). The intra assay coefficient of variation ranges between 3-6%. In our study vitamin D levels below 10ng/ml were considered as severe deficiency, levels between 10- 20ng/ml as deficiency and levels between 20 - 30ng/ml as insufficiency.

Ergometry tests

The jumping (SJ, CMJ) and sprinting ability (10m, 20m) of the soccer players were assessed with a jumping mat (Powertimer, Newtest Ltd., Oulu, Finland) and infrared photoelectric cells (Powertimer, Newtest Ltd., Oulu, Finland) respectively, according to standard procedures [24]. Maximal Oxygen Consumption (VO_2max) assessment was performed on a motorized treadmill using an automated gas-analysis system (VMAX29, Sensormedics, Yorba Linda, CA), with the use of set procedures of a standard protocol [24].

Statistical analysis

Statistical analysis was performed using the software program SPSS 17.0. Results are presented as means ±SD. The distribution of variables was tested by the Shapiro-Wilk

statistical method. Then, Pearson's (for normally distributed variables) and Spearman's (for non-normally distributed variables) correlation coefficients were used to assess the linear relationship between quantative variables. The changes between the experimental periods in the measured parameters within the groups were analyzed by the paired samples t-test for normally-distributed data, and by Mann-Whitney test for non-normally-distributed data. Statistical power analysis was performed (Stata® 13 software, StataCorp LP, USA) in order to attain 80% power. Analysis was carried out at a confidence level = 95% and confidence interval = 13,6 [26]. Our calculations showed that a sample size equal to 45, much lower than ours i.e. n=67, was needed to attain 80% power in order to detect any differences in changes of the measured variables between the two experimental sessions. The level of significance was set at $p<0.05$.

Results

Correlation between vitamin D levels and exercise performance parameters

The correlations between vitamin D levels and exercise performance parameters, during the beginning and the end of the 6-week transition period, are presented in table 1. Analysis of our results revealed a significant positive correlation between vitamin D levels and SJ, CM, and VO_2max values at the beginning and at the end of the experimental period (table 1). A significant negative correlation was observed between vitamin D levels and 10m, and 20m sprint times at the beginning and at the end of the study (table 1).

Changes in vitamin D levels, exercise performance parameters, and body composition

The values of vitamin D and exercise performance parameters at baseline and after the six-week transition period are presented in table 2. Vitamin D levels increased significantly during the off season period compared to baseline (table 2). In contrast, analysis of our data revealed significant reductions in SJ, CMJ, VO_2max, 10m, and 20m values at the end of the study compared to baseline (table 2). Lastly, there was evident a significant

increase in body weight (77,68±7,06 vs 79,08±7,24; p=0.013) and body fat (8,81±2,96 vs 10,05±3,56; p<0.001) at the end of the study compared to baseline.

Discussion

Our findings support our hypothesis. Analysis of our results showed that vitamin D levels are associated with neuromuscular performance and aerobic capacity in professional soccer players. Notably, to the best of our knowledge, for the first time our study provides evidence of a linear relationship between vitamin D serum levels, not only with jumping performance, but also with VO_2max and speed in non-supplemented soccer players. In addition, we have found that even the short off-season period of reduced training stress had a boosting effect on vitamin D levels. Interestingly, this increase in vitamin D levels was evident in parallel to a reduction in aerobic and neuromuscular performance parameters. The latter finding strengthens the well-documented concept that training plays the principal role for exercise adaptations and improvements in exercise performance, whereas all other parameters including vitamin D play a supportive role.

Vitamin D levels exhibited a positive linear relationship with the ergometric evaluation of muscular strength (SJ and CMJ) and speed at both experimental periods. Our observations are comparable to several studies showing that vitamin D is linearly associated with jumping ability and strength in pre-adolescent girls [2] and elderly individuals [7], [12], [27], and in agreement with the observation that 100m performance was enhanced after a single biodose of ultraviolet radiation in collegiate women [28]. Moreover, a recent vitamin D supplementation-study on professional soccer players revealed that inadequate vitamin D concentration was detrimental to jumping and sprinting ability, whereas supplementation counteracted this effect [29]. Notably, regarding musclular strength, Hamilton et al. [9] reported that vitamin D levels were not associated with lower limb isokinetic muscle function in soccer players. However, this finding was attributed to the different mode of exercise used since, as the authors suggested, vitamin D could preferentially affect muscle groups that were not evaluated in their study. It should be mentioned that both SJ and CMJ are considered to be

the most accurate field tests for the determination of the strength levels of the lower limps [23]. In order to perform SJ and CMJ the proximal muscles required are quadriceps, soleus, and gastrocnemius [7]. Those muscles have been found to be predominantly affected by vitamin D deficiency [7]. Furthermore, it is well established that sprint performance is linearly related with both SJ and CMJ [30], [31], suggesting a direct effect of strength levels on sprinting ability. Therefore, based on the aforementioned evidence, our findings indicate a possible effect of vitamin D on jumping ability and strength, which is in turn translated to an affected sprint performance in a similar manner.

The pathways via which vitamin D affects muscular strength (as measured by SJ and CMJ) and sprint performance are still hypothetical. However, there are several potential mechanisms conveying these effects. The ergogenic effects of vitamin D may be related to the regulation of muscle protein synthesis which could affect muscle mass, thanks to the presence of vitamin D receptors on skeletal myocytes [5], [31], [32]. Furthermore, alterations in vitamin D levels also affect its receptors at the expression and activation levels [5], [20], and thus affecting muscle mass [5], [33], neuromuscular coordination [18], and the relative number and the cross-sectional area of type II muscle fibers [16]. Since it well documented that the major determinants of jumping and sprinting ability are muscle strength [31], [34], type II muscle fibers [35], [36] and neuromuscular coordination, [19], [37], any potential effect of vitamin D on these parameters would in turn affect jumping and sprinting capacity in a similar manner.

Analysis of our data revealed a linear association between vitamin D and VO_2max in both experimental sessions. This finding is supported by an early study which reported increased aerobic capacity as a result of exposure to ultraviolet radiation in collegiate students [8]. Furthermore, a recent study on adolescents observed a positive relationship between vitamin D and aerobic performance on adolescents [38]. Since VO_2max is related to soccer performance, as indicated by the well-documented relationship between this parameter and the distance covered during a soccer game [15], our findings suggest that in order to perform

efficiently during a soccer game optimal vitamin D levels are needed. The observed association between vitamin D levels and VO_2max could be related to its protective effect on lung function. According to recently published evidence, low vitamin D levels are associated with lower indices of lung function [22] and increased airway reactivity [39]. Since exercise performance and especially aerobic capacity (VO_2max) is depended on optimal lung function [40], any protective and/or boosting effect of vitamin D on the function of this organ could beneficially influence aerobic performance during exercise [40]. Vitamin D could also influence VO_2max by affecting iron metabolism and erythropoietin [21], [41]. According to Li and associates [41] vitamin D deficiency results in dysregulation of innate immunity and inflammation which is affecting iron metabolism and contributes to erythropoietin resistance. It is well documented that erythropoietin is linearly associated with changes in red blood cells levels [42]. Thus, vitamin D could influence VO_2max via its effects on erythropoesis modifying the capacity of the oxygen supply to the exercising muscles and consequently affecting aerobic exercise performance [42].

Interestingly, analysis of our data showed that although vitamin D increased at the end of the off-season period all measured exercise performance parameters decreased. However, the observed linear correlation at baseline between vitamin D levels and performance was retained at the end of the study, suggesting that vitamin D is related to the ability to perform efficiently during exercise, irrespective the level of performance. These data clearly suggest that although vitamin D does seem to affect neuromuscular and aerobic performance, it does not play the primary role. Indeed, during periods of reduced training stimulus or training cessation (i.e. soccer transition period) there is deterioration in exercise performance [24]. Since this decline is widely accepted to be a result of the insufficient training stimulus [24], this finding demonstrates that the major determinant of exercise performance is the amount and the quality of training [15], [24]. The latter evidence documents that vitamin D plays a supportive role in exercise performance. However, this does not lessen the importance of vitamin D serum concentration since in elite soccer level even subtle changes in performance may determine the outcome of a competition.

In our study the six-week transition period had a boosting effect on vitamin D levels. Indeed, at the first experimental period, although none of our participants was vitamin D deficient (<20ng/ml) or severe deficient (<10 ng/ml), 55,22% of our players had insufficient vitamin D levels (<30ng/ml), whereas at the second one only 4,47% were found to be below 30 ng/ml. In regard to the first experimental session our findings are in agreement with studies on soccer players (~50% < 30ng/ml) [43], and members of the American national football league [44] (~ 51% < 30ng/ml). Furthermore, our values were at the lower level of the range observed (30-84% vitamin D insufficiency) in several athletic and non-athletic population [9], [43], [45]. The most plausible explanation for the elevation of vitamin D levels at the second experimental session could be the consequence of an increased exposure to UVB during the off-season period [9]. Indeed, this transition period in Greek Superleague takes place during June and at the beginning of July at a favorable latitude (35,9° N). During this period UVB reaches its peak [46], resulting in increased vitamin D production. It is well demonstrated the extremely importance that UVB plays on vitamin D synthesis [1], [3] and the observation that its effectiveness is among other parameters, season dependent [32]. Furthermore, this off-season phase is actually a holiday period for professional soccer players. This could indicate increased time spend under sunlight, and also an increased exposure of a larger proportion of the players' body to UVB. Since all these parameters are related with vitamin D production, the observed increased in its levels at the end of our study could be partly accounted to these factors [3], [47]. The above mentioned suggestions are further supported by the observation that 84% of 342 Qatar soccer players were vitamin D insufficient (<30ng/ml) [9], despite of the favorable latitude (25.4°N) and the period that the study was performed (i.e. July). This finding was attributed to inadequate exposure to the sun, since all outdoors training were performed after sunset, demonstrating the importance of UVB on vitamin D production.

Although our data reaffirm the importance of adequate exposure to UVB, recent evidence indicate that the exercise training -induced stress may play a regulatory role on vitamin D levels [14]. Andersen et al. [14] reported that intense military training resulted to

reduced vitamin D levels in female soldiers, although these activities were performed outdoors in the summer and early autumn. This finding was unanticipated by the authors since they expected vitamin D levels to increase or at least to remain constant due to the adequate daily exposure to UVB during the whole study. Further support is coming from two recent studies on soccer players [43], [48]. The authors observed much lower vitamin D levels (30,82±9,04 [42], 32,83±6,64 [48]) and insufficiency values (83%<30 ng/ml [42], 65% <30ng/ml [48]) during periods of training, compared to the ones we obtained after the six-week detraining period (45,67±13,70; 4,47<30 ng/ml). These low values were evident despite the fact that outdoor training sessions were performed at August [48] and during summer [43] under a sufficient daily exposure of a large proportion of their body to UVB. According to Holick [49], the participants of these two studies should have had sufficient vitamin D levels since the amount of sun exposure needed to maintain adequate vitamin D levels is approximately 5–30 minutes at least twice a week to the face, arms, and legs [49]. Based on the aforementioned evidence we could suggest that the exercise training-induced stress could play a regulatory role on vitamin D levels. Supporting to our hypothesis is the fact that our second testing was performed following a massive reduction of training stress, while the participants in these soccer studies were tested in pre-season and early in-season which are periods of high training stress (i.e. training sessions and games) [15]. This suggested impact of exercise-induced stress on vitamin D could be associated to the immune effects of the intense stress [50], since both vitamin D and exercise training are related with the immune system [50], [51]. Indeed, prolonged intense training sessions or intensified training periods, similar to the ones used repeatedly during a soccer season, suppresses athletes' immune system [50]. In our study, the first experimental session was performed at the end of competition season which according to the literature suppresses innate immunity [50]. On the contrary, the six-week period prior to the second testing our players were under minimal training stress, which could hypothetically have resulted in an enhanced innate immunity. Recently published data suggest a strong inverse correlation between inflammation and vitamin D concentration [8], [51], while elevated vitamin D levels

boost immunity [52]. Therefore, we could hypothesize that any positive alteration in soccer players' immunity due to reduced training stress could be evident in conjunction with increased vitamin D status.

Our study provides evidence of an association between not supplemented vitamin D levels and parameters of aerobic and neuromuscular exercise performance in soccer. In particular, our findings indicated a linear relationship between vitamin D levels and muscle strength as evaluated by SJ and CMJ, sprinting ability (10m, and 20m), and VO_2max in professional soccer players. Moreover, our results indicate that, apart from increased exposure to UVB, reductions in exercise training stress may also have beneficial effects on vitamin D levels in elite soccer players. However in order to confirm this hypothesis additional research is needed, also examining indices of inflammation. Furthermore, our findings indicate that vitamin D plays, among other parameters, a secondary supportive role in athletic performance. However, this does not lessen its importance, especially in highly competitive athletes, since in elite athletic level slight changes in performance e may determine the outcome of a competition. Our current data may provide an additional tool to sport scientists, coaches, and players to enhance soccer performance.

Supporting Information

File S1. Supplementary File Data.xlsx which include all performed vitamin D and performance parameters measurements in this study

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Tables

Table 1. Correlations (correlation coefficients and p-values) between Vitamin D levels and exercise performance parameters

Exercise Performance	Pre Vitamin D (ng/ml)	Post Vitamin D (ng/ml)
SJ (cm)	0.731 ($p<0.001$)	0.597 ($p<0.001$)
CMJ (cm)	0.740 ($p<0.001$)	0.476 ($p<0.001$)
VO_2max (ml/kg/min)	0.436 ($p<0.001$)	0.394 ($p=0.006$)
10m (sec)	-0.649 ($p<0.001$)	-0.410 ($p<0.001$)
20m (sec)	-0.673 ($p<0.001$)	-0.426 ($p<0.001$)

Pre: measurement prior to the beginning of the off-season transition period; Post: measurement at the end of the off-season transition period.

Table 2. Vitamin D and Performance values in the two experimental periods

Measurements	**Pre**	**Post**
Vitamin D (ng/ml)	34,41±7,08	47,24±13,50**
SJ (cm)	39,50±3,87	37,10±3,59**
CMJ (cm)	40,91±4,57	38,62±4,00**
VO_2max (ml/kg/min)	59,44±3,07	58,89±3,45**
10m (sec)	1,74±0,07	1,79±0,08**
20m (sec)	3,02±0,06	3,07±0,07**

Pre: measurement prior to the beginning of the off-season transition period; Post: measurement at the end of the off-season transition period.* significant difference at the level of significance $p<0.05$** , significant difference at the level of significance $p<0.01$.

CURRICULUM VITAE

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EDUCATION

1 **First degree** in the department of **Physical Education** in Athens Kapodistriako University
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3 **PhD** student in the **Medical School** of University of Crete with interest on **Exercise Physiology, Biochemistry, Heamatology, and Endocrinology of Soccer**

FOREIGN LANGUAGES

Proficiency Degree (English Language)

.

SCIENTIFIC PUBLICATIONS

1. **Koundourakis NE**, Androulakis N, Spyridaki EC, Castanas E, Malliaraki N, Tsatsanis C, Margioris AN. Effect of different seasonal strength training protocols on circulating androgen levels and performance parameters in professional soccer players. Hormones (Athens). 2014 Jan;13(1):578-83.

2. **Koundourakis NE**, Androulakis NE, Malliaraki N, Tsatsanis C, Venihaki M, Margioris AN. Discrepancy between exercise performance, body composition, and sex steroid response after a six-week detraining period in professional soccer players. PLoS One. 2014 Feb 19;9(2):e87803. doi: 10.1371/journal.pone.0087803. eCollection 2014.

3. **Vitamin D and exercise performance in professional soccer players** Nikolaos E Koundourakis, Nikolaos E Androulakis, Niki Malliaraki, Andrew N Margioris (Accepted for publication online at l June 3 2014. Plos One Journal.)

PRESENTATION IS SCIENTIFIC CONGRESSES

POSTER PRESENTATIONS

A. Sports Medicine – Exercise Physiology

1.Routine blood and urine tests in team athletes (football players): Are they necessary ?

Nikolaos Androulakis, **Nikolaos Koundourakis**, , Fotini Plati, Emmanouil Tsamis, Antonios Manidakis, & Niki Malliaraki

III International Congress on "People, sport and health". April 19-21 2007 St. Petersburg Russia.

2.Preventing Iron deficiency and anemia in professional and semi-professional football players.

[1]Nikolaos Androulakis, [3]**Nikolaos Koundourakis**, [2]Fotini Plati, [2]Emmanouil Tsamis, [4]Antonios Manidakis, & [2]Niki Malliaraki

III International Congress on "People, sport and health". 19-21 April 2007 St. Petersburg Russia

3.Alterations of specific Hormonal and Biochemical parameters during a soccer game.

Koundourakis N., Androulakis N., Mitrotasios M., Mpekris E., Manidakis A., Tsainis., Sotiropoulos A.

2^{o} Scientific World Congress on Soccer. 4-6 May 2007, Trikala, Greece.

4.Low Testosterone to Cortisol ratio and muscle injury in professiolan football players

N. Androulakis, N. Koundourakis, M. Tampakaki, K. Papoutsakis, A. Manidakis & J. Christoforakis

XXXI congress of the International Federation of Sports Medicine (FIMS),San Juan, Puerto Rico, 19 to 22 May, 2010.

5.B12 and Folate status in professional and semi-professional soccer players.

Androulakis Nikolaos**, Koundourakis Nikolaos**, MalliarakiNiki, Plati Fotini, Tsamis Emmanouil, Manidakis Antonios,Margioris Andrew

12th Annual Congress of the European College of Sports Sciense 11-14 July 2007, Jyvaskyla Finland

6.Sweat losses during game and practice in professional soccer players

Androulakis N, **Koundourakis N**, Christoforakis J, Kakavelaki K, Manidakis A, Manidaki A& Margioris A

30rd FIMS WORLD CONGRESS OF SPORTS MEDICINE ,18-23 November 2008, Barcelona, Spain.

7.Effects of pre-hydration on muscular power after intense soccer training

Koundourakis N,Androulakis N**,** Christoforakis J, Sassi R[3], Malliaraki N, Manidakis A, & Margioris A

30rd FIMS WORLD CONGRESS OF SPORTS MEDICINE 18-23 November 2008

8.The effects of different types of training on core temperature and sweat losses in adolescent soccer players

XXXIII congress of the International Federation of Sports Medicine (FIMS), Quebec, Canada, June 18-21, 2014.

Nikolaos E. Androulakis**, Nikolaos E. Koundourakis**, Nikolaos G. Tsakalis, Josef J. Christoforakis

9.The prevalence of anemia in professional and semiprofessional soccer players

XXXIII congress of the International Federation of Sports Medicine (FIMS), Quebec, Canada, June 18-21, 2014.

Nikolaos E. Androulakis**, Nikolaos E. Koundourakis**, Nikolaos G. Tsakalis, Maria Kokonozaki, Michael Alexandrakis.

B. Exercise Physiology – Training

1.The effects of a specific training program on the physical abilities of adolescent soccer players.

Koundourakis N., Tsainis I., Titomixelakis L., Mitrotasios M., Manidakis A., Sotiropoulos A. 2^{o} Scientific World Congress on Soccer. 4-6 May 2007, Trikala, Greece.

2.The effects of high intensity endurance interval training on VO_2max and soccer performance.

N. Koundourakis, N. Androulakis, A. Manidakis, N. Malliaraki, C. Zelenitsas, & A. Margioris. The VIIth World Congress on Science & Football 26 to 30 May 2011 Nagoya, Japan

ORAL PRESENTATIONS

(Presented by Nikolaos Koundourakis)

1.Preventing Iron deficiency and anemia in professional and semi-professional football players.

Nikolaos Androulakis, **Nikolaos Koundourakis**, Niki Malliaraki Fotini Plati, Emmanouil Tsamis, Antonios Manidakis, & George Katrinakis

VIIth World congress on science and football. January 16-20, 2007 Antalya, Turkey.

2. **The effects on strength training on androgens and soccer performace parameters in professional soccer players**

N.E. Koundourakis, N. Androulakis, M. Benixaki, N. Maliaraki, C Tsatsanis, A.N. Margioris

41st Hellenic Congress Endocrinology and Metabolism. 14-17 May 2014 Athens, Greece

Seminars – Congresses

TIME PERIOD	ORGANISATION	SUBJECT
16/1 - 20/1/2007	**Turkish Football Federation**	VIIth World congress on science and football
11/07-14/7 2007	**The state Russian Federal Agency of Physical Culture and Sport**	III International Congress on "People, sport and health".
04/05 – 06/05 2007	**Hellenic Football Federation** **&** **University of Thessaly** **Department of Physical Education**	2^{o} Scientific World Congress on Soccer.
16/05-17/05 2008	**Swiss Society of Sports Medicine**	3rd International Football Medicine Congress
19/05-22/05 2010	**The International Federation of Sports Medicine**	XXXI Fims Sport Medicine World Congress
26/05 – 30/05 2011	**Japanese Society of Science & Football**	7th World Congress on Science & Football 2011 & 9th Annual Conference of Japanese Society of Science & Football 2011
22 November 2013 -	**Greek Minister of Sports** **Basketball Coaching School**	Exercise Physiology lectures- Basic Principles (Speaker)
18/06/2014 - 21/06/2014	**The International Federation of Sports Medicine**	XXXIII FIMS World Congress of Sports Medicine

Attendance of Soccer Clubs Conditioning Trainings

1. FC Shcalke May 2009
2. FC Liverpool 2010
3. FC Utrecht January 2012

BUSINESS EXPERIENCE

PAST YEARS OCCUPATION			
PERIOD OF TIME	**ORGANIZATION**	**POSITION**	**JURISDICTION**
August 2013	Platanias Fc	Exercise Physiologist	Ergometry
August2012-	Episkopi FC (football league)	Exercise Physiologist	Ergometry Organization- Construction Of Training Programs Rehabilitation of Injured Players Sports Nutrition-Sports Supplements
October 2011 – October 2012	Ergotleis FC	Exercise Physiologist First Team, U20, and U17 Teams	Ergometry Soccer Training-Conditioning Coaching Rehabilitation of Injured Players Sports Nutrition-Sports Supplements
October 2011 – October 2012	Ergotleis FC Soccer Academy	Exercise Physiologist	Ergometry Training Control Rehabilitation of Injured Players
March 2012-	Lido Soccer Sports Center	Lido Soccer General Director	Sports Center Organization and Function Organization- Construction Of Training Programs

			Coordination Training
June 2006 – June 2011	OFI Crete FC	Conditioner Coach – Exercise Physiologist	Ergometry Soccer Training-Conditioning Coaching Rehabilitation of Injured Players Sports Nutrition-Sports Supplements
October 2006 – June 2007	Lido Soccer Sports Center	Lido Soccer General Director	Sports Center Organization and Function Organization- Construction Of Training Programs Coordination Training
July 2004 - August 2010	OFI Crete FC Soccer Academy	Exercise Physiologist Training control - organization	Ergometry (Training Control) Organization- Construction Of Training Programs
June 2005 –2011	Irodotos FC	Exercise Physiologist	Ergometry Training Control Sports Nutrition-Sports Supplements
June 2005 – July 2006	OF Ierapetras FC	Exercise Physiologist	Ergometry Training Control Sports Nutrition-Sports Supplements
April 2000 – June 2005	OFI Crete FC	Conditioner Coach – Exercise Physiologist	Ergometry Soccer Training-Conditioning Coaching Rehabilitation of Injured Players Sports Nutrition-Sports Supplements

May 2000 until today	Sports Clubs	Ergometry	***Football Teams***: Platanias FC, AOX FC, Apolon FC, Rhodes FC, Xersonisos FC, Poa FC, Agios Nikolaos FC, Asteras Rethimno FC, Eolikos FC ***Amateur Football Clubs***: AOT FC, PAOK FC etc. ***Football Camps:*** 2^{o} ,3^{o} ,4^{o}, 5^{o} Hellenic Football Camp ***Basketball Teams:*** Iracklio BC Rethimno BC OFI BC ***Track and Field :*** OFI Track and Field Department
July 2013		Conditioning Head Instructor	Geralds Soccer Camp
May 2000 until today	Soccer and athletic related individuals	Personal Conditioning Training – Rehabilitation- Ergometry –Sports nutrition	Top Level Soccer Players Amateur Soccer Players Adolescent Soccer Players Professional Basketball Players Track and Field Athletes Soccer referees