**UNIVERSITY OF CRETE**
**DEPARTMENT OF SCIENCES OF HEALTH**
**FACULTY OF MEDICINE**
**GRADUATE PROGRAM IN "THE MOLECULAR BASIS OF HUMAN DISEASES"**

# "EVALUATION OF THE NUTRITIONAL EFFECT ON THE IMMUNE PROFILE IN OBESE INDIVIDUALS"

# «ΧΑΡΑΚΤΗΡΙΣΜΟΣ ΤΗΣ ΕΠΙΔΡΑΣΗΣ ΤΗΣ ΔΙΑΤΡΟΦΗΣ ΣΤΟ ΑΝΟΣΟΛΟΓΙΚΟ ΠΡΟΦΙΛ ΠΑΧΥΣΑΡΚΩΝ ΑΤΟΜΩΝ»

## ANASTASOPOULOU DIMITRA

(Class of 2017-2019)

Laboratory of Biology, Department of Medicine, National and Kapodistrian University of Athens, Greece

**Main supervisor:**

Aristides G. Eliopoulos, PhD, Professor of Biology & Genetics, Head of Department of Biology, Medical School, National & Kapodistrian University of Athens

**Secondary supervisors:**

Georgios Chamilos, MD, Associate Professor, Clinical Microbiology and Microbial Pathogenesis Lab, School of Medicine, University of Crete

Christos Tsatsanis, PhD, Professor, Laboratory of Clinical Chemistry, School of Medicine, University of Crete, Director, Laboratory of Clinical Chemistry-Biochemistry, University Hospital of Heraklion, Heraklion 71003, Crete, Greece

HERAKLION 2019

# CONTENTS

## 1. ABSTRACT

Obesity is an emerging nutritional problem associated with low level chronic inflammation and deregulated immunity. Adipocytes that increase in number and size under obese condition are responsible for the production of an array of cytokines (adipokines) that trigger the inflammatory response and modify circulating immune cell numbers. Weight loss has a direct effect upon inflammation and can be achieved through personalized nutrition that is based on each person's characteristics. 34 obese volunteers (BMI= 34.45±1.08 kg/m$^2$) undergoing a weight loss program [personalized intervention group following a genotype- based diet (7.30±5.5% weight loss, n=4), conventional diet group following conventional low- calorie diet (8.76±3.82% weight loss, n=7)] and 15 lean volunteers (BMI= 22.64±0.52 kg/m$^2$) were recruited. PBMCs were isolated by density gradient centrifugation and leukocyte subpopulations were assessed before and after a 3-month intervention by flow cytometry. Statistical analysis was performed using the unpaired Mann- Whitney t- test (two- tailed) (differences in cell absolute counts and percentages between obese and lean participants) and two- way analysis of variance (ANOVA) (differences in PBMCs subtypes levels between genotype-based and conventional diet following the 3- month intervention). Our data demonstrate that obesity is associated with a significant increase in circulating PBMCs and memory T cells confirming the proinflammatory status of obese individuals. Moreover, a trend towards an increase in total leukocytes, neutrophils, CD3$^+$ cells, B lymphocytes, CD4$^+$ T cells, naïve and terminally differentiated effector memory (TEMRA) T cells, CD45RO$^+$CXCR3$^+$ T cells, T regulatory cells, as well as non- classical, intermediate and CD14$^{low}$CD16$^+$ monocytes was reported in obese participants. Conversely, a trend towards a reduction in basophils, eosinophils, classical monocytes, lymphocytes, natural killer (NK) cells, CD8$^+$ and CD45RA$^+$CXCR3$^+$ T cells was reported in the obese group. Furthermore, the genotype- based type of intervention induced a statistical significant reduction in naïve T cells and a statistical significant increase in TEMRA cells indicating that weight loss is in line with a more differentiated status of CD4$^+$ T cells. Thus, further studies are needed in order to confirm this trend in the intermediate stages, i.e. central and effector memory T cells.

## 1. ΠΕΡΙΛΗΨΗ

Η παχυσαρκία αποτελεί ένα διαρκώς αναπτυσσόμενο πρόβλημα και σχετίζεται με χαμηλού βαθμού χρόνια φλεγμονή. Στην παχυσαρκία, τα κύτταρα του λιπώδους ιστού αυξάνονται σε αριθμό και μέγεθος και παράγουν μεγάλο αριθμό κυτταροκινών οι οποίες ενεργοποιούν την φλεγμονώδη απόκριση και τροποποιούν τα επίπεδα των περιφερικών κυττάρων του ανοσοποιητικού συστήματος. Η απώλεια βάρους επηρεάζει άμεσα τη φλεγμονή και μπορεί να επιτευχθεί, μεταξύ άλλων, μέσω της εξατομικευμένης διατροφής. 34 παχύσαρκοι εθελοντές (BMI= 34.45±1.08 kg/m$^2$) οι οποίοι συμμετείχαν σε πρόγραμμα απώλειας βάρους [εξατομικευμένη διατροφή (7.30±5.5% απώλεια βάρους, n=4), κλασική υποθερμιδική δίαιτα (8.76±3.82% απώλεια βάρους, n=7)] και 15 νορμοβαρείς εθελοντές (BMI= 22.64±0.52 kg/m$^2$) συμπεριλήφθηκαν στη μελέτη. Για την απομόνωση των μονοπύρηνων κυττάρων του περιφερικού αίματος (PBMCs) χρησιμοποιήθηκε φυγοκέντρηση σε κλίση πυκνότητας και οι υποπληθυσμοί των λευκών αιμοσφαιρίων αξιολογήθηκαν πριν και μετά την τρίμηνη παρέμβαση με κυτταρομετρία ροής. Η στατιστική επεξεργασία των αποτελεσμάτων πραγματοποιήθηκε με Mann- Whitney t- test για την ανάδειξη των διαφορών στους απόλυτους αριθμούς και τα ποσοστά μεταξύ παχύσαρκων και νορμοβαρών εθελοντών και με ανάλυση διασποράς (ANOVA) για τις διαφορές στα επίπεδα των PBMCs στο τέλος της παρέμβασης μεταξύ των ατόμων που ακολούθησαν εξατομικευμένη και κλασική υποθερμιδική δίαιτα. Βάσει των αποτελεσμάτων μας, η παχυσαρκία σχετίζεται με μία στατιστικά σημαντική αύξηση στα επίπεδα των κυκλοφορούντων PBMCs και των Τ κυττάρων μνήμης επιβεβαιώνοντας με τον τρόπο αυτό την προ-φλεγμονώδη κατάσταση που χαρακτηρίζει τα παχύσαρκα άτομα. Επιπλέον, μία τάση για αυξημένη συχνότητα των λευκών αιμοσφαιρίων, ουδετερόφιλων, CD3$^+$ κυττάρων, Β λεμφοκυττάρων, CD4$^+$ Τ κυττάρων, αδιαφοροποίητων και τελικώς διαφοροποιημένων (TEMRA) Τ κυττάρων μνήμης, CD45RO$^+$CXCR3$^+$ Τ κυττάρων, Τ ρυθμιστικών κυττάρων, όπως επίσης και των μη-κλασικών, ενδιάμεσων και CD14$^{low}$CD16$^+$ μονοκυττάρων παρατηρήθηκε στους παχύσαρκους εθελοντές. Στην ίδια ομάδα εθελοντών καταγράφηκε μία τάση για μείωση στα επίπεδα των κυκλοφορούντων βασεόφιλων, ηωσινόφιλων, κλασικών μονοκυττάρων, λεμφοκυττάρων, φυσικών φονικών κυττάρων (NK), CD8$^+$ και CD45RA$^+$CXCR3$^+$ Τ κυττάρων. Επιπρόσθετα, η απώλεια βάρους χάρη στην εξατομικευμένη δίαιτα οδήγησε σε μία στατιστικά σημαντική μείωση στα επίπεδα των κυκλοφορούντων αδιαφοροποίητων Τ κυττάρων και σε αύξηση στα TEMRA κύτταρα υποδηλώνοντας ότι η απώλεια βάρους είναι συνυφασμένη με μία περισσότερο διαφοροποιημένη κατάσταση των Τ κυττάρων. Συνεπώς, περαιτέρω μελέτη απαιτείται για να επιβεβαιωθεί αυτή η τάση και για τα ενδιάμεσα στάδια των Τ κυττάρων μνήμης.

## 2. INTRODUCTION

### 2.1 Obesity

The worldwide epidemic of obesity is an emerging nutritional problem that, along with its related disease cluster represents a global public health challenge. Based on the criteria published by the US National Institutes of Health and the World Health Organization (WHO), individuals with a body mass index (BMI) 25-30 kg/m$^2$ are considered overweight, those with a BMI 30-40 kg/m$^2$ are considered obese and a BMI>40 kg/m$^2$ is considered morbid obesity, whereas normal weight individuals are those with a BMI 20-25 kg/m$^2$. Although BMI serves as a useful tool for the evaluation of obesity, the characterization of obesity state using BMI is not always effective because it usually underestimates fat mass in females and overestimates the total amount of fat in muscular individuals [1, 2].

According to the WHO, at least one billion adults are overweight, 300 million are obese, and an increase in those numbers is expected in the future without intervention [3]. Based on the latest statistics, overweight affects 30-70% and obesity affects 10-30% of the adult population in Europe [4], whereas in the United States, obesity is prevalent in 34.9% of adults [5]. Obesity is related to 4 million deaths and 120 million disability- adjusted life- years [6] and it is estimated to cost $147 billion dollars to the healthcare system annually [7].

It is now evident that obesity not only affects developed countries but also low-income countries where poverty and malnutrition are most widespread [8]. Both obesity/ overweight and childhood malnutrition/ underweight are among the top 10 causes of global mortality and disease [9-11]. The most worrying fact about obesity is that its prevalence has tripled in the past thirty years driving the occurrence of serious health problems that will affect their adult life [12].

#### 2.1.1 Cause of obesity

Increased energy intake and decreased energy expenditure is the leading cause of obesity resulting in increased number (hyperplasia) and size (hypertrophy) of adipocytes [13]. Those metabolic cells are responsible for the emergence of metabolic signals that trigger the inflammatory responses and the disruption of metabolic homeostasis. Physical activity, food consumption, and the dissipation of energy as

heat via inducible thermogenesis in beige adipocytes of white adipose tissue (WAT) and via constitutive thermogenesis in brown adipocytes of brown adipose tissue (BAT) regulate energy balance. Several factors including the sympathetic nervous system and mechanisms within the cells affect thermogenesis that is triggered in response to diet, exercise or clod exposure [14].

Lipid uptake and storage, as well as fatty acid mobilization in response to increased energetic requirements are the main responsibilities of WAT. Despite the storing role of this tissue, adipocytes also possess an endocrine role producing cytokines (adipokines), peptides and hormones and consequently affecting immunity and energy balance [15]. Adipokine production, including leptin, adiponectin, resistin and visfatin, contributes to the preservation of the inflammatory mechanism that modulates several physiopathological processes [16] (Figure 1).

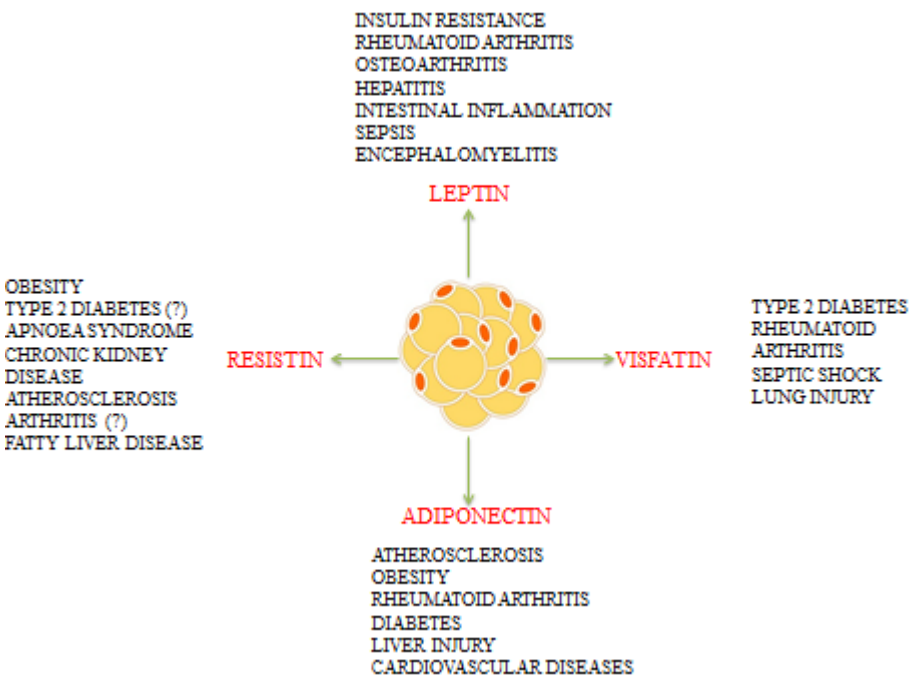



**Figure 1**. The effect of the production of major adipokines (adiponectin, leptin, resistin, visfatin) by adipocytes on the development of several inflammatory diseases [16].

During weight gain, an increase in adipocyte size takes place due to excess lipid storage leading to the disruption of normal cellular function. Since adipocytes are not

able to store excess lipids any more, the latter are redirected to the liver enhancing dyslipidemia along with plasma free fatty acids, low- density lipoprotein (LDL), and triglycerides increase, and a diminution in high- density lipoprotein (HDL) levels. Additionally, increased lipolysis of triglycerides in large adipocytes results in the formation of glycerol and free fatty acids, the latter of which contribute to the increase of their plasma levels and liver triglyceride synthesis [17].

In case of obesity, several proinflammatory factors including leptin, tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), are produced by adipocytes and are released into the circulation whereas the production of anti- inflammatory molecules such as adiponectin, is decreased compared to lean adipose tissue (AT) [18] (Figure 2). Interestingly, the circulating levels of the anti- inflammatory cytokine interleukin-10 (IL-10) have been found elevated during obesity [16]. Elevated numbers of M1-polarized proinflammatory macrophages and $CD8^+$ T cells which are thought to stimulate the development of insulin resistance derive from the excessive energy storage in obese AT [19].

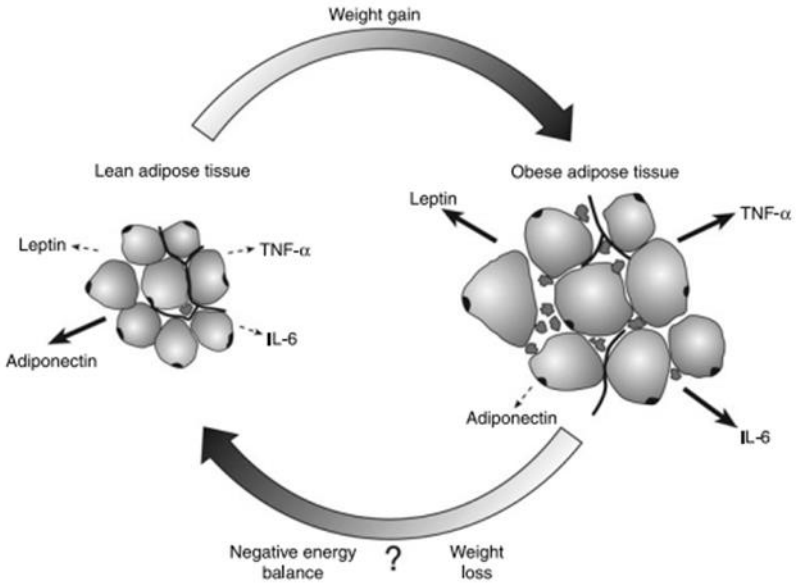



**Figure 2**. In obesity, adipocytes increase in size (hypertrophy) and they are responsible for the increased production of proinflammatory agents (including TNF-α, IL-6, and leptin) and the decreased production of the anti- inflammatory factor, adiponectin. This inflammatory condition can be reversed in case of weight loss most probably due to a negative energy balance period [18].

Insulin resistance is the state during which an increase in insulin levels does not cause increased uptake of glucose by liver and muscle, leading to increased insulin production by pancreatic beta cells. Increased abundance of circulating free fatty acids in obesity might lead to β- cell malfunction and type 2 diabetes (T2D) occurrence [17]. Obesity- induced inflammation might contribute to a variety of effects on several tissues, including dysbiosis and intestinal permeability (gut), elevated food intake (central nervous system), insulin insensitivity (liver, muscle, AT), and decreased insulin secretion from pancreatic islets [20] (Figure 3). Obesity- driven liver inflammation, leads to the development of non- alcoholic fatty liver disease (NAFLD), non- alcoholic steatohepatitis (NASH) and cirrhosis [19].

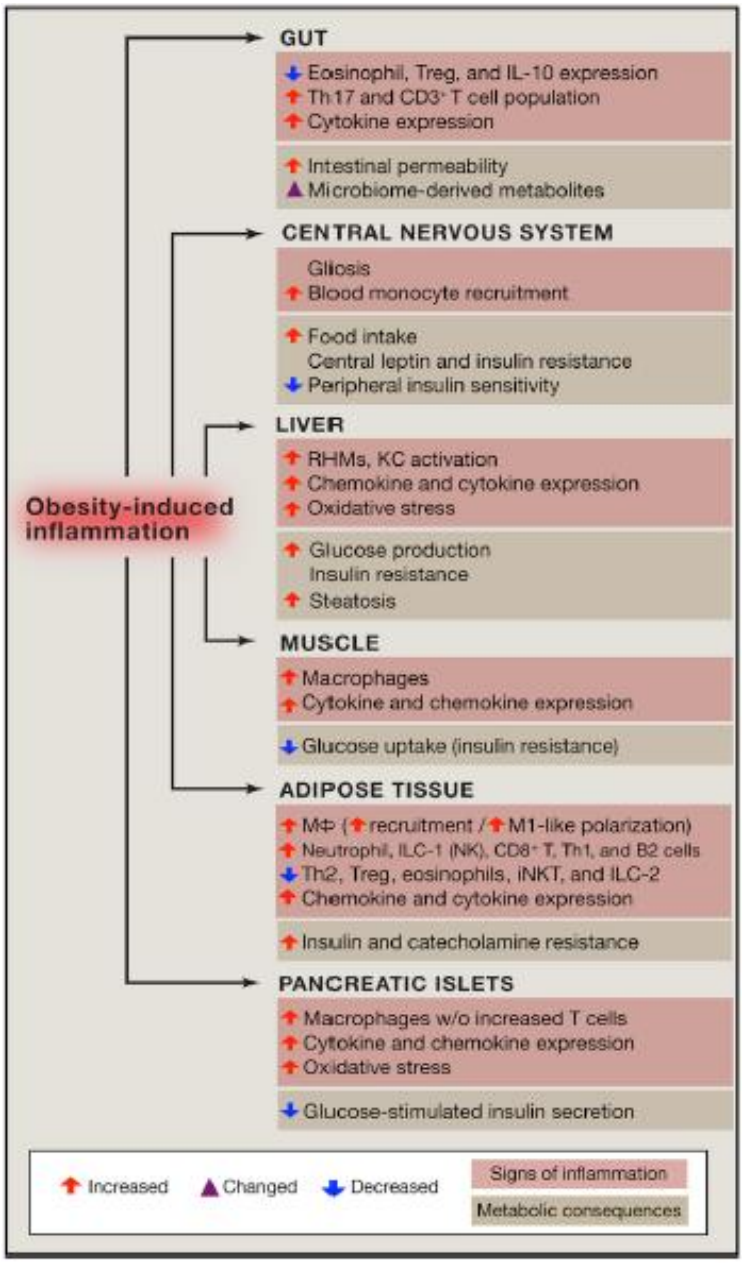


**Figure 3**. The immunomodulatory and metabolic effect of obesity- induced inflammation on several tissues (Treg: T regulatory cells, Th17: T helper 17 cells, RHMs: recruited hepatic macrophages, KC: Kupffer cells, MΦ: macrophages, ILC-1: innate lymphoid type 1 cells, Th1: T helper 1 cells, Th2: T helper 2 cells, iNKT: invariant natural killer cells, ILC-2: innate lymphoid type 2 cells) [20]

AT dysfunction under the state of excess body weight is further supported by the hypoxic environment of the tissue and the activation of the endoplasmic reticulum (ER) stress response [19]. Those events might affect several tissues and might act upon parenchymal cells to secrete an array of chemokines. The latter trigger chemotaxis and migration of several immune cells including macrophages into the inflamed tissue. Macrophages secrete a variety of proinflammatory factors that result in development of insulin resistance in liver, muscle and AT, as well as in decreased insulin secretion from β cells [20].

The increase in obesity- related inflammatory markers contributes to the orchestration of a state called chronic or low-grade systemic inflammation [18] in which inflamed vascular endothelium and activated immune cells are involved; both of them are potentially affected by nutrient excess, dyslipidemia, hyperglycemia or oxidative stress [21]. A more recent term used to describe the metabolically triggered inflammation that is characterized by the elevated levels of inflammatory factors observed in obesity is "meta-inflammation" (i.e. inflammation in metabolic tissues) [22]. The latter represents the absence of clinical manifestations of inflammation and a modest increase in circulating proinflammatory agents [23]. It is also noteworthy that dietary intake behavior has a direct effect on the composition of gut microbiota along with considerable consequences on immune response [24].

### 2.1.2 Inflammation & obesity- historical facts

The association between inflammation and metabolic perturbations, including obesity and insulin resistance, was first discussed in 1993, when Hotamisligil et al. [25] demonstrated the constitutive expression of the proinflammatory cytokine TNF-α by adipocytes and its increased expression in adipocytes of obese animals (ob/ob mouse lacking leptin secretion, db/db mouse lacking leptin receptor, and fa/fa Zucker rat).

This inflammatory phenotype was reversed when neutralization of TNF-α by soluble TNF-α receptor took place leading to an improvement in insulin resistance. These data were the first to reveal a potential link between the increased levels of a proinflammatory cytokine and insulin resistance. Further, TNF-α production is known to orchestrate glucose and lipid metabolism, inflammatory processes and immune response [26].

Later on, increased expression of TNF-α in human AT, as well as elevated plasma TNF-α levels in obese individuals were observed. Those levels decreased following weight loss indicating the association between TNF-α expression and BMI [27-29]. Obesity is also characterized by elevated levels of TNF receptor [30, 31]. However, infusions of TNF-α antibody were not effective against the reduction of TNF-α activity and amelioration of insulin resistance in human obesity [32].

The discovery of leptin, a 16-kDa non-glycosylated anorexia peptide, and its production by adipocytes in 1994 by Zhang et. al [33] shed light into the active endocrine role of AT, as it could no longer be regarded as an organ that solely stores fat. The levels of circulating leptin directly correlate with the amount of body fat stored and with adipocyte size [34]. This proportion can be attributed to the induction of energy expenditure and to the suppression of food intake triggered by overproduction of leptin in obesity that directly affects hypothalamic cell populations [35-38]. Upregulated metabolic rate and decreased food intake along with weight reduction are observed in lean or leptin deficient ob/ob mice that were administered with leptin [39]. From an immunological point of view, leptin enhances proliferation and inhibits apoptosis in several immune cell types, such as Th1 $CD4^+$ cells, as well as this peptide triggers the secretion of proinflammatory agents including TNF-α (early), IL-6 (late) and interleukin-1 (IL-1) by macrophages [40, 41].

Leptin production, at lower levels, is conducted by other tissues as well such as the bone marrow, skeletal mass, stomach, and placenta [42] and can be induced by inflammatory cytokines, including TNF-α, leukemia inhibitory factor and IL-1 [43]. Leptin receptors are broadly distributed in fat, muscle, brain, small intestine and colon, and their continuous stimulation by high leptin concentration during chronic obesity may lead to leptin resistance [44, 45]. Given the expression of the isoform of leptin receptor, obRb, involved in leptin signaling by nearly all cells of the innate

immune system (Figure 4), it is expected that the majority of immune cells are impaired in mice lacking leptin signaling [46].

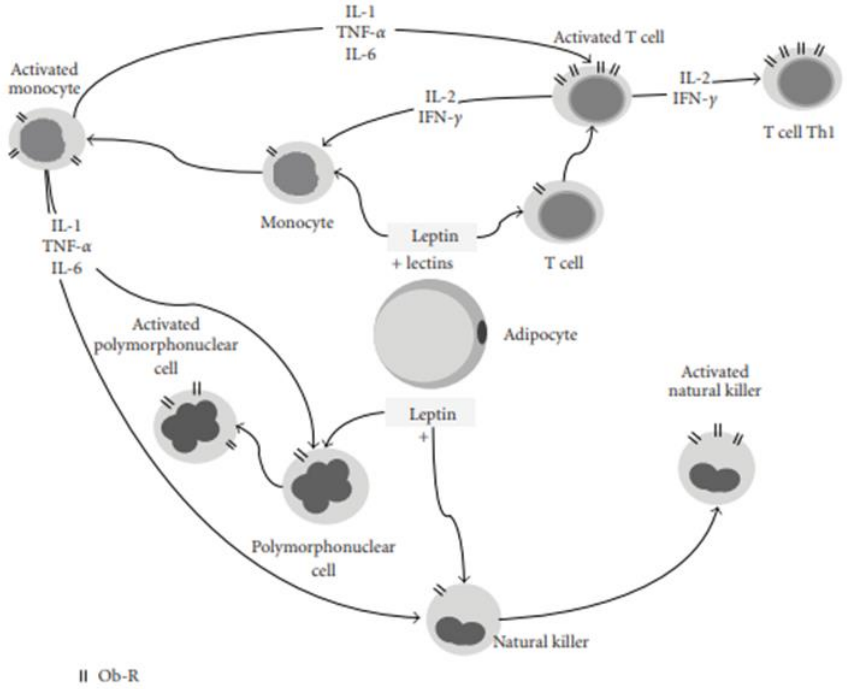



**Figure 4**. The crosstalk between leptin and cells of the immune system expressing leptin receptor (Ob-R) (IL-1: interleukin-1, IL-2: interleukin-2, TNF-α: tumor necrosis factor alpha IFN-γ: interferon- gamma) [15]

Leptin signaling is conducted through a Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway leading to signal transducer and activator of transcription 3 (STAT3) translocation into the nucleus and expression of leptin- induced genes, such as suppressor of cytokine signaling-3 [47]. The latter acts as a negative feedback mediator of JAK/STAT signaling pathway and may lead to central and peripheral leptin resistance [47]. Leptin resistance can be generally attributed to impaired leptin receptor signaling, impaired leptin transport across the blood- brain barrier and inhibition of neuronal circuits [48].

A genetic mutation inhibiting proper synthesis of leptin results in the development of morbid obesity and impaired immune response [49]. Lymphoid organ atrophy and immune dysfunction, along with decreased levels of circulating T and B cells, are reported in obese ob/ob and db/db mice in which truncation of the leptin receptor is

observed. Thus, leptin might play an active role in lymphopoiesis [39]. Moreover, leptin and leptin receptor deficiency are related to resistance in autoimmune manifestations, including multiple sclerosis, type 1 diabetes (T1D), and experimental colitis in mice, but also increased susceptibility to infections [50].

Several other proinflammatory factors are also elevated in the plasma of obese individuals, including plasminogen activator inhibitor-1 (PAI-1) [51], serum amyloid A (SAA), interleukin-8 (IL-8), C-C chemokine ligand 2 (CCL2)/ monocyte chemotactant protein 1 (MCP-1), CCL5/ regulated on activation, normal T cell expressed and secreted (RANTES) [21, 22, 52], IL-6 [53], and C- reactive protein (CRP) [1, 54-56], contributing to obesity- induced inflammation.

CRP belongs to the pentraxin family of proteins and its secretion by the liver is induced by IL-6 production in inflammatory states such as trauma and sepsis [57-59]. Its levels follow the trigger or resolution of inflammation and stimulate the production of pro- or anti- inflammatory cytokines through its interaction with immune cells. TNF-α stimulates the production of IL-6 which in turn induces CRP synthesis in the liver [54]. The levels of CRP have been observed to increase progressively with increasing BMI [60] and to decrease following weight loss regardless of the intervention used to induce body weight reduction [56, 61-64].

Mature adipocytes of WAT are responsible for the production of the anti-inflammatory peptide hormone adiponectin. The gene that codes for this hormone is located at a susceptibility locus for cardiovascular disease and diabetes [65]. Visceral obesity and insulin resistance are associated with decreased levels of adiponectin [66, 67] whereas this proportion could be reversed in case of weight loss and with the use of thiazolidinediones that promote insulin sensitivity [68]. Further, adiponectin treatment has been reported to improve insulin resistance [69]. This hormone could be also produced by non fat cells, such as endothelial cells, skeletal muscle cells and cardiac myocytes [70].

Adiponectin affects natural killer (NK) cell cytotoxicity [71] and stimulates the synthesis of anti- inflammatory cytokines [72] such as IL-1RA (receptor antagonist) and IL-10 by human dendritic cells, macrophages and monocytes [70]. Conversely, adiponectin inhibits the production of proinflammatory cytokines, including TNF-α

and interferon- γ (IFN-γ) [70], whereas the secretion of adiponectin is inhibited by proinflammatory cytokines including TNF-α [70, 73] and IL-6 [70, 74].

Weight loss has a direct effect upon inflammation. In that case, the levels of inflammatory markers, such as CRP, TNF-α, IL-6 and leptin, seem to decrease whereas the levels of the anti- inflammatory marker adiponectin increase. Forsythe et al. [18] concluded that the most outstanding improvements in inflammation were observed only when at least 10% weight loss was achieved and when weight loss was a result of low- calorie diet (with or without lifestyle changes) and gastric bypass surgery.

### 2.1.3 Co-morbidities

Obesity-related co-morbidities are subsequently linked to reduced life expectancy and premature death. Among them are hyperlipidemia, hypertension, as well as insulin resistance that leads to T2D, all being part of the metabolic syndrome [9, 75]. This association is further supported by a parallel increase in the prevalence of both obesity and diabetes during the last 20 years [76]. The rise in human obesity is closely related to an increase in several other diseases including cardiovascular disease, hepatic steatosis, neurodegeneration, airway disease, and biliary disease [22, 77] (Figure 5).

Moreover, obese individuals are at high risk of multiple forms of autoimmunity, including T1D, multiple sclerosis and thyroid autoimmunity [78-82]. Obesity has also been associated with various types of cancer (Figure 5), including thyroid, colorectal, gallbladder, pancreatic, esophageal, kidney, breast and endometrial, leukaemia, non-Hodgkin's lymphoma and multiple myeloma [83-86].

The link between obesity and increased susceptibility to several infectious diseases (Figure 5), such as Hepatitis C, pneumonia [87] and influenza [46, 88, 89] with respiratory, periodontal, nosocomial, surgical or post- operative nature [71] can be attributed to impaired host defense during obesity. Further, obese individuals seem to respond differently to vaccination and several drugs, including antibiotics [71].

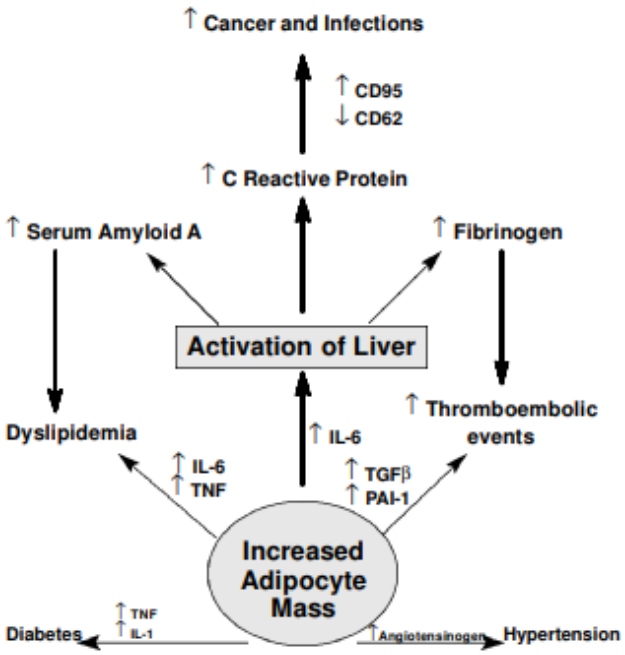



**Figure 5**. The association between increased adipocyte mass and possible existing co-morbidities, including diabetes, hypertension, dyslipidemia, thrombotic events, cancer and infections, under obese condition [90].

## 2.2 Immune response (peripheral blood leukocyte subclasses)

The active role of AT in the preservation of obesity- induced inflammation impacts on immune cell numbers that are recruited and on their circulating numbers. Both innate and adaptive immunity cooperate during the inflammatory process involved in obesity.

Several studies report elevated leukocyte numbers in obese children and adults [91] and a positive correlation between the number of total white blood cells and BMI [92].

### 2.2.1 Innate immunity

#### 2.2.1.1 Polymorphonuclear leukocytes

**Neutrophils**

Neutrophils are the first cells recruited to site of infection where they trigger strong inflammatory reactions through the production of several cytokines and chemokines including IL-1β, IL-8, TNF-α and MIP-1a [69]. There, they combat invading

pathogens either by phagocytosis or by degranulation of their antimicrobial factors [93]. The recruitment of cells participating in innate and adaptive immunity, such as dendritic cells, macrophages and lymphocytes, is regulated by neutrophils [94]. Although neutrophils only express the short form of leptin receptor [95], leptin signaling is enough to promote chemotaxis, the expression of CD11b, the production of reactive oxygen species (ROS) and neutrophil survival [15, 16, 71].

Obesity is associated with elevated neutrophil counts (i.e. neutrophilia) [96], increased AT infiltration by those cells [97] and greater activation of their status in plasma [98]. Their numbers are found elevated in response to high fat diet [24] and increase more rapidly than macrophages [99] that dominate in more chronic conditions [97]. Body weight loss results in decreased neutrophil levels regardless of the type of intervention [100]. The correlation between neutrophil levels and BMI has even been observed in children [101], while they are also related to an increase in inflammatory and insulin resistance markers [100]. Further, neutrophil inhibition results in amelioration of liver and AT inflammation, as well as in improvement in insulin resistance induced by obesity [102].

**Eosinophils**

Eosinophils are among the less abundant circulating granulocytes (Figure 6) and they play a vital role in allergic inflammation. They control AT inflammation by creating an anti- inflammatory environment through the secretion of IL-4 and IL-13 and adipose tissue macrophages (ATM) maintenance in M2 state [103]. Eosinophil's survival and function were shown to be induced by leptin action [100] that promotes the chemokinesis of eosinophils and triggers the production of inflammatory cytokines, such as IL-1β and IL-6, and chemokines, including IL-8, MCP-1 and growth-related oncogene-alpha. Leptin also stimulates the expression of the adhesion molecules ICAM-1 and CD18 on neutrophils cell surface, but inhibits L-selectin and ICAM-3 [15].

Data concerning eosinophils number during human obesity are inconsistent. No association between eosinophils levels (absolute count or percentage) and BMI/ metabolic syndrome has been reported by several studies [100]; conversely, eosinophil percentage of total leukocytes was elevated in a group of morbidly obese individuals with several comorbidities compared to the healthy lean group and this

percentage did not follow weight loss after Roux-en-Y gastric bypass (RYGB) surgery [104]. Moreover, eosinophils were decreased in number in VAT of obese mice [105] and mice that lack eosinophils are characterized by insulin resistance and increased inflammation [106].

**Basophils**

Basophils constitute the vast minority among granulocytes and all classes of total circulating leukocytes (Figure 6). They stimulate hypersensitivity inflammatory reactions by binding antigen-specific immunoglobulin E (IgE) that triggers the production of several factors involved in allergic reactions including histamine [100]. Leptin receptor expression on basophil's cell surface has been implicated in prolonged survival, activation, cytokine production, degranulation and migration [107]. The correlation between BMI/metabolic syndrome and basophils circulating levels has been found positive, negative or absent [100], indicating the lack of information concerning the role of basophils in obesity- induced inflammation.

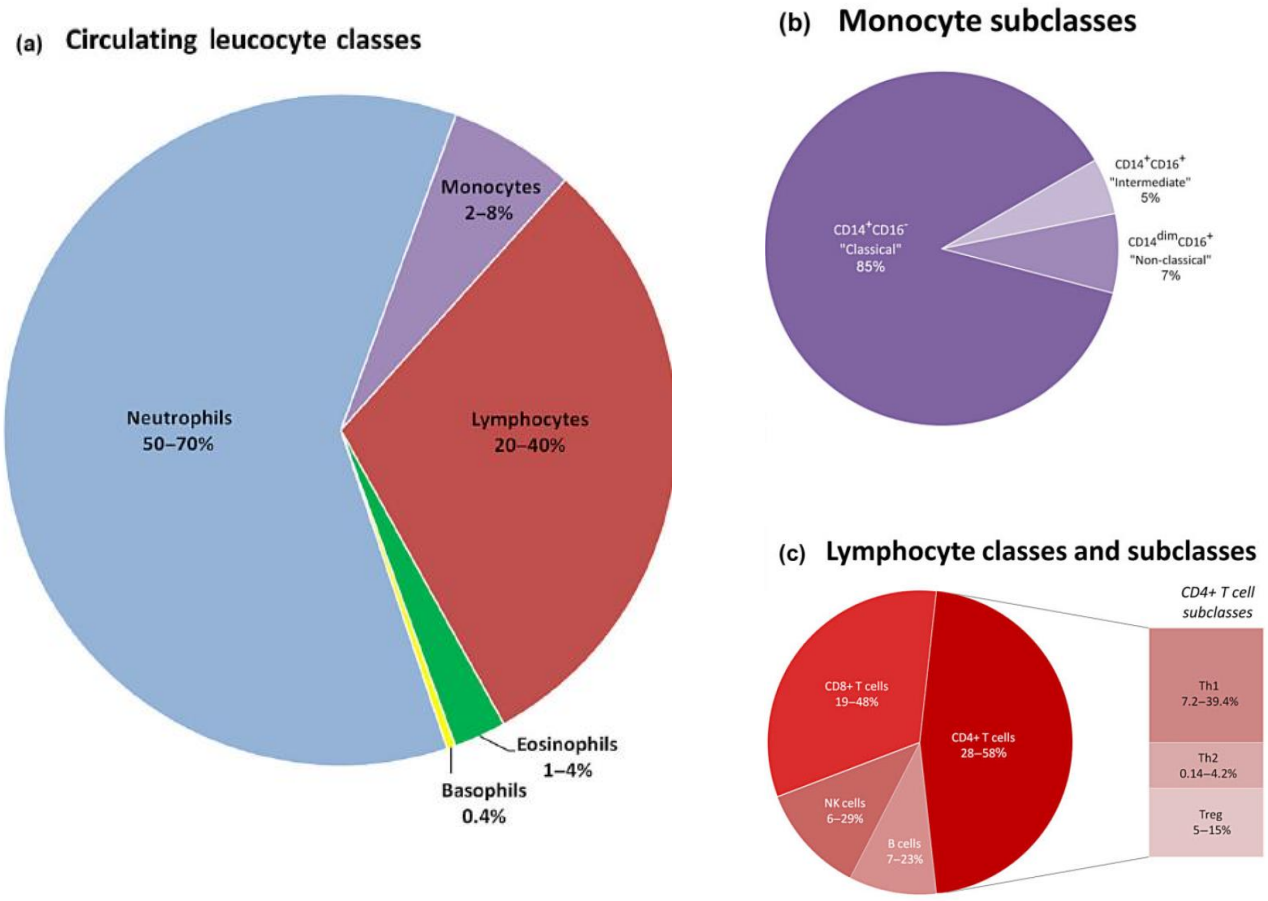


**Figure 6.** Percentages of the major leukocyte classes (a), monocyte subtypes based on the expression of the surface markers CD14 and CD16 (b), as well as lymphocytes classes and $CD4^+$ T cell subclasses (c) in the periphery [100].

#### 2.2.1.2 Natural killer (NK) cells

NK cells that belong to $CD3^-$ lymphocytes are implicated in exerting quick responses against virus- infected cells and tumor formation [108]. They comprise 10% of peripheral blood mononuclear cells (PBMCs) [109] and 6-29% of human lymphocytes [100] (Figure 6). Despite their presence in the circulation, NK cells are also encountered in several peripheral tissues and most importantly in the liver and uterus where they constitute up to 30 and 45%, respectively, of total lymphocytes [110, 111]. NK cells are involved in both innate and adaptive immunity [108]. NK cell contribution to adaptive immunity might be reflected on the production of chemokines and cytokines including TNF-α and IFN-γ that participate in macrophage recruitment and insulin resistance induction [112]. In mouse AT, NK cell frequency was lower in the epididymal fat pad of mice fed with a high- fat diet (HFD) [113].

Short and long form of leptin receptor is constitutively expressed by human NK cells [15]. It has been demonstrated that leptin levels are associated with NK cell activity [114]. Further, NK cells are directly affected by leptin administration that modulates NK cell maturation, proliferation, differentiation, activation, and cytotoxicity through the phosphorylation of signal transducer and activator of transcription-3 (STAT-3) and elevated expression at the transcription level of perforin and interleukin- 2 (IL-2) [115] (Figure 7). Leptin induces IL-12 and inhibits IL-15 expression in NK cells [116] whereas leptin has an indirect effect on NK function via regulation of the TNF-α, IL-1β and IL-6 production by monocytes and macrophages [117]. In a study including 8 normal weight (BMI=22.2 ± 0.6 $kg/m^2$) and 12 obese (BMI=38.5 ± 0.8 $kg/m^2$) participants, NK cells from the first group produced higher levels of IFN-γ compared to those from obese individuals following in vitro administration with leptin [118]. Moreover, impaired NK cell function has been observed in db/db mice [116, 119].

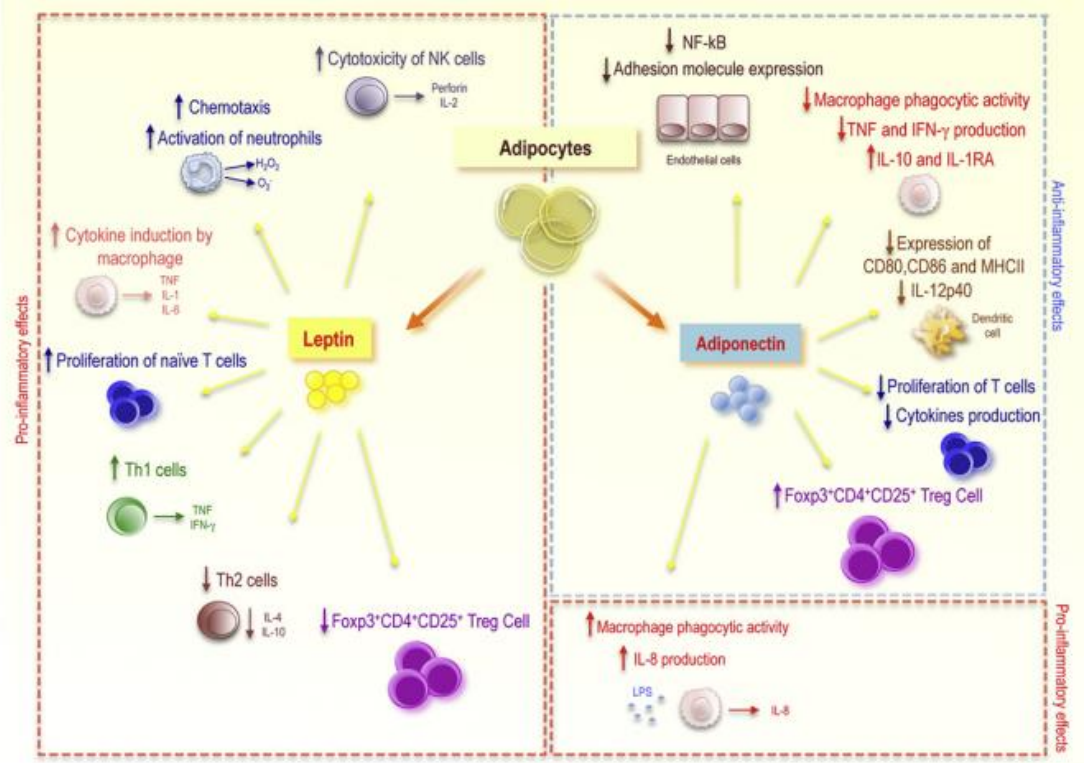



**Figure 7**. The effect of leptin and adiponectin production by adipocytes on survival, proliferation, activation and function of cells belonging to both innate and adaptive immunity [70].

In case of obesity, NK cells exhibit impaired functionality [120-124] and their numbers are found decreased in the circulation of obese individuals compared to lean controls [121, 125] (Figure 8). Similar reductions in NK cell levels have been reported in childhood obesity as well [126]. When an obese group was stratified into 26 metabolically healthy (MHO) and 26 age-, sex- and BMI matched metabolically unhealthy obese (MUO) individuals, the latter had 50% less circulating NK cells independent of BMI; their activation status was higher though [125]. In another study that included 116 obese and 41 lean women, no statistically significant difference was found in NK cells' frequency between the two groups [127]. Furthermore, NK cells' levels did not change significantly following weight loss regardless of the intervention type (diet, diet plus exercise, bariatric surgery) [128, 129] even when total leukocytes and specific subsets were decreased [128].

NK cells can be categorized into 3 subtypes based on the expression of CD56 and CD16 markers: $CD56^{bright}CD16^{-}$, $CD56^{dim}CD16^{-}$, and $CD56^{+}CD16^{+}$. CD56 or neural adhesion molecules are expressed on the surface of glia, neurons, skeletal muscle and NK cells. They mediate in the migration to areas of infection or inflammation and in cell- cell adhesion. Further, $CD56^{+}$ lymphocytes possess a critical role in the immune response against intracellular pathogens and tumor formation. CD16 molecules are expressed on various cells, including monocytes, macrophages, neutrophils, NK and NKT cells, and are implicated in cytotoxic responses [130]. $CD56^{+}CD16^{+}$ cells are the predominant subpopulation of NK cells in peripheral blood with important cytotoxic function [131, 132] which actively takes part in antibody- dependent cell-mediated cytotoxicity (ADCC) [133]. Conversely, $CD56^{+/-}CD16^{-}$ cells dominate lymphoid tissues and possess a significant immunoregulatory role via the secretion of anti- inflammatory cytokines and the regulation of T cell proliferation [134-137].

Increased counts of $CD56^{+}CD16^{+}$ NK cells in obese ($4x10^{5}$ cells) compared to control ($0.8x10^{5}$ cells) individuals were reported in a study examining the effect of polyphenols in PBMCs responses *in vitro* [138]. Conversely, no statistical difference in $CD3^{-}CD56^{+}CD16^{+}$ cells between obese and control women was detected in whole blood preparations by others [127, 139], whereas a negative correlation between the expression of NK cell marker $CD3^{-}CD16^{+}CD56^{+}$ and BMI was reported in another study [1140].

It is widely accepted that NK cell activity is modulated by several factors including metabolites and nutrients [100]. Based on *ex vivo* experiments, NK cells from MUO individuals had impaired cytotoxic capacity [121] and this obesity-related defective function was reversed six months after RYGB surgery reaching the activity level of control group. The last fact could be attributed to an increase in cytokines controlling NK cytotoxic function including IFN-γ, IL-12 and interleukin- 18 (IL-18) [129]. Moreover, in another study evaluating the expression of the early activation marker CD69 on NK cells, they found decreased time- dependent expression 12 months after weight- loss surgery [141]. Similar reduction in NK cytotoxicity was observed in obese women following an 8- week hypo- caloric diet [128]. As a result, it has been proposed that obesity- associated inflammation leads to NK functional impairment and activation- stimulated cell death [108]. Overall, taking published data into

account, NK cell counts/ capacity are found decreased in morbidly obese patients revealing a picture possibly not encountered in mildly obese subjects [100].

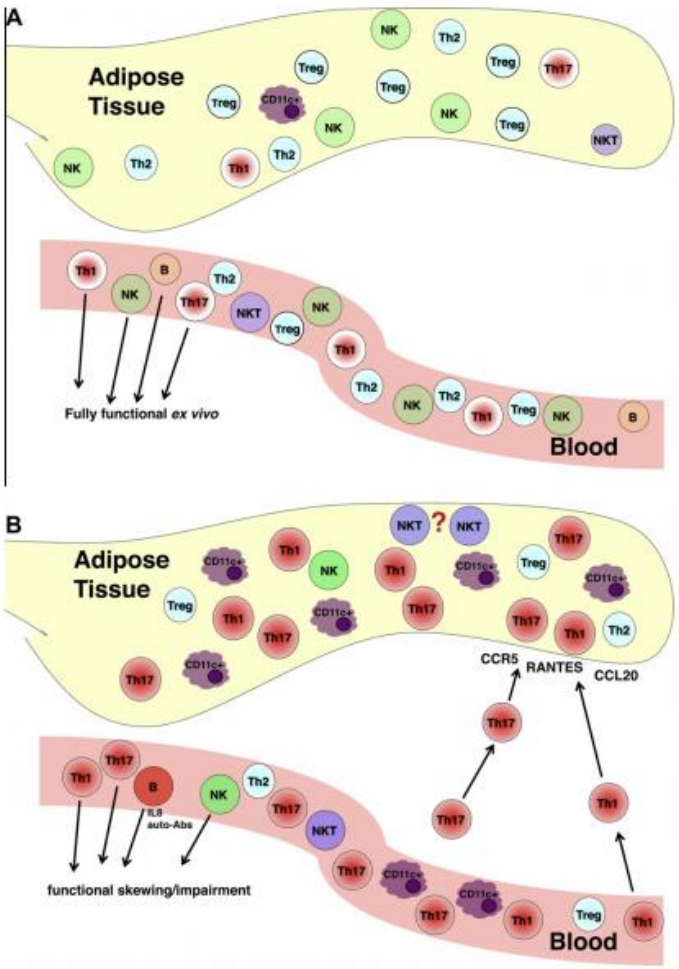



**Figure 8**. Differences in immune cell counts in adipose tissue and circulation under lean (A) and obese (B) state (NK: natural killer cells, NKT: natural killer T cells, Th1: T helper 1 cells, Th2: T helper 2 cells, Th17: T helper 17 cells, Treg: T regulatory cells, B: B lymphocytes, $CD11c^{+}$: antigen presenting cells, CCR5: C-C chemokine receptor type 5, RANTES: regulated on activation, normal T Cell expressed and secreted, CCL20: chemokine ligand 20) [108]

### 2.2.1.3 Monocytes

Blood monocytes are bone marrow-derived phagocytes and, as part of the innate immune system, they can differentiate into either tissue macrophages or myeloid lineage dendritic cells. They participate in the response against viruses, fungi, bacteria and parasites [142] via the production of inflammatory cytokines, phagocytosis and

secretion of nitric oxide (NO), ROS and myeloperoxidase. Monocytes' levels have been related to atherogenesis and, therefore, they have been implicated in cardiovascular disease and peripheral atherosclerosis. As regards metabolic disturbances, the frequency of monocytes has been positively associated with triglycerides, and negatively associated with HDL cholesterol [143].

Murine monocytes can be subdivided in two subsets. $Gr1^+$ monocytes, also termed as "inflammatory" monocytes, are responsible for the production of TNF-α, NO and ROS. Further, they secrete type 1 interferon in order to combat viruses, as well as low levels of IL-10 following in vivo infection with parasites or bacteria. The second subset or $Gr1^-$ monocytes are involved in tissue repair and they can differentiate into macrophages following tissue infiltration [142].

Human monocytes can be categorized into three groups based on the expression of the markers CD14, the bacterial lipopolysaccharide (LPS) co- receptor, and CD16, the low-affinity FcγIII receptor [100].

$CD14^{++}CD16^-$ or classical monocytes constitute the major subset of monocytes (80-90%) (Figure 6). On the basis of gene and surface molecules expression, they resemble murine $Gr1^+$ monocytes [142], and they express the chemokine receptor CCR2 at a high degree and CX3CR1, as well as C-C chemokine receptor type 5 (CCR5), at lower levels [143, 144]. Classical monocytes produce IL-6, IL-8 and ROS in response to LPS, whereas they secrete high levels of the anti- inflammatory cytokine IL-10 but low levels of TNF-α *in vitro* [142] revealing a possible anti-inflammatory character of this type of cells.

$CD14^+CD16^+$ or intermediate monocytes comprise 5% of total monocytes (Figure 6). They differ from $CD14^+CD16^-$ cells in the expression levels of markers CD43, CD45RA, HLA-DR, epidermal growth factor module-containing mucinlike receptor 2 (EMR2), Ig-like transcript 4 (ILT-4) [145], CD11c, CD16, CD62L, CD163, and CX3CR1. They also resemble murine $Gr1^+$ cells in that they possess similar functional properties such as phagocytosis and the secretion of TNF-α and IL-1 in response to LPS stimulation. Moreover, it has been proposed that CD16 expression by $CD14^+$ monocytes coincides with activation and differentiation of these cells [142]. Increased $CD14^+$ monocyte counts and their activation status were correlated with atherosclerosis and hyperglycemia in obese adults and children [91, 146].

$CD14^-CD16^+$ or non-classical monocytes have a smaller size and granularity, and comprise 7% of total monocytes (Figure 6). They express CCR5 at a lower level compared to intermediate monocytes [144], as well as CD11b and CD163 at a lower degree. Although non- classical monocytes possess anti- inflammatory properties in a steady state, they highly secrete proinflammatory factors including IL-1β, TNF-α and CCL3 in response to nucleic acids and viruses through a proinflammatory TLR7-TLR 8- MyD88-MEK pathway. Further, they produce IL-1RA but not ROS, as well as lysozyme and myeloperoxidase at low or zero levels [142]. Non- classical monocytes have been implicated in atherogenesis given their positive correlation with atherogenic lipoproteins and their negative correlation with HDL cholesterol [147]. Moreover, it has been demonstrated that their population is under the control of increased glycemia in morbidly obese patients [143].

Both intermediate and non- classical monocytes, i.e. $CD16^+$ monocytes, are considered as cells capable to trigger inflammatory reaction and their numbers have been found increased in inflammatory situations such as rheumatoid arthritis, sepsis, chronic kidney disease, and infections [145]. $CD16^+$ monocytes possess an enhanced ability of antigen presentation, and increased expression of MHC-II and TNF-α, whereas decreased expression of IL-10 has been detected in $CD16^+$ compared to $CD16^-$ monocytes [143]. Other studies report that $CD16^+$ cells, including $CD14^+CD16^+$, resemble murine $Gr1^-$ cells and they respond to LPS and to an array of toll- like receptors (TLRs) through the secretion of IL- 1β and TNF-α [144, 148, 149]. Although $CD16^-$ and $CD16^+$ monocytes efficiently face *Aspergillus fumigatus* conidia via phagocytosis, they follow different strategy against conidial germination and the secretion of TNF-α [142].

Considerable recruitment of monocytes into VAT via several candidate chemokine pathways including MCP-1 signaling has been reported [105, 150]. Increased expression of CCR2 by peripheral $CD14^+CD16^+$ cells and CCR5 by peripheral $CD14^+CD16^-$ cells in obese women may reveal increased recruitment of those cells into obese AT [150]. AT- recruited monocytes differentiate into adipose tissue macrophages (ATMs) that exhibit high chemokine and cytokine production and stimulate further recruitment of monocytes into VAT [69]. Those ATMs belong to either M1 subtype that predominate in obese AT, or to M2 subtype that is widely distributed in lean AT [138].

Under overweight and obese condition, monocyte count is increased in peripheral circulation compared to lean subjects [127, 151, 152] and a diminution in their numbers follows fat body reduction [138]. However, this is not a universal finding and the number of study participants should be taken into account when seeking changes in cell counts between lean and obese state [100]. Interestingly, positive [143], no [153, 154] or even negative [155] association between BMI and monocyte counts has been reported.

Further classification of monocytes revealed an elevated number of $CD14^{+}CD16^{+}$ and $CD14^{dim}CD16^{+}$ cells in obese compared to non- obese subjects that positively correlated with BMI, fat mass and inflammatory factors such as hsCRP [104, 143, 150, 154]. As for $CD14^{+}CD16^{-}$ subset, currently available studies report either similar or decreased levels of these cells in obese compared to lean individuals revealing an inconsistent picture concerning this type of cells [143, 154]. Monocyte numbers decreased following a 6- week very low-energy diet (VLED) intervention in obese subjects compared with baseline [156]. A significant reduction in the number and percentage of non- classical and intermediate subtype was induced following fat mass loss of at least 5% [143].

Despite monocyte numbers, obesity also impacts on their function given the elevated phagocytosis and oxidative burst monocytes exhibit [157]. Monocytes from obese individuals produced ROS at high levels [158] and exhibited an elevated surface expression of integrin CD11b compared to those from lean subjects indicating a more activated phenotype of these cells [155]. Others reported increased expression of the proinflammatory markers IL-6 and TNF-α and decreased expression of the anti-inflammatory IL-10 by monocytes from obese non- diabetic patients compared to their lean counterparts [159]. Further, triglycerides levels detected in the serum of obese adolescents with or without metabolic syndrome have been associated with the expression levels of TNF-α in peripheral monocytes [160]. LPS- induced and basal production of TNF-α by PBMC- isolated monocytes from obese subjects with no other comorbidities was increased compared to those from lean individuals [26]. To this line, PBMCs- isolated monocytes from obese subjects produced lower levels of TNF-α following a VLED weight loss intervention and *in vitro* stimulation with LPS compared to baseline [26].

*In vitro* experiments have shown that human leptin induces the proliferation and activation of human circulating monocytes via the expression of activation markers, such as CD25 (IL-2 receptor a-chain), CD38, CD69, and CD71 (transferrin receptor) [161], and their fate is directly affected by leptin via apoptosis regulation [157]. Moreover, leptin enhances the secretion of interferon-gamma-inducible protein (IP-10) and interleukin 1 receptor antagonist [15], as wells as the secretion of proinflammatory cytokines including TNF-α, IL-6 and IL-12 by monocytes, and their ability to phagocytose [39, 71, 162]. Additionally, healthy overweight men treated with leptin had their monocyte levels reduced [163]. However, it has been reported that oxidative burst in monocytes previously activated is downregulated by human leptin [164].

### 2.2.2 Adaptive immunity

**Lymphocytes**

Lymphocytes are the main cell type encountered in lymph and can be identified by the large size of their nucleus. They can be subdivided in three major subsets, T cells (adaptive immunity), B cells (adaptive immunity) and natural killer cells (innate immunity). Under obese and overweight condition, increased circulating lymphocyte numbers have been reported [60] and have been shown to positively correlate with elevated BMI following adjustment for several characteristics, such as ethnicity, age, sex and smoking. However, similar levels of inflammatory cytokine- secretion by lymphocytes induced by stimulation with LPS were observed in overweight and lean young adults [100].

#### 2.2.2.1 T cells

T cells that are generally distinguished by the expression of the CD3 marker are produced in bone marrow and they fully mature in thymus. They orchestrate adaptive immunity and they shift from naive to effector state during an immune response [165]. Based on the expression of surface markers, T cells can be subdivided in CD4 and CD8 T cells. CD4 T cells can be further subdivided into T helper and T regulatory cells, whereas CD8 T cells are characterized as T cytotoxic cells depending on their different functions. T helper cells or $CD4^+$ cells are further separated in several subtypes mostly based on the production of different cytokines, including Th1 that produce IFN-γ and TNF-α, Th2 that release IL- 4, IL- 5 and IL- 13, Th17 that synthesize IL- 17, IL- 21 and IL- 22, as well as Th22 that produce IL- 22 in the

absence of IL- 17 [69, 166] (Figure 9). T lymphocytes orchestrate obesity- related inflammation via both their ability to produce proinflammatory factors and their cytotoxic activity [167].

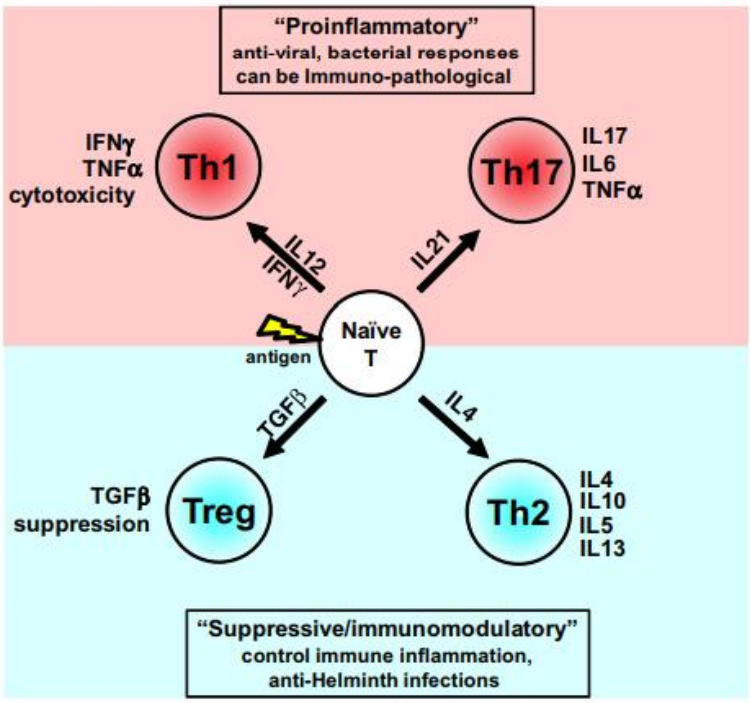



**Figure 9**. The presence of cytokines in the microenvironment directs naïve T cell differentiation into several subtypes (either proinflammatory or suppressive) following their contact with an antigen [108].

$CD3^+$ T cells along with macrophages are the most abundant cells observed in AT. The implication of T lymphocytes in obesity- related inflammation of AT [105], as well as the development of insulin resistance along with lymphocyte infiltration of AT prior to macrophages [105, 168, 169] have been reported. The last fact is supported by the decreased AT infiltration by macrophages and improved insulin signaling following T- cell depletion in HFD- fed mice [169, 170]. In addition, a positive correlation between AT T lymphocyte counts and BMI has been reported based on studies in obese and lean subjects [171].

Nevertheless, data concerning circulating T cell numbers are not so clear. Studies have reported increased levels of circulating T cells by 15- 50% in morbidly obese compared to lean subjects and a positive correlation with adiposity regardless of the

type of studied cohort [40, 92, 101, 127, 172]. However, other studies reported similar $CD3^+$ counts between obese and lean adults [153] and children [173], increased $CD3^+$ numbers in obese but not in morbidly obese subjects compared to lean individuals [140], and no correlation with BMI in female students [172]. Additionally, similar $CD3^+$ T cells percentages were observed between lean, obese and formerly obese women subjected to gastric banding surgery [174], whereas others reported elevated $CD3^+$ cell levels in lean compared to obese people [26]. Those variations concerning circulating T cell numbers during obesity could be attributed to differences related to the heterogeneity of T cell pool, the estimation of T cell function and cohort characteristics [100].

**$CD4^+$ T cells**

CD4 T cells are activated by class 2 MHC antigens that are present on the surface of APCs like macrophages and dendritic cells and they trigger the recruitment of other immune cells to the site of inflammation via cytokine production. Their circulating numbers are found elevated in overweight [40], obese [40, 128, 139, 172, 174] and morbidly obese [40, 92, 157, 175] individuals and they positively correlate with BMI [92, 157]. Further, a positive correlation between $CD4^+$ T cell numbers and insulin sensitivity (glucose-to-insulin ratio and fasting insulin levels) in non-diabetic morbid obese women has been shown [92]. The increase in $CD4^+$ T cell counts may be attributed to an increased proliferative capacity as has been demonstrated in vitro [92]. Importantly, in some cases, their percentage or number did not change following diet- or/ and surgery- induced weight loss [155, 174].

Regulatory T cells (Tregs) are responsible for the maintenance of immune homeostasis through the regulation of immune cell activation and the production of the anti-inflammatory cytokines IL-10 and TGF-β (Figure 9). They usually constitute only 5-15% of circulating $CD4^+$ T cell population in mice and humans [41] (Figure 6). These cells actively participate in allergies and peripheral immune tolerance [176], and contribute to the arrangement of an anti- inflammatory environment by inducing M2 polarization via IL- 4, IL- 10 and IL- 13 production [177], reducing M1 polarization and improving insulin sensitivity [178, 179]. Treg cells are differentiated by the regulatory action of transcription factor fox P3 (fork head box P3- foxp3) [180, 181].

During obesity, the number of T reg cells is decreased in rodent [178] and human AT [182] (Figure 8) resulting in the reduction of anti- inflammatory cytokines and a subsequent obesity- induced insulin resistance [169, 170, 178]. Similarly, their circulating levels have been found decreased in T2D patients [183, 184] as well as in obese compared to lean individuals (Figure 8) and have been negatively associated with body weight, BMI and plasma leptin levels [185-187]. As a result, the Th1/Tregs ratio is positively associated with BMI [183].

Treg cells depletion using foxp3- DTR results in an increased transcription of inflammatory genes in VAT and development of insulin insensitivity [178], whereas treatment with Thioridazone (TZD) is associated with increased numbers of Treg cells in AT and a subsequent amelioration of inflammation and insulin resistance. TZD is an agonist of peroxisome proliferator-activated receptor gamma (PPAR-γ); the latter possesses an anti- inflammatory role, controls Tregs accumulation in VAT and adipocyte differentiation, and it is highly expressed in VAT but not in peripheral Tregs. Moreover, IL- 2 administration results in upregulation of regulatory T cells and improves insulin signaling during obesity [188]. Conversely, a direct inhibition of Treg- cell anti- inflammatory function by elevated levels of insulin during obesity was described, thus inducing TNF-α production by macrophages and subsequent inflammation [189].

Other studies report increased mRNA expression of the transcription factor Foxp3, a marker used to identify Treg cells, in the AT of obese compared to lean individuals accompanied by an increase in cytokines produced by Treg cells, IL- 10 and TGF-β [190, 191]. Another study reported increased circulating Treg cell numbers in morbidly obese patients and a positive association with BMI [92], whereas no difference among weight groups in children was suggested by others [192].

It has been suggested that Treg cells percentage might serve as a metabolic state index and individuals having Treg cells > 1.06% are at a 9.6- fold higher risk of developing an inflammatory obese phenotype [185]. Further, mouse and human Treg cells generation and proliferation are directly affected by leptin [176] and a negative correlation between leptin levels and circulating Treg percentage has been reported (Figure 7) [185]. Inhibition of proper leptin signaling leads to enhanced proliferation of Tregs along with loss of their suppressive character, whereas resistance to leptin

signaling might lead to increased levels of Tregs [71]. Conversely, adiponectin has an indirect positive effect on Tregs proliferation (Figure 7) since an increase in $CD4^+CD25^+Foxp3^+$ Treg cells frequency and a diminution in T cell proliferation and IL- 2 production have been observed in co- culture experiments of dendritic cells treated with adiponectin and T cells [193].

$CD4^+$ T cells can be also characterized by the expression of the leukocyte common antigen isoforms, CD45RA and CD45RO, and the expression of the homing chemokine receptor CCR7 and can be subdivided into four subtypes: naïve (N, $CD45RA^+CCR7^+$), central memory (CM, $CD45RO^+CCR7^+$), effector memory (EM, $CD45RO^+CCR7^-$) and terminally differentiated effector memory cells (TEMRA, $CD45RA^+CCR7^-$). CCR7 has been involved in the stimulation and maintenance of central and peripheral tolerance, as well as in the migration of dendritic, T and B cells into secondary lymphoid organs [194]. Importantly, a sequential differentiation of T cells through the stages Naïve→ CM→ EM→ TEMRA takes place, the latter of which cannot differentiate further.

Alterations in the expression of CD45 isoforms have been associated with metabolic diseases, including T2D and hypothyroidism [195], alcoholic cirrhosis and hepatitis C [196, 197]. A reduction in $CD4^+CD45RA^+$ ($29.45 \pm 8.90$ NASH vs. $43.29 \pm 6.62$ CNT; $p = 0.008$) whereas an increase in $CD4^+CD45RO^+$ ($72.99 \pm 5.09$ NASH vs. $60.38 \pm 5.88$ CNT; $p = 0.013$) compartment was reported in NASH patients compared to healthy subjects. NASH development is accompanied by the development of insulin resistance and the presence of risk factors for the metabolic syndrome, including central obesity [198]. Another study reported a positive correlation between $CD4^+$ and $CD4^+CD45RO^+$ T cells with characteristics of metabolic syndrome in a sample of 439 apparently healthy men [153].

Elevated levels of memory ($CD4^+CD45RO^+$) cells positively correlated with protection against T1D in a study that included identical twins [199]. A study including 54 T1D and 12 healthy children reported similar expression of $CD45RA^+$, $CD45RO^+$ and $CCR7^+$ in $CD4^+$ cells between diabetic children and controls. A positive correlation between $CD4^+CD45RA^+$ and $CD4^+CCR7^+$ in both T1D and healthy children was observed. However, a negative correlation between

$CD4^+CD45RO^+$ cells and $CD4^+CD45RA^+$/ $CD4^+CCR7^+$ cells was reported in healthy children [194].

Koch et al. (2008) reported an age- associated but not statistically significant diminution in naïve $CD4^+$ T cells, as well as an increase in CM but not in TEMRA cells. However, a marked increase in TEMRA cells and a reduction in naïve cells from the elderly were observed in $CD8^+$ T cells [200].

Studies discussing the presence of naïve/ memory $CD4^+$ T cell compartment exclusively during obesity with no other existing co-morbidities are limited in number. Decreased $CD4^+CD45RO^+$ T cell counts in obese compared to healthy individuals ($0.52 \pm 0.18$ $x10^3$cells/$mm^3$ OB vs. $0.66 \pm 0.20$ $x10^3$cells/$mm^3$ CNT; $p < 0,01$) were reported in a study that included 34 obese and 50 healthy subjects, whereas their levels increased following VLED- induced weight loss in 23 obese subjects. Differences in $CD4^+CD45RA^+$ cell counts between obese- healthy individuals and their levels following weight reduction did not reach statistical significance [26]. Further, obesity positively correlated with lower percentages of naïve T cells ($CD4^+CD45RA^+CCR7^+$) and higher percentages of effector memory T cells ($CD4^+CD45RO^+CCR7^-$) in a study that included 187 subjects [60].

Both $CD4^+CD45RA^+$ and $CD4^+CD45RO^+$ T cells express the long isoform of leptin receptor and are directly affected by leptin signaling. More specifically, leptin regulates the proliferation of naïve T cells and IL-2 production by those cells [41, 70]. Conversely, leptin affects the proliferation of memory T cells to a lesser extent via Th1- cell responses induction [70] whereas others suggest the prevention of memory T cells proliferation by leptin [41].

Chemokine receptor CXCR3, also known as GPR9, is a 38 kD transmembrane G-protein- coupled receptor that binds to the chemokines CXCL9, CXCL10, and CXCL11 [201, 202]. CXCR3 belongs to the CXC chemokine receptor family and is highly expressed by Th1 cells, NK cells, dendritic cells, alveolar macrophages, eosinophils, mast cells, and human airway epithelial cells, and at low levels by Th2 cells [203]. CXCR3 expression on $CD4^+$ and $CD8^+$ T cells takes place during their activation state [204, 205]. The interaction between CXCR3 ligands and CXCR3 promotes the polarization of effector T cell recruitment into inflamed tissue, and mediates Th1 trafficking and maturation. As a result, several cellular processes are

triggered, including changes in the cytoskeleton, integrin activation and chemotactic migration. CXCR3 trafficking has been implicated in several inflammatory and autoimmune diseases, such as rheumatoid arthritis, Crohn's disease, multiple sclerosis, and chronically inflamed liver.

Studies in mice have shed light into the role of CXCR3 in T cell recruitment into VAT during obesity. CXCR3 knockout mice fed with a HFD had fewer T cells in VAT compared to control mice on the same diet. Moreover, diet- induced obese CXCR3 deficient mice were more insulin sensitive, and expressed lower mRNA levels of various proinflammatory genes in AT, such as MCP-1, and higher mRNA levels of anti- inflammatory genes, including Foxp3, IL-10 and arginase-1, than their obese littermates [206]. Another study reported no difference in weight gain between CXCR3 deficient and control mice both fed with a HFD for 20 weeks; the former were characterized by decreased VAT infiltration of immature myeloid cells and by improved glucose tolerance, though [207].

Downregulation of CXCR3 and its ligands (CXCL9-11) was observed in cultured progenitor cells derived from 18 obese African- American women compared to their control littermates revealing a disturbance of the CXCR3 pathway under obese condition [208]. Moreover, Hueso et al. [209] reported elevated systemic levels of the CXCR3 ligands CXCL10 and CXCL11 that were associated with increased BMI and insulin resistance in 25 morbidly obese patients who had undergone laparoscopic Roux-Y-gastric bypass surgery.

In a study using human blood samples from healthy donors, it was demonstrated that upregulation of CXCR3 on memory $CD8^+$ T cells mediates their migration to sites of inflammation, as well as their differentiation. Moreover, higher expression of CXCR3 by $CD8^+$ (~70%) than by $CD4^+$ (~20%) T cell compartment was detected [210]. In a group of newly diagnosed T1D patients and diabetic children, Lohmann [211] and Hedman [194], respectively, reported, among others, decreased percentages of $CD4^+CXCR3^+$ cells in diabetic patients compared to healthy controls revealing a possible reduced function of Th1 cells in T1D. Lower percentages of $CXCR3^+$ T cells in circulation and lymph nodes along with higher levels of those cells at sites of inflammation may reveal a critical role of this receptor in the infiltration of inflammatory sites by pathogenic T cells [212].

**$CD8^+$ T cells**

T cytotoxic or $CD8^+$ cells actively participate in anti- viral and anti- tumoral immunity via their cytolytic activity (perforin, granzyme, and Fas- induced apoptosis) and secretion of proinflammatory cytokines including TNF-α and IFN-γ [100]. They are activated by class 1 MHC molecules presented by APCs [213] and their numbers have been positively associated with BMI [40]. Three- to four- fold increase in CD8 T cell numbers has been reported in obese compared to lean individuals whereas CD8-produced IFN-γ is also elevated in obesity. Increased IFN-γ stimulates macrophage infiltration and M1 polarization contributing to insulin resistance development [69]. Conversely, insulin sensitivity is associated with a reduction in $CD8^+$ T cells levels under obese condition [169].

Memory and local effector $CD8^+$ T cell numbers have been found elevated in human and rodent obese AT [169, 171, 214-216] and agents produced by these cells contribute to macrophage AT infiltration, differentiation and chemotaxis [169, 217]. $CD8^+$ effector T cells depletion that precedes mouse obesity induction results in inhibition of AT infiltration by M1 macrophages and the subsequent enhanced glucose tolerance with no evident effect on AT resident Th1 cells and M2 macrophages numbers or adiposity. Moreover, immunological depletion of $CD8^+$ effector cells ameliorated AT inflammation and insulin sensitivity. However, adoptive transfer of $CD8^+$ cells into HFD- fed CD8- deficient mice promoted the production of proinflammatory cytokines, VAT infiltration by macrophages, as well as development of insulin insensitivity. Data obtained from *in vitro* studies showed that macrophages had a greater migratory capacity when they were co- cultured with $CD8^+$ cells isolated from obese mice compared to those ones isolated from lean mice [169]. Jiang et al. [216] reported that VAT $CD8^+$ T cell activation is stimulated by Th1 cytokine IFN- γ *in vitro*.

Despite the observed $CD8^+$ increase under obese condition, reduced $CD8^+$ T cells in 34 obese compared to 50 lean individuals [26], as well as in 10 morbidly obese compared to 10 healthy, normal weight women [157] have been reported in two different studies. Similarly, low levels of $CD8^+$ cells have been reported in several other studies [125, 174, 218]; those results may be attributed to the small sample size though. It is also worth mentioning that $CD8^+$ T lymphocytes were more abundant in MHO than in MUO individuals [125]. Further, other studies reported no difference in

$CD8^+$ T lymphocyte numbers in obese and normal weight subjects [92, 127, 219]. Weight loss interventions based on either very low calorie diet or surgery did not significantly impact on $CD8^+$ T cell number [125] or percentage [220], respectively.

#### 2.2.2.2 B cells

B lymphocytes participate in humoral immunity by secreting antibodies and constitute a far smaller population of lymphocytes in both adipose tissue [221] and circulation [100] (Figure 6) compared to T cells.

The contribution of leptin in hematopoiesis and lymphopoiesis has been demonstrated indicating its pivotal role in the orchestration of immune system function [15]. Reduced numbers of peripheral B and T lymphocytes have been observed in obese ob/ob and db/db mice that exhibit lymphoid organ atrophy and immune dysregulation [222]. When recombinant leptin was supplied to obese mice for seven days, normal myelopoiesis was induced and a twofold increase of the levels of B cells was observed accompanied by a twofold and threefold increase in the numbers of pre- B and immature B cells, respectively [223]. Another study reports a minor increase in $CD19^+$ B cells enhanced by leptin treatment that supports the beneficial effects of weight loss on proinflammatory factors [163]. However, no correlation between leptin and B or T lymphocyte counts in postmenopausal obese women has been reported elsewhere [224].

The remarkable presence of several types of lymphocytes including B cells in obese AT has been demonstrated [9, 20, 215]. B cell counts have been found elevated in mouse VAT during diet- induced obesity and have been associated with the development of insulin resistance [221].

Data revealing the association between obesity and circulating B cell levels are inconsistent. Elevated frequency of B cells has been observed in overweight [140] and obese subjects [127, 140, 225] compared to lean control sample. Moreover, B cell levels were related to increased risk for metabolic syndrome and obesity [225]. The most profound correlation between BMI and B cell frequency has been observed in women [140]. However, a lack of association between B cell numbers and BMI during obesity has been reported in other studies [139, 172].

As regards B cell capacity, it has been demonstrated that B cells are less functional and produce more proinflammatory and less anti- inflammatory agents in both young and elderly obese subjects compared to lean individuals based on *ex vivo* data [226]. A similar pattern regarding the production of cytokines by B lymphocytes has been demonstrated by Jagannathan et al. [227]. More specifically, increased secretion of the proinflammatory cytokine/chemokine IL-8 and reduced production of the anti-inflammatory cytokine IL-10 by B cells of obese T2D patients have been reported.

B cells also contribute to the production of the proinflammatory cytokines IFN-γ and IL-17 by T cells [226] and play a significant role in the development of insulin resistance based on data obtained from DIO mouse models [221]. Further, decreased levels of the inflammatory IFN-γ and IL-6, increased levels of the anti- inflammatory IL-10, elevated counts of Treg cells in VAT and protection against insulin resistance have been observed in obese B cell deficient mice compared to obese WT ones [228]. Based on these data, it could be proposed that VAT infiltration by macrophages and T cells follows the presence of B cells in VAT that drive T cell and ATM activation and subsequent inflammation during obesity [9, 20, 168].

#### 2.2.2.3 Natural Killer T (NK T) cells

NK T cells possess properties that lie between innate and adaptive immunity and share similar characteristics to that of NK and T cells. More specifically, they are specialized T cells expressing NK1 and CD3 markers that recognize lipid antigens presented by CD1d molecules, but not peptide antigens presented by MHC-1 and MHC-2 molecules [69, 93, 105, 229]. Based on the type of T- cell receptor (TCR) they express and on their antigen specificity, NK T cells can be categorized in three groups: invariant NK T (iNKT), type II NK T, and NK T-like lymphocytes [166]. They produce a variety of proinflammatory cytokines such as TNF-α and IFN-γ, and anti- inflammatory cytokines including IL-4 and IL-13 [69] thus mediating Th1 and Th2 responses [97]. NK T cells are involved in a variety of pathological conditions, including autoimmune diseases (e.g. multiple sclerosis, rheumatoid arthritis and T1D) [230], microbial infection, metabolic syndrome and cancer [108, 231] and they are typically found in the liver and, to a lesser extent, in circulation, WAT and spleen [105].

Although VAT is enriched in iNKT lymphocytes (15% of total leucocytes) compared to blood samples [166], their number in peripheral circulation, AT and liver is reduced during obesity [105, 230-232]. Others suggest that, although there is a decrease in NKT cells number in the liver under obese condition, ATMs stimulate a slight increase in their levels in AT [233].

According to a recent study, iNKT circulating levels are increased in a group of MHO individuals (i.e. obese subjects who do not suffer from chronic inflammation and insulin resistance) compared to MUO (i.e. obese patients with metabolic abnormalities, chronic inflammation and insulin resistance) and metabolically unhealthy normal- weight individuals (i.e. patients who suffer from metabolic abnormalities and insulin resistance but have normal weight), whereas their numbers are decreased compared to metabolically healthy normal- weight subjects (i.e. individuals protected from metabolic disabilities). Those observations indicate that fat accumulation and its related chronic inflammation but not body weight affect iNKT percentage [229]. Furthermore, weight loss triggers an increase in their numbers in both humans and mice [230].

The frequency of NK T lymphocytes is regulated by leptin that has a negative effect on their numbers based on results obtained from healthy overweight men treated with leptin [163]. It is noteworthy that AT iNKT cells enhance the proliferation of Tregs and the activation of anti- inflammatory macrophages [234]. However, iNKT depletion in combination with high- fat diet provision in mouse models had contradictory effects on insulin resistance and AT inflammation [93] making their role in obesity- induced inflammation unclear.

### 2.3 Personalized nutrition

The term “personalized nutrition” refers to an approach that aims at the design and subsequent development of specific nutritional advice, products, or services based on each person’s characteristics in order to achieve health increase or preservation. The nature of those characteristics may be genetic, medical, nutritional or phenotypic. Several related terms including precision nutrition, nutritional genomics, nutrigenomics, and nutrigenetics are often used instead of the term “personalized nutrition” [235]. Nutrigenomics or nutrigenetics are used to describe the impact of

genetic variations on individual's response to diet regarding several aspects of health [236].

The application of personalized nutrition is favored either when improvement of public health is needed or when groups requiring specific dietary management (e.g. old people, pregnant women) or suffering from specific diseases need to be nutritionally guided. At personal level, a person's goals regarding performance in competitive sports, desired food preferences, or body dimensions (e.g. through muscle building) could also be achieved through personalized nutrition [235]. It is of great importance that, except for genetic variation, environmental factors should be taken into account during dietary planning, especially in the case of diet- related chronic diseases [237].

Several studies about the use of personalized information in dietary planning have been published so far. In 2015, Zeevi et al [238] designed a machine- learning algorithm using a 800- person cohort and recording responses to 46,898 meals, that requires data concerning biochemical and anthropometric characteristics, personal habits and gut microbiota in order to predict a subject's postprandial glycemic response to real-life meals. The results of the use of this algorithm in a randomized controlled dietary study suggested that personalized diets may contribute to alterations in elevated postprandial blood glucose levels and subsequent beneficial metabolic effects. Another study from the same group [239] suggested that microbiome based information obtained prior to test meal consumption can be used to predict glycemic response to that meal.

In 2016, maybe one of the most outstanding randomized controlled studies [240] concerning personalized nutrition was published, namely the Food4Me Study. This study paved the way for the effective use of the internet to deliver dietary advice. More than 1600 individuals from seven European countries participated in the study and were randomized into four different groups (individual baseline diet, individual baseline diet plus phenotype [blood biomarkers and anthropometry], individual baseline diet plus phenotype plus genotype, and conventional diet) in order to determine the effectiveness of personalized nutrition in changing diet and the aspect of personalization that mostly affects that change. Nutritional advice was delivered to participants via internet and, after a 6- month intervention, it was demonstrated that

any aspect of personalized nutrition was more effective than conventional “one size fit al” approach. The fact that dietary advice was personalized urged participants to develop and sustain a healthier lifestyle highlighting the need for developing cost effective interventions to improve public health.

A limitation of the Food4Me Study is that outcomes of any kind of intervention were restricted to a 3- or a 6- month intervention. However, Lara et al. [241] reported that dietary changes observed at 6 months of intervention may be sustained for at least a year.

Except for the Food4Me Study, the effectiveness of the delivery of personalized nutritional advice via internet has been also widely discussed [242-246]. Increased consumption of fruits and vegetables [247], as well as body weight reduction [2748250] were the outcomes of personalized intervention compared to non-personalized advice reported in several reviews and meta- analyses.

Few studies have focused on genotype- based interventions and their impact on a healthier lifestyle. Joost [251] and Hietaranta-Luoma [252] both concluded that further research is needed in order to model dietary advice on genotype, whereas the limited number and quality of the available studies make the effect of genotype knowledge on successful intervention unclear [253]. Similarly, a prospective longitudinal cohort study including 2,240 individuals reported that knowledge of the results of genomic testing does not affect exercise or fat intake after 3 or 12 months [254].

BMI has a strong genetic background (40-80% heritability) as indicated by family and twin studies, and is affected by genes expressed in the hypothalamus and are involved in appetite regulation [255]. One such gene is fat- mass and obesity- associated (FTO) whose introns 1 and 2 contain 89 common variants [14] (Figure 10).

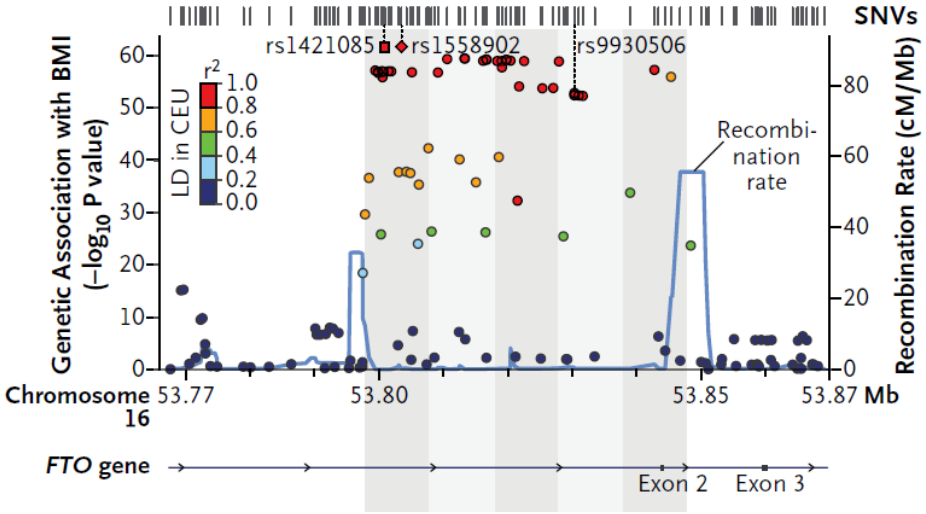



**Figure 10.** Genetic association for FTO locus variants with BMI (LD: linkage disequilibrium, CEU: population of Utah residents with western and northern European ancestry, gray shading describes consecutive 10-kb parts) [14]

The strong association of variants in the first intron of the FTO gene with obesity has been reported in several studies [256]. Homozygosity for the FTO risk allele AA (rs9939609) resulted in a 3 kg increase in body weight and in a 1.7- fold increased probability of being obese than homozygosity for the lower- risk allele TT [257]. The effect of FTO SNP rs9939609 on changes in BMI and obesity is evident by the age of 7 and continue to exist in the prepubertal period and beyond [257]. Moreover, physical activity has been shown to affect the impact of FTO genetic susceptibility on obesity [256].

In a prospective study including 51 overweight or obese American veterans [258], subjects received one of four types of diet (balanced, low-carbohydrate, low-fat, or Mediterranean) based on their genotype regarding 7 obesity- related polymorphisms [ApoA2, Adiponectin, C1Q and Collagen Domain Containing (ADIPOQ), FTO, Potassium Channel Tetramerization Domain Containing 10 (KCTD10), Lipase C, Hepatic Type (LIPC), Methylmalonic Aciduria (Cobalamin Deficiency) CbIB Type (MMAB), and PPARG], whereas 21 out of 51 individuals served as the control group (standard balanced diet). No difference in weight loss due to nutrigenetic- guided and conventional diet was observed. Further, individuals with low- risk obesity-associated SNPs had a greater weight loss at 8 weeks (5.0% vs 2.9%, respectively; P=

0.02) and BMI reduction at 24 weeks (6.4% vs 3.6% respectively; P= 0.03) compared to all other participants.

On the contrary, a Canadian randomized controlled trial including 138 individuals aged 20-35 years reported that nutritional advice based on the ACE genotype successfully led to significant changes in sodium intake at 12 months in individuals with the ACE risk allele compared to the group receiving general population- based recommendation (-287.3 ± 114.1 mg/day vs. 129.8 ± 118.2 mg/day, p = 0.008) [236]. This was the first study to examine the impact of genotype- based advice on modification of dietary lifestyle.

Qi et al. [259] examined the role of the insulin receptor substrate 1 (IRS1) variant rs2943641 in improvement of insulin resistance and weight loss in response to four diets. Weight loss interventions were part of the Pounds Lost Trial [260] and varied in macronutrient components. 738 overweight adults were genotyped and were assigned to one of the four diets for 2 years. As a result, participants with the CC genotype showed greater weight loss and decreases in insulin levels than those without CC genotype when they followed a high- carbohydrate/ low- fat diet. It is worth-mentioning that this was the first study to evaluate the effect of gene- diet interactions on improvement of insulin resistance along with weight loss in a long- term randomized clinical trial and highlights the significance of an intervention based on personalized nutrition in preventing obesity- related diseases.

Arkadianos et al. [261] compared the effects of a genetically-guided and a traditional weight loss diet on BMI and blood glucose levels. For that reason, 50 participants in the intervention group were screened for 24 variants in 19 genes related to metabolism, whereas 43 individuals who did not receive a nutrigenetic test served as the control group. It was demonstrated that personalized diet resulted in improvements in fasting blood glucose levels, better dietary adherence and longer-term maintenance of weight loss as compared to conventional diet.

Meisel et al. [262] reported that healthy individuals being informed about their FTO status in the weight- control recommendation were more confident to control their weight; no effect on behavior change was observed, though. In a group of 683 participants from the Food4Me randomized controlled trial, it was reported that individuals carrying the FTO risk allele and being informed about their genotype

significantly had their body weight (21.34 compared with 22.28 kg, respectively; P = 0.045) and waist circumference (22.82 compared with 24.34 cm, respectively; P = 0.046) reduced compared to the non-personalized control group who were not informed about their genotype [256]. Further, in a 1- year intervention study including 107 healthy adults, it was found that genetic information regarding apoE gene may impact on dietary fat quality and promote a healthier lifestyle [252].

Significant conclusions regarding the association of gene variants and diet with cardiovascular disease manifestations [263] and T2D incidence [264] were drawn out in two studies including >7,000 participants.

Public interest around personal genomics and, more specifically, nutrigenomics, is noteworthy [265]. In a survey conducted in six European countries with 5,967 participants, 66% of responders were willing to undergo nutrigenomics testing and 27% to participate in a personalized intervention [294]. Nielsen and El- Sohemy (2012) in a randomized controlled study with 149 participants [267] reported that individuals find genotype- based dietary recommendations more useful and understandable than general population based dietary advice and they would be more willing to make changes to their usual diet if genetics- based advice was provided to them. Other studies demonstrate that knowledge of genetic information based on genome analysis affects dietary lifestyle even though the genetic information was not related to any dietary change [268, 269].

It is interesting to mention that in a double- blind, randomized controlled trial with 138 participants, it was demonstrated that individuals prefer to be informed about their personal genetic results by a health care professional or a university research lab than a genetic testing company [265]. The important role of the involvement of healthcare professionals in the communication of genetic results has also been discussed in other studies [270, 271]. Individuals would also prefer to receive personalized nutrition recommendation by registered dietitians [265].

## 3. OBJECTIVE

This study was designed to identify differences in circulating levels of leukocyte and lymphocyte subclasses between obese and non- obese individuals. Furthermore, the effect of weight loss due to personalized or non- personalized intervention on the frequency of circulating immune cells was also evaluated.

## 4. METHODS

### 4.1 Study design

Two groups of participants were included in this study. In the first group, 34 obese volunteers (BMI >30 kg/m$^2$) with no other existing co- morbidities, undergoing a weight loss program were included. Obese participants were assigned to either a personalized intervention group (n=4) who followed a diet based on their genetic background or a conventional diet group (n=7) who followed conventional low-calorie diet. Leukocyte subpopulations were assessed before (n=34) and after a 3-month genotype- based or conventional diet intervention (n=11).

The second group included 15 lean (BMI<25 kg/m$^2$) healthy volunteers.

Informed consent was obtained from all volunteers and venous blood was drawn into 10mL EDTA tubes. Leukocyte subpopulations count was determined either by a cell counter or by means of flow cytometry (Figure 11).

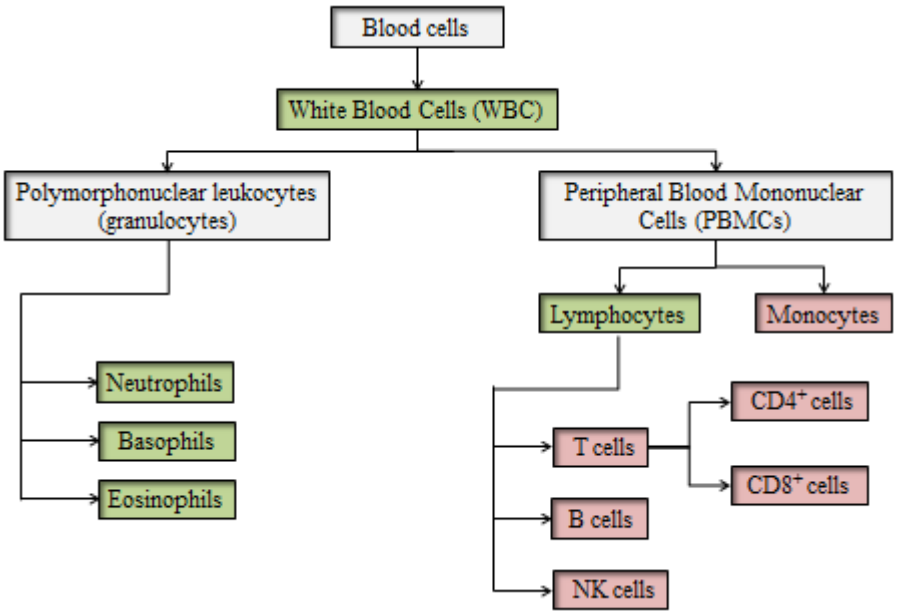



**Figure 11**. Schematic illustration of immune cells assessed in this study either by cell counter (green boxes) or by means of flow cytometry (red boxes). PBMC subpopulations were further analyzed by flow cytometry.

### 4.2 Isolation of peripheral blood mononuclear cells (PBMCs)

PBMCs exposure to systemic factors, including inflammatory agents and nutrients might render those cells probable biomarkers to predict early homeostatic energy imbalance [272]. The most commonly used method for the isolation of peripheral blood mononuclear cells (PBMCs), namely monocytes and lymphocytes, is by density gradient centrifugation. This method takes advantage of the property that immune cells possess different densities (Figure 12) and are separated in distinct layers during centrifugation in the presence of a density gradient media, such as Ficoll- Paque medium. Thus, cells possessing a high density will migrate through ficoll whereas cells with a lower density will be found on top of the ficoll layer following centrifugation. As a result, PBMCs- containing layer can be easily extracted and purified.

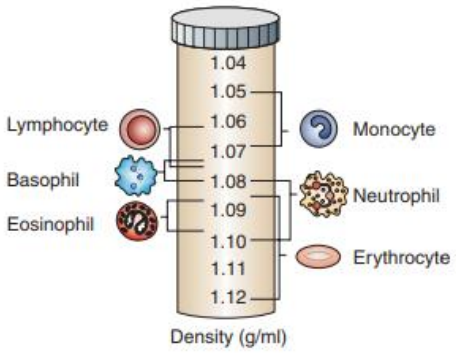



**Figure 12**. Different densities of human blood cells [273]

PBMCs were isolated from venous blood according to the following protocol.

- Peripheral blood was drawn in the presence of the anti-coagulant ethylenediamine-tetraacetic acid (EDTA) and then transferred to 50 ml falcon tube.

- 10-15 ml of Ficoll-Paque were prepared in a falcon tube.

- Peripheral blood was diluted 1:1 with Roswell Park Memorial Institute 1640 (RPMI).

- Ficoll was overlaid with diluted blood slowly against the wall of tube (10 ml Ficoll for <30 ml diluted blood).

- Cells were separated by spinning at 900g for 20min at 18$^{o}$C (no brake).

- Following spin, a Pasteur's pipette was used to collect the lymphocytes layer into a fresh tube (Figure 13). It was important to slowly suck and move in circular motion in order to ensure all possible white cloudy part was sucked up into a new falcon tube.

- Lymphocytes layer was topped up to 50 ml with RPMI and spinned at 300g for 10min at 4$^{o}$C, with brakes. Lymphocytes were washed 2 more times.

- Cells were counted prior last wash using Neubauer's chamber by trypan blue exclusion.

- After determining the number of cells, they were resuspended in appropriate volume of pyrogen- free saline, namely PBS$^{+}$ (1% Human serum, 2mM EDTA) to be used for flow cytometry profiling.

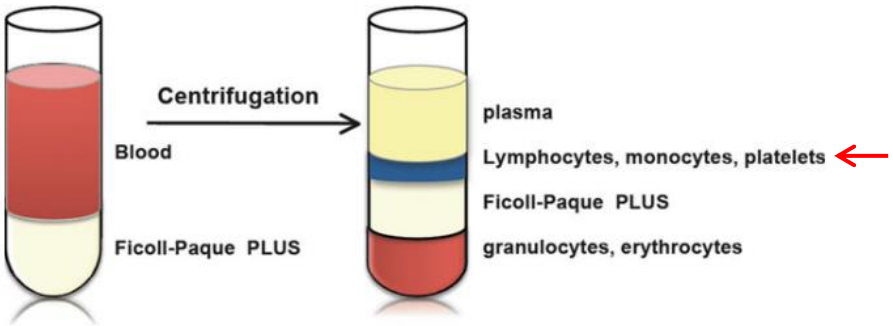



**Figure 13**. Experimental procedure for PBMCs isolation. First, peripheral blood is layered over ficoll (left part). Then, after centrifugation, blood components are separated into four layers (right part): (1) granulocytes, erythrocytes, (2) ficoll, (3) lymphocytes, monocytes (i.e. PBMCs), platelets (red arrow), and (4) plasma [274].

### 4.3 Immune profiling using flow cytometry

Flow cytometry is a widely used laser- based technique used for the analysis of the expression of cell surface and intracellular molecules, the definition of cell size and volume, the detection of cell viability, and the characterization of different cell types in a mixed cell population. It was first used in the 1950s for the estimation of cell volume in a rapidly flowing fluid stream during cell passing in front of a viewing

hole. Nowadays, flow cytometers allow the simultaneous detection of an increased number of fluorescent parameters on the same cell (from 1 up to >30) and the extremely rapid collection of information from millions of cells.

The simultaneous multi- parameter analysis of single cells is the most powerful application of flow cytometry. Its functional principle is based on the measurement of fluorescence intensity emitted by fluorescent- labeled antibodies detecting ligands or proteins that bind to cell- associated factors. In order to prepare samples for flow cytometry analysis, a single- cell suspension from tissue samples or cell culture is incubated with fluorochrome- labeled or unlabeled antibodies and are then analyzed.

The three main components of a cytometer are (Figure 14):

- the fluidics system, that is responsible for sample transportation from the container to the flow cell,
- the optical system, that includes excitation light sources, lenses and filters responsible for light collection and spread around the cytometer, as well as the detection system that gives rise to photocurrent generation,
- the electronics, in which the photocurrent is digitized and prepared for further analysis

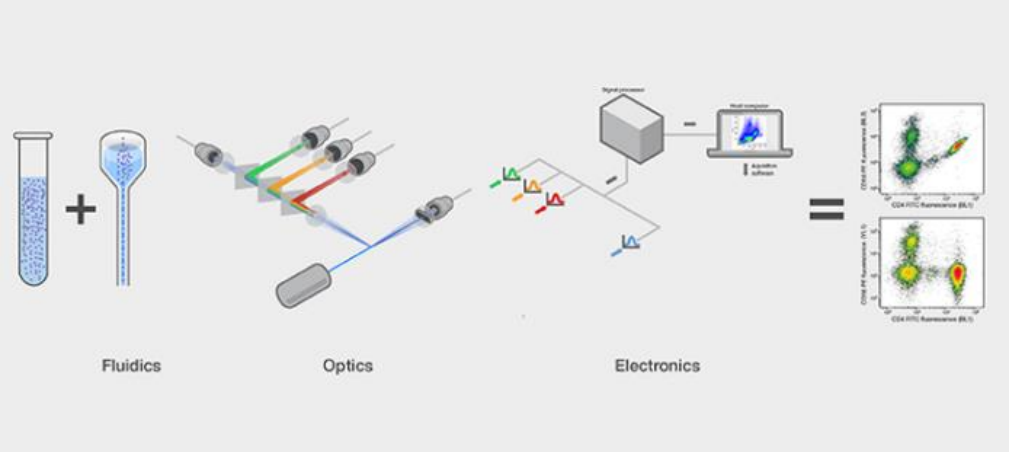



**Figure 14**. Schematic illustration of the three main parts of a flow cytometer [275]

It is important to mention that instruments used for sorting cells into different containers in real time (Fluorescence activated cell sorting [FACS]), are also consisted by a cell sorting component (Figure 15).

The sample is drawn from the tube or multi- well plate and is injected into the flow chamber. There, laminar stream of sheath fluid focuses the cells through a nozzle and allows only the single pass of cells through the laser light at a time. Cells or particles scatter the light following their route through the laser beam. Forward scatter (FS) is measured by a detector placed in front of the light beam and is indicative of the size of the cell or particle passing in front of the laser. Side scatter (SS) is measured by several detectors placed to the side of the light beam and detects changes in optical density and complexity of the objects. Fluorescence produced by stained cells is detected by fluorescence detectors (Figure 15).

Except for fluorescent dyes used in the experiment, fluorescence may also originate from autofluorescence within a cell. In order to determine the real signal emitted by an antibody, estimation of the background fluorescence is conducted by using unstained cells or isotype antibodies. Isotypes are antibodies with a binding domain that does not target any cell molecule and a constant region that may bind to some receptors on cell surface and dead cells. The isotype control should ideally be the same isotype, in terms of species, heavy and light chain, conjugated to the same fluorophore and have the same F:P ratio (i.e. a ratio indicative of the number of fluorescent molecules detected on an antibody) as the experimental antibody.

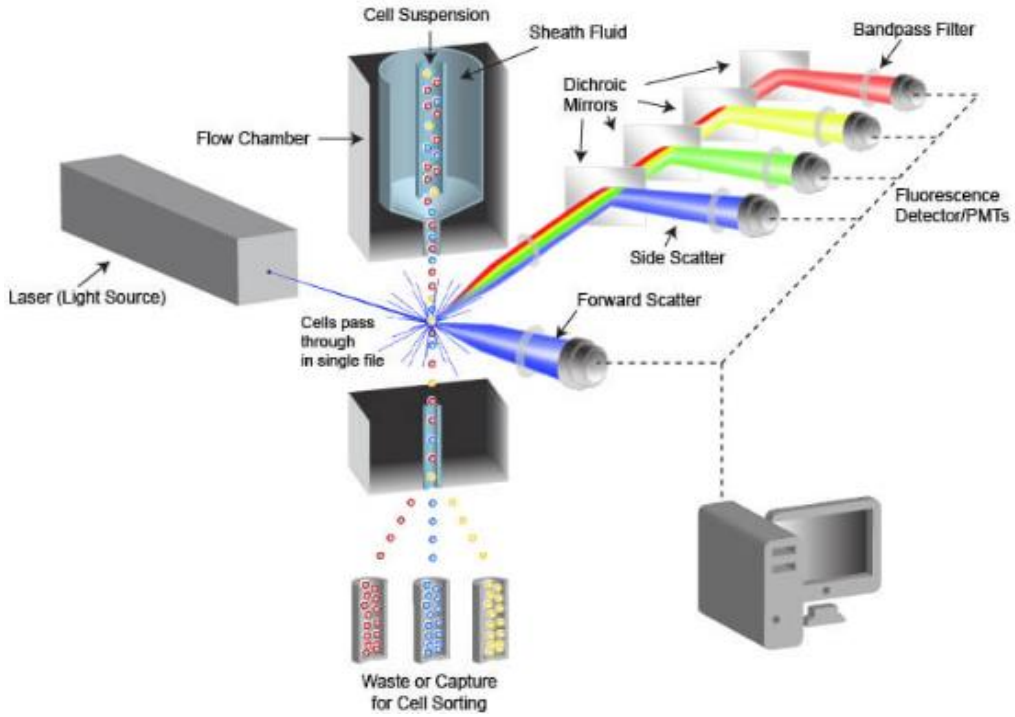



**Figure 15**. Schematic illustration of flow cytometry system and main parts [276]

We stained isolated PBMCs for flow cytometry profiling according to the following protocol:

- $2x10^6$ cell suspension in $PBS^+$ were dispensed in three different tubes.

- Antibody mastermix was prepared and 6μL of antibody mixture were added in the appropriate tube (Table 1). The dilution of each monoclonal antibody in each tube was 1:10. Singles and isotype controls were also prepared.

**Table 1**. Antibody mixture used for each staining (St1, St2, St3) for the detection of monocytes, NK cells and T cell subsets

| **St1** | **St2** | **St3** |
|---|---|---|
| **T cells** | **$CD8^+$ T cells/ Tregs** | **Monocytes/ NK cells** |

| | | |
|---|---|---|
| CD3 FITC | CD3 FITC | CD45 PE/Dazzle |
| CXCR3 PE | CD4 PE | CD 14 PE |
| CD45RO APC | | CD16 FITC |
| CD4 PercPCy5.5 | CD127 PECy5 | CD15 PECy7 |
| CD45RA PECy7 | CD25 PECy7 | HLA-DR APCCy7 |
| CCR7 APCCy7 | CD8 APCCy7 | CD56 APC |

- After 30min of incubation on ice in the dark, tubes were centrifuged at 1500rpm for 7min at $4^{o}$C.

- Supernatants were discarded and cells were resuspended in 150 μL $PBS^{+}$. DAPI (dilution 1:1000) was added when needed in order to exclude dead cells.

- Cells were then analyzed by flow cytometry (FACS Aria).

- Data acquisition and analysis were performed with the FACS Diva Software version 6.1.3.

### 4.4 Statistical analysis

GraphPad Prism 6 was used for graph construction and the statistical analysis of results. WBC percentages are expressed as mean± standard error of the mean (SEM). Statistical analysis was performed using the unpaired Mann- Whitney t- test (two-tailed) when comparing cell absolute counts and percentages derived from obese and lean participants. Two- way analysis of variance (ANOVA) was used to assess the statistical significance of the differences in PBMCs subtypes levels between genotype-based and conventional diet following the 3- month intervention. Sidac multiple comparison test assuming that each comparison is independent of the others, was used for post hoc comparison. The threshold for significance was set at $p< 0.05$.

## 5. RESULTS

### 5.1 Participants' characteristics

The baseline characteristics of the 49 study participants are depicted in Table 2. As described in "Methods" section, participants were classified according to their BMI and were defined as obese (BMI= 34.45±1.08 kg/$m^2$, n=34) or lean (BMI= 22.64±0.52 kg/$m^2$, n=15). 71% of them were female (60% non- obese and 76% obese) and mean age was 32.60±2.70 years for lean and 38.21±1.58 years for obese subjects.

**Table 2**. Baseline characteristics of the participants included in the study (*: mean±SEM).

| | **Lean** | **Obese** |
|---|---|---|
| **No.** | 15 | 34 |
| **Sex ratio, F/M** | 9/6 | 26/8 |
| **Age, years*** | 32.60±2.70 | 38.21±1.58 |
| **Weight, kg*** | 65.30±2.10 | 96.17±3.41 |
| **BMI, kg/$m^2$*** | 22.64±0.52 | 34.45±1.08 |

### 5.2 Gating strategy in flow cytometry profiling

In order to evaluate the number of immune cells present during obesity, three gating strategies were followed.

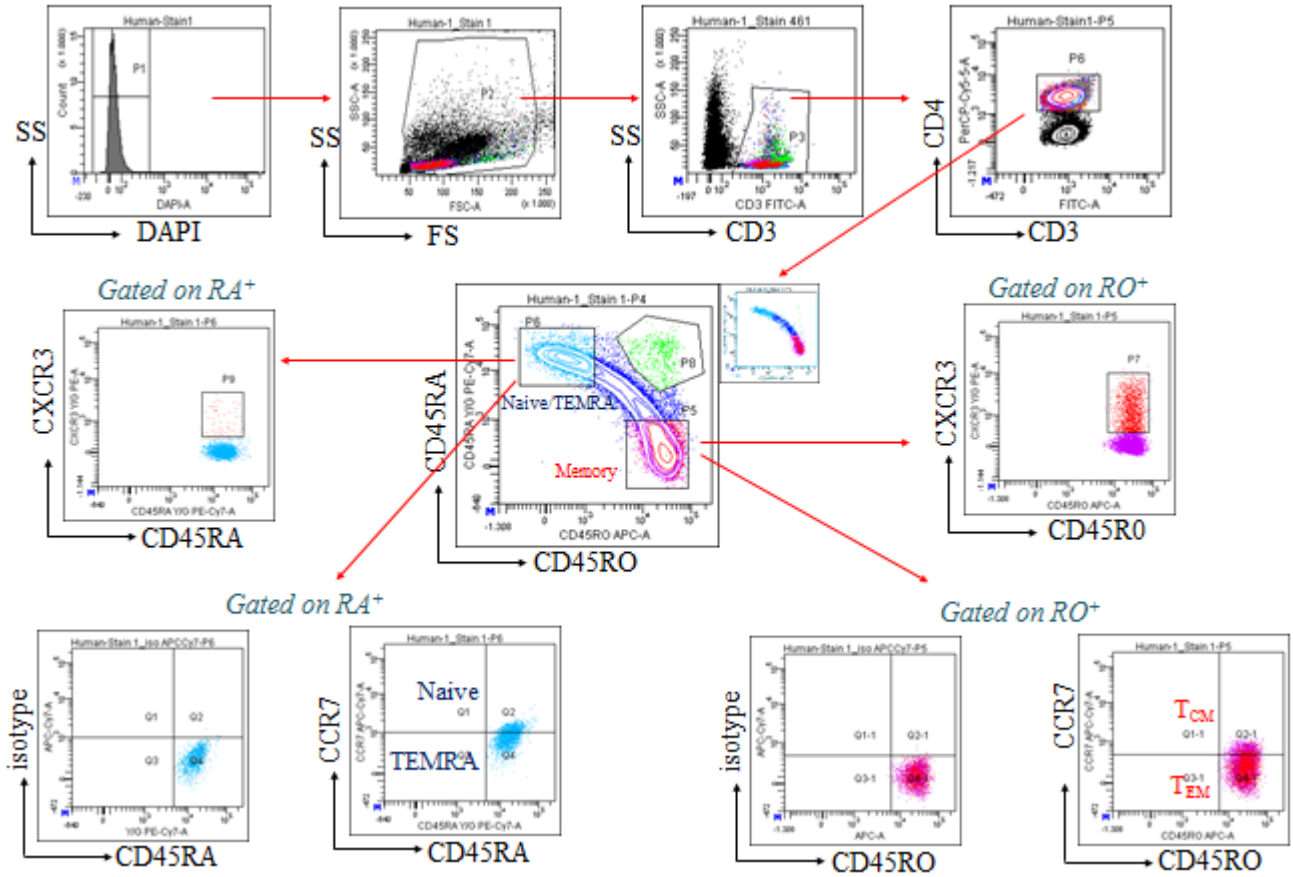
#### Staining 1

In this strategy, the following cell populations were assessed (Figure 16):

- T cells ($CD3^+$)
- $CD4^+$ T cells
- Naïve T cells ($CD45RA^+CCR7^+$)
- Central memory T cells ($T_{CM}$) ($CD45RO^+CCR7^+$)

- Effector memory T cells ($T_{EM}$) ($CD45RO^{+}CCR7^{-}$)
- Terminally differentiated effector memory cells (TEMRA) ($CD45RA^{+}CCR7^{-}$)
- $CD45RA^{+}CD45RO^{+}$ T cells
- $CD45RA^{+}CXCR3^{+}$ T cells
- $CD45RO^{+}CXCR3^{+}$ T cells

**Figure 16**. Gating strategy followed for the assessment of $CD4^{+}$ T cells subtypes



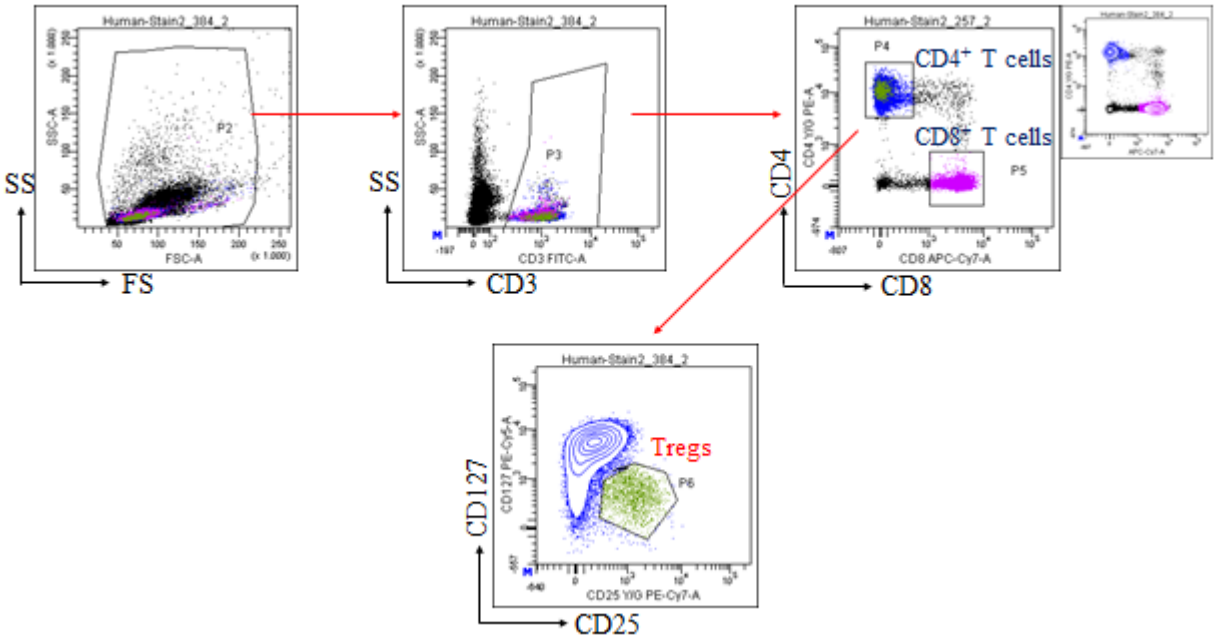

(Staining 1)

**Staining 2**

In this strategy, the following cell populations were assessed (Figure 17):

- T cells ($CD3^{+}$)
- $CD4^{+}$ T cells
- T cytotoxic cells ($CD8^{+}$)
- Regulatory T cells (Tregs) ($CD127^{-}CD25^{+}$)

**Figure 17**. Gating strategy followed for the assessment of $CD8^+$ T cells and T




regulatory cells (Tregs) (Staining 2)

### Staining 3

In this strategy, the following cell populations were evaluated (Figure 18):

- Monocytes
  - Classical ($CD14^+CD16^-$)
  - Intermediate ($CD14^+CD16^+$)
  - $CD14^{low}CD16^+$
  - Non- classical ($CD14^-CD16^+$)
- NK cells
  - $CD56^+CD16^+$
  - $CD56^{bright}CD16^-$
  - $CD56^{dim}CD16^-$
- B lymphocytes ($CD16^-CD14^-HLA\text{-}DR^+$)

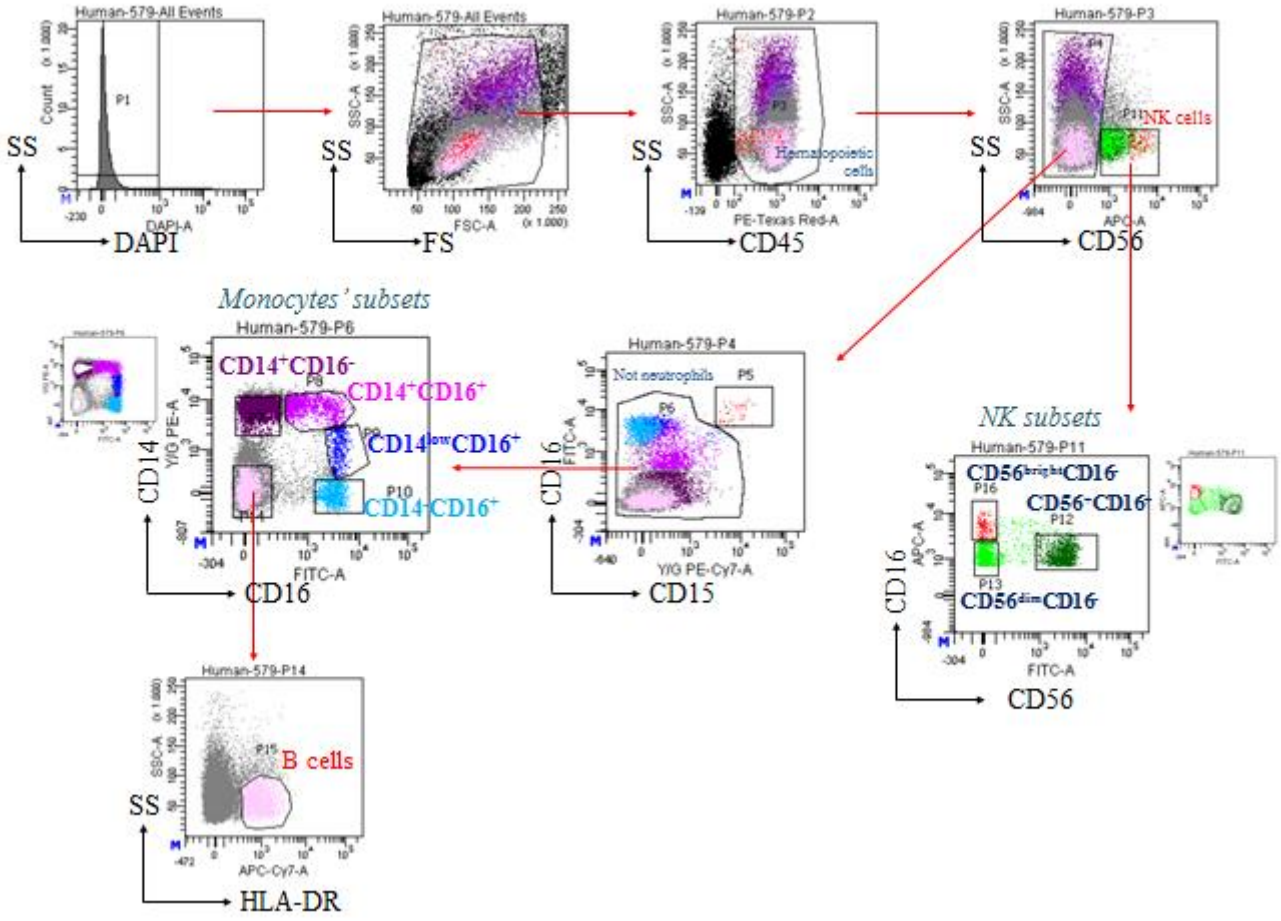



**Figure 18**. Gating strategy followed for the assessment of B lymphocytes, monocyte and NK subsets (Staining 3)

### 5.3 Differences in immune cell numbers between lean and obese individuals

Differences in absolute numbers and percentages of immune cells between obese and non- obese participants are summarized in Table 3.

**Table 3**. Leukocyte and lymphocyte subset counts or percentages in lean and obese individuals. Values are presented as mean± standard error of the mean (SEM). (*: p values in bold represent statistical significant differences between the obese and control group, $p<0.05$)

| Type of cell | Lean | Obese | |
|---|---|---|---|
| | mean±SEM | | p value* |
| **White blood cells (cells/µL)** | 6423±308.4 | 6809±439 | 0.9045 |
| **Basophils (% total cells)** | 0.79±0.1426 | 0.6543±0.07273 | 0.4612 |
| **Eosinophils (% total cells)** | 2.75±0.5272 | 2.628±0.2561 | 0.6211 |
| **Neutrophils (% total cells)** | 54.15±2.252 | 54.98±1.556 | 0.7033 |
| **PBMCs (total cells)** | $1.87x10^7$±$1.957x10^6$ | $2.654x10^7$±$1.533x10^6$ | **0.0037** |
| **Classical monocytes ($CD14^+CD16^-$) (% total PBMCs)** | 10.09±1.297 | 8.475±0.5443 | 0.4636 |
| **Intermediate monocytes ($CD14^+CD16^+$) (% total PBMCs)** | 1.26±0.1848 | 1.461±0.1768 | 0.7056 |
| **Non-classical monocytes ($CD14^-CD16^+$) (% total PBMCs)** | 2.42±0.4900 | 3.402±0.6301 | 0.6309 |
| **$CD14^{low}CD16^+$ monocytes (% total PBMCs)** | 0.5779±0.09208 | 0.7293±0.08834 | 0.3861 |
| **Lymphocytes (% total cells)** | 35.74±1.560 | 34.83±1.364 | 0.4956 |
| **T ($CD3^+$) lymphocytes (% total PBMCs)** | 43.73±2.388 | 48.51±1.395 | 0.0506 |
| **B lymphocytes (% total PBMCs)** | 6.304±0.6829 | 6.481±0.4483 | >0.9999 |
| **NK cells (% total PBMCs)** | 8.438±0.6743 | 7.224±0.5447 | 0.1072 |
| **$CD56^{bright}CD16^-$ NK cells (% total PBMCs)** | 0.2679±0.03839 | 0.3117±0.04647 | 0.4064 |
| **$CD56^{bright}CD16^-$ NK cells (% NK cells)** | 3.7±0.6942 | 4.443±0.5208 | 0.1446 |

| | | | |
|---|---|---|---|
| **$CD56^{dim}CD16^{-}$ NK cells (% total PBMCs)** | 1.281±0.2568 | 1.46±0.2241 | 0.5617 |
| **$CD56^{dim}CD16^{-}$ NK cells (% NK cells)** | 15.71±2.456 | 20.35±2.635 | 0.2161 |
| **$CD56^{+}CD16^{+}$ NK cells (% total PBMCs)** | 6.287±0.6456 | 4.897±0.4450 | 0.0664 |
| **$CD56^{+}CD16^{+}$ NK cells (% NK cells)** | 72.41±3.591 | 66.88±3.410 | 0.3753 |
| **$CD3^{+}CD4^{+}$ (% total PBMCs)** | 28.89±2.177 | 32.61±1.637 | 0.1481 |
| **$CD3^{+}CD4^{+}$ (% $CD3^{+}$ cells)** | 61.27±1.776 | 63.58±1.608 | 0.48 |
| **$CD3^{+}CD8^{+}$ (% total PBMCs)** | 12.63±0.8096 | 12.4±0.7214 | 0.7541 |
| **$CD3^{+}CD8^{+}$ (% $CD3^{+}$ cells)** | 27.77±1.853 | 24.47±1.080 | 0.1173 |
| **$CD3^{+}CD4^{+}CD45RA^{+}CD45RO^{-}$ (% total PBMCs)** | 10.04±1.418 | 9.206±0.6649 | 0.7704 |
| **$CD3^{+}CD4^{+}CD45RA^{+}CD45RO^{-}$ (% $CD4^{+}$)** | 35.74±3.385 | 29.92±1.710 | 0.2466 |
| **$CD3^{+}CD4^{+}CD45RA^{-}CD45RO^{+}$ (% total PBMCs)** | 10.92±0.8434 | 13.96±0.6497 | **0.0124** |
| **$CD3^{+}CD4^{+}CD45RA^{-}CD45RO^{+}$ (% total $CD4^{+}$)** | 43.39±3.248 | 45.9±1.550 | 0.5408 |
| **$CD3^{+}CD4^{+}CD45RA^{+}CD45RO^{+}$ (% total PBMCs)** | 0.5667±0.5667 | 0.6106±0.06858 | 0.4816 |
| **$CD3^{+}CD4^{+}CD45RA^{+}CD45RO^{+}$ (% total $CD4^{+}$)** | 1.893±0.3098 | 2.032±0.2053 | 0.7031 |
| **$CD3^{+}CD4^{+}CD45RA^{+}CCR7^{+}$ (Naïve) (% total PBMCs)** | 1.617±0.2635 | 2.22±0.2686 | 0.3142 |
| **$CD3^{+}CD4^{+}CD45RA^{+}CCR7^{+}$ (Naïve) (% $CD4^{+}$)** | 6.827±1.243 | 7.438±0.8730 | 0.8117 |
| **$CD3^{+}CD4^{+}CD45RA^{+}CCR7^{-}$ (TEMRA) (% total PBMCs)** | 10.63±1.600 | 12.01±0.9013 | 0.3549 |

| | | | |
|---|---|---|---|
| **$CD3^+CD4^+CD45RA^+CCR7^-$ (TEMRA) (% $CD4^+$)** | 46.1±4.053 | 45.96±2.001 | 0.9023 |
| **$CD3^+CD4^+CD45RO^+CCR7^+$ (CM) (% total PBMCs)** | 0.3336±0.05293 | 0.5962±0.08440 | 0.1772 |
| **$CD3^+CD4^+CD45RO^+CCR7^+$ (CM) (% $CD4^+$)** | 1.304±0.2253 | 2.079±0.3258 | 0.3363 |
| **$CD3^+CD4^+CD45RO^+CCR7^-$ (EM) (% total PBMCs)** | 13.76±1.065 | 16.94±1.183 | 0.1361 |
| **$CD3^+CD4^+CD45RO^+CCR7^-$ (EM) (% $CD4^+$)** | 41.56±3.024 | 43.51±1.525 | 0.7179 |
| **$CD3^+CD4^+CD45RA^+CXCR3^+$ (% total PBMCs)** | 0.366±0.06918 | 0.3265±0.03911 | 0.8595 |
| **$CD3^+CD4^+CD45RA^+CXCR3^+$ (% $CD4^+$)** | 1.255±0.1832 | 1.083±0.1346 | 0.3749 |
| **$CD3^+CD4^+CD45RO^+CXCR3^+$ (% total PBMCs)** | 5.032±0.5299 | 5.316±0.4552 | 0.7156 |
| **$CD3^+CD4^+CD45RO^+CXCR3^+$ (% $CD4^+$)** | 19.52±1.724 | 17.28±1.266 | 0.362 |
| **$CD3^+CD4^+CD127^-CD25^+$ (T regs) (% total PBMCs)** | 1.996±0.1423 | 2.157±0.1446 | 0.6406 |
| **$CD3^+CD4^+CD127^-CD25^+$ (T regs) (% $CD4^+$)** | 7.06±0.3254 | 6.559±0.2522 | 0.1616 |

**White blood cells (WBC)**

Increased white blood cell counts were observed in obese (mean± SEM= 6809±439 cells/μL) compared to non- obese participants (mean± SEM= 6423±308.4 cells/μL) but the difference did not reach the levels of statistical significance (Figure 19A).

### 5.3.1 Polymorphonuclear leukocytes (granulocytes)

Regarding polymorphonuclear leukocytes, a reduction in basophil levels (mean± SEM= 0.65±0.07% vs. 0.79±0.14%) (Figure 19B) and a slight increase in neutrophils' levels (mean± SEM= 54.98±1.56% vs. 54.15±2.25%) (Figure 19C) were observed in obese compared to lean individuals. Furthermore, the percentage of eosinophils in the

obese group was lower than that of controls (mean± SEM= 2.63±0.26% vs. 2.75±0.53%) (Figure 19D).

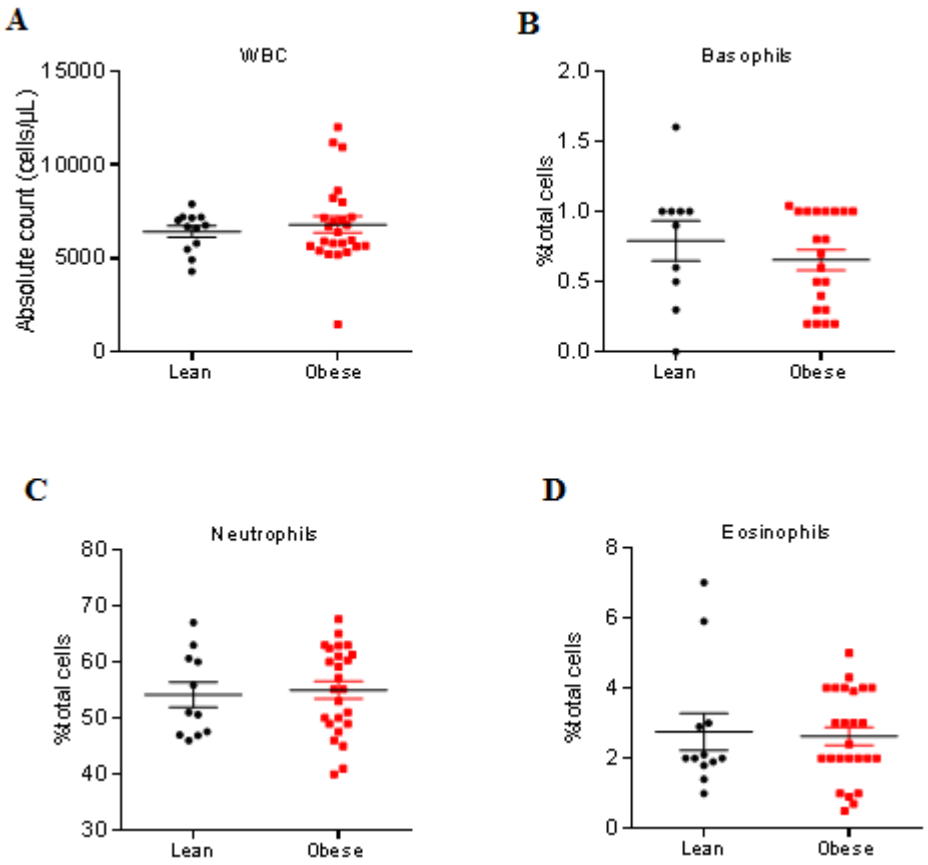



**Figure 19**. Differences in white blood cell (WBC) counts (n=12 lean/ 25 obese) (A) and granulocytes' [basophils (B) (n=10 lean/ 21 obese), neutrophils (C) (n=11 lean/ 25 obese), eosinophils (D) (n=12 lean/ 25 obese)] percentages between lean and obese participants. Comparisons between groups were performed using a Mann- Whitney t-test. In all cases $p>0.05$.

### 5.3.2 Peripheral Blood Mononuclear Cells (PBMCs)

Circulating numbers of PBMCs were significantly higher ($p=0.0037$) in obese (mean± SEM= $2.65x10^7$±$1.53x10^6$ cells) compared to non- obese (mean± SEM= $1.87x10^7$±$1.96x10^6$ cells) individuals (Figure 20A).

#### 5.3.2.1 Monocytes

As for monocytes' total percentages, an increase in $CD14^-CD16^+$ (non- classical) (mean± SEM= 3.40±0.63% vs. 2.42±0.49%) (Figure 20B), $CD14^+CD16^+$

(intermediate) (mean± SEM= 1.46±0.18% vs. 1.26±0.18%) and $CD14^{low}CD16^{+}$ (mean± SEM= 0.73±0.09% vs. 0.58±0.09%) (Figure 20C) subsets in obese compared to lean individuals were observed. However, $CD14^{+}CD16^{-}$ (classical) monocytes (Figure 20B) were found decreased in obese (mean± SEM= 8.47±0.54%) compared to non- obese (mean± SEM= 10.09±1.3%) individuals.

### 5.3.2.2 Lymphocytes

Relative to non- obese subjects (mean± SEM= 35.74±1.56%), obese participants experienced a slight reduction in lymphocytes' total percentage (mean± SEM= 34.83±1.36%) (Figure 20D).

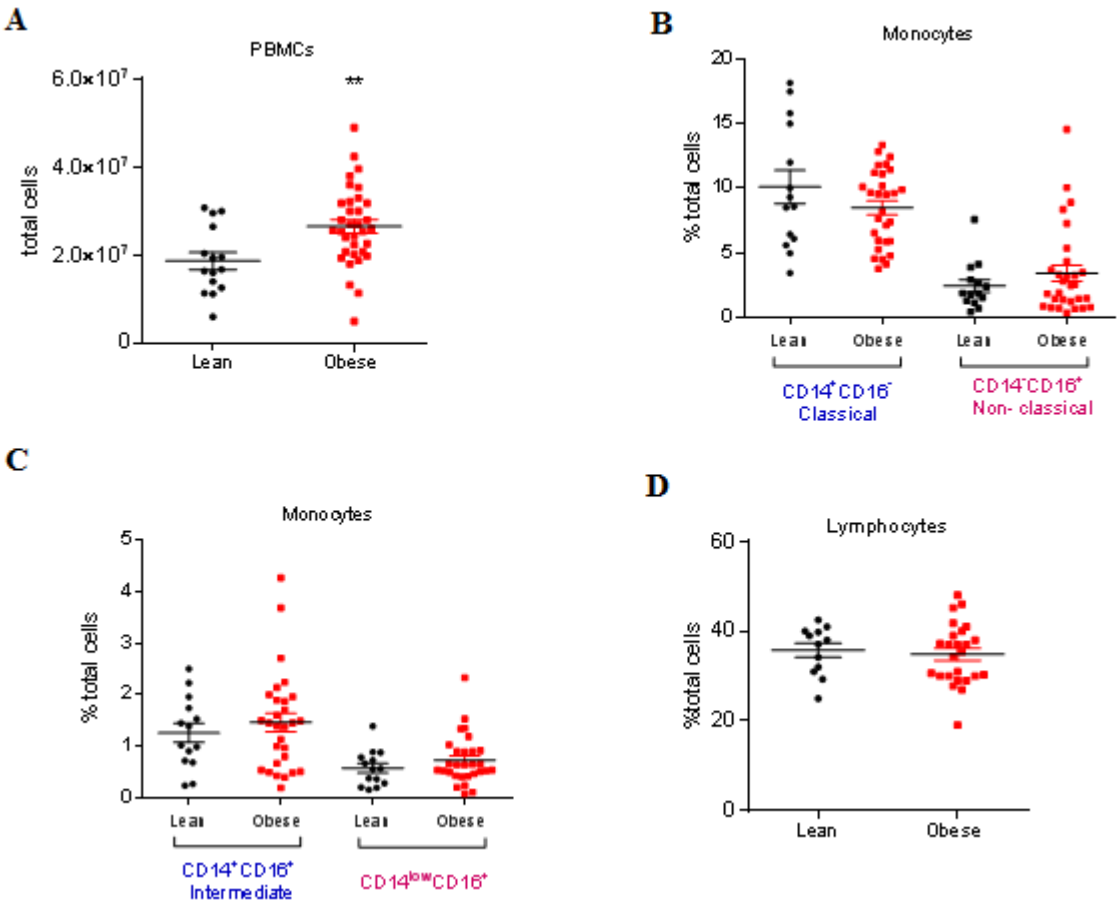



**Figure 20**. Differences in PBMCs counts (n=15 lean/ 34 obese) (A), as well as in monocytes' subsets [$CD14^{+}CD16^{-}$ (classical), $CD14^{-}CD16^{+}$ (non- classical) (B), $CD14^{+}CD16^{+}$ (intermediate), $CD14^{low}CD16^{+}$ (C)] (n=14 lean/ 29 obese) and lymphocytes' percentages (n=12 lean/ 25 obese) (D) between lean and obese

individuals. Comparisons between groups were performed using a Mann- Whitney t-test. **: p=0.0037

**Lymphocyte compartment**

Elevated T cell ($CD3^+$) (mean± SEM= 48.51±1.39% vs. 43.73±2.39%) (p= 0.0506) (Figure 21A), decreased NK cell (mean± SEM= 7.22±0.54% vs. 8.44±0.67%) (Figure 21B) and similar levels of B cells (mean± SEM= 6.48±0.45% vs. 6.30±0.68%) (Figure 21C) were observed in obese compared to lean individuals.

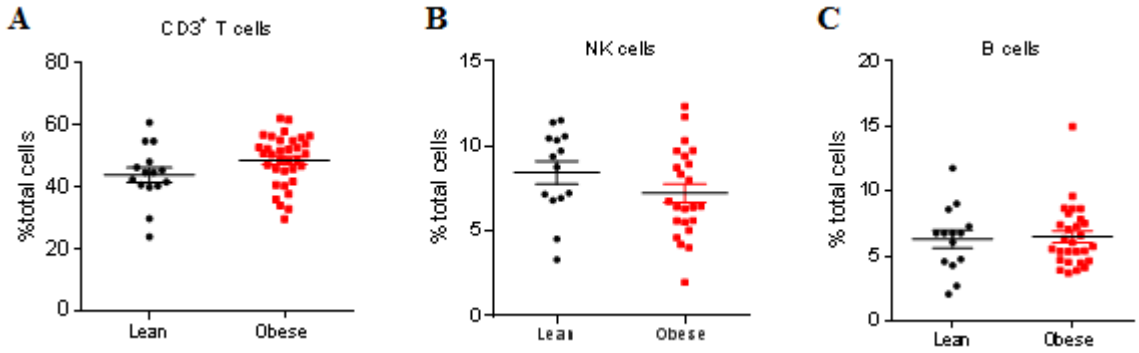



**Figure 21**. Differences in T lymphocytes ($CD3^+$) (n=15 lean/ 34 obese) (A), NK cells (n=14 lean/ 23 obese) (B) and B lymphocytes (n=14 lean/ 28 obese) (C) levels between lean and obese individuals. Comparisons between groups were performed using a Mann- Whitney t- test. In all cases p>0.05.

**NK cell compartment**

Total percentages of $CD56^{bright}CD16^{-}$ (mean± SEM=0.31±0.05% vs. 0.27±0.04%) (Figure 22A) and $CD56^{dim}CD16^{-}$ (mean± SEM= 1.46±0.22% vs. 1.28±0.26%) (Figure 22B) NK cells were elevated in obese individuals. A reduction in $CD56^{+}C16^{+}$ levels (Figure 22C) in the same group (mean± SEM= 4.9±0.44%) compared to non- obese participants (mean± SEM= 6.29±0.64%) was observed though (p=0.0664). Similar observations were reported even when cell percentages regarding NK cell compartment were examined ($CD56^{bright}CD16^{-}$:4.44±0.52% vs. 3.7±0.69%, $CD56^{dim}CD16^{-}$: 20.35±2.63% vs. 15.71±2.46%, $CD56^{+}C16^{+}$: 66.88±3.41% vs. 72.41±3.59%) (Figure 22D-F).

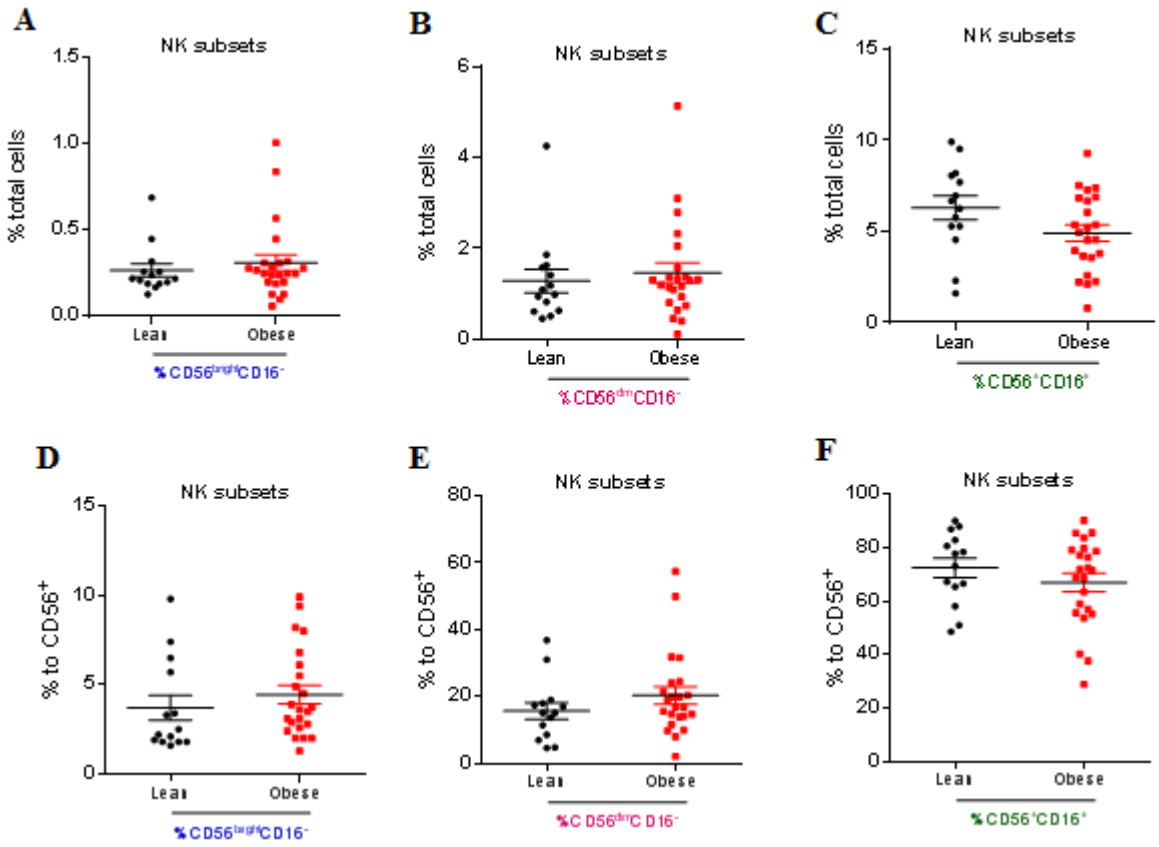



**Figure 22**. Total and % percentage to total NK cells ($CD56^{+}$) of $CD56^{bright}CD16^{-}$ (A, D), $CD56^{dim}CD16^{-}$ (B, E), and $CD56^{+}C16^{+}$ (C, F) NK cells, respectively, in obese (n=23) and lean (n=14) group. Comparisons between groups were performed using a Mann- Whitney t- test. In all cases p>0.05.

**T lymphocyte compartment**

In obese individuals, total levels of $CD4^+$ T cells were higher (mean± SEM= 32.61±1.64% vs. 28.89±2.18%) (Figure 23A) whereas total percentage of $CD8^+$ T cells was slightly lower (mean± SEM= 12.40±0.72% vs. 12.63±0.81%) (Figure 23B) than those of controls. Regarding $CD3^+$ T cell compartment, $CD4^+$ T cells were increased (mean± SEM= 63.58±1.61% vs. 61.27±1.78%) (Figure 23C) and $CD8^+$ T cells were decreased (mean± SEM= 24.47±1.08% vs. 27.77±1.85%) (Figure 23D) in obese compared to non- obese participants.

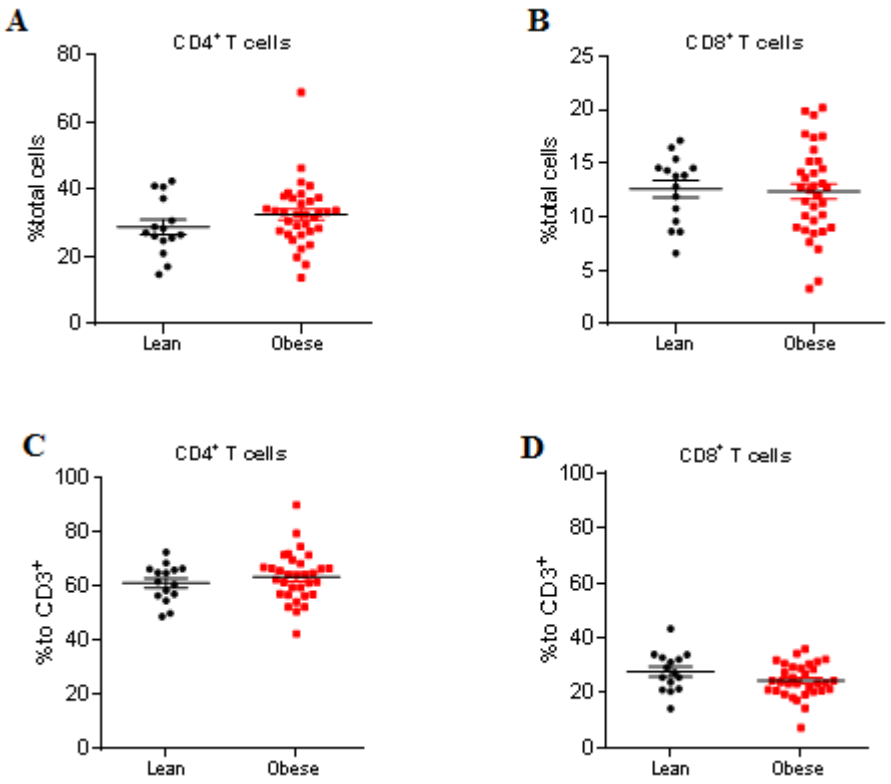



**Figure 23**. Total (n=15 lean/ 34 obese) and % percentage to T lymphocytes ($CD3^+$) (n=15 lean/ 32 obese) of $CD4^+$ (A, C) and $CD8^+$ (B, D) T cells, respectively, in obese and lean participants. Comparisons between groups were performed using a Mann-Whitney t- test. In all cases $p>0.05$.

**$CD4^+$ T cells compartment**

$CD4^+$ T cells were further assessed based on the expression of CD45RA/CD45RO markers. More specifically, total $CD4^+CD45RA^+$ T cells (Naïve/ TEMRA) were

decreased in obese individuals (mean± SEM= 9.21±0.66% vs. 10.04±1.42%) (Figure 24A). The levels of $CD4^+CD45RO^+$ T cells (Memory) were elevated in obese participants (mean± SEM= 13.96±0.65% vs. 10.92±0.84%) and the difference between the obese and control group reached the levels of statistical significance (p=0.0124) (Figure 24B). Furthermore, total percentage of $CD4^+CD45RA^+CD45RO^+$ T cells was slightly increased in the obese group (mean± SEM= 0.61±0.07% vs. 0.57±0.12%) (Figure 24C).

When cell levels were examined regarding their distribution in $CD4^+$ T cell compartment, decreased levels of $CD4^+CD45RA^+$ (mean± SEM= 29.92±1.71% vs. 35.74±3.38%) (Figure 24D) and increased levels of $CD4^+CD45RO^+$ (mean± SEM= 45.90±1.55% vs. 43.39±3.25%) (Figure 24E) and $CD4^+CD45RA^+CD45RO^+$ (mean± SEM= 2.03±0.21% vs. 1.89±0.31%) (Figure 24F) T cells were observed in obese compared to lean individuals.

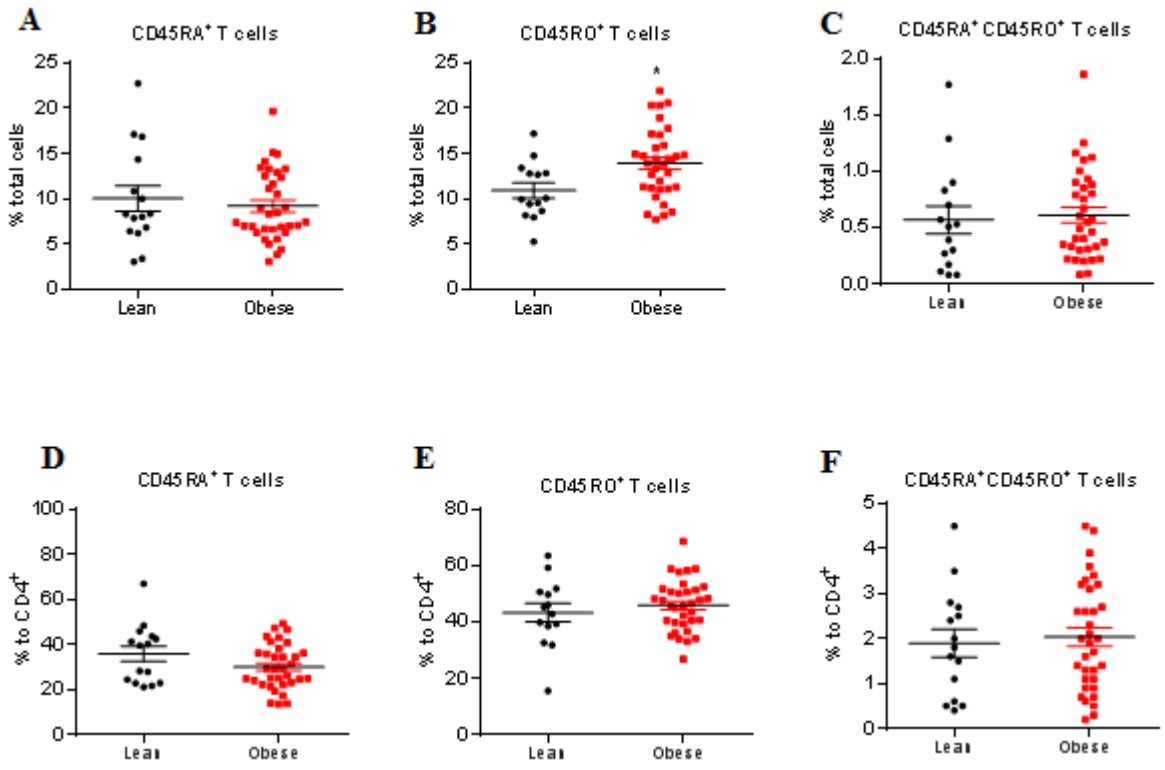



**Figure 24**. Total and % percentage to $CD4^+$ T lymphocytes of $CD4^+CD45RA^+$ (n=15 lean/ 34 obese) (A, D), $CD4^+CD45RO^+$ (n=14 lean/ 34 obese) (B, E) and $CD4^+CD45RA^+CD45RO^+$ (n=15 lean/ 34 obese) (C, F) T cells, respectively, in obese

and lean individuals. Comparisons between groups were performed using a Mann-Whitney t- test. *: p= 0.0124

The expression of the marker CCR7 was used for the classification of cells belonging to CD45RA and CD45RO compartment.

Relative to non- obese individuals, obese subjects experienced noteworthy increases in total levels of $CD4^+CD45RA^+CCR7^+$ (Naïve) (mean± SEM= 2.22±0.27% vs. 1.62±0.26%) (Figure 25A) and $CD4^+CD45RA^+CCR7^-$ (TEMRA) (mean± SEM= 12.01±0.90% vs. 10.63±1.6%) (Figure 25B) T cells. Regarding their distribution in $CD4^+$ T cell compartment, naïve T cells were increased in obese group (mean± SEM= 7.44±0.87% vs. 6.83±1.24%) (Figure 25C) whereas TEMRA T cells were about the same at both groups (mean± SEM= 45.96±2.00% vs. 46.10±4.05%) (Figure 25D).

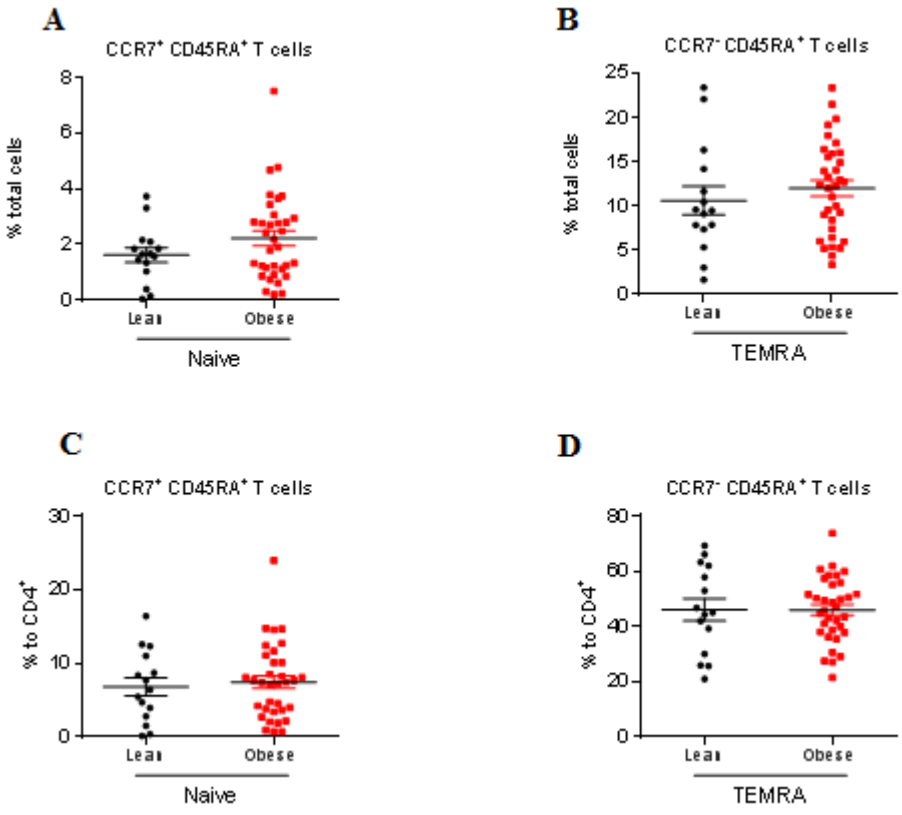



**Figure 25**. Total and % percentage to $CD4^+$ T lymphocytes of $CD4^+CD45RA^+CCR7^+$ (Naïve) (A, C) and $CD4^+CD45RA^+CCR7^-$ (TEMRA) (B, D) T cells, respectively, in obese (n=34) and lean (n=15) participants. Comparisons between groups were performed using a Mann- Whitney t- test. In all cases p>0.05.

As for memory cells, total $CD4^+CD45RO^+CCR7^+$ (CM) (mean± SEM= 0.60±0.08% vs. 0.33±0.05%) (Figure 26A) and $CD4^+CD45RO^+CCR7^-$ (EM) (mean± SEM= 16.94±1.18% vs. 13.76±1.06%) (Figure 26B) were elevated in obese participants compared to the control group. This increase in both groups was reserved even when the classification in $CD4^+$ T cell compartment was evaluated (CM: 2.08±0.32% vs. 1.30±0.22%, EM: 43.51±1.52% vs. 41.56±3.02%) (Figure 26C, D).

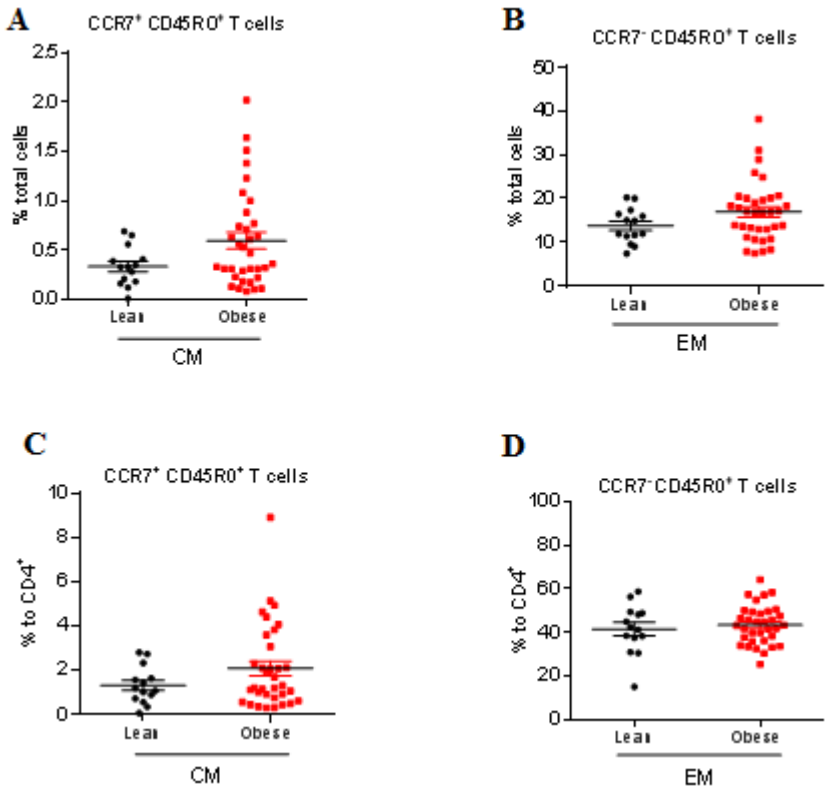



**Figure 26**. Total and % percentage to $CD4^+$ T lymphocytes of $CD4^+CD45RO^+CCR7^+$ (CM) (A, C) and $CD4^+CD45RO^+CCR7^-$ (EM) (B, D) T cells, respectively, in obese (n=34) and control (n=14) group. Comparisons between groups were performed using a Mann- Whitney t- test. In all cases $p>0.05$.

The expression of the CXCR3 marker by $CD4^+CD45RA^+$ and $CD4^+CD45RO^+$ T cells was also evaluated. Total $CD4^+CD45RA^+CXCR3^+$ T cells were decreased (mean± SEM= 0.33±0.04% vs. 0.37±0.07%) (Figure 27A) whereas $CD4^+CD45RO^+CXCR3^+$ were increased (mean± SEM= 5.32±0.45% vs. 5.03±0.53%) (Figure 27B) in obese

compared to lean participants. Furthermore, regarding their distribution in $CD4^+$ T cell compartment, both $CD45RA^+CXCR3^+$ (mean± SEM= 1.08±01.3% vs. 1.25±0.18%) (Figure 27C) and $CD45RO^+CXCR3^+$ (mean± SEM= 17.28±1.27% vs. 19.52±1.72%) (Figure 27D) were decreased in the obese group.

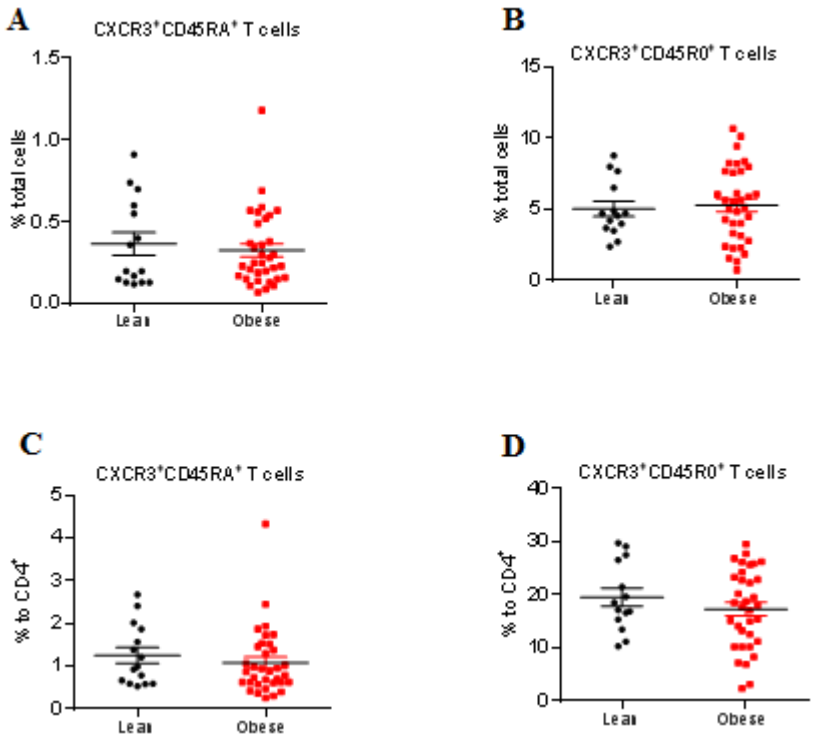



**Figure 27**. Total and % percentage to $CD4^+$ T lymphocytes of $CD4^+CD45RA^+CXCR3^+$ (n=15 lean/ 34 obese) (A, C) and $CD4^+CD45RO^+CXCR3^+$ (n=14 lean/ 34 obese) (B, D) T cells, respectively, in obese and control participants. Comparisons between groups were performed using a Mann- Whitney t- test. In all cases $p>0.05$.

The last subtype of $CD4^+$ T lymphocytes examined was the anti- inflammatory Treg cells. Their total levels were elevated in obese compared to lean participants (mean± SEM= 2.16±0.14% vs. 2.00±0.14%) (Figure 28A). However, their levels were decreased in the obese group regarding their distribution in $CD4^+$ T cell compartment (mean± SEM= 6.56±0.25% vs. 7.06±0.32%) (Figure 28B).

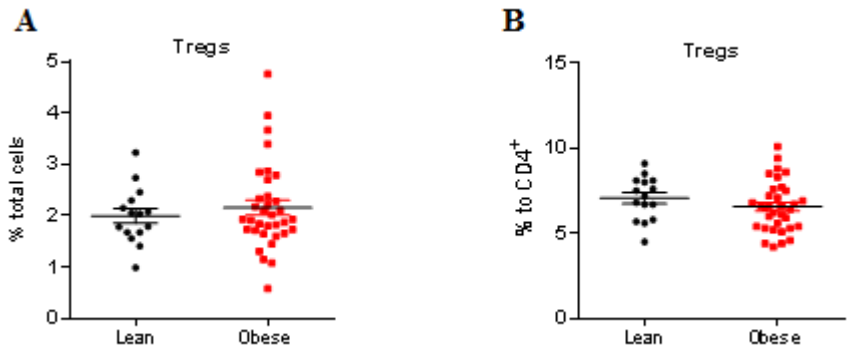



**Figure 28**. Total (A) and % percentage to $CD4^+$ T lymphocytes (B) of Treg cells in obese (n=34) and control (n=15) group. Comparisons between groups were performed using a Mann- Whitney t- test. In all cases $p>0.05$.

### 5.4 Effect of weight reduction on immune phenotype induced by genotype- based and conventional diet

The effect of genotype- based diet on the expression of inflammatory and anti-inflammatory markers by immune cells compared to conventional intervention was evaluated (Table 4).

Marked weight reduction (8.23±4.29%) was observed throughout the 3- month period of intervention. More specifically, 8.76±3.82% and 7.30±5.5% weight loss was reported in participants who followed conventional and personalized intervention, respectively, but the difference between the two groups did not reach the levels of statistical significance.

**Table 4**. Leukocyte and lymphocyte subset counts or percentages before and after conventional and genotype- based intervention. Values are presented as mean± standard error of the mean (SEM). (ns: not significant, *: $p\leq0.05$, **: $p\leq0.01$)

| Type of cell | Conventional diet | Genotype- based diet | Significance (convent |
|---|---|---|---|

| | Baseline | | 3 months | | Significance | Baseline | | 3 months | | Significance | ional vs. genotype - based diet) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | | Mean | SEM | Mean | SEM | | |
| **PBMCs (total cells)** | 2E+07 | 3E+06 | 2E+07 | 2E+06 | ns | 4E+07 | 4E+06 | 3E+07 | 5E+06 | ns | ** at baseline |
| **Classical monocytes ($CD14^+CD16^-$) (% total PBMCs)** | 9.333 | 1.423 | 10.74 | 2.167 | ns | 8.358 | 1.42 | 5.81 | 1.381 | ns | ns |
| **Intermediate monocytes ($CD14^+CD16^+$)** | 1.373 | 0.434 | 2.137 | 1.165 | ns | 1.445 | 0.364 | 1.298 | 0.49 | ns | ns |
| **Non-classical monocytes ($CD14^-CD16^+$)** | 5.137 | 1.89 | 2.42 | 0.444 | ns | 3.768 | 1.17 | 5.7 | 2.374 | ns | ns |
| **$CD14^{low}CD16^+$ monocytes (% total PBMCs)** | 0.777 | 0.123 | 0.45 | 0.127 | ns | 0.718 | 0.245 | 0.67 | 0.204 | ns | ns |
| **B lymphocytes (% total PBMCs)** | 9.01 | 1.062 | 6.13 | 1.135 | ns | 9.49 | 3.196 | 7.817 | 2.268 | ns | ns |
| **T ($CD3^+$) lymphocytes (% total PBMCs)** | 44.41 | 5.058 | 46.77 | 3.824 | ns | 62.34 | 16.88 | 47.15 | 6.201 | ns | ns |
| **$CD3^+CD4^+$ (% total PBMCs)** | 29.95 | 3.632 | 33.28 | 3.851 | ns | 40.77 | 9.542 | 32.72 | 5.707 | ns | ns |
| **$CD3^+CD4^+$ (% $CD3^+$ cells)** | 64.77 | 5.635 | 68.47 | 5.446 | ns | 67.63 | 2.266 | 62.4 | 7.17 | ns | ns |
| **$CD3^+CD8^+$ (% total PBMCs)** | 9.729 | 1.672 | 9.633 | 1.138 | ns | 11.72 | 2.611 | 9.03 | 1.101 | ns | ns |
| **$CD3^+CD8^+$ (% $CD3^+$ cells)** | 20.37 | 2.832 | 21.28 | 3.49 | ns | 20.37 | 1.761 | 21.53 | 4.191 | ns | ns |
| **$CD3^+CD4^+CD45RA^+$ $CD45RO^-$ (% total PBMCs)** | 8.55 | 1.232 | 12.58 | 1.308 | ns | 8.313 | 1.658 | 10.43 | 1.919 | ns | ns |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **$CD3^+CD4^+CD45RA^+$ $CD45RO^-$ (% $CD4^+$)** | 30.21 | 4.395 | 38.96 | 3.38 | ns | 27 | 5.062 | 32.23 | 3.065 | ns | ns |
| **$CD3^+CD4^+CD45RA^-$ $CD45RO^+$ (% total PBMCs)** | 13.03 | 1.726 | 14.34 | 2.155 | ns | 15.38 | 2.038 | 15.26 | 2.615 | ns | ns |
| **$CD3^+CD4^+CD45RA^-$ $CD45RO^+$ (% total $CD4^+$)** | 45.67 | 4.763 | 42.01 | 2.937 | ns | 49.85 | 5.358 | 48.2 | 6.259 | ns | ns |
| **$CD3^+CD4^+CD45RA^+$ $CD45RO^+$ (% total PBMCs)** | 0.531 | 0.131 | 0.547 | 0.215 | ns | 0.613 | 0.205 | 0.375 | 0.106 | ns | ns |
| **$CD3^+CD4^+CD45RA^+$ $CD45RO^+$ (% total $CD4^+$)** | 2.043 | 0.54 | 1.5 | 0.437 | ns | 2.1 | 0.756 | 1.175 | 0.229 | ns | ns |
| **$CD3^+CD4^+CD45RA^+$ $CCR7^+$ (Naïve) (% total PBMCs)** | 2.367 | 0.682 | 2.349 | 0.589 | ns | 3.76 | 1.279 | 0.68 | 0.11 | * | ns |
| **$CD3^+CD4^+CD45RA^+$ $CCR7^+$ (Naïve) (% $CD4^+$)** | 7.763 | 2.047 | 6.374 | 1.362 | ns | 12.05 | 3.982 | 2.295 | 0.49 | * | ns |
| **$CD3^+CD4^+CD45RA^+$ $CCR7^-$ (TEMRA) (% total PBMCs)** | 9.341 | 1.111 | 13.88 | 1.909 | ns | 9.11 | 1.44 | 16.33 | 4.239 | ns | ns |
| **$CD3^+CD4^+CD45RA^+$ $CCR7^-$ (TEMRA) (% $CD4^+$)** | 44.53 | 3.952 | 56.61 | 2.762 | ns | 37.53 | 3.635 | 63.22 | 7.727 | ** | ns |
| **$CD3^+CD4^+CD45RO^+$ $CCR7^+$ (CM) (% total PBMCs)** | 0.776 | 0.255 | 0.566 | 0.149 | ns | 0.375 | 0.166 | 0.198 | 0.027 | ns | ns |
| **$CD3^+CD4^+CD45RO^+$ $CCR7^+$ (CM) (% $CD4^+$)** | 2.537 | 0.696 | 1.579 | 0.347 | ns | 1.148 | 0.493 | 0.625 | 0.075 | ns | ns |
| **$CD3^+CD4^+CD45RO^+$ $CCR7^-$ (EM) (% total PBMCs)** | 18.52 | 4.344 | 19.51 | 3.724 | ns | 19.17 | 3.051 | 20.39 | 4.503 | ns | ns |
| **$CD3^+CD4^+CD45RO^+$ $CCR7^-$ (EM) (% $CD4^+$)** | 42.89 | 4.31 | 40.09 | 2.939 | ns | 47.85 | 6.542 | 47.4 | 6.135 | ns | ns |
| **$CD3^+CD4^+CD45RA^+$ $CXCR3^+$ (% total PBMCs)** | 0.353 | 0.06 | 0.457 | 0.115 | ns | 0.25 | 0.099 | 0.518 | 0.171 | ns | ns |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **$CD3^+CD4^+CD45RA^+$ $CXCR3^+$ (% $CD4^+$)** | 1.279 | 0.25 | 1.279 | 0.198 | ns | 0.823 | 0.311 | 1.703 | 0.528 | ns | ns |
| **$CD3^+CD4^+CD45RO^+$ $CXCR3^+$ (% total PBMCs)** | 5.699 | 0.993 | 5.401 | 1.148 | ns | 3.955 | 0.945 | 5.545 | 0.746 | ns | ns |
| **$CD3^+CD4^+CD45RO^+$ $CXCR3^+$ (% $CD4^+$)** | 19.65 | 2.536 | 15.58 | 2.406 | ns | 12.55 | 2.483 | 18.5 | 3.584 | ns | ns |
| **$CD3^+CD4^+CD127^-$ $CD25^+$ (T regs) (% total PBMCs)** | 1.864 | 0.244 | 2.19 | 0.36 | ns | 2.605 | 0.736 | 2.713 | 0.427 | ns | ns |
| **$CD3^+CD4^+CD127^-$ $CD25^+$ (T regs) (% $CD4^+$)** | 6.186 | 0.507 | 6.657 | 0.73 | ns | 6.225 | 0.397 | 8.375 | 0.246 | ns | ns |

## Peripheral Blood Mononuclear Cells (PBMCs)

Participants who followed a 3- month genotype- based diet had their circulating levels of PBMCs decreased (baseline: $3.83x10^7 \pm 3.61x10^6$ cells, 3 months: $2.77x10^7 \pm 4.58x10^6$ cells) whereas those who followed conventional diet had a mild increase in PBMCs total count (baseline: $1.96x10^7 \pm 3.32x10^6$ cells, 3 months: $2.12x10^7 \pm 2.05x10^6$ cells) (Figure 29). However, the difference between the two groups reached the levels of statistical significance only at the beginning of the intervention ($p \leq 0.05$).

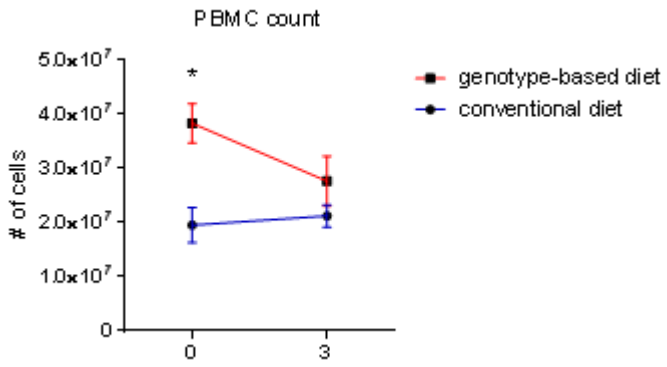



**Figure 29**. Differences in PBMC total count in genotype- based (n=4) and conventional (n=7) group. X axis: months after the beginning of the intervention.

Comparisons between groups were performed using two- way analysis of variance (ANOVA). *: $p \leq 0.05$

### 5.4.1 Monocytes

Personalized intervention resulted in a reduction in total levels of classical (baseline: 8.36±1.42%, 3 months: 5.81±1.38%) (Figure 30A) and intermediate (baseline: 1.44±0.36%, 3 months: 1.30±0.49%) (Figure 30B) monocytes. Conversely, individuals who followed conventional diet had their classical (baseline: 9.33±1.42%, 3 months: 10.74±2.17%) (Figure 30A) and intermediate (baseline: 1.37±0.43%, 3 months: 2.14±1.16%) (Figure 30B) monocytes increased throughout the 3- month period.

Furthermore, total percentage of non- classical monocytes was found increased in individuals who followed genotype- based diet (baseline: 3.77±1.17%, 3 months: 5.7±2.37%) and decreased in the conventional group (baseline: 5.14±1.89%, 3 months: 2.42±0.44%) (Figure 30C). At the end of the intervention, the personalized group had more non- classical monocytes than the non- personalized group.

A slight reduction in $CD14^{low}CD16^{+}$ monocytes was observed in participants who followed genotype- based intervention (baseline: 0.72±0.24%, 3 months: 0.67±0.20%). Similarly, the non- personalized group had their total levels of the same monocyte subset decreased (baseline: 0.78±0.12%, 3 months: 0.45±0.13%) and their levels were lower at 3 months than the genotype- based group (Figure 30D).

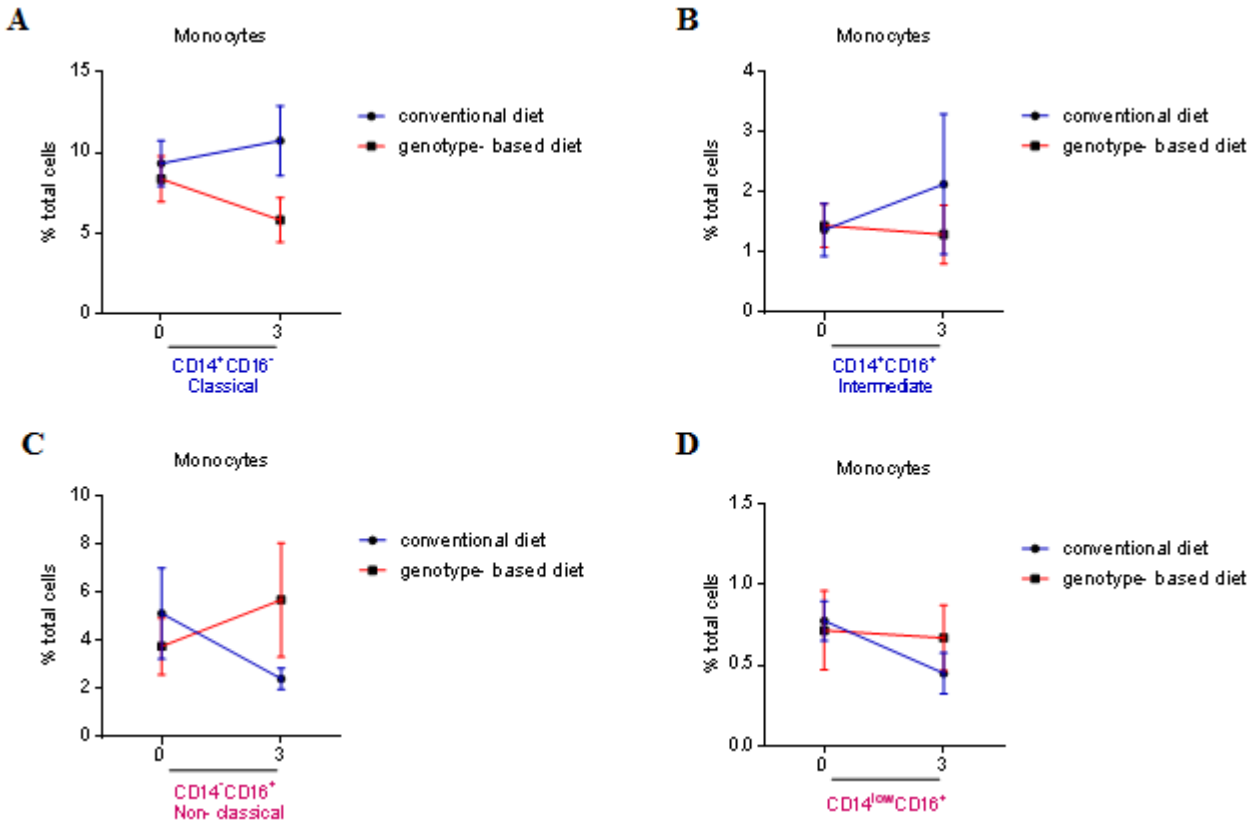



**Figure 30**. Total percentages of monocytes' subtypes [$CD14^{+}CD16^{-}$ (classical) (A), $CD14^{+}CD16^{+}$ (intermediate) (B), $CD14^{-}CD16^{+}$ (non- classical) (C), $CD14^{low}CD16^{+}$ (D)] in personalized (n=4) and conventional (n=3) group. X axis: months after the beginning of the intervention. Comparisons between groups were performed using two- way analysis of variance (ANOVA). In all cases $p>0.05$.

### 5.4.2 Lymphocytes

#### 5.4.2.1 B lymphocytes

At the beginning of the intervention, both groups had similar total levels of B lymphocytes (genotype- based: 9.49±3.20%, conventional: 9.01±1.06%) (Figure 31A). After the 3- month diet, both groups had their B cells decreased, but a milder decrease in their levels was observed in individuals belonging to the genotype- based group (7.82±2.27%) compared to the conventional group (6.13±1.13%).

### 5.4.2.2 CD3$^+$ T cells

Individuals who were assigned to the genotype- based intervention group had their total CD3$^+$ T cell levels decreased throughout the 3- month period (baseline: 62.34±16.88%, 3 months: 47.15±6.20%). Conversely, a slight increase in those cells in the non- personalized group was reported (baseline: 44.41±5.06%, 3 months: 46.77±3.82%). Similar T lymphocyte levels between the two intervention groups were observed at 3 months (personalized: 47.15±6.20%, conventional: 46.77±3.82%) (Figure 31B).

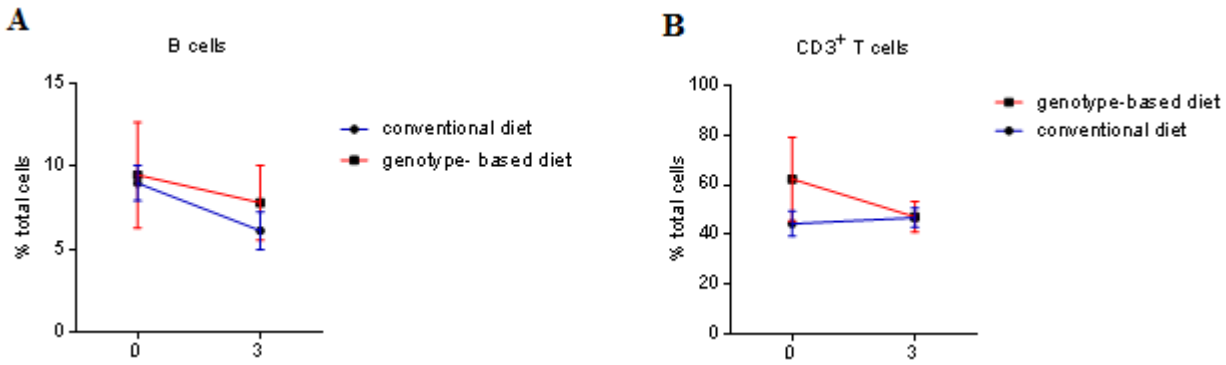



**Figure 31**. Differences in B lymphocytes' (n=3 conventional/ 3 personalized) (A) and CD3$^+$ T cell total percentage (n=6 conventional/ 3 personalized) (B) in genotype-based and conventional group. X axis: months after the beginning of the intervention. Comparisons between groups were performed using two- way analysis of variance (ANOVA). In all cases p>0.05.

### CD4$^+$ and CD8$^+$ T cells

After dieting, participants belonging in the personalized group had marked decreases in total percentages of CD4$^+$ (baseline: 40.76±9.54%, 3 months: 32.72±5.71%) (Figure 32A) and CD8$^+$ T lymphocytes (baseline: 11.72±2.61%, 3 months: 9.03±1.10%) (Figure 32B). An increase in CD4$^+$ T cell levels was reported in the conventional group (baseline: 29.95±3.63%, 3 months: 33.28±3.85%) but at the end of the intervention cell percentages were similar in personalized (32.72±5.71%) and non- personalized group (33.28±3.85%) (Figure 32A). Moreover, no difference in CD8$^+$ T cells was observed in the conventional group throughout the 3- month intervention (baseline: 9.73±1.67%, 3 months: 9.63±1.14%) (Figure 32B).

Relative to $CD3^+$ T cell compartment, $CD4^+$ T cell levels differed at baseline and the genotype- based group had increased levels of those cells (genotype-based: 67.63±2.26%, conventional: 64.77±5.63%). After the 3- month intervention, $CD4^+$ T cell percentage increased in the conventional group (68.47±5.44%), decreased in the genotype- based group (62.4±7.17%), and cell levels of the latter were lower compared to those of the conventional group (Figure 32C). As for $CD8^+$ T cell levels relative to $CD3^+$ T cells, an increase was reported in participants belonging to both groups (genotype- based: 20.37±1.76% at baseline, 21.53±4.19% at 3 months, conventional: 20.37±2.83% at baseline, 21.28±3.49% at 3 months). It is noteworthy that the percentage of $CD8^+$ cells to $CD3^+$ lymphocytes was similar at the beginning and at the end of the intervention in both groups (Figure 32D).

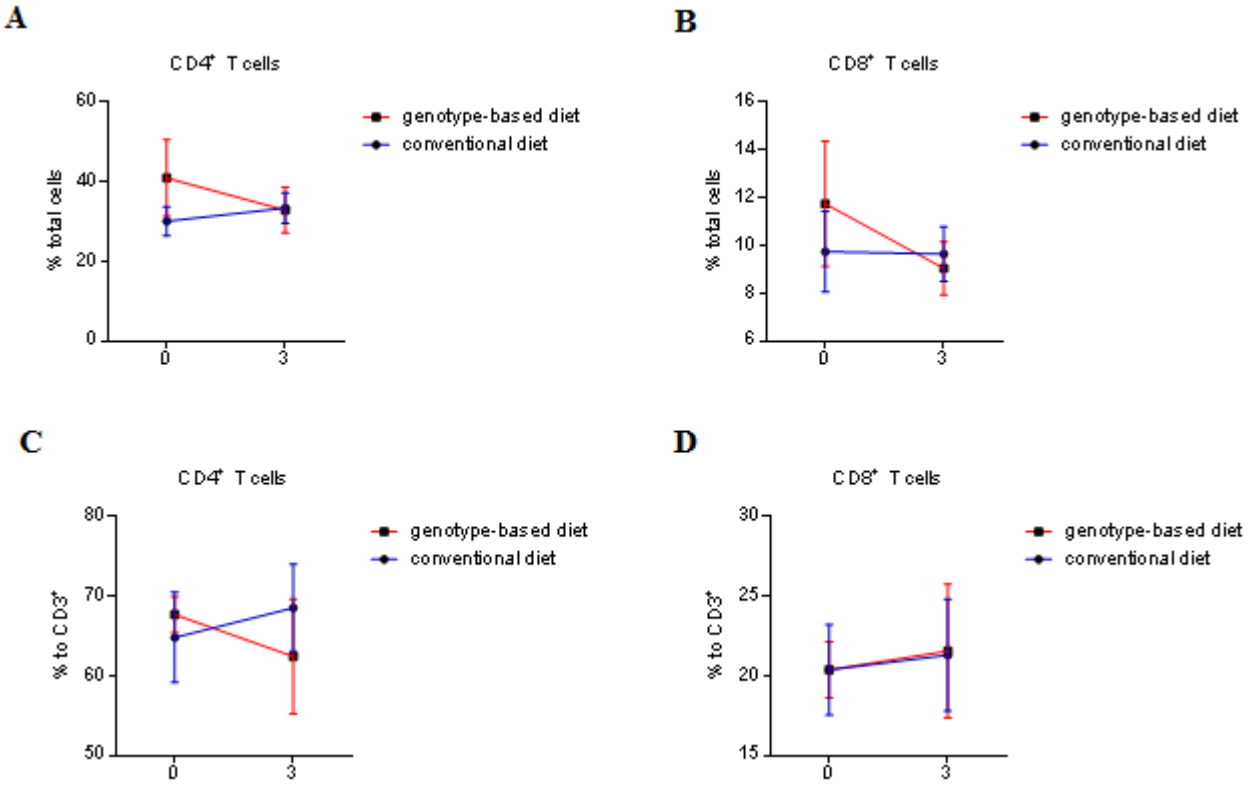



**Figure 32**. Total (n=7 conventional/ 4 personalized) and % percentage to T lymphocytes ($CD3^+$) (n=6 conventional/ 3 personalized) of $CD4^+$ (A, C) and $CD8^+$ (B, D) T cells, respectively, in genotype- based and conventional group. X axis: months after the beginning of the intervention. Comparisons between groups were performed using two- way analysis of variance (ANOVA). In all cases $p>0.05$.

**$CD4^+$ T cells compartment**

Taking $CD4^+$ T cell classification into account based on the expression of the CD45RA/CD45RO markers, a marked increase in total $CD4^+CD45RA^+$ (Naïve/ TEMRA) T cells was reported in both groups (genotype- based: 8.31±1.66% at baseline, 10.42±1.92% at 3 months, conventional: 8.55±1.23% at baseline, 12.58±1.31% at 3 months) (Figure 33A). The increase in the non- personalized group was more prominent but the values between the two groups remained not significantly different.

Similarly, participants who followed conventional intervention had their $CD4^+CD45RO^+$ (Memory) T cells increased throughout the 3- month period (baseline: 13.03±1.73%, 3 months: 14.34±2.15%). A slight reduction in those cells was observed in the genotype- based group, though (baseline: 15.38±2.04%, 3 months: 15.26±2.61%), but their total levels remained elevated compared to those of the non- personalized group (Figure 33B).

Furthermore, subjects who followed personalized diet experienced a marked reduction in $CD4^+CD45RA^+CD45RO^+$ T cell levels (baseline: 0.61±0.20%, 3 months: 0.37±0.11%), whereas those cells were about the same in the conventional group before (0.53±0.13%) and after (0.55±0.21%) the intervention (Figure 33C). At the end of the 3- month period, participants following the conventional diet had more $CD45RA^+CD45RO^+$ T cells than those who followed genotype- based intervention.

Regarding $CD4^+$ T cell compartment, the relative percentage of $CD4^+CD45RA^+$ T cells to $CD4^+$ cells was increased in both groups following the 3- month diet period (genotype- based: 27.00±5.06% at baseline, 32.22±3.06% at 3 months, conventional: 30.21±4.39% at baseline, 38.96±3.38% at 3 months) (Figure 33D). As in the case of naïve/ TEMRA T cells total percentage, their final levels regarding $CD4^+$ T cells were elevated in the conventional group.

During the intervention period, relative percentage of memory T cells to $CD4^+$ T cells markedly decreased in participants belonging to both groups (genotype- based: 49.85±5.36% at baseline, 48.2±6.26% at 3 months, conventional: 45.67±4.76% at baseline, 42.01±2.94% at 3 months) (Figure 33E) with a greater reduction in the conventional group.

As opposed to absolute percentages $CD4^+CD45RA^+CD45RO^+$ T cells, their relative percentages to $CD4^+$ T cells were decreased in both dieting groups. It is noteworthy that similar baseline levels were reported (genotype-based: 2.1±0.76%, conventional: 2.04±0.54%) but participants who followed genotype- based diet had lower levels of those cells at the end of the intervention (genotype-based: 1.17±0.23%, conventional: 1.5±0.44%) (Figure 33F).

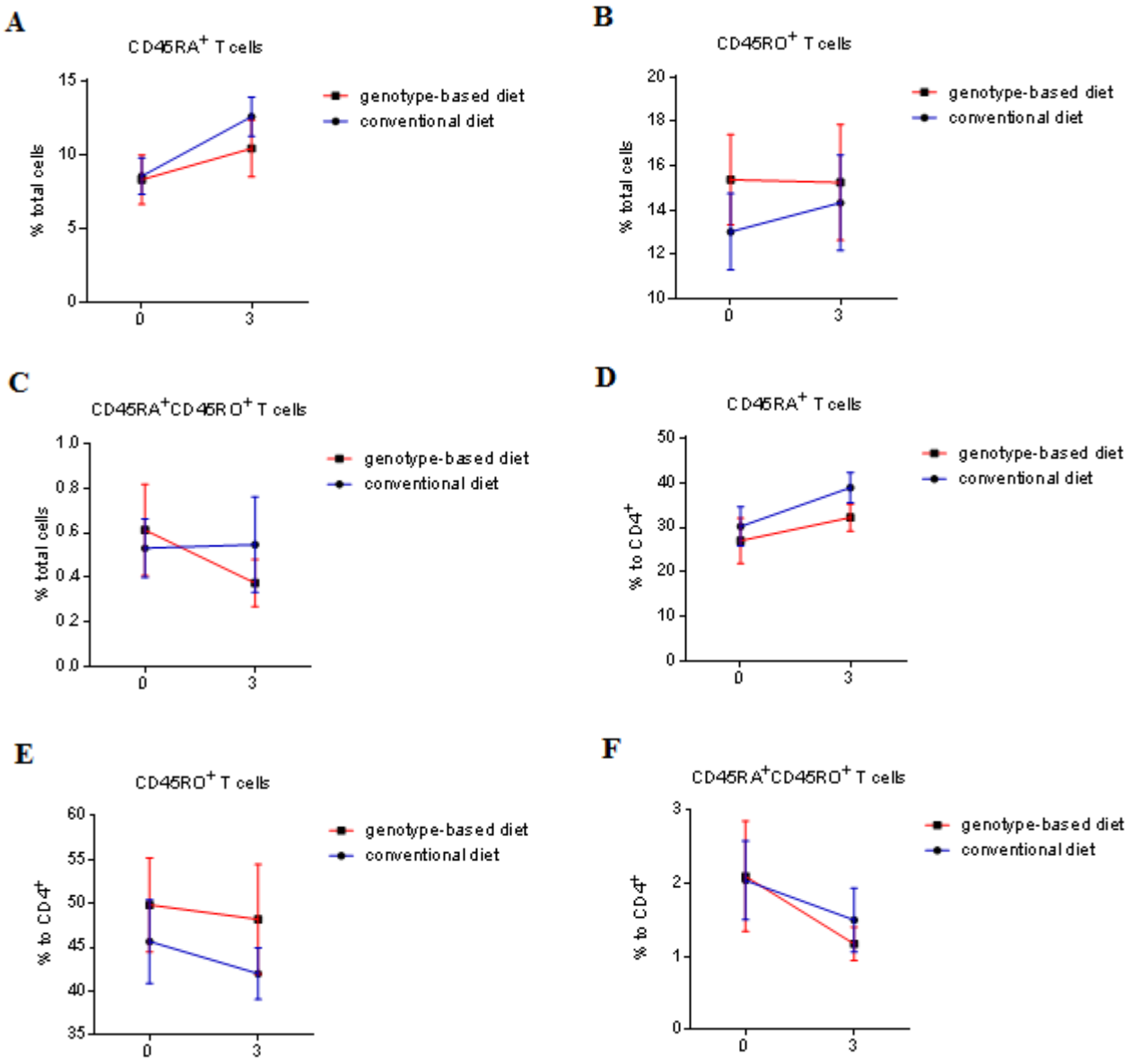



**Figure 33**. Total and % percentage to $CD4^+$ T lymphocytes of $CD4^+CD45RA^+$ (A, D) $CD4^+CD45RO^+$ (B, E) and $CD4^+CD45RA^+CD45RO^+$ (C, F) T cells, respectively, in participants following genotype- based (n=4) and conventional (n=7) intervention. X axis: months after the beginning of the intervention. Comparisons between groups were performed using two- way analysis of variance (ANOVA). In all cases $p>0.05$.

Baseline total levels of $CD4^+CD45RA^+CCR7^+$ (Naïve) T cells were similar to those at the end of the intervention in subjects who followed conventional diet (baseline: 2.37±0.68%, 3 months: 2.35±0.59%). However, total levels of naïve T cells were significantly lower ($p \leq 0.05$) at the end of the 3- month period in participants following personalized diet (baseline: 3.76±1.28%, 3 months: 0.68±0.11%) (Figure 34A).

Regarding total $CD4^+CD45RA^+CCR7^-$ (TEMRA) T cells, both groups had similar levels at the beginning of the intervention (genotype-based: 9.11±1.44%, conventional: 9.34±1.11%). A more profound increase was observed in the genotype-based group at the end of the intervention resulting in higher final TEMRA levels compared to the conventional group (genotype-based: 16.32±4.24%, conventional: 13.88±1.91%) (Figure 34B).

A significant reduction ($p \leq 0.05$) in relative percentage of naïve T cells to $CD4^+$ cells was reported in participants who followed personalized intervention throughout the 3-month period (baseline: 12.04±3.98%, 3 months: 2.29±0.49%). Furthermore, a slight reduction in relative percentage of those cells was observed in the non- personalized group (baseline: 7.76±2.05%, 3 months: 6.37±1.36%). At the end of the intervention, naïve T cell percentage to $CD4^+$ T cells was elevated in the conventional group compared to genotype- based group (Figure 34C).

Conversely, relative percentage of TEMRA T cells to $CD4^+$ cells increased significantly ($p \leq 0.01$) with intervention in subjects following a personalized diet (baseline: 37.53±3.63%, 3 months: 63.21±7.23%) (Figure 34D). The reduction in TEMRA T cell relative levels to $CD4^+$ cells observed in the conventional group comparing baseline and 3- month intervention values (baseline: 44.53±3.95%, 3 months: 56.61±2.76%) did not reach the levels of statistical significance. It is interesting to mention that TEMRA baseline levels were lower and TEMRA final levels were higher in the personalized group compared to the non- personalized.

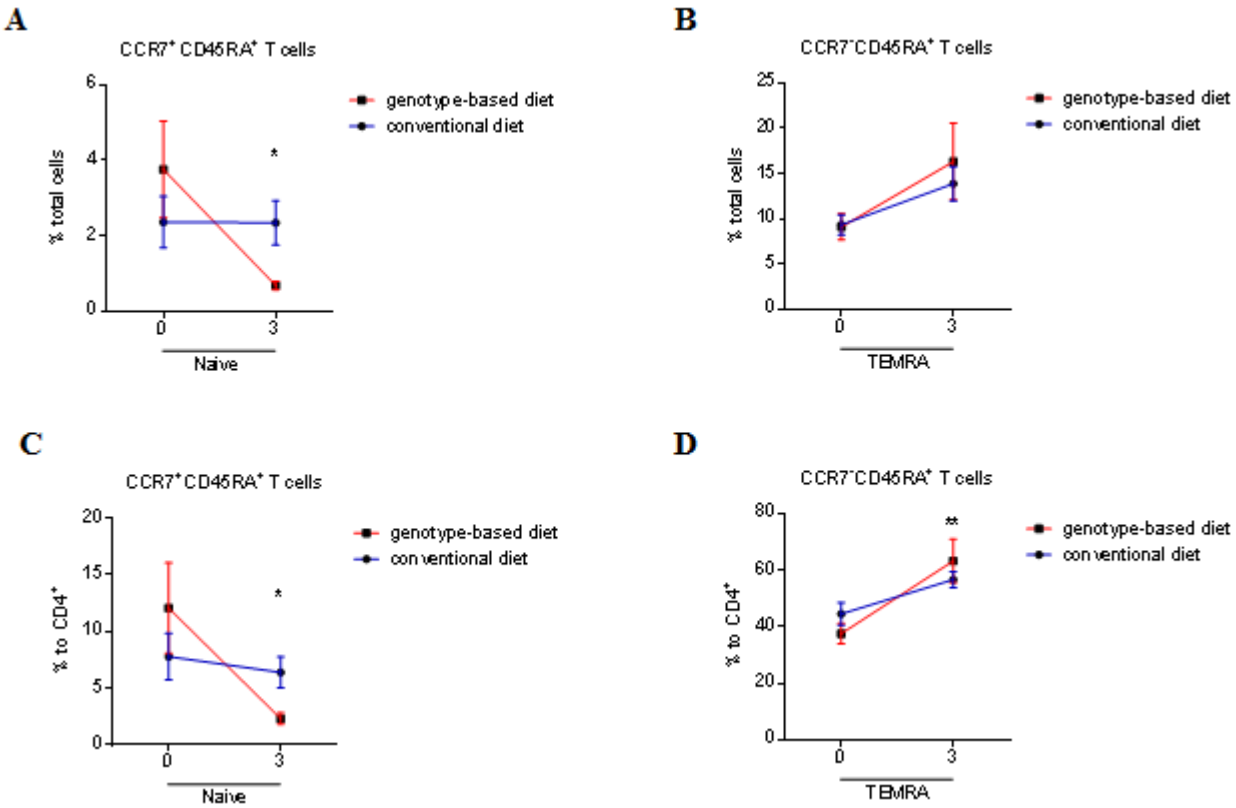



**Figure 34**. Total and % percentage to $CD4^+$ T lymphocytes of $CD4^+CD45RA^+CCR7^+$ (Naïve) (A, C) and $CD4^+CD45RA^+CCR7^-$ (TEMRA) (B, D) T cells, respectively, in genotype- based (n=4) and conventional (n=7) group. X axis: months after the beginning of the intervention. Comparisons between groups were performed using two- way analysis of variance (ANOVA). *: $p \leq 0.05$, **: $p \leq 0.01$

During the 3- month diet intervention period, total (genotype- based: 0.37±0.16% at baseline, 0.20±0.03% at 3 months, conventional: 0.77±0.25% at baseline, 0.56±0.15% at 3 months) (Figure 35A) and relative percentage to $CD4^+$ cells (genotype- based: 1.15±0.49% at baseline, 0.62±0.07% at 3 months, conventional: 2.54±0.69% at baseline, 1.58±0.35% at 3 months) (Figure 35B) of $CD4^+CD45RO^+CCR7^+$ (CM) cells markedly decreased in personalized and conventional group. It is noteworthy that baseline and final levels of CM cells were lower in subjects who followed personalized diet (Figure 35A, B).

Conversely, individuals following both types of diet had their total $CD4^+CD45RO^+CCR7^-$ (EM) T cell levels increased at the end of the intervention (genotype- based: 19.16±3.05% at baseline, 20.38±4.50% at 3 months, conventional:

18.52±4.34% at baseline, 19.50±3.72% at 3 months) (Figure 35C) with a more profound increase observed in the personalized group. Regarding $CD4^+$ T cell compartment, similar relative EM T cell percentage to $CD4^+$ cells was observed in genotype- based group at the beginning and at the end of the intervention (baseline: 47.85±6.54%, 3 months: 47.40±6.13%). A reduction in those cells was reported in the conventional group at 3 months, though (baseline: 42.89±4.31%, 3 months: 40.09±2.94%) (Figure 35D). Both baseline and final relative levels of EM cell to $CD4^+$ cells were lower at non- personalized compare to the personalized group.

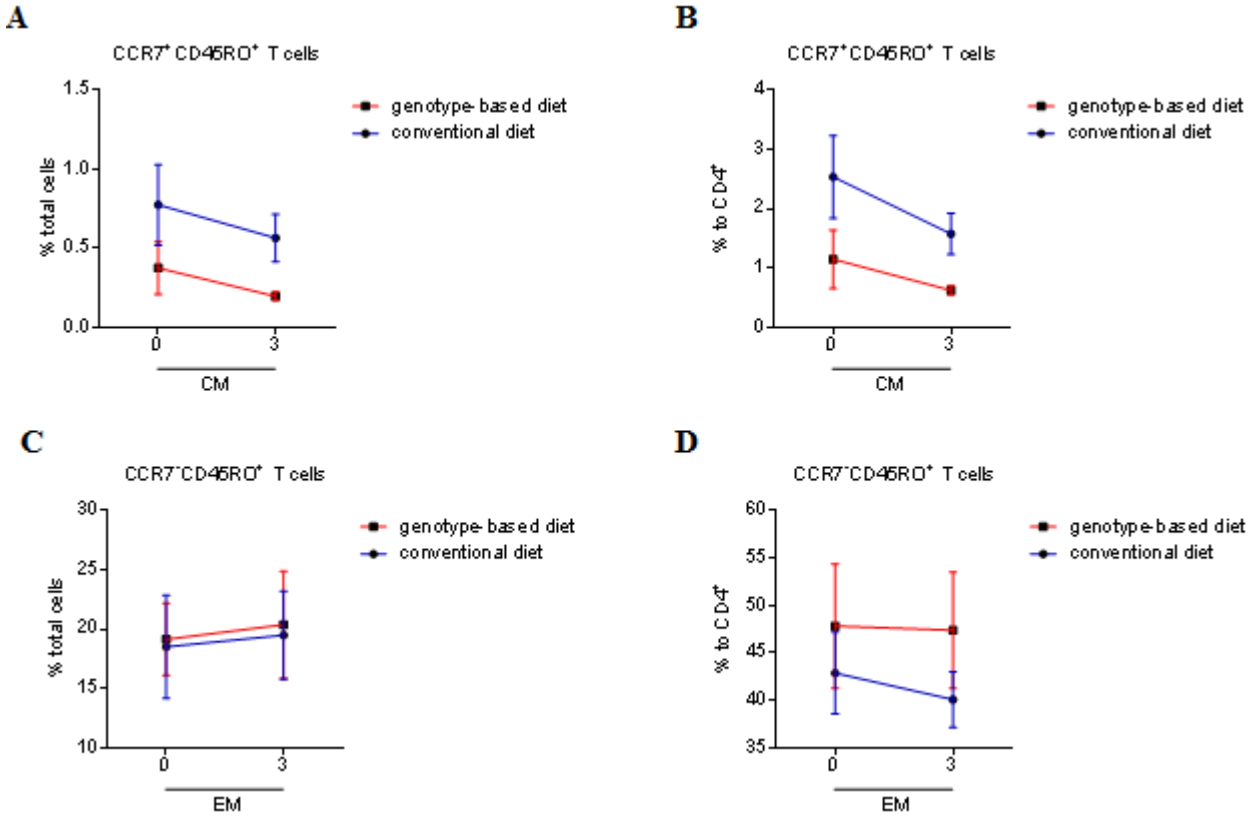



**Figure 35**. Total and % percentage to $CD4^+$ T lymphocytes of $CD4^+CD45RO^+CCR7^+$ (CM) (A, B) and $CD4^+CD45RO^+CCR7^-$ (EM) (C, D) T cells, respectively, in subjects following personalized (n=4) and conventional (n=7) diet. X axis: months after the beginning of the intervention. Comparisons between groups were performed using two- way analysis of variance (ANOVA). In all cases $p>0.05$.

Genotype- based intervention resulted in a marked increase in total levels of $CD4^+CD45RA^+CXCR3^+$ (baseline: 0.25±0.10%, 3 months: 0.52±0.17%) (Figure 36A) and $CD4^+CD45RO^+CXCR3^+$ (baseline: 3.96±0.94%, 3 months: 5.54±0.74%)

(Figure 36B) T cells. A less prominent increase in $CD4^+CD45RA^+CXCR3^+$ cells was observed in subjects following conventional diet (baseline: 0.35±0.06%, 3 months: 0.46±0.11%) (Figure 36A) and resulted in lower final levels of those cells compared to the personalized group. Lower total levels of $CD4^+CD45RO^+CXCR3^+$ cells were reported in conventional group at the end of the intervention (baseline: 5.70±0.99%, 3 months: 5.40±1.15%) (Figure 36B) but both intervention groups had similar total levels of those cells at 3 months. Those facts contributed to an immune phenotype characterized by increased $CD4^+CD45RA^+CXCR3^+$ cells in personalized group compared to the conventional group at the end of the 3- month intervention.

Similarly, relative percentage of $CD4^+CD45RA^+CXCR3^+$ (baseline: 0.82±0.31%, 3 months: 1.70±0.53%) (Figure 36C) and $CD4^+CD45RO^+CXCR3^+$ (baseline: 12.55±2.48%, 3 months: 18.50±3.58%) (Figure 36D) cells to $CD4^+$ cells increased with genotype- based intervention. Relative levels of $CD4^+CD45RA^+CXCR3^+$ cells were similar to those of baseline in the conventional group (baseline: 1.28±0.25%, 3 months: 1.28±0.20%) (Figure 36C). Participants belonging to the conventional group had their relative percentage of $CD4^+CD45RO^+CXCR3^+$ cells to $CD4^+$ cells decreased throughout the 3- month period (baseline: 19.65±2.54%, 3 months: 15.57±2.41%) (Figure 36D). At the end of the intervention, both cell levels were increased in the personalized group.

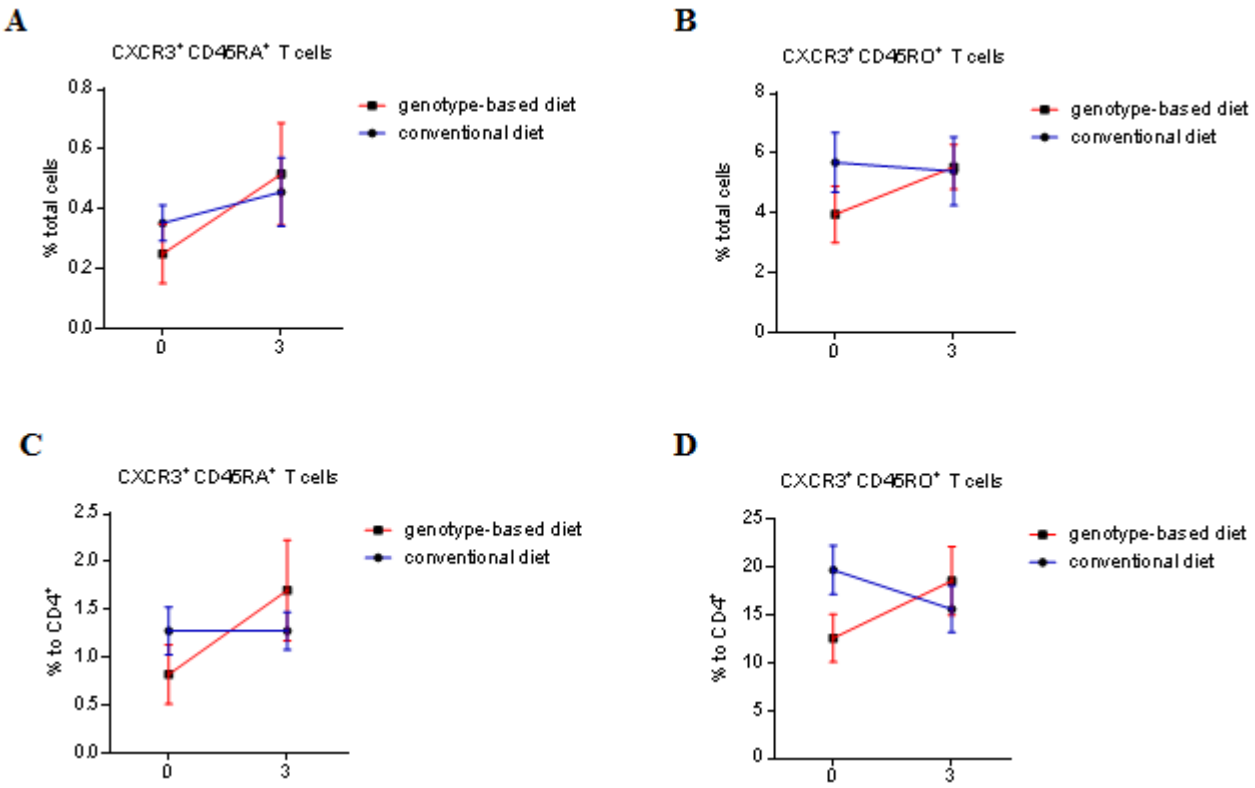



**Figure 36**. Total and % percentage to $CD4^+$ T lymphocytes of $CD4^+CD45RA^+CXCR3^+$ (A, C) and $CD4^+CD45RO^+CXCR3^+$ (B, D) T cells, respectively, in personalized (n=4) and conventional (n=7) group. X axis: months after the beginning of the intervention. Comparisons between groups were performed using two- way analysis of variance (ANOVA). In all cases $p>0.05$.

Finally, total levels of Treg cells increased in participants who followed both the 3-month genotype- based (baseline: 2.60±0.74%, 3 months: 2.71±0.43%) and conventional diet (baseline: 1.86±0.24%, 3 months: 2.19±0.36%) (Figure 37A). Although this increase was less profound in the personalized group, higher total levels of Treg cells were observed in individuals following personalized diet.

Regarding $CD4^+$ T cell compartment, both groups had similar relative Tregs percentage to $CD4^+$ cells at the beginning of the intervention (genotype- based: 6.22±0.40, conventional: 6.19±0.51%). Personalized diet resulted in a more profound increase in Tregs relative percentage (8.37±0.25%) compared to conventional diet (6.66±0.73%) (Figure 37B).

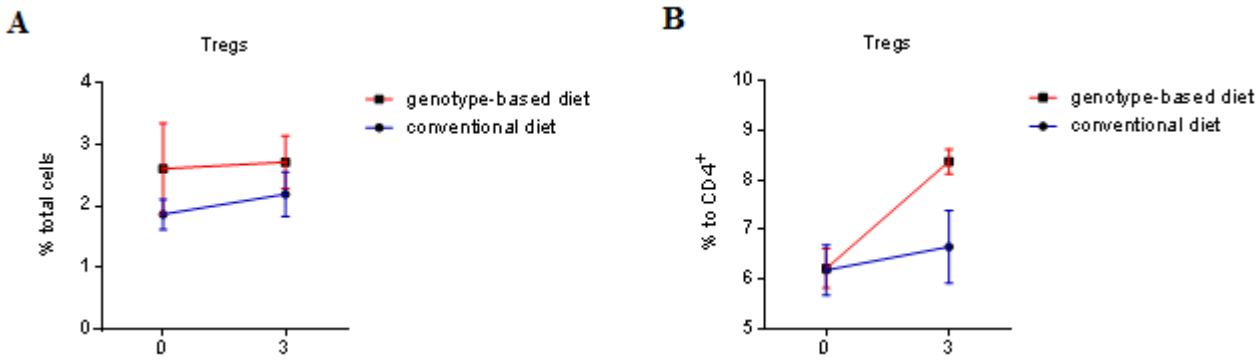



**Figure 37**. Total (A) and % percentage to $CD4^+$ T lymphocytes (B) of Treg cells in participants following personalized (n=4) and conventional (n=7) intervention. X axis: months after the beginning of the intervention. Comparisons between groups were performed using two- way analysis of variance (ANOVA). In all cases $p>0.05$.

## 6. DISCUSSION

Obesity is associated with low level chronic inflammation and deregulated immunity. This link is highlighted by the elevated levels of various proinflammatory factors including TNF-α, IL- 6 and CRP, in the serum of obese individuals, the dysregulation of leptin that mediates the cross- talk between immune function and physiologic mechanisms that control body weight and by the increased incidence of several types of cancer among obese individuals [157].

The involvement of lymphocyte dysregulation in the pathogenesis of diabetes, atherosclerosis and steatohepatitis, i. e. co- morbidities related to obesity, along with the impaired cellular immune function observed in obesity- related cancer, reveal the effect of variations in lymphocyte number and/ or function on immune response during obesity. To this line, leptin, the levels of which are upregulated in the case of excess body weight, modulates T- cell proliferation and apoptosis, as well as monocyte apoptosis, revealing a profound role of this hormone in the regulation of immunity [157].

Our results regarding WBC counts in obesity are in line with literature (Table 5) suggesting a positive correlation with BMI. Few studies report no correlation between WBC numbers and adiposity but this fact may be attributed to the limited number of participants examined (<20 lean and obese participants in 3 out of 4 studies, Table 5).

**Table 5**. Summary of studies evaluating the effect of human obesity on major classes of circulating leukocytes and T cell subclasses (↑: increased cell numbers or percentages in obese compared to non- obese individuals, ↓: decreased cell numbers or percentages in obese compared to non- obese individuals, -: no statistical difference between obese and non- obese individuals)

| Type of cell | Overall change of circulating cell population frequency and/ or numbers during obesity | References |
|---|---|---|

| | | |
|---|---|---|
| **White blood cells** | ↑ | 40, 91, 127, 139, 140, 151, 152, 172, 174, 175, 218, 283 |
| | – | 92, 150, 219, 229 |
| **Basophils** | ↑ | 175, 283 |
| | – | 91, 151, 152, 172 |
| **Eosinophils** | ↑ | 104, 175 |
| | – | 91, 151, 152, 172 |
| **Neutrophils** | ↑ | 127, 139, 140, 152, 172, 175, 283, 284 |
| | – | 91, 151, 229 |
| **Monocytes** | ↑ | 91, 127, 140, 151, 152, 175, 219, 283 |
| | – | 92, 139, 144, 150, 172 |
| | ↓ | 155 |
| **Classical monocytes ($CD14^+CD16^-$)** | – | 144 |
| | ↓ | 143, 150 |
| **Intermediate monocytes ($CD14^+CD16^+$)** | ↑ | 104, 143, 150 |
| | – | 144 |
| **Non-classical monocytes ($CD14^-CD16^+$)** | ↑ | 143 |
| **Lymphocytes** | ↑ | 40, 60, 127, 152, 172, 174, 175, 283 |
| | – | 91, 92, 139, 151, 219, 229 |
| **T lymphocytes ($CD3^+$)** | ↑ | 92, 127, 138, 139 |
| | – | 172, 174, 219 |

| | | |
|---|---|---|
| | ↓ | 26 |
| **B lymphocytes** | ↑ | 127, 140, 225 |
| | – | 92, 139, 172 |
| **NK cells ($CD56^+$)** | ↓ | 121, 125 |
| **$CD56^+C16^+$ NK cells** | ↑ | 138 |
| | – | 92, 127, 139, 172 |
| | ↓ | 140 |
| **$CD3^+CD4^+$ cells** | ↑ | 40, 92, 127, 138, 157, 172, 174 |
| | – | 219 |
| | ↓ | 26 |
| **$CD3^+CD8^+$ cells** | ↑ | 40, 138 |
| | – | 92, 127, 172, 219 |
| | ↓ | 26, 157, 174, 218, 125 |
| **$CD3^+CD4^+CD45RA^+CD45RO^-$ cells** | – | 26 |
| **$CD3^+CD4^+CD45RA^-CD45RO^+$ cells** | ↓ | 26 |
| **$CD3^+CD4^+CD45RA^+CCR7^+$ (Naïve) cells** | ↑ | 92 |
| | ↓ | 60 |
| **$CD3^+CD4^+CD45RA^+CCR7^-$ (TEMRA) cells** | ↑ | 92 |
| **$CD3^+CD4^+CD45RO^+CCR7^+$ (CM) cells** | ↑ | 92 |
| **$CD3^+CD4^+CD45RO^+CCR7^-$ (EM) cells** | ↑ | 60 |

| | | |
|---|---|---|
| | – | 92 |
| **T reg cells** | ↑ | 92 |
| | ↓ | 185, 186, 187, 231, 285 |

The involvement of granulocytes in immune dysfunction during obesity is not clear. Neutrophils, the most abundant type of polymorphonuclear leukocytes, were found slightly increased in obese participants. This upregulation in neutrophils levels is supported by several other studies whereas an absence of correlation between obesity and cell numbers are reported by few of them (Table 5). The conflicting relationship between excess body weight and granulocytes' levels might be attributed to basophils and eosinophils. Despite the fact that the percentage of basophils and eosinophils was found decreased in obese individuals in our study, published data report either positive or no correlation with increasing BMI for both subsets of granulocytes (Table 5). Although obesity and both eosinophils and basophils are related to allergic inflammation, current information about circulating levels of those cells in the case of excess body weight are rather inconsistent.

A statistical significant increase in PBMCs count reported in obese participants reveals an upsurge in cells that constitute the lymphocyte and monocyte compartment. Conclusions regarding the effect of different types of intervention could not be easily drawn because baseline levels of PBMCs differed a lot between the two intervention groups. Generally speaking, personalized diet was more efficient at the improvement of obesity- related inflammation represented by the reduction of total PBMCs counts.

As for monocyte compartment, the majority of studies report an increase in monocytes' numbers and/ or percentages along with increasing adiposity. A small number of studies report no association and one study reports a negative association between monocyte levels and BMI. It is noteworthy that only a limited number of participants are included in that study (16 obese and 12 normal- weight individuals) (Table 5).

Monocytes represent a heterogeneous population and their classification based on the expression of the CD14 and CD16 surface markers reveals important alterations during obesity.

Regarding classical monocytes, our results are in line with published data reporting a reduction in this type of monocytes in obese compared to lean individuals (Table 5). A lack of association between those cells and increasing BMI is reported in a study published by de Matos in which only 9 obese and 8 lean participants were included [144]. When the type of diet was examined, personalized diet resulted in a reduction whereas conventional intervention contributed to an increase in classical monocytes. Those results indicate that conventional diet is more efficient in the enforcement of the anti – inflammatory character of immunity represented by classical monocytes.

Conversely, intermediate monocytes were found elevated in obese participants and this increase is reported by several other studies (Table 5). As mentioned above, de Matos et al. reported no difference between lean and obese subjects regarding $CD14^{+}CD16^{+}$ but this observation may be due to the limited number of participants included in this study [144]. These cells along with non- classical monocytes are related to the initiation of immune response in case of an inflammatory situation. Consequently, they are expected to be upregulated in obesity. Similar to classical monocytes, intermediate monocytes were found elevated due to conventional diet compared with baseline whereas personalized diet resulted in a moderate reduction in their levels.

A marked increase in $CD14^{-}CD16^{+}$ and $CD14^{low}CD16^{+}$ monocytes was observed in obese participants compared with lean subjects emphasizing the proinflammatory role of those cells in obesity- related low grade systemic inflammation. In 2011, Poitou et al. [143] reported a 2- fold increase of $CD14^{dim}CD16^{+}$ monocytes in obese individuals with noteworthy additional effects of diabetes. Weight loss due to non- personalized diet resulted in a reduction in $CD14^{-}CD16^{+}$ and $CD14^{low}CD16^{+}$ monocyte levels, whereas personalized diet contributed to a marked increase in non- classical monocytes and to a less profound reduction in $CD14^{-}CD16^{+}$ cells. Consequently, in the case of $CD14^{-}CD16^{+}$ and $CD14^{low}CD16^{+}$ monocytes, personalized intervention seems to be more efficient in the restriction of the proinflammatory character of obese state represented by those cells.

Regarding the second subtype of PBMCs, the majority of studies report an upregulation in lymphocyte levels during obesity although in some cases the difference between lean and obese individuals did not reach the levels of statistical significance (Table 5). Our results are not in accordance with published data given the slight reduction in obese participants but this observation may be due to the limited number of participants included in our study.

When lymphocyte classification is taken into account, a marked reduction in NK cell ($CD56^+$) total levels was reported in the obese group. Those observations are in line with a study including 52 morbidly obese [26 MHO (BMI= 46.8±7.3 $kg/m^2$), 26 MUO (BMI= 47.5±12.2 $kg/m^2$)] and 11 lean controls [125]. NK cells were less abundant in severely obese participants compared with lean controls (9.1% versus 12.3% of total peripheral lymphocytes, p=0.03). Similarly, O' Shea et al. [121] reported a significant reduction in $CD56^+$ NK cell levels in morbidly obese patients (n=40) compared to lean healthy (n=20) subjects (7.6% versus 16.6%, p=0.0008).

When the expression of the CD16 marker was evaluated in order to further classify NK cells, the major NK subtype, i. e. $CD56^+CD16^+$ cells, were found decreased in obese compared to lean individuals. This reduction is responsible for the total reduction of NK cells in the obese group given the reported increase in relative levels of the other two NK subtypes ($CD56^{bright}CD16^-$, $CD56^{dim}CD16^-$) in obese compared to lean participants. As for $CD56^+CD16^+$ NK cells in obesity, current literature reveals a complex picture reporting elevated [138], similar [92, 127, 139, 172] or even decreased [140] levels of those cells in obese subjects (Table 5). Due to the participation of NK cells in both innate immunity against infection and in immune response against malignancy, studying those cells is of great interest given the fact that obesity predisposes to elevated risk of several types of cancer. According to currently published data, reduction in NK numbers/ function might be more prominent in morbidly but not in mildly obese individuals compared to lean subjects [100] but this association has to be further addressed.

The second component of the lymphocyte compartment, i. e. B cells, was found slightly elevated in obese participants and genotype- based diet resulted in a less profound reduction in their numbers following the 3- month intervention period. To this line, obesity was associated with elevated B cell numbers in several studies [127,

140, 225]. Nevertheless, others [92, 172, 139] reported similar levels of B cells in the obese and non- obese group and found no correlation with BMI [172].

An increase in $CD3^+$ T cells, i. e. the third component of lymphocyte compartment, was reported in obese participants. Several other groups reported an upregulation of those cells in case of excess body weight (Table 5). Nevertheless, others reported similar levels of those cells in obese individuals but only ≤25 participants were included in those studies (Table 5). Weight reduction did not induce significant changes in total percentages of T cells and a more profound reduction was reported in the genotype- based group. It is interesting to mention that Tanaka et al. [26] reported a statistical significant upregulation ($p<0.001$) in $CD3^+$ cells in non- obese (n=50) compared to obese (n=34) subjects. Furthermore, 23 obese participants were subjected to a VLED that resulted in a marked weight reduction and a significant increase ($p<0.05$) in T lymphocytes compared to the baseline value.

Further classification of $CD3^+$ T cells revealed an upregulation in total percentage of $CD4^+$ cells in obese individuals that is in line with current literature (Table 5). In contrast, Tanaka et al. [26] reported a reduction in those cells in obese subjects who also had their $CD4^+$ cells increased following VLED ($p<0.05$). Moreover, in a study published by Fink et al. [219], no difference between lean and obese individuals regarding $CD4^+$ cells was reported but only 22 participants (9 obese and 13 non-obese) were included in this study. Our results regarding the type of diet used for the induction of weight loss indicate an increase and a reduction in non- personalized and in personalized group, respectively, whereas both groups have similar final levels of $CD4^+$ cells.

A not significant reduction in the second subset of T lymphocytes, i. e. $CD8^+$ cells, was reported in obese participants. The majority of studies report either decreased or similar $CD8^+$ numbers between obese and non- obese state (Table 5). Nevertheless, Magrone et al. [138] reported an upregulation in obese subjects ($7x10^5$ versus $2x10^5$, $p<0.0001$), whereas morbid obesity was associated with a higher $CD8^+$ count in another study [40]. A noteworthy reduction in total $CD8^+$ cells was observed in obese participants who followed genotype- based intervention. The latter is in line with a study [215] in which 9 obese women following a very- low- calorie diet program were

enrolled. After dieting, obese subjects had both their $CD8^+$ total number (from 467/m1 to 336/m1, $p<0.05$) and percentage (from 21% to l6%, $p<0.02$) decreased.

Those inconsistencies in literature regarding $CD8^+$ numbers in obesity may originate from the more destructive behavior of these cells once they get activated as compared to $CD4^+$ T lymphocytes. The latter cells respond to several signals induced by increased adiposity, including cytokines and adipokines that stimulate their differentiation and proliferation. On the other hand, $CD8^+$ cells are less prone to adipokine activation thus they only get activated following $CD4^+$ T cell activation by fat- induced adipokine production. As a result, it is more possible to detect the effect of fat accumulation on $CD8^+$ T cell number in extreme BMI condition as compared to lean state [40].

As for $CD4^+$ and $CD8^+$ cell distribution in T lymphocyte compartment, the increase reported in overall $CD3^+$ T cells could be attributed to the increase in $CD4^+$ cells given the reduction reported in $CD8^+$ cells. Similar to total percentages, a trend towards the reduction of $CD4^+$ cells to $CD3^+$ lymphocytes is observed in participants following personalized intervention. Conversely, results regarding $CD8^+$ cells to $CD3^+$ cells are similar in the personalized and non- personalized group.

When the classification of $CD4^+$ T cells based on the expression of the markers CD45RA/CD45RO was examined, a statistical significant increase of memory T cells in the obese group was reported. In contrast, obese subjects had significantly lower memory T cells than lean control subjects in a study published by Tanaka et al. [26] (See "Introduction" section). Among the functional features of memory cells, they have already been exposed to the antigen and still exist in its absence; they also possess enhanced activity in case of re- exposure and solicit immune response [277], and they have been strongly associated with obese condition [60]. Furthermore, 3-month genotype- based type of diet induced a reduction in memory cell levels (total percentage and relative percentage to $CD4^+$ T cells), whereas conventional diet resulted in an increase in total percentage of those cells. Similarly, in the study mentioned above, weight reduction resulted in a significant increase in $CD4^+CD45RO^+$ cells ($0.56\pm0.22$ $x10^3$ cells/$mm^3$ at baseline, $0.73\pm 0.32x10^3$ cells/$mm^3$ at refeeding, $p<0.001$) [26].

$CD45RA^+$ T cells, representing both the first and final stage of differentiation of T cells, i. e. naïve/ TEMRA cells, were found elevated in lean individuals and were increased to a greater extent due to conventional intervention. Others reported no difference in $CD4^+CD45RA^+$ cells between lean and obese individuals and weight loss did not cause significant alterations in their levels [26].

$CD4^+CD45RA^+CD45RO^+$ cells are among the less studied cells included in our study according to current literature. They resemble naïve T cells in the inhibition of immunoglobulin production by B lymphocytes, and they resemble memory T cells in the expression of mRNA and the production of IFN-γ. $CD4^+CD45RA^+CD45RO^+$ cells were found slightly upregulated in obese participants and personalized diet induced a marked reduction in their levels following the 3- month intervention period. Given the fact that those cells are a transient stage of differentiation in the process of transformation from naïve to memory cells, they are expected to be found at greater numbers in secondary lymphoid organs than in the periphery. In circulation, their frequency is <1% and the expression of CD45 isoforms by the majority of intermediate cells is reported at low density [278].

Within the $CD4^+$ T cell compartment, $CD45RA^+$ cells were found decreased in obese participants. The overall increase in $CD4^+$ T cells in the obese group could be attributed to the increase reported in $CD45RA^-CD45RO^+$ and $CD45RA^+CD45RO^+$ cells.

Studying the expression of the CCR7 marker by $CD4^+$ T cells revealed noteworthy observations regarding the sequential differentiation of $CD4^+$ cells from naïve to TEMRA cells, with central memory and effector memory representing the intermediate stages.

Our results regarding the total percentage of naïve T cells are in line with a study published by Van der Weerd [92] reporting a significant increase in morbidly obese (n=8) compared to lean (n=11) individuals. However, Mauro et al. [60] observed a marked reduction in naïve T cells in obese individuals in a much larger group of participants (n=187). Naïve T lymphocytes represent cells responding to pathogens that the immune system has not yet encountered and may further differentiate into memory cells. Given the strong inflammatory response that is activated during obesity, we expect that fewer cells will remain in the undifferentiated stage. It is

noteworthy that participants following the genotype- based diet significantly had their naïve T cells decreased (total percentage and relative percentage to $CD4^+$ T cells).

Central memory T cells possessing little or no effector function proliferate and differentiate into effector cells in response to stimulation by antigens. A noteworthy increase in total percentage of central memory T cells was observed in obese subjects and similar observations are reported in another study [92]. Both types of diet (i.e. personalized and non- personalized) resulted in a marked reduction in total and relative to $CD4^+$ cells levels following the 3- month intervention period.

Effector memory T cells possess immediate effector function. No lymph- node homing receptors are reported in those cells and they are mostly encountered in tissues and circulation. According to our results, obese participants had elevated percentages of this type of cells compared to lean subjects. Similarly, Mauro et al. [60] demonstrated a significant association between increased effector memory T cells and obese condition. In contrast, effector memory T cell counts in obese individuals did not significantly differ from controls in another study [92]. Given the chronic systemic inflammation reported in obesity, the differentiation of naïve T cells into memory T cells and the subsequent marked upsurge of effector memory T cells in obese individuals are expected. In addition, except for the reduction of effector memory cell percentage to $CD4^+$ T cells in response to conventional diet, no other marked changes regarding the type of intervention used to induce weight loss were reported.

TEMRA T cells are cytolytic cells possessing lower homeostatic proliferation capacity and shorter telomeres than naïve, central memory and effector memory T cells. In our study, TEMRA cells were found elevated in the obese group. Our results are line with those from a study published by Van der Weerd [92] reporting significant increases in TEMRA cells in obese participants compared to lean controls. Generally, weight reduction induced an upregulation in TEMRA T cell levels with a statistical significant increase reported in the group following the genotype- based 3-month intervention (% percentage to $CD4^+$ T cells).

The overall increase reported in total $CD4^+$ T cells in obese individuals could be predominantly attributed to the increase in central memory T cells and to a lesser extent to the increase reported in naïve and effector memory T cells. TEMRA T cells

did not exhibit any difference between lean and obese participants regarding their distribution in $CD4^+$ T cell compartment.

The expression of the CXCR3 marker in obesity has not been extensively studied. Except for a study examining plasma levels of the CXCR3 ligand, CXCL10, in morbidly obese patients [209], no studies investigating the peripheral expression of CXCR3 in case of obesity with no other existing co-morbidities have been published so far. Our results indicate a reduction of naïve/ TEMRA cells and an upregulation of memory cells expressing the CXCR3 marker in obese participants. Thus, memory cells more than naïve/ TEMRA cells possess a possible active role in the orchestration of the accumulation of inflammatory T cells in fat sites in obesity. In addition, following weight loss, personalized intervention results in an overall increase in both $CXCR3^+$ memory and $CXCR3^+$ naïve/ TEMRA cells, whereas non- personalized intervention results in a moderate upregulation in naïve/ TEMRA cells and in a less profound reduction in memory cells.

The extensive investigation of Treg cells in obesity is supported by the fact that low grade systemic inflammation observed in obese individuals might be indicative of impaired Tregs function and/ or number [279]. The involvement of the anti-inflammatory Treg cells in obesity has been largely evaluated by several studies. The majority of them report a significant reduction in circulating Treg cell total count or percentage in obese compared to non- obese subjects (Table 5). This reduction might be attributed to their active recruitment to sites of inflammation [185].

In spite of the reported downregulation, Van der Weerd [92] reported an increase in circulating Treg cell counts in morbidly obese adults and a positive correlation with BMI. Our results are line with the latter study indicating an upregulation in Tregs total percentage in the obese group. However, Tregs percentage to $CD4^+$ T cells was decreased in obese compared to non- obese subjects. Similarly, Wagner [185] and Agabiti- Rosei [186] reported that the percentage of Treg cells from $CD4^+$ T cells was lower in obese compared to non- obese individuals (median: 1.2% versus 0.73%, $p=0.038$, and $4.11\pm1.60\%$ versus $2.69\pm1.81\%$ OB, $p<0,01$, respectively). Furthermore, both types of diet induced an upregulation in Tregs levels following weight loss with genotype- based type of diet resulting in a more marked increase in their relative levels regarding $CD4^+$ T cells. Those results indicate that personalized

intervention is more efficient at the enhancement of the anti- inflammatory mechanism represented by Treg cells via limitation of obesity- related inflammation.

It is widely accepted that individuals respond differently to dietary factors. According to results from several studies, the delivery of personalized intervention results in outstanding improvements in dietary intake behavior compared to "one fits all" approach, and forces individuals to sustain healthy changes to their usual lifestyle. However, the number of well- designed randomized controlled trials reflecting the efficacy of personalized intervention is limited and noteworthy results obtained until now mostly derive from observational studies with a low level of reproducibility. Thus, larger and better- quality studies are needed in order to elucidate the exact role of personalized recommendation, especially the one based on genetic information, in diet and lifestyle modifications.

Similarly, there is a lack of knowledge regarding the factors that cause interindividual differences in response to pieces of diet and their persistence over time. Results from intervention studies based on personalized information seem to be useful and they should be definitely be conducted in a larger scale in order to draw the right conclusions about the role of interindividual differences in responses to dietary interventions.

Today, most of the available services in the area of personalized nutrition are based on limited published evidence regarding their safety or efficacy [235], and their clinical utility and analytical validity are a matter of concern. For that reason, world-wide government bodies make an effort to effectively control the distribution and familiarize consumers with genetic tests [280-282].

It is evident that current literature is characterized by conflicting data regarding most of the cells included in our study. Those variations might be attributed to differences in the BMI of participants. More specifically, a dose- dependent effect of BMI on the levels of circulating leukocytes, lymphocytes and T cells has been reported [26, 127]. Furthermore, existing co-morbidities and intake of multiple medications by obese patients might also serve as confounding factors that affect the frequency and function of immune cells, and contribute to differences between published data [157].

Well- designed studies evaluating immune cell frequency and function from circulation and adipose tissue from the same person are indispensable in order to examine whether circulating immune cells might be used as biomarkers for short-term clinical trial outcomes [108]. Understanding how immune cells are altered in obesity will probably lead to treatment for diseases in which dietary intake behavior regulates clinical outcome.

**In summary, it is evident that obesity directly affects the regulation of the inflammatory and immune response. Our data demonstrate that obesity is associated with an increased frequency of circulating PBMCs and memory T cells confirming the proinflammatory status of obese individuals. Furthermore, the 3- month genotype- based type of intervention induced a statistical significant reduction in naïve T cells (total and % percentage to $CD4^+$ T cells) and a statistical significant increase in TEMRA cells (relative percentage to $CD4^+$ T cells). That observation indicates that weight loss is in line with a more differentiated status of $CD4^+$ T cells but further studies are needed in order to confirm this trend in the intermediate stages, i.e. central and effector memory T cells.**

## 7. REFERENCES


1. Visser, M., Bouter, L. M., McQuillan, G. M., Wener, M. H., and Harris, T. B. (1999) Elevated C-reactive protein levels in overweight and obese adults. *J. Am. Med. Assoc.* 10.1001/jama.282.22.2131
2. Gallagher, D., Visser, M., Sepúlveda, D., Pierson, R. N., Harris, T., and Heymsfieid, S. B. (1996) How useful is body mass index for comparison of body fatness across age, sex, and ethnic groups? *Am. J. Epidemiol.* 10.1093/oxfordjournals.aje.a008733
3. WHO. 2010. WHO Fact Files: Ten facts on obesity. Geneva: WHO. http://www.who.int/features/factfiles/obesity/en/index.html
4. Flegal, K. M., Carroll, M. D., Ogden, C. L., and Curtin, L. R. (2010) Prevalence and trends in obesity among US adults, 1999-2008. *JAMA - J. Am. Med. Assoc.* 10.1001/jama.2009.2014
5. Ogden, C. L., Carroll, M. D., Kit, B. K., and Flegal, K. M. (2014) Prevalence of childhood and adult obesity in the United States, 2011-2012. *JAMA - J. Am. Med. Assoc.* 10.1001/jama.2014.732
6. Afshin, A., Forouzanfar, M. H., Reitsma, M. B., Sur, P., Estep, K., Lee, A., Marczak, L., Mokdad, A. H., Moradi-Lakeh, M., Naghavi, M., Salama, J. S., Vos, T., Abate, K. H., Abbafati, C., Ahmed, M. B., Al-Aly, Z., Alkerwi, A., Al-Raddadi, R., Amare, A. T., Amberbir, A., Amegah, A. K., Amini, E., Amrock, S. M., Anjana, R. M., Ärnlöv, J., Asayesh, H., Banerjee, A., Barac, A., Baye, E., Bennett, D. A., Beyene, A. S., Biadgilign, S., Biryukov, S., Bjertness, E., Boneya, D. J., Campos-Nonato, I., Carrero, J. J., Cecilio, P., Cercy, K., Ciobanu, L. G., Cornaby, L., Damtew, S. A., Dandona, L., Dandona, R., Dharmaratne, S. D., Duncan, B. B., Eshrati, B., Esteghamati, A., Feigin, V. L., Fernandes, J. C., Fürst, T., Gebrehiwot, T. T., Gold, A., Gona, P. N., Goto, A., Habtewold, T. D., Hadush, K. T., Hafezi-Nejad, N., Hay, S. I., Horino, M., Islami, F., Kamal, R., Kasaeian, A., Katikireddi, S. V., Kengne, A. P., Kesavachandran, C. N., Khader, Y. S., Khang, Y. H., Khubchandani, J., Kim, D., Kim, Y. J., Kinfu, Y., Kosen, S., Ku, T., Defo, B. K., Kumar, G. A., Larson, H. J., Leinsalu, M., Liang, X., Lim, S. S., Liu, P., Lopez, A. D., Lozano, R., Majeed, A., Malekzadeh, R., Malta, D. C., Mazidi, M., McAlinden, C., McGarvey, S. T., Mengistu, D. T., Mensah, G. A., Mensink, G. B. M., Mezgebe, H. B., Mirrakhimov, E. M., Mueller, U. O., Noubiap, J. J., Obermeyer, C. M., Ogbo, F. A., Owolabi, M. O., Patton, G. C., Pourmalek, F., Qorbani, M., Rafay, A., Rai, R. K., Ranabhat, C. L., Reinig, N., Safiri, S., Salomon, J. A., Sanabria, J. R., Santos, I. S., Sartorius, B., Sawhney, M., Schmidhuber, J., Schutte, A. E., Schmidt, M. I., Sepanlou, S. G., Shamsizadeh, M., Sheikhbahaei, S., Shin, M. J., Shiri, R., Shiue, I., Roba, H. S., Silva, D. A. S., Silverberg, J. I., Singh, J. A., Stranges, S., Swaminathan, S., Tabarés-Seisdedos, R., Tadese, F., Tedla, B. A., Tegegne, B. S., Terkawi, A. S., Thakur, J. S., Tonelli, M., Topor-Madry, R., Tyrovolas, S., Ukwaja, K. N., Uthman, O. A., Vaezghasemi, M., Vasankari, T., Vlassov, V. V., Vollset, S. E., Weiderpass, E., Werdecker, A., Wesana, J., Westerman, R., Yano, Y., Yonemoto, N., Yonga, G., Zaidi, Z., Zenebe, Z. M., Zipkin, B., and Murray, C. J. L. (2017) Health effects of overweight and obesity in 195 countries over 25 years. *N. Engl. J. Med.* 10.1056/NEJMoa1614362
7. Finkelstein, E. A., Trogdon, J. G., Cohen, J. W., and Dietz, W. (2009) Annual medical spending attributable to obesity: Payer-and service-specific estimates. *Health Aff.* 10.1377/hlthaff.28.5.w822
8. Hossain, P., Kawar, B., and El Nahas, M. (2007) Obesity and diabetes in the developing world - A growing challenge. *N. Engl. J. Med.* 10.1056/NEJMp068177
9. Gerriets, V. A., and MacIver, N. J. (2014) Role of T cells in malnutrition and obesity. *Front. Immunol.* 10.3389/fimmu.2014.00379
10. Magrone, T., and Jirillo, E. (2015) Childhood obesity: Immune response and nutritional approaches. *Front. Immunol.* 10.3389/fimmu.2015.00076
11. Mitchell, S., and Shaw, D. (2015) The worldwide epidemic of female obesity. *Best Pract. Res. Clin. Obstet. Gynaecol.* 10.1016/j.bpobgyn.2014.10.002

12. Ogden, C. L., Carroll, M. D., Curtin, L. R., Lamb, M. M., and Flegal, K. M. (2010) Prevalence of high body mass index in US children and adolescents, 2007-2008. *JAMA - J. Am. Med. Assoc.* 10.1001/jama.2009.2012
13. Gregor, M. F., and Hotamisligil, G. S. (2011) Inflammatory Mechanisms in Obesity. *Annu. Rev. Immunol.* 10.1146/annurev-immunol-031210-101322
14. Claussnitzer, M., Dankel, S. N., Kim, K. H., Quon, G., Meuleman, W., Haugen, C., Glunk, V., Sousa, I. S., Beaudry, J. L., Puviindran, V., Abdennur, N. A., Liu, J., Svensson, P. A., Hsu, Y. H., Drucker, D. J., Mellgren, G., Hui, C. C., Hauner, H., and Kellis, M. (2015) FTO obesity variant circuitry and adipocyte browning in humans. *N. Engl. J. Med.* 10.1056/NEJMoa1502214
15. Sánchez-Margalet, V., Fernández-Riejos, P., Najib, S., Santos-Alvarez, J., Martín-Romero, C., Pérez-Pérez, A., and González-Yanes, C. (2010) Role of leptin in the activation of immune cells. *Mediators Inflamm.* 10.1155/2010/568343
16. Lago, F., Dieguez, C., Gómez-Reino, J., and Gualillo, O. (2007) The emerging role of adipokines as mediators of inflammation and immune responses. *Cytokine Growth Factor Rev.* 10.1016/j.cytogfr.2007.04.007
17. Bjørndal, B., Burri, L., Staalesen, V., Skorve, J., and Berge, R. K. (2011) Different adipose depots: Their role in the development of metabolic syndrome and mitochondrial response to hypolipidemic agents. *J. Obes.* 10.1155/2011/490650
18. Forsythe, L. K., Wallace, J. M. W., and Livingstone, M. B. E. (2008) Obesity and inflammation: The effects of weight loss. *Nutr. Res. Rev.* 10.1017/S0954422408138732
19. Norata, G. D., Caligiuri, G., Chavakis, T., Matarese, G., Netea, M. G., Nicoletti, A., O'Neill, L. A. J., and Marelli-Berg, F. M. (2015) The Cellular and Molecular Basis of Translational Immunometabolism. *Immunity*. 10.1016/j.immuni.2015.08.023
20. Lee, Y. S., Wollam, J., and Olefsky, J. M. (2018) An Integrated View of Immunometabolism. *Cell*. 10.1016/j.cell.2017.12.025
21. Dalmas, E., Rouault, C., Abdennour, M., Rovere, C., Rizkalla, S., Bar-Hen, A., Nahon, J. L., Bouillot, J. L., Guerre-Millo, M., Clément, K., and Poitou, C. (2011) Variations in circulating inflammatory factors are related to changes in calorie and carbohydrate intakes early in the course of surgery-induced weight reduction1-3. *Am. J. Clin. Nutr.* 10.3945/ajcn.111.013771
22. Hotamisligil, G. (2006) Inflammation and metabolic disorders. *Nature*. 444, 860–867
23. Medzhitov, R. (2008) Origin and physiological roles of inflammation. *Nature*. 10.1038/nature07201
24. Wypych, T. P., Marsland, B. J., and Ubags, N. D. J. (2017) The impact of diet on immunity and respiratory diseases. *Ann. Am. Thorac. Soc.* 10.1513/AnnalsATS.201703-255AW
25. Hotamisligil, G. S., Shargill, N. S., and Spiegelman, B. M. (1993) Adipose expression of tumor necrosis factor-α: Direct role in obesity-linked insulin resistance. *Science (80-. ).* 10.1126/science.7678183
26. Tanaka, S. I., Isoda, F., Ishihara, Y., Kimura, M., and Yamakawa, T. (2001) T lymphopaenia in relation to body mass index and TNF-α in human obesity: Adequate weight reduction can be corrective. *Clin. Endocrinol. (Oxf).* 10.1046/j.1365-2265.2001.1139/cn2155.x
27. Kern, P. A., Saghizadeh, M., Ong, J. M., Bosch, R. J., Deem, R., and Simsolo, R. B. (1995) The expression of tumor necrosis factor in human adipose tissue: Regulation by obesity, weight loss, and relationship to lipoprotein lipase. *J. Clin. Invest.* 10.1172/JCI117899
28. Dandona, P., Weinstock, R., Thusu, K., Abdel-Rahman, E., Aljada, A., and Wadden, T. (1998) Tumor necrosis factor-α in sera of obese patients: Fall with weight loss. *J. Clin. Endocrinol. Metab.* 10.1210/jc.83.8.2907
29. Bruun, J. M., Pedersen, S. B., Kristensen, K., and Richelsen, B. (2002) Opposite regulation of interleukin-8 and tumor necrosis factor-α by weight loss. *Obes. Res.* 10.1038/oby.2002.68
30. Mantzoros, C. S., Moschos, S., Avramopoulos, I., Kaklamani, V., Liolios, A., Doulgerakis, D. E., Griveas, I., Katsilambros, N., and Flier, J. S. (1997) Leptin concentrations in relation to

body mass index and the tumor necrosis factor-α system in humans. *J. Clin. Endocrinol. Metab.* 10.1210/jc.82.10.3408
31. Tsukui, S., Kanda, T., Nara, M., Nishino, M., Kondo, T., and Kobayashi, I. (2000) Moderate-intensity regular exercise decreases serum tumor necrosis factor-α and HbA(1c) levels in healthy women. *Int. J. Obes.* 10.1038/sj.ijo.0801373
32. Ofei, F., Hurel, S., Newkirk, J., Sopwith, M., and Taylor, R. (1996) Effects of an engineered human anti-TNF-α antibody (CDP571) on insulin sensitivity and glycemic control in patients with NIDDM. *Diabetes*
33. Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., and Friedman, J. M. (1994) Positional cloning of the mouse obese gene and its human homologue. *Nature*. 10.1038/372425a0
34. Houseknecht, K. L., Baile, C. A., Matteri, R. L., and Spurlock, M. E. (1998) The Biology of Leptin: A Review. *J. Anim. Sci.* 10.2527/1998.7651405x
35. Ahlma, R. S., Prabakaran, D., Mantzoros, C., Qu, D., Lowell, B., Maratos-Flier, E., and Flier, J. S. (1996) Role of leptin in the neuroendocrine response to fasting. *Nature*. 10.1038/382250a0
36. Lin, S., Thomas, T. C., Storlien, L. H., and Huang, X. F. (2000) Development of high fat diet-induced obesity and leptin resistance in C57B1/6J mice. *Int. J. Obes.* 10.1038/sj.ijo.0801209
37. Chan, J. L., Heist, K., DePaoli, A. M., Veldhuis, J. D., and Mantzoros, C. S. (2003) The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men. *J. Clin. Invest.* 10.1172/JCI200317490
38. Huang, X. F., Xin, X., McLennan, P., and Storlien, L. (2004) Role of fat amount and type in ameliorating diet-induced obesity: Insights at the level of hypothalamic arcuate nucleus leptin receptor, neuropeptide Y and pro-opiomelanocortin mRNA expression. *Diabetes, Obes. Metab.* 10.1111/j.1463-1326.2004.00312.x
39. Sánchez-Margalet, V., Martín-Romero, C., Santos-Alvarez, J., Goberna, R., Najib, S., and Gonzalez-Yanes, C. (2003) Role of leptin as an immunomodulator of blood mononuclear cells: Mechanisms of action. *Clin. Exp. Immunol.* 10.1046/j.1365-2249.2003.02190.x
40. Womack, J., Tien, P. C., Feldman, J., Shin, J. H., Fennie, K., Anastos, K., Cohen, M. H., Bacon, M. C., and Minkoff, H. (2007) Obesity and immune cell counts in women. *Metabolism.* 10.1016/j.metabol.2007.03.008
41. Matarese, G., Procaccini, C., De Rosa, V., Horvath, T. L., and La Cava, A. (2010) Regulatory T cells in obesity: the leptin connection. *Trends Mol. Med.* 10.1016/j.molmed.2010.04.002
42. Friedman, J. M., and Halaas, J. L. (1998) Leptin and the regulation of body weight in mammals. *Nature*. 10.1038/27376
43. Gualillo, O., Eiras, S., Lago, F., Diéguez, C., and Casanueva, F. F. (2000) Elevated serum leptin concentrations induced by experimental acute inflammation. *Life Sci.* 10.1016/S0024-3205(00)00827-4
44. Huang, X. F., Koutcherov, I., Lin, S., Wang, H. Q., and Storlien, L. (1996) Localization of leptin receptor mRNA expression in mouse brain. *Neuroreport.* 10.1097/00001756-199611040-00045
45. White, D. W., and Tartaglia, L. A. (1996) Leptin and OB-R: body weight regulation by a cytokine receptor. *Cytokine Growth Factor Rev.* 7: 303–309
46. Milner, J. J., and Beck, M. A. (2012) The impact of obesity on the immune response to infection. in *Proceedings of the Nutrition Society*, 10.1017/S0029665112000158
47. Bjørbæk, C., El-Haschimi, K., Frantz, J. D., and Flier, J. S. (1999) The role of SOCS-3 in leptin signaling and leptin resistance. *J. Biol. Chem.* 10.1074/jbc.274.42.30059
48. Flier, J. S. (2004) Obesity Wars: Molecular Progress Confronts an Expanding Epidemic. *Cell.* 10.1016/S0092-8674(03)01081-X
49. Sadaf Farooqi, I., Matarese, G., Lord, G. M., Keogh, J. M., Lawrence, E., Agwu, C., Sanna, V., Jebb, S. A., Perna, F., Fontana, S., Lechler, R. I., Depaoli, A. M., and O'Rahilly, S. (2002) Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and

neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *J. Clin. Invest.* 10.1172/JCI200215693
50. La Cava, A., and Matarese, G. (2004) The weight of leptin in immunity. *Nat. Rev. Immunol.*
51. Lundgren, C. H., Brown, S. L., Nordt, T. K., Sobel, B. E., and Fujii, S. (1996) Elaboration of type-1 plasminogen activator inhibitor from adipocytes: A potential pathogenetic link between obesity and cardiovascular disease. *Circulation*. 10.1161/01.CIR.93.1.106
52. Poitou, C., Viguerie, N., Cancello, R., De Matteis, R., Cinti, S., Stich, V., Coussieu, C., Gauthier, E., Courtine, M., Zucker, J. D., Barsh, G. S., Saris, W., Bruneval, P., Basdevant, A., Langin, D., and Clément, K. (2005) Serum amyloid A: Production by human white adipocyte and regulation by obesity and nutrition. *Diabetologia*. 10.1007/s00125-004-1654-6
53. Mohamed-Ali, V., Goodrick, S., Rawesh, A., Katz, D. R., Miles, J. M., Yudkin, J. S., Klein, S., and Coppack, S. W. (1997) Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-α, in vivo. *J. Clin. Endocrinol. Metab.*
54. Yudkin, J. S., Stehouwer, C. D. A., Emeis, J. J., and Coppack, S. W. (1999) C-reactive protein in healthy subjects: Associations with obesity, insulin resistance, and endothelial dysfunction: A potential role for cytokines originating from adipose tissue? *Arterioscler. Thromb. Vasc. Biol.* 10.1161/01.ATV.19.4.972
55. Visser, M., Bouter, L. M., McQuillan, G. M., Wener, M. H., and Harris, T. B. (2001) Low-grade systemic inflammation in overweight children. *Pediatrics*
56. Tchernof, A., Nolan, A., Sites, C. K., Ades, P. A., and Poehlman, E. T. (2002) Weight loss reduces C-reactive protein levels in obese postmenopausal women. *Circulation*. 10.1161/hc0502.103331
57. Weinhold, B., and Rüther, U. (1997) Interleukin-6-dependent and -independent regulation of the human C-reactive protein gene. *Biochem. J.* 10.1042/bj3270425
58. Du Clos TW (2011) Function of C-reactive protein. *Ann Med.* 10.1360/zd-2013-43-6-1064
59. Das, U. N. (2001) Is obesity an inflammatory condition? *Nutrition*. 10.1016/S0899-9007(01)00672-4
60. Mauro, C., Smith, J., Cucchi, D., Coe, D., Fu, H., Bonacina, F., Baragetti, A., Cermenati, G., Caruso, D., Mitro, N., Catapano, A. L., Ammirati, E., Longhi, M. P., Okkenhaug, K., Norata, G. D., and Marelli-Berg, F. M. (2017) Obesity-Induced Metabolic Stress Leads to Biased Effector Memory CD4+ T Cell Differentiation via PI3K p110δ-Akt-Mediated Signals. *Cell Metab.* 10.1016/j.cmet.2017.01.008
61. Bastard, J. P., Jardel, C., Bruckert, E., Blondy, P., Capeau, J., Laville, M., Vidal, H., and Hainque, B. (2000) Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. *J. Clin. Endocrinol. Metab.* 10.1210/jc.85.9.3338
62. Heilbronn, L. K., Noakes, M., and Clifton, P. M. (2001) Energy restriction and weight loss on very-low-fat diets reduce C-reactive protein concentrations in obese, healthy women. *Arterioscler. Thromb. Vasc. Biol.* 10.1161/01.ATV.21.6.968
63. Laimer, M., Ebenbichler, C. F., Kaser, S., Sandhofer, A., Weiss, H., Nehoda, H., Aigner, F., and Patsch, J. R. (2002) Markers of chronic inflammation and obesity: A prospective study on the reversibility of this association in middle-aged women undergoing weight loss by surgical intervention. *Int. J. Obes.* 10.1038/sj.ijo.0801970
64. Ziccardi, P., Nappo, F., Giugliano, G., Esposito, K., Marfella, R., Cioffi, M., D'Andrea, F., Molinari, A. M., and Giugliano, D. (2002) Reduction of inflammatory cytokine concentrations and improvement of endothelial functions in obese women after weight loss over one year. *Circulation*. 10.1161/hc0702.104279
65. Saito, K., Tobe, T., Minoshima, S., Asakawa, S., Sumiya, J., Yoda, M., Nakano, Y., Shimizu, N., and Tomita, M. (1999) Organization of the gene for gelatin-binding protein (GBP28). *Gene*. 10.1016/S0378-1119(99)00041-4
66. Arita, Y., Kihara, S., Ouchi, N., Takahashi, M., Maeda, K., Miyagawa, J. I., Hotta, K., Shimomura, I., Nakamura, T., Miyaoka, K., Kuriyama, H., Nishida, M., Yamashita, S.,

Okubo, K., Matsubara, K., Muraguchi, M., Ohmoto, Y., Funahashi, T., and Matsuzawa, Y. (1999) Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem. Biophys. Res. Commun.* 10.1006/bbrc.1999.0255

67. Xu, A., Tso, A. W. K., Cheung, B. M. Y., Wang, Y., Wat, N. M. S., Fong, C. H. Y., Yeung, D. C. Y., Janus, E. D., Sham, P. C., and Lam, K. S. L. (2007) Circulating adipocyte-fatty acid binding protein levels predict the development of the metabolic syndrome: A 5-year prospective study. *Circulation*. 10.1161/CIRCULATIONAHA.106.647503
68. Maeda, N., Takahashi, M., Funahashi, T., Kihara, S., Nishizawa, H., Kishida, K., Nagaretani, H., Matsuda, M., Komuro, R., Ouchi, N., Kuriyama, H., Hotta, K., Nakamura, T., Shimomura, I., and Matsuzawa, Y. (2001) PPARγ Ligands Increase Expression and Plasma Concentrations of Adiponectin, an Adipose-Derived Protein. *Diabetes*. 10.2337/diabetes.50.9.2094
69. Asghar, A., and Sheikh, N. (2017) Role of immune cells in obesity induced low grade inflammation and insulin resistance. *Cell. Immunol.* 10.1016/j.cellimm.2017.03.001
70. Carbone, F., La Rocca, C., and Matarese, G. (2012) Immunological functions of leptin and adiponectin. *Biochimie*. 10.1016/j.biochi.2012.05.018
71. Milner, J. J., and Beck, M. A. (2012) Micronutrients, immunology and inflammation: The impact of obesity on the immune response to infection. *Proc Nutr Soc*. 10.1017/S0029665112000158
72. Wolf, A. M., Wolf, D., Rumpold, H., Enrich, B., and Tilg, H. (2004) Adiponectin induces the anti-inflammatory cytokines IL-10 and IL-1RA in human leukocytes. *Biochem. Biophys. Res. Commun.* 10.1016/j.bbrc.2004.08.145
73. Maeda, N., Shimomura, I., Kishida, K., Nishizawa, H., Matsuda, M., Nagaretani, H., Furuyama, N., Kondo, H., Takahashi, M., Arita, Y., Komuro, R., Ouchi, N., Kihara, S., Tochino, Y., Okutomi, K., Horie, M., Takeda, S., Aoyamaa, T., Funahashi, T., and Matsuzawa, Y. (2002) Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat. Med.* 10.1038/nm724
74. Fasshauer, M., Kralisch, S., Klier, M., Lossner, U., Bluher, M., Klein, J., and Paschke, R. (2003) Adiponectin gene expression and secretion is inhibited by interleukin-6 in 3T3-L1 adipocytes. *Biochem. Biophys. Res. Commun.* 10.1016/S0006-291X(03)00090-1
75. Li, Z., Bowerman, S., and Heber, D. (2005) Health ramifications of the obesity epidemic. *Surg. Clin. North Am.* 10.1016/j.suc.2005.04.006
76. Mokdad, A. H., Bowman, B. A., Ford, E. S., Vinicor, F., Marks, J. S., and Koplan, J. P. (2001) The continuing epidemics of obesity and diabetes in the United States. *J. Am. Med. Assoc.* 10.1001/jama.286.10.1195
77. Mauro, C., and Marelli-Berg, F. M. (2012) T cell immunity and cardiovascular metabolic disorders: Does metabolism fuel inflammation? *Front. Immunol.* 10.3389/fimmu.2012.00173
78. Marzullo, P., Minocci, A., Tagliaferri, M. A., Guzzaloni, G., Di Blasio, A., De Medici, C., Aimaretti, G., and Liuzzi, A. (2010) Investigations of thyroid hormones and antibodies in obesity: Leptin levels are associated with thyroid autoimmunity independent of bioanthropometric, hormonal, and weight-related determinants. *J. Clin. Endocrinol. Metab.* 10.1210/jc.2009-2798
79. Lukens, J. R., Dixit, V. D., and Kanneganti, T. D. (2011) Inflammasome activation in obesity-related inflammatory diseases and autoimmunity. *Discov. Med.*
80. Marrie, R. A., Horwitz, R. I., Cutter, G., Tyry, T., and Vollmer, T. (2011) Association between comorbidity and clinical characteristics of MS. *Acta Neurol. Scand.* 10.1111/j.1600-0404.2010.01436.x
81. Duntas, L. H., and Biondi, B. (2013) The interconnections between obesity, thyroid function, and autoimmunity: The multifold role of leptin. *Thyroid*. 10.1089/thy.2011.0499
82. Harpsøe, M. C., Basit, S., Andersson, M., Nielsen, N. M., Frisch, M., Wohlfahrt, J., Nohr, E. A., Linneberg, A., and Jess, T. (2014) Body mass index and risk of autoimmune diseases: A study within the Danish National Birth Cohort. *Int. J. Epidemiol.* 10.1093/ije/dyu045

83. Huang, X. F., and Chen, J. Z. (2009) Obesity, the PI3K/Akt signal pathway and colon cancer. *Obes. Rev*. 10.1111/j.1467-789X.2009.00607.x
84. Wolin, K. Y., Carson, K., Colditz, G. A. (2010) Obesity and cancer. *Oncologist*. 15:556–65.doi:10.1634/theoncologist.2009-0285
85. Faulds, M. H., and Dahlman-Wright, K. (2012) Metabolic diseases and cancer risk. *Curr. Opin. Oncol.*
86. Schottenfeld, D., Beebe-Dimmer, J. L., Buffler, P. A., and Omenn, G. S. (2013) Current Perspective on the Global and United States Cancer Burden Attributable to Lifestyle and Environmental Risk Factors. *Annu. Rev. Public Health*. 10.1146/annurev-publhealth-031912-114350
87. Baik, I., Curhan, G. C., Rimm, E. B., Bendich, A., Willett, W. C., and Fawzi, W. W. (2000) A prospective study of age and lifestyle factors in relation to community-acquired pneumonia in US men and women. *Arch. Intern. Med.* 10.1001/archinte.160.20.3082
88. Dávalos Moscol, M. (2010) Indications for treatment in chronic HCV infection. *Ann. Hepatol.* 10.1016/s1665-2681(19)31724-7
89. Morgan, O. W., Bramley, A., Fowlkes, A., Freedman, D. S., Taylor, T. H., Gargiullo, P., Belay, B., Jain, S., Cox, C., Kamimoto, L., Fiore, A., Finelli, L., Olsen, S. J., and Fry, A. M. (2010) Morbid obesity as a risk factor for hospitalization and death due to 2009 pandemic influenza A(H1N1) disease. *PLoS One*. 10.1371/journal.pone.0009694
90. Cottam, D. R., Mattar, S. G., Barinas-Mitchell, E., Eid, G., Kuller, L., Kelley, D. E., and Schauer, P. R. (2004) The chronic inflammatory hypothesis for the morbidity associated with morbid obesity: Implications and effect of weight loss. *Obes. Surg.* 10.1381/096089204323093345
91. Yoshimura, A., Ohnishi, S., Orito, C., Kawahara, Y., Takasaki, H., Takeda, H., Sakamoto, N., and Hashino, S. (2015) Association of peripheral total and differential leukocyte counts with obesity-related complications in young adults. *Obes. Facts*. 10.1159/000373881
92. Van Der Weerd, K., Dik, W. A., Schrijver, B., Schweitzer, D. H., Langerak, A. W., Drexhage, H. A., Kiewiet, R. M., Van Aken, M. O., Van Huisstede, A., Van Dongen, J. J. M., Van Der Lelij, A. J., Staal, F. J. T., and Van Hagen, P. M. (2012) Morbidly obese human subjects have increased peripheral blood CD4 + T cells with skewing toward a Treg- and Th2-dominated phenotype. *Diabetes*. 10.2337/db11-1065
93. Mraz, M., and Haluzik, M. (2014) The role of adipose tissue immune cells in obesity and low-grade inflammation. *J. Endocrinol.* 10.1530/JOE-14-0283
94. Elgazar-Carmon, V., Rudich, A., Hadad, N., and Levy, R. (2008) Neutrophils transiently infiltrate intra-abdominal fat early in the course of high-fat feeding. *J. Lipid Res.* 10.1194/jlr.M800132-JLR200
95. Zarkesh-Esfahani, H., Pockley, A. G., Wu, Z., Hellewell, P. G., Weetman, A. P., and Ross, R. J. M. (2004) Leptin Indirectly Activates Human Neutrophils via Induction of TNF-α. *J. Immunol.* 10.4049/jimmunol.172.3.1809
96. Herishanu, Y., Rogowski, O., Polliack, A., and Marilus, R. (2006) Leukocytosis in obese individuals: Possible link in patients with unexplained persistent neutrophilia. *Eur. J. Haematol.* 10.1111/j.1600-0609.2006.00658.x
97. Lee, B. C., and Lee, J. (2014) Cellular and molecular players in adipose tissue inflammation in the development of obesity-induced insulin resistance. Biochim. Biophys. Acta - Mol. Basis Dis. 10.1016/j.bbadis.2013.05.017
98. Nijhuis, J., Rensen, S. S., Slaats, Y., Van Dielen, F. M. H., Buurman, W. A., and Greve, J. W. M. (2009) Neutrophil activation in morbid obesity, chronic activation of acute inflammation. *Obesity*. 10.1038/oby.2009.113
99. Nguyen, K. D., Qiu, Y., Cui, X., Goh, Y. P. S., Mwangi, J., David, T., Mukundan, L., Brombacher, F., Locksley, R. M., and Chawla, A. (2011) Alternatively activated macrophages produce catecholamines to sustain adaptive thermogenesis. *Nature*. 10.1038/nature10653

100. Pecht, T., Gutman-Tirosh, A., Bashan, N., and Rudich, A. (2014) Peripheral blood leucocyte subclasses as potential biomarkers of adipose tissue inflammation and obesity subphenotypes in humans. *Obes. Rev.* 10.1111/obr.12133
101. Zaldivar, F., McMurray, R. G., Nemet, D., Galassetti, P., Mills, P. J., and Cooper, D. M. (2006) Body fat and circulating leukocytes in children. *Int. J. Obes.* 10.1038/sj.ijo.0803227
102. Rothenberg, M. E., Hogan, S. P. (2006) The eosinophil. *Annu.Rev.Immunol*. 24, pp. 147-174
103. Wu, D., AB, M., HE, L., RR, R.-G., HA, J., JK, B., A, C., and RM, L. (2011) Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. *Science (80-. ).*
104. Cottam, D. R., Schaefer, P. A., Shaftan, G. W., Velcu, L., and Angus, L. D. G. (2002) Effect of surgically-induced weight loss on leukocyte indicators of chronic inflammation in morbid obesity. *Obes. Surg.* 10.1381/096089202321088101
105. Cildir, G., Akincilar, S. C., and Tergaonkar, V. (2013) Chronic adipose tissue inflammation: All immune cells on the stage. Trends Mol. Med. 10.1016/j.molmed.2013.05.001
106. Huh, J. Y., Park, Y. J., Ham, M., and Kim, J. B. (2014) Crosstalk between adipocytes and immune cells in adipose tissue inflammation and metabolic dysregulation in obesity. *Mol. Cells*. 10.14348/molcells.2014.0074
107. Suzukawa, M., Nagase, H., Ogahara, I., Han, K., Tashimo, H., Shibui, A., Koketsu, R., Nakae, S., Yamaguchi, M., and Ohta, K. (2011) Leptin Enhances Survival and Induces Migration, Degranulation, and Cytokine Synthesis of Human Basophils. *J. Immunol.* 10.4049/jimmunol.1004054
108. Snyder-Cappione, J. E., and Nikolajczyk, B. S. (2013) When diet and exercise are not enough, think immunomodulation. *Mol. Aspects Med.* 10.1016/j.mam.2012.10.003
109. Moretta, A., Bottino, C., Mingari, M. C., Biassoni, R., and Moretta, L. (2002) What is a natural killer cell? *Nat. Immunol.* 10.1038/ni0102-6
110. Marquardt, N., Béziat, V., Nyström, S., Hengst, J., Ivarsson, M. A., Kekäläinen, E., Johansson, H., Mjösberg, J., Westgren, M., Lankisch, T. O., Wedemeyer, H., Ellis, E. C., Ljunggren, H.-G., Michaëlsson, J., and Björkström, N. K. (2015) Cutting Edge: Identification and Characterization of Human Intrahepatic CD49a + NK Cells . *J. Immunol.* 10.4049/jimmunol.1402756
111. Ivarsson, M. A., Stiglund, N., Marquardt, N., Westgren, M., Gidlöf, S., and Björkström, N. K. (2017) Composition and dynamics of the uterine NK cell KIR repertoire in menstrual blood. *Mucosal Immunol.* 10.1038/mi.2016.50
112. Andoniou, C. E., Coudert, J. D., and Degli-Esposti, M. A. (2008) Killers and beyond: NK-cell-mediated control of immune responses. *Eur. J. Immunol.* 10.1002/eji.200838882
113. Caspar-Bauguil, S., Cousin, B., Galinier, A., Segafredo, C., Nibbelink, M., André, M., Casteilla, L., and Pénicaud, L. (2005) Adipose tissues as an ancestral immune organ: Site-specific change in obesity. *FEBS Lett.* 10.1016/j.febslet.2005.05.031
114. Motivala, S. J., Dang, J., Obradovic, T., Meadows, G. G., Butch, A. W., and Irwin, M. R. (2003) Leptin and Cellular and Innate Immunity in Abstinent Alcoholics and Controls. *Alcohol. Clin. Exp. Res.* 10.1097/01.ALC.0000093741.21492.02
115. Matarese, G., Moschos, S., and Mantzoros, C. S. (2005) Leptin in Immunology. *J. Immunol.* 10.4049/jimmunol.174.6.3137
116. Zhao, Y., Sun, R., You, L., Gao, C., and Tian, Z. (2003) Expression of leptin receptors and response to leptin stimulation of human natural killer cell lines. *Biochem. Biophys. Res. Commun.* 10.1016/S0006-291X(02)02838-3
117. Napoleone, E., Di Santo, A., Amore, C., Baccante, G., Di Febbo, C., Porreca, E., De Gaetano, G., Donati, M. B., and Lorenzet, R. (2007) Leptin induces tissue factor expression in human peripheral blood mononuclear cells: A possible link between obesity and cardiovascular risk? *J. Thromb. Haemost.* 10.1111/j.1538-7836.2007.02578.x

118. Laue, T., Wrann, C. D., Hoffmann-Castendiek, B., Pietsch, D., Hübner, L., and Kielstein, H. (2015) Altered NK cell function in obese healthy humans. *BMC Obes.* 10.1186/s40608-014-0033-1
119. Tian, Z., Sun, R., Wei, H., and Gao, B. (2002) Impaired natural killer (NK) cell activity in leptin receptor deficient mice: Leptin as a critical regulator in NK cell development and activation. *Biochem. Biophys. Res. Commun.* 10.1016/S0006-291X(02)02462-2
120. Nave, H., Mueller, G., Siegmund, B., Jacobs, R., Stroh, T., Schueler, U., Hopfe, M., Behrendt, P., Buchenauer, T., Pabst, R., and Brabant, G. (2008) Resistance of Janus kinase-2 dependent leptin signaling in natural killer (NK) Cells: A novel mechanism of NK cell dysfunction in diet-induced obesity. *Endocrinology*. 10.1210/en.2007-1516
121. O'Shea, D., Cawood, T. J., O'Farrelly, C., and Lynch, L. (2010) Natural killer cells in obesity: Impaired function and increased susceptibility to the effects of cigarette smoke. *PLoS One*. 10.1371/journal.pone.0008660
122. O'Rourke, R. W., Gaston, G. D., Meyer, K. A., White, A. E., and Marks, D. L. (2013) Adipose tissue NK cells manifest an activated phenotype in human obesity. *Metabolism.* 10.1016/j.metabol.2013.07.011
123. Kobayashi, T., and Mattarollo, S. R. (2017) Natural killer cell metabolism. *Mol. Immunol.* 10.1016/j.molimm.2017.11.021
124. Viel, S., Besson, L., Charrier, E., Marçais, A., Disse, E., Bienvenu, J., Walzer, T., and Dumontet, C. (2017) Alteration of Natural Killer cell phenotype and function in obese individuals. *Clin. Immunol.* 10.1016/j.clim.2016.01.007
125. Lynch, L. A., O'Connell, J. M., Kwasnik, A. K., Cawood, T. J., O'Farrelly, C., and O'Shea, D. B. (2009) Are natural killer cells protecting the metabolically healthy obese patient? *Obesity*. 10.1038/oby.2008.565
126. Tobin, L. M., Mavinkurve, M., Carolan, E., Kinlen, D., O'Brien, E. C., Little, M. A., Finlay, D. K., Cody, D., Hogan, A. E., and O'Shea, D. (2017) NK cells in childhood obesity are activated, metabolically stressed, and functionally deficient. *JCI Insight*. 10.1172/jci.insight.94939
127. Nieman, D. C., Henson, D. A., Nehlsen-Cannarella, S. L., Ekkens, M., Utter, A. C., Butterworth, D. E., and Fagoaga, O. R. (1999) Influence of obesity on immune function. *J. Am. Diet. Assoc.* 10.1016/S0002-8223(99)00077-2
128. Scanga, C. B., Verde, T. J., Paolone, A. M., Andersen, R. E., and Wadden, T. A. (1998) Effects of weight loss and exercise training on natural killer cell activity in obese women. *Med. Sci. Sports Exerc.* 10.1097/00005768-199812000-00002
129. Moulin, C. M., Marguti, I., Peron, J. P. S., Halpern, A., and Rizzo, L. V. (2011) Bariatric surgery reverses natural killer (NK) cell activity and NK-related cytokine synthesis impairment induced by morbid obesity. *Obes. Surg.* 10.1007/s11695-010-0250-8
130. Mousavi, T., Farnia, P., Tajik, N., and Soofi, M. (2010) Elevation of CD56brightCD16- lymphocytes in MDR pulmonary tuberculosis. *Iran. J. Immunol.*
131. Moretta, L., Ferlazzo, G., Bottino, C., Vitale, M., Pende, D., Mingari, M. C., and Moretta, A. (2006) Effector and regulatory events during natural killer-dendritic cell interactions. *Immunol. Rev.* 10.1111/j.1600-065X.2006.00450.x
132. De Maria, A., Bozzano, F., Cantoni, C., and Moretta, L. (2011) Revisiting human natural killer cell subset function revealed cytolytic CD56dimCD16+ NK cells as rapid producers of abundant IFN-γ on activation. *Proc. Natl. Acad. Sci. U. S. A.* 10.1073/pnas.1012356108
133. Bayigga, L., Nabatanzi, R., Sekiziyivu, P. N., Mayanja-Kizza, H., Kamya, M. R., Kambugu, A., Olobo, J., Kiragga, A., Kirimunda, S., Joloba, M., and Nakanjako, D. (2014) High CD56++CD16- natural killer (NK) cells among suboptimal immune responders after four years of suppressive antiretroviral therapy in an African adult HIV treatment cohort. *BMC Immunol.* 10.1186/1471-2172-15-2

134. Schepis, D., Gunnarsson, I., Eloranta, M. L., Lampa, J., Jacobson, S. H., Kärre, K., and Berg, L. (2009) Increased proportion of CD56bright natural killer cells in active and inactive systemic lupus erythematosus. *Immunology*. 10.1111/j.1365-2567.2008.02887.x
135. Laroni, A., Gandhi, R., Beynon, V., and Weiner, H. L. (2011) IL-27 imparts immunoregulatory function to human NK cell subsets. *PLoS One*. 10.1371/journal.pone.0026173
136. Fu, B., Li, X., Sun, R., Tong, X., Ling, B., Tian, Z., and Wei, H. (2013) Natural killer cells promote immune tolerance by regulating inflammatory TH17 cells at the human maternal-fetal interface. *Proc. Natl. Acad. Sci. U. S. A.* 10.1073/pnas.1206322110
137. Morandi, F., Horenstein, A. L., Chillemi, A., Quarona, V., Chiesa, S., Imperatori, A., Zanellato, S., Mortara, L., Gattorno, M., Pistoia, V., and Malavasi, F. (2015) CD56 bright CD16 − NK Cells Produce Adenosine through a CD38-Mediated Pathway and Act as Regulatory Cells Inhibiting Autologous CD4 + T Cell Proliferation . *J. Immunol.* 10.4049/jimmunol.1500591
138. Magrone, T., Jirillo, E., Spagnoletta, A., Magrone, M., Russo, M. A., Fontana, S., Laforgia, F., Donvito, I., Campanella, A., Silvestris, F., and De Pergola, G. (2017) Immune Profile of Obese People and in Vitro Effects of Red Grape Polyphenols on Peripheral Blood Mononuclear Cells. *Oxid. Med. Cell. Longev.* 10.1155/2017/9210862
139. Nieman, D. C., Nehlsen-Cannarella, S. L., Henson, D. A., Butterworth, D. E., Fagoaga, O. R., Warren, B. J., and Rainwater, M. K. (1996) Immune response to obesity and moderate weight loss. *Int. J. Obes.*
140. Ilavská, S., Horváthová, M., Szabová, M., Nemessányi, T., Jahnová, E., Tulinská, J., Líšková, A., Wsolová, L., Staruchová, M., and Volkovová, K. (2012) Association between the human immune response and body mass index. *Hum. Immunol.* 10.1016/j.humimm.2012.02.023
141. Cottam, D. R., Schaefer, P. A., Shaftan, G. W., and Angus, L. D. G. (2003) Dysfunctional immune-privilege in morbid obesity: Implications and effect of gastric bypass surgery. *Obes. Surg.* 10.1381/096089203321136584
142. Cros, J., Cagnard, N., Woollard, K., Patey, N., Zhang, S. Y., Senechal, B., Puel, A., Biswas, S. K., Moshous, D., Picard, C., Jais, J. P., D'Cruz, D., Casanova, J. L., Trouillet, C., and Geissmann, F. (2010) Human CD14dim Monocytes Patrol and Sense Nucleic Acids and Viruses via TLR7 and TLR8 Receptors. *Immunity*. 10.1016/j.immuni.2010.08.012
143. Poitou, C., Dalmas, E., Renovato, M., Benhamo, V., Hajduch, F., Abdennour, M., Kahn, J. F., Veyrie, N., Rizkalla, S., Fridman, W. H., Sautès-Fridman, C., Clément, K., and Cremer, I. (2011) CD14dimCD16+ and CD14+CD16+ monocytes in obesity and during weight loss: Relationships with fat mass and subclinical atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* 10.1161/ATVBAHA.111.230979
144. de Matos, M. A., Duarte, T. C., Ottone, V. de O., Sampaio, P. F. d. M., Costa, K. B., de Oliveira, M. F. A., Moseley, P. L., Schneider, S. M., Coimbra, C. C., Brito-Melo, G. E. A., Magalhães, F. de C., Amorim, F. T., and Rocha-Vieira, E. (2016) The effect of insulin resistance and exercise on the percentage of CD16+ monocyte subset in obese individuals. *Cell Biochem. Funct.* 10.1002/cbf.3178
145. Ziegler-Heitbrock, L. (2007) The CD14+ CD16+ blood monocytes: their role in infection and inflammation. *J. Leukoc. Biol.* 10.1189/jlb.0806510
146. Schipper, H. S., Nuboer, R., Prop, S., Van Den Ham, H. J., De Boer, F. K., Kesmir, Ç., Mombers, I. M. H., Van Bekkum, K. A., Woudstra, J., Kieft, J. H., Hoefer, I. E., De Jager, W., Prakken, B., Van Summeren, M., and Kalkhoven, E. (2012) Systemic inflammation in childhood obesity: Circulating inflammatory mediators and activated CD14++ monocytes. *Diabetologia*. 10.1007/s00125-012-2641-y
147. Rothe, G., Gabriel, H., Kovacs, E., Klucken, J., Stöhr, J., Kindermann, W., and Schmitz, G. (1996) Peripheral blood mononuclear phagocyte subpopulations as cellular markers in hypercholesterolemia. *Arterioscler. Thromb. Vasc. Biol.* 10.1161/01.ATV.16.12.1437

148. Belge, K.-U., Dayyani, F., Horelt, A., Siedlar, M., Frankenberger, M., Frankenberger, B., Espevik, T., and Ziegler-Heitbrock, L. (2002) The Proinflammatory CD14 + CD16 + DR ++ Monocytes Are a Major Source of TNF . *J. Immunol.* 10.4049/jimmunol.168.7.3536
149. Ingersoll, M. A., Spanbroek, R., Lottaz, C., Gautier, E. L., Frankenberger, M., Hoffmann, R., Lang, R., Haniffa, M., Collin, M., Tacke, F., Habenicht, A. J. R., Ziegler-Heitbrock, L., and Randolph, G. J. (2010) Comparison of gene expression profiles between human and mouse monocyte subsets (Blood (2010) 115, 3 (e10-e19)). *Blood*. 10.1182/blood-2010-06-290122
150. Krinninger, P., Ensenauer, R., Ehlers, K., Rauh, K., Stoll, J., Krauss-Etschmann, S., Hauner, H., and Laumen, H. (2014) Peripheral monocytes of obese women display increased chemokine receptor expression and migration capacity. *J. Clin. Endocrinol. Metab.* 10.1210/jc.2013-2611
151. Kullo, I. J., Hensrud, D. D., and Allison, T. G. (2002) Comparison of numbers of circulating blood monocytes in men grouped by body mass index (<25, 25 to <30, ≥30). *Am. J. Cardiol.* 10.1016/S0002-9149(02)02366-4
152. Marzullo, P., Minocci, A., Giarda, P., Marconi, C., Tagliaferri, A., Walker, G. E., Scacchi, M., Aimaretti, G., and Liuzzi, A. (2014) Lymphocytes and immunoglobulin patterns across the threshold of severe obesity. *Endocrine*. 10.1007/s12020-013-0006-z
153. Tanigawa, T., Iso, H., Yamagishi, K., Muraki, I., Kawamura, N., Nakata, A., Sakurai, S., Ohira, T., and Shimamoto, T. (2004) Association of lymphocyte sub-populations with clustered features of metabolic syndrome in middle-aged Japanese men. *Atherosclerosis*. 10.1016/j.atherosclerosis.2003.12.019
154. Rogacev, K. S., Ulrich, C., Blömer, L., Hornof, F., Oster, K., Ziegelin, M., Cremers, B., Grenner, Y., Geisel, J., Schlitt, A., Köhler, H., Fliser, D., Girndt, M., and Heine, G. H. (2010) Monocyte heterogeneity in obesity and subclinical atherosclerosis. *Eur. Heart J.* 10.1093/eurheartj/ehp308
155. Viardot, A., Heilbronn, L. K., Samocha-Bonet, D., Mackay, F., Campbell, L. V., and Samaras, K. (2012) Obesity is associated with activated and insulin resistant immune cells. *Diabetes. Metab. Res. Rev.* 10.1002/dmrr.2302
156. Field, C. J., Gougeon, R., and Marliss, E. B. (1991) Changes in circulating leukocytes and mitogen responses during very-low-energy all-protein reducing diets. *Am. J. Clin. Nutr.* 10.1093/ajcn/54.1.123
157. O'Rourke, R. W., Kay, T., Scholz, M. H., Diggs, B., Jobe, B. A., Lewinsohn, D. M., and Bakke, A. C. (2005) Alterations in T-cell subset frequency in peripheral blood in obesity. *Obes. Surg.* 10.1381/096089205774859308
158. Degasperi, G. R., Denis, R. G. P., Morari, J., Solon, C., Geloneze, B., Stabe, C., Pareja, J. C., Vercesi, A. E., and Velloso, L. A. (2009) Reactive oxygen species production is increased in the peripheral blood monocytes of obese patients. *Metabolism.* 10.1016/j.metabol.2009.04.002
159. Satoh, N., Shimatsu, A., Himeno, A., Sasaki, Y., Yamakage, H., Yamada, K., Suganami, T., and Ogawa, Y. (2010) Unbalanced M1/M2 phenotype of peripheral blood monocytes in obese diabetic patients: Effect of pioglitazone. *Diabetes Care*. 10.2337/dc09-1315
160. Hardy, O. T., Kim, A., Ciccarelli, C., Hayman, L. L., and Wiecha, J. (2013) Increased Toll-like receptor (TLR) mRNA expression in monocytes is a feature of metabolic syndrome in adolescents. *Pediatr. Obes.* 10.1111/j.I2047T-6310.201Y2.00098.x
161. Santos-Alvarez, J., Goberna, R., and Sánchez-Margalet, V. (1999) Human leptin stimulates proliferation and activation of human circulating monocytes. *Cell. Immunol.* 10.1006/cimm.1999.1490
162. Kiguchi, N., Maeda, T., Kobayashi, Y., Fukazawa, Y., and Kishioka, S. (2009) Leptin enhances CC-chemokine ligand expression in cultured murine macrophage. *Biochem. Biophys. Res. Commun.* 10.1016/j.bbrc.2009.04.121
163. Hukshorn, C. J., Lindeman, J. H. N., Toet, K. H., Saris, W. H. M., Eilers, P. H. C., Westerterp-Plantenga, M. S., and Kooistra, T. (2004) Leptin and the proinflammatory state associated with human obesity. *J. Clin. Endocrinol. Metab.* 10.1210/jc.2003-030803

164. Sánchez-Pozo, C., Rodriguez-Baño, J., Domínguez-Castellano, A., Muniain, M. A., Goberna, R., and Sánchez-Margalet, V. (2003) Leptin stimulates the oxidative burst in control monocytes but attenuates the oxidative burst in monocytes from HIV-infected patients. *Clin. Exp. Immunol.* 10.1111/j.1365-2249.2003.02321.x
165. Jäger, A., and Kuchroo, V. K. (2010) Effector and regulatory T-cell subsets in autoimmunity and tissue inflammation. *Scand. J. Immunol.* 10.1111/j.1365-3083.2010.02432.x
166. Touch, S., Clément, K., and André, S. (2017) T Cell Populations and Functions Are Altered in Human Obesity and Type 2 Diabetes. *Curr. Diab. Rep.* 10.1007/s11892-017-0900-5
167. Hamann, A., Klugewitz, K., Austrup, F., and Jablonski-Westrich, D. (2000) Activation induces rapid and profound alterations in the trafficking of T cells. *Eur. J. Immunol.* 10.1002/1521-4141(200011)30:11<3207::AID-IMMU3207>3.0.CO;2-L
168. Duffaut, C., Galitzky, J., Lafontan, M., and Bouloumié, A. (2009) Unexpected trafficking of immune cells within the adipose tissue during the onset of obesity. *Biochem. Biophys. Res. Commun.* 10.1016/j.bbrc.2009.05.002
169. Nishimura, S., Manabe, I., Nagasaki, M., Eto, K., Yamashita, H., Ohsugi, M., Otsu, M., Hara, K., Ueki, K., Sugiura, S., Yoshimura, K., Kadowaki, T., and Nagai, R. (2009) CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nat. Med.* 10.1038/nm.1964
170. Winer, S., Chan, Y., Paltser, G., Truong, D., Tsui, H., Bahrami, J., Dorfman, R., Wang, Y., Zielenski, J., Mastronardi, F., Maezawa, Y., Drucker, D., Engleman, E., Winer, D., and Dosch, H. (2009) Normalization of obesity-associated insulin resistance through immunotherap...: Discovery Service for Endeavour College of Natural Health Library. *Nat. Med.*
171. Duffaut, C., Zakaroff-Girard, A., Bourlier, V., Decaunes, P., Maumus, M., Chiotasso, P., Sengenès, C., Lafontan, M., Galitzky, J., and Bouloumié, A. (2009) Interplay between human adipocytes and T lymphocytes in obesity: CCL20 as an adipochemokine and T lymphocytes as lipogenic modulators. *Arterioscler. Thromb. Vasc. Biol.* 10.1161/ATVBAHA.109.192583
172. Al-Sufyani, A. A., and Mahassni, S. H. (2011) Obesity and immune cells in Saudi females. *Innate Immun.* 10.1177/1753425910372536
173. Pacifico, L., Di Renzo, L., Anania, C., Osborn, J. F., Ippoliti, F., Schiavo, E., and Chiesa, C. (2006) Increased T-helper interferon-γ-secreting cells in obese children. *Eur. J. Endocrinol.* 10.1530/eje.1.02138
174. Hanusch-Enserer, U., Cauza, E., Spak, M., Dunky, A., Rosen, H. R., Wolf, H., Prager, R., and Eibl, M. M. (2003) Acute-phase response and immunological markers in morbid obese patients and patients following adjustable gastric banding. *Int. J. Obes.* 10.1038/sj.ijo.0802240
175. Laurson, K. R., McCann, D. A., and Senchina, D. S. (2011) Age, sex, and ethnicity may modify the influence of obesity on inflammation. *J. Investig. Med.* 10.2310/JIM.0b013e318200151a
176. De Rosa, V., Procaccini, C., Calì, G., Pirozzi, G., Fontana, S., Zappacosta, S., La Cava, A., and Matarese, G. (2007) A Key Role of Leptin in the Control of Regulatory T Cell Proliferation. *Immunity*. 10.1016/j.immuni.2007.01.011
177. Tiemessen, M. M., Jagger, A. L., Evans, H. G., Van Herwijnen, M. J. C., John, S., and Taams, L. S. (2007) CD4+CD25+Foxp3+ regulatory T cells induce alternative activation of human monocytes/macrophages. *Proc. Natl. Acad. Sci. U. S. A.* 10.1073/pnas.0706832104
178. Feuerer, M., Herrero, L., Cipolletta, D., Naaz, A., Wong, J., Nayer, A., Lee, J., Goldfine, A. B., Benoist, C., Shoelson, S., and Mathis, D. (2009) Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. *Nat. Med.* 10.1038/nm.2002
179. Deiuliis, J., Shah, Z., Shah, N., Needleman, B., Mikami, D., Narula, V., Perry, K., Hazey, J., Kampfrath, T., Kollengode, M., Sun, Q., Satoskar, A. R., Lumeng, C., Moffatt-Bruce, S., and Rajagopalan, S. (2011) Visceral adipose inflammation in obesity is associated with critical alterations in tregulatory cell numbers. *PLoS One*. 10.1371/journal.pone.0016376

180. Sakaguchi, S., Yamaguchi, T., Nomura, T., Ono, M. (2008) Regulatory T cells and immune tolerance. *Cell*. 133 pp. 775-787
181. Long, S. A., and Buckner, J. H. (2011) CD4 + FOXP3 + T Regulatory Cells in Human Autoimmunity: More Than a Numbers Game . *J. Immunol.* 10.4049/jimmunol.1003224
182. Esser, N., L'Homme, L., De Roover, A., Kohnen, L., Scheen, A. J., Moutschen, M., Piette, J., Legrand-Poels, S., and Paquot, N. (2013) Obesity phenotype is related to NLRP3 inflammasome activity and immunological profile of visceral adipose tissue. *Diabetologia*. 10.1007/s00125-013-3023-9
183. Jagannathan-Bogdan, M., McDonnell, M. E., Shin, H., Rehman, Q., Hasturk, H., Apovian, C. M., and Nikolajczyk, B. S. (2011) Elevated Proinflammatory Cytokine Production by a Skewed T Cell Compartment Requires Monocytes and Promotes Inflammation in Type 2 Diabetes. J. Immunol. 10.4049/jimmunol.1002615
184. Zeng, C., Shi, X., Zhang, B., Liu, H., Zhang, L., Ding, W., and Zhao, Y. (2012) The imbalance of Th17/Th1/Tregs in patients with type 2 diabetes: Relationship with metabolic factors and complications. *J. Mol. Med.* 10.1007/s00109-011-0816-5
185. Wagner, N. M., Brandhorst, G., Czepluch, F., Lankeit, M., Eberle, C., Herzberg, S., Faustin, V., Riggert, J., Oellerich, M., Hasenfuss, G., Konstantinides, S., and Schäfer, K. (2013) Circulating regulatory T cells are reduced in obesity and may identify subjects at increased metabolic and cardiovascular risk. *Obesity*. 10.1002/oby.20087
186. Agabiti-Rosei, C., Trapletti, V., Piantoni, S., Airo, P., Tincani, A., De Ciuceis, C., Rossini, C., Mittempergher, F., Titi, A., Portolani, N., Caletti, S., Coschignano, M. A., Porteri, E., Tiberio, G. A. M., Pileri, P., Solaini, L., Kumar, R., Ministrini, S., Rosei, E. A., and Rizzoni, D. (2018) Decreased circulating t regulatory lymphocytes in obese patients undergoing bariatric surgery. *PLoS One*. 10.1371/journal.pone.0197178
187. Donninelli, G., Cornò, M. Del, Pierdominici, M., Scazzocchio, B., Varì, R., Varano, B., Pacella, I., Piconese, S., Barnaba, V., D'Archivio, M., Masella, R., Conti, L., and Gessani, S. (2017) Distinct blood and visceral adipose tissue regulatory T cell and innate lymphocyte profiles characterize obesity and colorectal cancer. *Front. Immunol.* 10.3389/fimmu.2017.00643
188. Cipolletta, D., Feuerer, M., Li, A., Kamei, N., Lee, J., Shoelson, S. E., Benoist, C., and Mathis, D. (2012) PPAR-γ is a major driver of the accumulation and phenotype of adipose tissue T reg cells. *Nature*. 10.1038/nature11132
189. Han, J. M., Patterson, S. J., Speck, M., Ehses, J. A., and Levings, M. K. (2014) Insulin Inhibits IL-10–Mediated Regulatory T Cell Function: Implications for Obesity. *J. Immunol.* 10.4049/jimmunol.1302181
190. Pereira, S., Teixeira, L., Aguilar, E., Oliveira, M., Savassi-Rocha, A., Pelaez, J. N., Capettini, L., Diniz, M. T., Ferreira, A., and Alvarez-Leite, J. (2014) Modulation of adipose tissue inflammation by FOXP3+ Treg cells, IL-10, and TGF-β in metabolically healthy class III obese individuals. *Nutrition*. 10.1016/j.nut.2013.11.023
191. Travers, R. L., Motta, A. C., Betts, J. A., Bouloumié, A., and Thompson, D. (2015) The impact of adiposity on adipose tissue-resident lymphocyte activation in humans. *Int. J. Obes.* 10.1038/ijo.2014.195
192. Łuczyński, W., Stasiak-Barmuta, A., Wawrusiewicz-Kurylonek, N., Kowalczuk, O., Iłendo, E., Głowińska-Olszewska, B., Urban, R., Szczepański, W., Urban, M., Křtowski, A., and Chyczewski, L. (2010) Disturbances in some gene expression in T regulatory cells separated from children with metabolic syndrome. *Scand. J. Immunol.* 10.1111/j.1365-3083.2009.02354.x
193. Tsang, J. Y. S., Li, D., Ho, D., Peng, J., Xu, A., Lamb, J., Chen, Y., and Tam, P. K. H. (2011) Novel immunomodulatory effects of adiponectin on dendritic cell functions. *Int. Immunopharmacol.* 10.1016/j.intimp.2010.11.009

194. Hedman, M., Faresjö, M., Axelsson, S., Ludvigsson, J., and Casas, R. (2008) Impaired CD4+ and CD8+ T cell phenotype and reduced chemokine secretion in recent-onset type 1 diabetic children. *Clin. Exp. Immunol.* 10.1111/j.1365-2249.2008.03720.x
195. Li, Q. F., Li, Y. P., Cheng, P. S. (1989) Peripheral T-lymphocyte subsets changes in patients with endocrine and metabolic diseases. *Zhonghua Nei Ke Za Zhi*. 28(7):410–2
196. Arizcorreta, A., Márquez, M., Fernández-Gutiérrez, C., Guzmán, E. P., Brun, F., Rodríguez-Iglesias, M., and Girón-González, J. A. (2006) T cell receptor excision circles (TRECs), CD4+, CD8+ and their CD45RO+ and CD45RA+ subpopulations in hepatitis C virus (HCV)-HIV-co-infected patients during treatment with interferon alpha plus ribavirin: Analysis in a population on effective antiretroviral therapy. *Clin. Exp. Immunol.* 10.1111/j.1365-2249.2006.03220.x
197. Romo, E. M., Muñoz-Robles, J. A., Castillo-Rama, M., Meneu, J. C., Moreno-Elola, A., Pérez-Saborido, B., Mancebo, E., Calleja-Antolín, S. M., Bernardo, I., Allende, L. M., and Paz-Artal, E. (2007) Peripheral blood lymphocyte populations in end-stage liver diseases. *J. Clin. Gastroenterol.* 10.1097/01.mcg.0000248000.42581.35
198. Inzaugarat, M. E., Ferreyra Solari, N. E., Billordo, L. A., Abecasis, R., Gadano, A. C., and Cherñavsky, A. C. (2011) Altered phenotype and functionality of circulating immune cells characterize adult patients with nonalcoholic steatohepatitis. *J. Clin. Immunol.* 10.1007/s10875-011-9571-1
199. Peakman, M., Alviggi, L., Hussain, M. J., Lo, S. S. S., Hawa, M., Leslie, R. D. G., and Vergani, D. (1994) Increased expression of T-cell markers of immunological memory associated with protection from type I diabetes: A study of identical twins. *Diabetes*. 10.2337/diab.43.5.712
200. Koch, S., Larbi, A., Derhovanessian, E., Özcelik, D., Naumova, E., and Pawelec, G. (2008) Multiparameter flow cytometric analysis of CD4 and CD8 T cell subsets in young and old people. *Immun. Ageing*. 10.1186/1742-4933-5-6
201. Loetscher, M., Gerber, B., Loetscher, P., Jones, S. A., Piali, L., Clark-Lewis, I., Baggiolini, M., and Moser, B. (1996) Chemokine receptor specific for IP10 and Mig: Structure, function, and expression in activate T-Lymphocytes. *J. Exp. Med.* 10.1084/jem.184.3.963
202. Cole, K. E., Strick, C. A., Paradis, T. J., Ogborne, K. T., Loetscher, M., Gladue, R. P., Lin, W., Boyd, J. G., Moser, B., Wood, D. E., Sahagan, B. G., and Neote, K. (1998) Interferon-inducible T cell alpha chemoattractant (I-TAC): A novel non- ELR CXC chemokine with potent activity on activated T cells through selective high affinity binding to CXCR3. *J. Exp. Med.* 10.1084/jem.187.12.2009
203. Sallusto, F., Lenig, D., Mackay, C. R., and Lanzavecchia, A. (1998) Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. *J. Exp. Med.* 10.1084/jem.187.6.875
204. Rabin, R. L., Park, M. K., Liao, F., Farber, J. M., Swofford, R., and Stephany, D. (1999) Chemokine receptor responses on T cells are achieved through regulation of both receptor expression and signaling. *J. Immunol.*
205. Rabin, R. L., Alston, M. A., Sircus, J. C., Knollmann-Ritschel, B., Moratz, C., Ngo, D., and Farber, J. M. (2003) CXCR3 Is Induced Early on the Pathway of CD4 + T Cell Differentiation and Bridges Central and Peripheral Functions . *J. Immunol.* 10.4049/jimmunol.171.6.2812
206. Rocha, V. Z., Folco, E. J., Ozdemir, C., Sheikine, Y., Christen, T., Sukhova, G. K., Tang, E. H. C., Bittencourt, M. S., Santos, R. D., Luster, A. D., Cohen, D. E., and Libby, P. (2014) CXCR3 controls T-cell accumulation in fat inflammation. *Arterioscler. Thromb. Vasc. Biol.* 10.1161/ATVBAHA.113.303133
207. Deiuliis, J. A., Oghumu, S., Duggineni, D., Zhong, J., Rutsky, J., Banerjee, A., Needleman, B., Mikami, D., Narula, V., Hazey, J., Satoskar, A. R., and Rajagopalan, S. (2014) CXCR3 modulates obesity-induced visceral adipose inflammation and systemic insulin resistance. *Obesity*. 10.1002/oby.20642

208. Pemu, P. E., Anderson, L., Gee, B. E., Ofili, E. O., and Ghosh, S. (2012) Early alterations of the immune transcriptome in cultured progenitor cells from obese African-American women. *Obesity*. 10.1038/oby.2011.370
209. Hueso, L., Ortega, R., Selles, F., Wu-Xiong, N. Y., Ortega, J., Civera, M., Ascaso, J. F., Sanz, M. J., Real, J. T., and Piqueras, L. (2018) Upregulation of angiostatic chemokines IP-10/CXCL10 and I-TAC/CXCL11 in human obesity and their implication for adipose tissue angiogenesis. *Int. J. Obes.* 10.1038/s41366-018-0102-5
210. Kobayashi, N. (2006) Functional and phenotypic analysis of human memory CD8+ T cells expressing CXCR3. *J. Leukoc. Biol.* 10.1189/jlb.1205725
211. Lohmann, T., Laue, S., Nietzschmann, U., Kapellen, T. M., Lehmann, I., Schroeder, S., Paschke, R., and Kiess, W. (2002) Reduced expression of Th1-associated chemokine receptors on peripheral blood lymphocytes at diagnosis of type 1 diabetes. *Diabetes*. 10.2337/diabetes.51.8.2474
212. Qin, S., Rottman, J. B., Myers, P., Kassam, N., Weinblatt, M., Loetscher, M., Koch, A. E., Moser, B., and Mackay, C. R. (1998) The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. *J. Clin. Invest.* 10.1172/JCI1422
213. Sun, K., Kusminski, C. M., and Scherer, P. E. (2011) Adipose tissue remodeling and obesity. *J. Clin. Invest.* 10.1172/JCI45887
214. Rausch, M. E., Weisberg, S., Vardhana, P., and Tortoriello, D. V. (2008) Obesity in C57BL/6J mice is characterized by adipose tissue hypoxia and cytotoxic T-cell infiltration. *Int. J. Obes.* 10.1038/sj.ijo.0803744
215. Sell, H., and Eckel, J. (2010) Adipose tissue inflammation: Novel insight into the role of macrophages and lymphocytes. *Curr. Opin. Clin. Nutr. Metab. Care*. 10.1097/MCO.0b013e32833aab7f
216. Jiang, E., Perrard, X. D., Yang, D., Khan, I. M., Perrard, J. L., Smith, C. W., Ballantyne, C. M., and Wu, H. (2014) Essential role of CD11a in CD8+ T-cell accumulation and activation in adipose tissue. *Arterioscler. Thromb. Vasc. Biol.* 10.1161/ATVBAHA.113.302077
217. Chatzigeorgiou, A., Karalis, K. P., Bornstein, S. R., and Chavakis, T. (2012) Lymphocytes in obesity-related adipose tissue inflammation. Diabetologia. 10.1007/s00125-012-2607-0
218. Han, S. N., Jeon, K. J., Kim, M. S., Kim, H. K., and Lee, A. J. (2011) Obesity with a body mass index under 30 does not significantly impair the immune response in young adults. *Nutr. Res.* 10.1016/j.nutres.2011.04.002
219. Fink, S., Eckert, E., Mitchell, J., Crosby, R., and Pomeroy, C. (1996) T-lymphocyte subsets in patients with abnormal body weight: Longitudinal studies in anorexia nervosa and obesity. *Int. J. Eat. Disord.* 10.1002/(SICI)1098-108X(199611)20:3<295::AID-EAT9>3.0.CO;2-J
220. Viardot, A., Lord, R. V., and Samaras, K. (2010) The effects of weight loss and gastric banding on the innate and adaptive immune system in type 2 diabetes and prediabetes. J. Clin. Endocrinol. Metab. 10.1210/jc.2009-2371
221. Winer, D. A., Winer, S., Shen, L., Wadia, P. P., Yantha, J., Paltser, G., Tsui, H., Wu, P., Davidson, M. G., Alonso, M. N., Leong, H. X., Glassford, A., Caimol, M., Kenkel, J. A., Tedder, T. F., McLaughlin, T., Miklos, D. B., Dosch, H. M., and Engleman, E. G. (2011) B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. Nat. Med. 10.1038/nm.2353
222. Lord, G., Howard, J., Matarese, G., Baker, R., Bloom, S., and Lechler, R. (1999) The Role of Leptin in the Cell-Mediated Immune Response and T Lymphocyte Development. *Clin. Sci.* 10.1042/cs097010pa
223. Claycombe, K., King, L. E., and Fraker, P. J. (2008) A role for leptin in sustaining lymphopoiesis and myelopoiesis. *Proc. Natl. Acad. Sci. U. S. A.* 10.1073/pnas.0712053105
224. Meyers, J. A., Liu, A. Y., McTiernan, A., Wener, M. H., Wood, B., Weigle, D. S., Sorensen, B., Chen-Levy, Z., Yasui, Y., Boynton, A., Potter, J. D., and Ulrich, C. M. (2008) Serum

leptin concentrations and markers of immune function in overweight or obese postmenopausal women. *J. Endocrinol.* 10.1677/JOE-07-0569

225. Phillips, A. C., Carroll, D., Gale, C. R., Drayson, M., Thomas, G. N., and Batty, G. D. (2010) Lymphocyte sub-population cell counts are associated with the metabolic syndrome and its components in the Vietnam Experience Study. *Atherosclerosis*. 10.1016/j.atherosclerosis.2010.08.047
226. Frasca, D., Ferracci, F., Diaz, A., Romero, M., Lechner, S., and Blomberg, B. B. (2016) Obesity decreases B cell responses in young and elderly individuals. *Obesity*. 10.1002/oby.21383
227. Jagannathan, M., McDonnell, M., Liang, Y., Hasturk, H., Hetzel, J., Rubin, D., Kantarci, A., Van Dyke, T. E., Ganley-Leal, L. M., and Nikolajczyk, B. S. (2010) Toll-like receptors regulate B cell cytokine production in patients with diabetes. *Diabetologia*. 10.1007/s00125-010-1730-z
228. DeFuria, J., Belkina, A. C., Jagannathan-Bogdan, M., Snyder-Cappione, J., Carr, J. D., Nersesova, Y. R., Markham, D., Strissel, K. J., Watkins, A. A., Zhu, M., Allen, J., Bouchard, J., Toraldo, G., Jasuja, R., Obin, M. S., McDonnell, M. E., Apovian, C., Denis, G. V., and Nikolajczyk, B. S. (2013) B cells promote inflammation in obesity and type 2 diabetes through regulation of T-cell function and an inflammatory cytokine profile. *Proc. Natl. Acad. Sci. U. S. A.* 10.1073/pnas.1215840110
229. Wang, X. L., Chang, X. Y., Tang, X. X., Chen, Z. G., Zhou, T., and Sun, K. (2016) Peripheral invariant natural killer T cell deficiency in metabolically unhealthy but normal weight versus metabolically healthy but obese individuals. *J. Int. Med. Res.* 10.1177/0300060516663778
230. Lynch, L., Nowak, M., Varghese, B., Clark, J., Hogan, A. E., Toxavidis, V., Balk, S. P., O'Shea, D., O'Farrelly, C., and Exley, M. A. (2012) Adipose Tissue Invariant NKT Cells Protect against Diet-Induced Obesity and Metabolic Disorder through Regulatory Cytokine Production. *Immunity*. 10.1016/j.immuni.2012.06.016
231. Berger, S., Ceccarini, G., Scabia, G., Barone, I., Pelosini, C., Ferrari, F., Magno, S., Dattilo, A., Chiovato, L., Vitti, P., Santini, F., and Maffei, M. (2017) Lipodystrophy and obesity are associated with decreased number of T cells with regulatory function and pro-inflammatory macrophage phenotype. *Int. J. Obes.* 10.1038/ijo.2017.163
232. Lynch, L. (2014) Adipose invariant natural killer T cells. *Immunology*. 10.1111/imm.12269
233. Mantell, B. S., Stefanovic-Racic, M., Yang, X., Dedousis, N., Sipula, I. J., and O'Doherty, R. M. (2011) Mice lacking NKT cells but with a complete complement of CD8+ T-Cells are not protected against the metabolic abnormalities of diet-induced obesity. *PLoS One*. 10.1371/journal.pone.0019831
234. Lynch, L., Michelet, X., Zhang, S., Brennan, P. J., Moseman, A., Lester, C., Besra, G., Vomhof-Dekrey, E. E., Tighe, M., Koay, H. F., Godfrey, D. I., Leadbetter, E. A., Sant'Angelo, D. B., Von Andrian, U., and Brenner, M. B. (2015) Regulatory iNKT cells lack expression of the transcription factor PLZF and control the homeostasis of T reg cells and macrophages in adipose tissue. *Nat. Immunol.* 10.1038/ni.3047
235. Ordovas, J. M., Ferguson, L. R., Tai, E. S., and Mathers, J. C. (2018) Personalised nutrition and health. *BMJ*. 10.1136/bmj.k2173
236. Nielsen, D. E., and El-Sohemy, A. (2014) Disclosure of genetic information and change in dietary intake: A randomized controlled trial. *PLoS One*. 10.1371/journal.pone.0112665
237. Wesselius, A., Zeegers, M. P. (2013) Direct-to-consumer genetic testing. *OA Epidemiology*. 30;1(1):4.
238. Zeevi, D., Korem, T., Zmora, N., Israeli, D., Rothschild, D., Weinberger, A., Ben-Yacov, O., Lador, D., Avnit-Sagi, T., Lotan-Pompan, M., Suez, J., Mahdi, J. A., Matot, E., Malka, G., Kosower, N., Rein, M., Zilberman-Schapira, G., Dohnalová, L., Pevsner-Fischer, M., Bikovsky, R., Halpern, Z., Elinav, E., and Segal, E. (2015) Personalized Nutrition by Prediction of Glycemic Responses. *Cell*. 10.1016/j.cell.2015.11.001

239. Tal Korem, David Zeevi, Niv Zmora, Avraham A. Levy, Eran Elinav, and Eran Segal (2017) Bread Affects Clinical Parameters and Induces Gut Microbiome-Associated Personal Glycemic Responses. *Cell Metab.*
240. Celis-Morales, C., Livingstone, K. M., Marsaux, C. F. M., Macready, A. L., Fallaize, R., O'Donovan, C. B., Woolhead, C., Forster, H., Walsh, M. C., Navas-Carretero, S., San-Cristobal, R., Tsirigoti, L., Lambrinou, C. P., Mavrogianni, C., Moschonis, G., Kolossa, S., Hallmann, J., Godlewska, M., Surwiłło, A., Traczyk, I., Drevon, C. A., Bouwman, J., Van Ommen, B., Grimaldi, K., Parnell, L. D., Matthews, J. N. S., Manios, Y., Daniel, H., Martinez, J. A., Lovegrove, J. A., Gibney, E. R., Brennan, L., Saris, W. H. M., Gibney, M., and Mathers, J. C. (2017) Effect of personalized nutrition on health-related behaviour change: Evidence from the Food4Me European randomized controlled trial. *Int. J. Epidemiol.* 10.1093/ije/dyw186
241. Lara, J., Evans, E. H., O'Brien, N., Moynihan, P. J., Meyer, T. D., Adamson, A. J., Errington, L., Sniehotta, F. F., White, M., and Mathers, J. C. (2014) Association of behaviour change techniques with effectiveness of dietary interventions among adults of retirement age: A systematic review and meta-analysis of randomised controlled trials. *BMC Med.* 10.1186/s12916-014-0177-3
242. Hurling, R., Catt, M., De Boni, M., Fairley, B. W., Hurst, T., Murray, P., Richardson, A., and Sodhi, J. S. (2007) Using internet and mobile phone technology to deliver an automated physical activity program: Randomized controlled trial. *J. Med. Internet Res.* 10.2196/jmir.9.2.e7
243. Alexander, G. L., McClure, J. B., Calvi, J. H., Divine, G. W., Stopponi, M. A., Rolnick, S. J., Heimendinger, J., Tolsma, D. D., Resnicow, K., Campbell, M. K., Strecher, V. J., and Johnson, C. C. (2010) A randomized clinical trial evaluating online interventions to improve fruit and vegetable consumption. *Am. J. Public Health*. 10.2105/AJPH.2008.154468
244. Hansen, A. W., Grønbæk, M., Helge, J. W., Severin, M., Curtis, T., and Tolstrup, J. S. (2012) Effect of a web-based intervention to promote physical activity and improve health among physically inactive adults: A population-based randomized controlled trial. *J. Med. Internet Res.* 10.2196/jmir.2109
245. Celis-Morales, C., Marsaux, C. F. M., Livingstone, K. M., Navas-Carretero, S., San-Cristobal, R., O'Donovan, C. B., Forster, H., Woolhead, C., Fallaize, R., Macready, A. L., Kolossa, S., Hallmann, J., Tsirigoti, L., Lambrinou, C. P., Moschonis, G., Godlewska, M., Surwiłło, A., Grimaldi, K., Bouwman, J., Manios, Y., Traczyk, I., Drevon, C. A., Parnell, L. D., Daniel, H., Gibney, E. R., Brennan, L., Walsh, M. C., Gibney, M., Lovegrove, J. A., Martinez, J. A., Saris, W. H. M., and Mathers, J. C. (2016) Physical activity attenuates the effect of the FTO genotype on obesity traits in European adults: The Food4Me study. *Obesity*. 10.1002/oby.21422
246. Livingstone, K. M., Celis-Morales, C., Navas-Carretero, S., San-Cristoba, R., MacReady, A. L., Fallaize, R., Forster, H., Woolhead, C., O'Donovan, C. B., Marsaux, C. F. M., Kolossa, S., Tsirigoti, L., Lambrinou, C. P., Moschonis, G., Godlewska, M., Surwio, A., Drevon, C. A., Manios, Y., Traczyk, I., Gibney, E. R., Brennan, L., Walsh, M. C., Lovegrove, J. A., Saris, W. H., Daniel, H., Gibney, M., Martinez, J. A., and Mathers, J. C. (2016) Effect of an Internet-based, personalized nutrition randomized trial on dietary changes associated with the Mediterranean diet: The Food4Me Study. *Am. J. Clin. Nutr.* 10.3945/ajcn.115.129049
247. Celis-Morales, C., Lara, J., and Mathers, J. C. (2015) Personalising nutritional guidance for more effective behaviour change. in *Proceedings of the Nutrition Society*, 10.1017/S0029665114001633
248. Kodama, S., Saito, K., Tanaka, S., Horikawa, C., Fujiwara, K., Hirasawa, R., Yachi, Y., Iida, K. T., Shimano, H., Ohashi, Y., Yamada, N., and Sone, H. (2012) Effect of web-based lifestyle modification on weight control: A meta-analysis. *Int. J. Obes.* 10.1038/ijo.2011.121
249. Hutchesson, M. J., Rollo, M. E., Krukowski, R., Ells, L., Harvey, J., Morgan, P. J., Callister, R., Plotnikoff, R., and Collins, C. E. (2015) eHealth interventions for the prevention and

treatment of overweight and obesity in adults: A systematic review with meta-analysis. *Obes. Rev.* 10.1111/obr.12268

250. Levine, D. M., Savarimuthu, S., Squires, A., Nicholson, J., and Jay, M. (2015) Technology-Assisted Weight Loss Interventions in Primary Care: A Systematic Review. *J. Gen. Intern. Med.* 10.1007/s11606-014-2987-6
251. Joost, H. G., Gibney, M. J., Cashman, K. D., Görman, U., Hesketh, J. E., Mueller, M., van Ommen, B., Williams, C. M., and Mathers, J. C. (2007) Personalised nutrition: Status and perspectives. *Br. J. Nutr.* 10.1017/S0007114507685195
252. Hietaranta-Luoma, H. L., Tahvonen, R., Iso-Touru, T., Puolijoki, H., and Hopia, A. (2014) An intervention study of individual, apoe genotype-based dietary and physical-activity advice: Impact on health behavior. *J. Nutrigenet. Nutrigenomics*. 10.1159/000371743
253. Marteau, T. M., French, D. P., Griffin, S. J., Prevost, A. T., Sutton, S. R., and Watkinson, C. (2010) Effects of communicating DNA-based disease risk estimates on risk-reducing behaviours. *Cochrane Database Syst. Rev.* 10.1002/14651858.CD007275
254. Bloss, C. S., Wineinger, N. E., Darst, B. F., Schork, N. J., and Topol, E. J. (2013) Impact of direct-to-consumer genomic testing at long term follow-up. *J. Med. Genet.* 10.1136/jmedgenet-2012-101207
255. Speliotes, E. K., Willer, C. J., Berndt, S. I., Monda, K. L., Thorleifsson, G., Jackson, A. U., Allen, H. L., Lindgren, C. M., Luan, J., Mägi, R., Randall, J. C., Vedantam, S., Winkler, T. W., Qi, L., Workalemahu, T., Heid, I., Steinthorsdottir, V., Stringham, H., Weedon, M. N., Wheeler, E., Wood, A. R., Ferreira, T., Weyant, R. J., Segrè, A. V., Estrada, K., Liang, L., Nemesh, J., Park, J. H., Gustafsson, S., Kilpeläinen, T. O., Yang, J., Bouatia-Naji, N., Esko, T., Feitosa, M. F., Kutalik, Z., Mangino, M., Raychaudhuri, S., Scherag, A., Smith, A. V., Welch, R., Zhao, J. H., Aben, K. K., Absher, D. M., Amin, N., Dixon, A. L., Fisher, E., Glazer, N., Goddard, M. E., Heard-Costa, N., Hoesel, V., Hottenga, J. J., Johansson, Å., Johnson, T., Ketkar, S., Lamina, C., Li, S., Moffatt, M. F., Myers, R. H., Narisu, N., Perry, J. R. B., Peters, M. J., Preuss, M., Ripatti, S., Rivadeneira, F., Sandholt, C., Scott, L. J., Timpson, N. J., Tyrer, J. P., Van Wingerden, S., Watanabe, R., White, C. C., Wiklund, F., Barlassina, C., Chasman, D. I., Cooper, M. N., Jansson, J. O., Lawrence, R. W., Pellikka, N., Prokopenko, I., Shi, J., Thiering, E., Alavere, H., Alibrandi, M. T. S., Almgren, P., Arnold, A., Aspelund, T., Atwood, L. D., Balkau, B., Balmforth, A. J., Bennett, A. J., Ben-Shlomo, Y., Bergman, R., Bergmann, S., Biebermann, H., Blakemore, A. I. F., Boes, T., Bonnycastle, L., Bornstein, S. R., Brown, M. J., Buchanan, T. A., Busonero, F., Campbell, H., Cappuccio, F. P., Cavalcanti-Proença, C., Ida Chen, Y. D., Chen, C. M., Chines, P., Clarke, R., Coin, L., Connell, J., Day, I., Den Heijer, M., Duan, J., Eebrahim, S., Eelliott, P., Eelosua, R., Eeiriksdottir, G., Eerdos, M. R., Eeriksson, J. G., Facheris, M. F., Felix, S. B., Fischer-Posovszky, P., Folsom, A. R., Friedrich, N., Freimer, N. B., Fu, M., Gaget, S., Gejman, P. V., Geus, E. J., Gieger, C., Gjesing, A. P., Goel, A., Goyette, P., Grallert, H., Gräßler, J., Greenawalt, D., Groves, C. J., Gudnason, V., Guiducci, C., Hartikainen, A. L., Hassanali, N., Hall, A., Havulinna, A., Hayward, C., Heath, A., Hengstenberg, C., Hicks, A. A., Hinney, A., Hofman, A., Homuth, G., Hui, J., Igl, W., Iribarren, C., Isomaa, B., Jacobs, K. B., Jarick, I., Jewell, E., John, U., Jørgensen, T., Jousilahti, P., Jula, A., Kaakinen, M., Kajantie, E., Kaplan, L., Kathiresan, S., Kettunen, J., Kinnunen, L., Knowles, J., Kolcic, I., König, I. R., Koskinen, S., Kovacs, P., Kusisto, J., Kraft, P., Kvaløy, K., Laitinen, J., Lantieri, O., Lanzani, C., Launer, L. J., Lecoeur, C., Lehtimäki, T., Lettre, G., Liu, J., Lokki, M. L., Lorentzon, M., Luben, R., Ludwig, B., Magic, Manunta, P., Marek, D., Marre, M., Martin, N. G., McArdle, W., McCarthy, A., McKnight, B., Meitinger, T., Melander, O., Meyre, D., Midthjell, K., Montgomery, G., Morken, M. A., Morris, A. P., Mulic, R., Ngwa, J., Nelis, M., Neville, M. J., Nyholt, D. R., O'Ddonnell, C. J., O'Rahilly, S., Ong, K., Ostra, B., Paré, G., Parker, A., Perola, M., Pichler, I., Pietiläinen, K. H., Platou, C. P., Polasek, O., Pouta, A., Rafelt, S., Raitakari, O., Rayner, N., Ridderstråle, M., Rief, W., Ruokonen, A., Robertson, N. R., Rzehak, P., Salomaa, V., Sanders, A. R., Sandhu, M., Sanna, S., Saramies, J., Savolainen, M.

J., Scherag, S., Schipf, S., Schreiber, S., Schunkert, H., Silander, K., Sinisalo, J., Siscovick, D. S., Smit, J. H., Soranzo, N., Sovio, U., Stephens, J., Surakka, I., Swift, A. J., Tammesoo, M. L., Tardif, J. C., Teder-Laving, M., Teslovich, T., Thompson, J. R., Thomson, B., Tönjes, A., Tuomi, T., Van Meurs, J. B. J., Van OMen, G. J., Vatin, V., Viikari, J., Visvikis-Siest, S., Vitart, V., Vogel, C. I., Voight, B. F., Waite, L., Wallaschofski, H., Walters, B., Widen, E., Wiegand, S., Wild, S. H., Willemsen, G., Witte, D. R., Witteman, J., Xu, J., Zhang, Q., Zgaga, L., Ziegler, A., Zitting, P., Beilby, J. P., FarOqi, I. S., Hebebrand, J., Huikuri, H. V., James, A., Kähönen, M., Levinson, D. F., MacCiardi, F., Nieminen, M. S., Ohlsson, C., Palmer, L. J., Ridker, P., Stumvoll, M., Beckmann, J., Boeing, H., Boerwinkle, E., BOmsma, D. I., Caulfield, M. J., Chanock, S. J., Collins, F., Cupples, L. A., Smith, G. D., Eerdmann, J., Frogue, P., Grönberg, H., Gyllensten, U., Hall, P., Hansen, T., Harris, T. B., Hattersley, A. T., Hayes, R. B., Heinrich, J., Hu, F. B., Hveem, K., Illig, T., Jarvelin, M. R., Kaprio, J., Karpe, F., Khaw, K. T., Kiemeney, L. A., Krude, H., Laakso, M., Lawlor, D. A., Metspalu, A., Munroe, P. B., Ouwehand, W. H., Pedersen, O., Penninx, B. W., Peters, A., Pramstaller, P. P., Quertermous, T., Reinehr, T., Rissanen, A., Rudan, I., Samani, N. J., Schwarz, P. E. H., Shuldiner, A. R., Spector, T. D., Tuomilehto, J., Uda, M., Uitterlinden, A., Valle, T., Wabitsch, M., Waeber, G., Wareham, N. J., Watkins, H., Wilson, J. F., Wright, A. F., Zillikens, M. C., ChatterjE, N., McCarroll, S. A., Purcell, S., Schadt, E., Visscher, P., Assimes, T. L., Borecki, I. B., Deloukas, P., Fox, C. S., Groop, L. C., Haritunians, T., Hunter, D. J., Kaplan, R., Mohlke, K., O'ConneL, J. R., Peltonen, L., SchleSinger, D., P Strachan, D. P., Van Duijn, C., Wichmann, H. E., Frayling, T. M., Thorsteinsdottir, U., Abecasis, G. R., Barroso, I., Boehnke, M., StefanSon, K., North, K. E., McArthy, M. I., Hirschhorn, J. N., IngelSon, E., and Loos, R. J. F. (2010) Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat. Genet.* 10.1038/ng.686

256. Celis-Morales, C., Marsaux, C. F. M., Livingstone, K. M., Navas-Carretero, S., San-Cristobal, R., Fallaize, R., Macready, A. L., O'Donovan, C., Woolhead, C., Forster, H., Kolossa, S., Daniel, H., Moschonis, G., Mavrogianni, C., Manios, Y., Surwillo, A., Traczyk, I., Drevon, C. A., Grimaldi, K., Bouwman, J., Gibney, M. J., Walsh, M. C., Gibney, E. R., Brennan, L., Lovegrove, J. A., Martinez, J. A., Saris, W. H. M., and Mathers, J. C. (2017) Can genetic-based advice help you lose weight? Findings from the Food4Me European randomized controlled trial. *Am. J. Clin. Nutr.* 10.3945/ajcn.116.145680

257. Frayling, T. M., Timpson, N. J., Weedon, M. N., Zeggini, E., Freathy, R. M., Lindgren, C. M., Perry, J. R. B., Elliott, K. S., Lango, H., Rayner, N. W., Shields, B., Harries, L. W., Barrett, J. C., Ellard, S., Groves, C. J., Knight, B., Patch, A. M., Ness, A. R., Ebrahim, S., Lawlor, D. A., Ring, S. M., Ben-Shlomo, Y., Jarvelin, M. R., Sovio, U., Bennett, A. J., Melzer, D., Ferrucci, L., Loos, R. J. F., Barroso, I., Wareham, N. J., Karpe, F., Owen, K. R., Cardon, L. R., Walker, M., Hitman, G. A., Palmer, C. N. A., Doney, A. S. F., Morris, A. D., Smith, G. D., Hattersley, A. T., and McCarthy, M. I. (2007) A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science (80-. ).* 10.1126/science.1141634

258. Frankwich, K. A., Egnatios, J., Kenyon, M. L., Rutledge, T. R., Liao, P. S., Gupta, S., Herbst, K. L., and Zarrinpar, A. (2015) Differences in Weight Loss Between Persons on Standard Balanced vs Nutrigenetic Diets in a Randomized Controlled Trial. *Clin. Gastroenterol. Hepatol.* 10.1016/j.cgh.2015.02.044

259. Qi, Q., Bray, G. A., Smith, S. R., Hu, F. B., Sacks, F. M., and Qi, L. (2011) Insulin receptor substrate 1 gene variation modifies insulin resistance response to weight-loss diets in a 2-year randomized trial the preventing overweight using novel dietary strategies (POUNDS LOST) Trial. *Circulation.* 10.1161/CIRCULATIONAHA.111.025767

260. Sacks, F. M., Bray, G. A., Carey, V. J., Smith, S. R., Ryan, D. H., Anton, S. D., McManus, K., Champagne, C. M., Bishop, L. M., Laranjo, N., Leboff, M. S., Rood, J. C., De Jonge, L., Greenway, F. L., Loria, C. M., Obarzanek, E., and Williamson, D. A. (2009) Comparison of

weight-loss diets with different compositions of fat, protein, and carbohydrates. *Obstet. Gynecol. Surv.* 10.1097/01.ogx.0000351673.32059.13

261. Arkadianos, I., Valdes, A. M., Marinos, E., Florou, A., Gill, R. D., and Grimaldi, K. A. (2007) Improved weight management using genetic information to personalize a calorie controlled diet. *Nutr. J.* 10.1186/1475-2891-6-29
262. Meisel, S. F., Beeken, R. J., Van Jaarsveld, C. H. M., and Wardle, J. (2015) Genetic susceptibility testing and readiness to control weight: Results from a randomized controlled trial. *Obesity*. 10.1002/oby.20958
263. Corella, D., Carrasco, P., Sorlí, J. V., Estruch, R., Rico-Sanz, J., Martínez-González, M. Á., Salas-Salvadó, J., Covas, M. I., Coltell, O., Arós, F., Lapetra, J., Serra-Majem, L., Ruiz-Gutiérrez, V., Warnberg, J., Fiol, M., Pintó, X., Ortega-Azorín, C., Muñoz, M. Á., Martínez, J. A., Gómez-Gracia, E., González, J. I., Ros, E., and Ordovás, J. M. (2013) Mediterranean diet reduces the adverse effect of the TCF7L2-rs7903146 polymorphism on cardiovascular risk factors and stroke incidence: A randomized controlled trial in a high-cardiovascular-risk population. *Diabetes Care*. 10.2337/dc13-0955
264. Corella, D., Asensio, E. M., Coltell, O., Sorlí, J. V., Estruch, R., Martínez-González, M. Á., Salas-Salvadó, J., Castañer, O., Arós, F., Lapetra, J., Serra-Majem, L., Gómez-Gracia, E., Ortega-Azorín, C., Fiol, M., Espino, J. D., Díaz-López, A., Fitó, M., Ros, E., and Ordovás, J. M. (2016) CLOCK gene variation is associated with incidence of type-2 diabetes and cardiovascular diseases in type-2 diabetic subjects: Dietary modulation in the PREDIMED randomized trial. *Cardiovasc. Diabetol.* 10.1186/s12933-015-0327-8
265. Nielsen, D. E., Shih, S., and El-Sohemy, A. (2014) Perceptions of genetic testing for personalized nutrition: A randomized trial of DNA-based dietary advice. *J. Nutrigenet. Nutrigenomics*. 10.1159/000365508
266. Stewart-Knox, B. J., Bunting, B. P., Gilpin, S., Parr, H. J., Pinhão, S., Strain, J. J., de Almeida, M. D. V., and Gibney, M. (2009) Attitudes toward genetic testing and personalised nutrition in a representative sample of European consumers. *Br. J. Nutr.* 10.1017/S0007114508055657
267. Nielsen, D. E., and El-Sohemy, A. (2012) A randomized trial of genetic information for personalized nutrition. *Genes Nutr.* 10.1007/s12263-012-0290-x
268. Maher, B. (2011) Nature readers flirt with personal genomics. *Nature*. 10.1038/478019a
269. Kaufman, D. J., Bollinger, J. M., Dvoskin, R. L., and Scott, J. A. (2012) Risky business: Risk perception and the use of medical services among customers of DTC personal genetic testing. *J. Genet. Couns.* 10.1007/s10897-012-9483-0
270. Morin, K. (2009) Knowledge and attitudes of Canadian consumers and health care professionals regarding nutritional genomics. *Omi. A J. Integr. Biol.* 10.1089/omi.2008.0047
271. Almeling, R., and Gadarian, S. K. (2014) Public opinion on policy issues in genetics and genomics. *Genet. Med.* 10.1038/gim.2013.175
272. Leite, F., Lima, M., Marino, F., Cosentino, M., and Ribeiro, L. (2016) Dopaminergic Receptors and Tyrosine Hydroxylase Expression in Peripheral Blood Mononuclear Cells: A Distinct Pattern in Central Obesity. *PLoS One*. 10.1371/journal.pone.0147483
273. Munoz, N. M., and Leff, A. R. (2007) Highly purified selective isolation of eosinophils from human peripheral blood by negative immunomagnetic selection. *Nat. Protoc.* 10.1038/nprot.2006.340
274. Bharadwaj, M., Mifsud, N. A., and McCluskey, J. (2012) Detection and characterisation of alloreactive t cells. *Methods Mol. Biol.* 10.1007/978-1-61779-842-9_18
275. https://www.thermofisher.com/gr/en/home/life-science/cell-analysis/cell-analysis-learning-center/molecular-probes-school-of-fluorescence/flow-cytometry-basics/flow-cytometry-fundamentals/how-flow-cytometer-works.html
276. https://www.cellsignal.com/contents/_/overview-of-flow-cytometry/flow-cytometry-overview
277. Rosenblum, M. D., Sing Way, S., and Abbas, A. K. (2016) Regulatory T cell memory HHS Public Access. *Nat Rev Immunol*. 10.1038/nri.2015.1

278. Hamann, D., Baars, P. A., Hooibrink, B., and Van Lier, R. A. W. (1996) Heterogeneity of the human CD4+ T-cell population: Two distinct CD4+ T-cell subsets characterized by coexpression of CD45RA and CD45RO isoforms. *Blood*
279. Švec, P., Vásárhelyi, B., Pászthy, B., Körner, A., Kovács, L., Tulassay, T., and Treszl, A. (2007) Do regulatory T cells contribute to Th1 skewness in obesity? *Exp. Clin. Endocrinol. Diabetes*. 10.1055/s-2007-960494
280. Human Genetics Commission (2010) A common framework of principles for direct-to-consumer genetic testing services. *London Hum. Genet. Comm.*
281. Wagner, J. K. (2010) Understanding FDA regulation of DTC genetic tests within the context of administrative law. *Am. J. Hum. Genet.* 10.1016/j.ajhg.2010.09.003
282. Borry, P., Van Hellemondt, R. E., Sprumont, D., Jales, C. F. D., Rial-Sebbag, E., Spranger, T. M., Curren, L., Kaye, J., Nys, H., and Howard, H. (2012) Legislation on direct-to-consumer genetic testing in seven European countries. *Eur. J. Hum. Genet.* 10.1038/ejhg.2011.278
283. Johannsen, N. M., Priest, E. L., Dixit, V. D., Earnest, C. P., Blair, S. N., and Church, T. S. (2010) Association of white blood cell subfraction concentration with fitness and fatness. *Br. J. Sports Med.* 10.1136/bjsm.2008.050682
284. Mortensen, O. H., Nielsen, A. R., Erikstrup, C., Plomgaard, P., Fischer, C. P., Krogh-Madsen, R., Lindegaard, B., Petersen, A. M., Taudorf, S., and Philip, C. (2009) Calprotectin - A novel marker of obesity. *PLoS One*. 10.1371/journal.pone.0007419
285. Yun, J. M., Jialal, I., and Devaraj, S. (2010) Effects of epigallocatechin gallate on regulatory T cell number and function in obese v. lean volunteers. *Br. J. Nutr.* 10.1017/S000711451000005X

# 8. APPENDIX

## 8.1 STATISTICAL ANALYSIS

Statistical significant results obtained from the statistical analysis (see “Methods” section) are presented below.

### 8.1.1 Differences in immune cell levels between lean and obese individuals

#### 8.1.1.1 Peripheral blood mononuclear cells (PBMCs)

| t test | | |
|---|---|---|
| 1 | Table Analyzed | Total cells |
| 2 | | |
| 3 | Column B | Obese |
| 4 | vs. | vs. |
| 5 | Column A | Lean |
| 6 | | |
| 7 | Mann Whitney test | |
| 8 | P value | 0.0037 |
| 9 | Exact or approximate P value? | Exact |
| 10 | P value summary | ** |
| 11 | Significantly different? (P < 0.05) | Yes |
| 12 | One- or two-tailed P value? | Two-tailed |
| 13 | Sum of ranks in column A,B | 244.0 , 981.0 |
| 14 | Mann-Whitney U | 124.0 |
| 15 | | |
| 16 | Difference between medians | |
| 17 | Median of column A | 1.680e+007 |
| 18 | Median of column B | 2.580e+007 |
| 19 | Difference: Actual | 9.000e+006 |
| 20 | Difference: Hodges-Lehmann | 8.100e+006 |

### 8.1.1.2 $CD3^{+}CD4^{+}CD45RA^{-}CD45RO^{+}$ T cells (Memory)

| t test | |
|---|---|
| 1 | Table Analyzed | %CD45RA-CD45R0+ tota |
| 2 | | |
| 3 | Column B | Obese |
| 4 | vs. | vs. |
| 5 | Column A | Lean |
| 6 | | |
| 7 | Mann Whitney test | |
| 8 | P value | 0.0124 |
| 9 | Exact or approximate P value? | Exact |
| 10 | P value summary | * |
| 11 | Significantly different? (P < 0.05) | Yes |
| 12 | One- or two-tailed P value? | Two-tailed |
| 13 | Sum of ranks in column A,B | 234.0 , 942.0 |
| 14 | Mann-Whitney U | 129.0 |
| 15 | | |
| 16 | Difference between medians | |
| 17 | Median of column A | 10.01 |
| 18 | Median of column B | 14.07 |
| 19 | Difference: Actual | 4.065 |
| 20 | Difference: Hodges-Lehmann | 2.950 |

## 8.1.2 Effect of genotype- based diet- induced weight reduction on immune phenotype

### 8.1.2.1 Peripheral blood mononuclear cells (PBMCs)

| 2way ANOVA<br>Multiple comparisons | A<br>Data Set-A | B<br>Data Set-B | C<br>Data Set-C | D<br>Data Set-D |
|---|---|---|---|---|
| | Y | Y | Y | Y |
| 1 | Compare each cell mean with the other cell mean in that row. | | | | |
| 2 | | | | | |
| 3 | Number of families | 1 | | | |
| 4 | Number of comparisons per family | 2 | | | |
| 5 | Alpha | 0.05 | | | |
| 6 | | | | | |
| 7 | Sidak's multiple comparisons test | Mean Diff. | 95% CI of diff. | Significant? | Summary |
| 8 | | | | | |
| 9 | conventional diet - genotype-based diet | | | | |
| 10 | 0 | -1.878e+007 | -3.029e+007 to -7.265e+006 | Yes | ** |
| 11 | 3 | -6.443e+006 | -1.796e+007 to 5.070e+006 | No | ns |

### 8.1.2.2 $CD4^+CD45RA^+CCR7^+$ cells (Naïve) (% total PBMCs)

| 2way ANOVA<br>Multiple comparisons | A<br>Data Set-A | B<br>Data Set-B | C<br>Data Set-C | D<br>Data Set-D |
|---|---|---|---|---|
| | Y | Y | Y | Y |
| Compare each cell mean with the other cell mean in that column. | | | | |
| | | | | |
| Number of families | 1 | | | |
| Number of comparisons per family | 2 | | | |
| Alpha | 0.05 | | | |
| | | | | |
| Sidak's multiple comparisons test | Mean Diff. | 95% CI of diff. | Significant? | Summary |
| | | | | |
| 0 - 3 | | | | |
| conventional diet | 0.01857 | -2.237 to 2.274 | No | ns |
| genotype-based diet | 3.080 | 0.09647 to 6.064 | Yes | * |

### 8.1.2.3 $CD4^+CD45RA^+CCR7^+$ cells (Naïve) (% $CD4^+$)

| 2way ANOVA<br>Multiple comparisons | A<br>Data Set-A | B<br>Data Set-B | C<br>Data Set-C | D<br>Data Set-D |
|---|---|---|---|---|
| | Y | Y | Y | Y |
| Compare each cell mean with the other cell mean in that column. | | | | |
| | | | | |
| Number of families | 1 | | | |
| Number of comparisons per family | 2 | | | |
| Alpha | 0.05 | | | |
| | | | | |
| Sidak's multiple comparisons test | Mean Diff. | 95% CI of diff. | Significant? | Summary |
| | | | | |
| 0 - 3 | | | | |
| conventional diet | 1.389 | -5.109 to 7.886 | No | ns |
| genotype-based diet | 9.750 | 1.155 to 18.35 | Yes | * |

### 8.1.2.4 $CD4^+CD45RA^+CCR7^-$ cells (TEMRA) (% $CD4^+$)

| 2way ANOVA<br>Multiple comparisons | A<br>Data Set-A | B<br>Data Set-B | C<br>Data Set-C | D<br>Data Set-D |
|---|---|---|---|---|
| | Y | Y | Y | Y |
| Compare each cell mean with the other cell mean in that column. | | | | |
| | | | | |
| Number of families | 1 | | | |
| Number of comparisons per family | 2 | | | |
| Alpha | 0.05 | | | |
| | | | | |
| Sidak's multiple comparisons test | Mean Diff. | 95% CI of diff. | Significant? | Summary |
| | | | | |
| 0 - 3 | | | | |
| conventional diet | -12.08 | -25.30 to 1.141 | No | ns |
| genotype-based diet | -25.68 | -43.17 to -8.193 | Yes | ** |

## 8.2 INSTRUMENTS- DEVICES

Equipment and instruments- devices that were used in our study are summarized in the following table.

| INSTRUMENT-DEVICE | MODEL | MANUFACTURER |
|---|---|---|
| Blood collection tubes | REF 368841 | BD Vacutainer |
| Laminar flow hood | NU-437 | Labgard |
| P20 pipetman | Cat. number: F123600 | Gilson |
| P200 pipetman | Cat. number: F123601 | Gilson |
| P1000 pipetman | Cat. number: F123602 | Gilson |
| Centrifuge | Multifuge 3SR | Heraeus |
| Light microscope | DM IRE2 | Leica |
| Cell sorter | FACS ARIA III | BD |

### 8.3 REAGENTS

Reagents that were used in our study are summarized in the following table.

| Experimental procedure | Reagent | Manufacturer | Catalog number |
|---|---|---|---|
| **PBMCs isolation** | RPMI Medium1640 (1X) + GlutaMAX | gibco | 61870-010 |
| | Ficoll-Paque PLUS | GE Healthcare Bio-Sciences AB | 17-1440-03 |
| **PBMCs counting** | Trypan Blue solution | Sigma- Aldrich | 72571 |
| **Flow cytometry** | PBS pH 7.2 (10X) Phosphate Buffered Saline | gibco | 70013-016 |
| | PBS pH 7.4 (1X) Phosphate Buffered Saline | gibco | 10010-015 |
| | Distilled Water | gibco | 15230-097 |
| | Ethylenediaminetetraaceti c acid disodium salt solution (EDTA) | Sigma-aldrich | E7889 |
| | FITC anti-human CD3 | BioLegend | 317306 |
| | PE anti-human CD183 (CXCR3) | BioLegend | 353706 |
| | APC anti-human CD45RO | BioLegend | 304210 |
| | PerCP/Cy5.5 anti-human CD4 | BioLegend | 300530 |
| | PE/Cy7 anti-human CD45RA | BioLegend | 304126 |
| | APC/Cy7 anti-human CD197 (CCR7) | BioLegend | 353212 |
| | PE anti-human CD4 | BioLegend | 300508 |
| | PE/Cy5 anti-human CD127 (IL-7Ra) | BioLegend | 351324 |
| | PE/Cy7 anti-human CD25 | BioLegend | 356107 |
| | APC/Cy7 anti-human CD8 | BioLegend | 344714 |
| | APC/Cy7 Mouse IgG2a, κ isotype Ctrl | BioLegend | 400230 |
| | APC anti-human CD56 (NCAM) | BioLegend | 318309 |
| | APC/Cy7 anti-human HLA-DR | BioLegend | 307618 |
| | PE/Cy7 anti-human CD15 (SSEA-1) | BioLegend | 323030 |

| | | | |
|---|---|---|---|
| | FITC anti-human CD16 | BioLegend | 302006 |
| | PE anti-human CD14 | BioLegend | 325606 |
| | PE-CF594 Mouse Anti-Human CD45 | BD Horizon | 562279 |
| | DAPI | Calbiochem | 268298 |