UNIVERSITY OF CRETE – FACULTY OF MEDICINE

M.Sc Public Health & Health Care Management

Acetaminophen use during pregnancy and child cardiometabolic traits at the age of 4 years in Crete

The Rhea Mother-Child Study, Crete

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"We live on an island of knowledge surrounded by a sea of ignorance. As our island of knowledge grows, so does the shore of our ignorance"

John Archibald Wheeler

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Περίληψη Μεταπτυχιακής Εργασίας

Τίτλος:*Σχέση λήψης ακεταμινοφαίνης κατά την κύηση και εμφάνιση καρδιομεταβολικών συμπτωμάτων σε παιδιά ηλικίας 4 ετών*

Εισαγωγή:Η ακεταμινοφαίνη είναι ένα ευρέως χρησιμοποιούμενο μη συνταγογραφούμενο φάρμακο με αντιπυρετικέςκαι αναλγητικές ιδιότητες,το οποίοχρησιμοποιείται από το 65% των εγκύων, καθώςηχρήση της θεωρείται σχετικά ασφαλής.Πρόσφατες μελέτες δείχνουν ότι αυτό πιθανόν να μην ισχύει, καταδεικνύοντας τη θετική συσχέτιση της έκθεσης σε παρακεταμόλη στην εμβρυική και πρώιμη βρεφική ηλικία με πληθώρα διαταραχών.

Σκοπός:Σκοπός της παρούσας μελέτης ήταν η διερεύνηση της σχέσηςτης έκθεσης σε ακεταμινοφαίνη κατά την εγκυμοσύνη με καρδιομεταβολικούςδείκτεςσε παιδιά προσχολικής ηλικίας.Επιπλέον σε έναν υποπληθυσμό της μελέτης διερευνήθηκε η πιθανή συσχέτισης μεταξύτης έκθεσης σε ακεταμινοφαίνη κατά την εγκυμοσύνημετο μεταβολικό προφίλ του παιδιού στην γέννηση.

Μέθοδοι:Για την παρούσα μελέτη χρησιμοποιήθηκαν δεδομένα από την προοπτική μελέτη Μητέρας-Παιδιού Κρήτης, «Μελέτη Ρέα» όπου συμπεριλήφθηκαν 625 ζεύγημητέρας-παιδιού. Ως έκθεση ορίστηκε η λήψη ακεταμινοφαίνης κατά την κύηση, ενώ στις εκβάσεις συμπεριλήφθηκαν καρδιομεταβολικοί δείκτες (ολική χοληστερόλη, υψηλής πυκνότητας λιποπρωτεϊνη HDL,συστολική και διαστολική αρτηριακή πίεση, λεπτίνη, αδιπονεκτίνη), δείκτες παχυσαρκίας (δείκτης μάζας σώματος ΔΜΣ, περίμετρος μέσης, άθροισμα δερματικών πτυχών, αναλογία λίπους) και τα επίπεδα φλεγονωδώνπαραγόντων (C-reactiveproteincrp, ιντερφερόνη-γ, ιντερλευκίνες ΙΛ -1β, -6, -8, -10, -17α, MIP1α). Επιπλέον, σε διερευνητική ανάλυση, μετρήθηκαν μεταβολίτες σε ομφαλικό αίμα και συσχετίστηκαν με την χρήση ακεταμινοφαίνης κατά την εγκυμοσύνη. Χρησιμοποιήθηκαν μοντέλα πολλαπλής παλινδρόμησης για την διερεύνηση των συσχετίσεων μεταξύ της ενδομήτριας έκθεσης σεακεταμινοφαίνη και της συχνότητας εμφάνισης καρδιομεταβολικών συμπτώματων σε παιδιά προσχολικής ηλικίας.

Αποτελέσματα:Το 16.6% των γυναικών της μελέτης χρησιμοποίησε ακεταμινοφαίνη συνολικά κατά την εγκυμοσύνη, όπου το 13.3% κατά το 1^{ο} τρίμηνο και το 4.6% κατά το 2^{ο} τρίμηνο. Μέσωπολυπαραγοντικών μοντέλων παλινδρόμησης διαπιστώθηκε ότι η λήψη ακεταμινοφαίνηςκατά το 1ο ή το 2ο τρίμηνο της κύησης, αυξάνει τον κίνδυνο για υψηλά επίπεδα IFN-γ (RR:1.58; 95%CI:1.19-2.09), IL-1b (RR: 1.37; 95%CI:1.01-1.86), IL-17a(RR:1.43; 95%CI:1.07-1.90),και MIP1a (RR: 1.40; 95%CI:1.04-1.89), καισυσχετίστηκε με αυξημένο κίνδυνο για χαμηλή HDLχοληστερόλη(RR:1.62; 95%CI: 1.05-2.49) και υψηλά επίπεδα λεπτίνης (RR: 1.33; 95%CI: 1.01-1.75). Χρήση της ακεταμινοφαίνης κατά το 1ο τρίμηνο συσχετίστηκε με αυξημένο κίνδυνο για υψηλά επίπεδα IFN-γ (RR: 1.45; 95%CI: 1.06, 1.98), υψηλά επίπεδα IL-17α (RR: 1.57; 95%CI: 1.17, 2.10) και υψηλά επίπεδα MIP1α (RR: 1.48; 95%CI: 1.09, 2.03). Χρήση κατά το 2ο τρίμηνο της εγκυμοσύνης συσχετίστηκε με αύξηση της αναλογίας λίπους του παιδιού (β-coeff.: 2.51; 95%CI: 0.36, 4.65). Επιπλέον, η προγεννητική έκθεση σε ακεταμινοφαίνη κατά το 2^{ο} τρίμηνο φαίνεται να συσχετίζεται με διπλάσιο κίνδυνο για εμφάνιση κεντρικού τύπου παχυσαρκίας(RR:2.07; 95%CI: 0.99, 4.33) και σχεδόν διπλάσιο κίνδυνο για υψηλά επίπεδα IFN-γ (RR: 1.79; 95%CI: 1.21, 2.65).

Συμπεράσματα: Η χρήση ακεταμινοφαίνης κατά την κύηση συσχετίστηκε με καρδιομεταβολικούς δείκτες σε παιδιά προσχολικής ηλικίας και αύξηση του κινδύνου για υψηλά επίπεδα δεικτών φλεγμονής. Η χρήση της κατά την κύηση ενδεχομένως να λειτουργεί σαν ενδοκρινικός διαταρράκτης, παρεμβαίνοντας στην παραγωγή και λειτουργία διαφόρων ορμονών. Απ' όσο γνωρίζουμε, αυτή είναι η πρώτη μελέτη συσχέτισης καρδιομεταβολικών δεικτών σε παιδιά με την ενδομήτρια έκθεση σε ακεταμινοφαίνη και απαιτούνται περαιτέρω μελέτες ώστε να επιβεβαιώσουν αυτά τα αποτελέσματα. Περαιτέρω διερεύνηση χρειάζεται και ο βιολογικός μηχανισμός μέσω του οποίου δρα η ακεταμινοφαίνη κατά την ενδομήτρια ζωή, καταλήγοντας στην αύξηση των επιπέδων καρδιομεταβολικώ δεικτών.

Λέξεις κλειδιά: ακεταμινοφαίνη, εγκυμοσύνη, καρδιομεταβολικοί δείκτες, παιδιά, μεταβολικό προφίλ

Abstract

Title:*Acetaminophen use during pregnancy and child cardiometabolic traits at the age of 4 years in Crete*

Introduction: Acetaminophen is a widely used non-prescription drug with antipyretic and analgesic properties that is used by 65% of pregnant women as its use is considered relatively safe. Recent studies indicate that this may not be the case, demonstrating the positive association of acetaminophen exposure in embryonic and early infancy with a variety of disorders.

Objectives: The purpose of this study was to investigate the association of prenatal exposure to acetaminophen andcardiometabolictraits in preschool children. In addition, in a subpopulation of the study, we investigated the possible association between exposure to acetaminophen during pregnancy and the metabolic profile of the child at birth.

Methods: For the present study, data from the Mother-Child Prospective Cohort Study, "Rhea Study", were used, including 625 mother-child pairs. Exposure was defined as use of acetaminophen during pregnancy, while cardiometabolictraits (total cholesterol, high density lipoprotein HDL, systolic and diastolic blood pressure, leptin, adiponectin), obesity (body mass index BMI, waist circumference, sum of skinfolds, fat proportion) and inflammatory markers (C-reactive protein crp, interfero-g, interleukins IL- 1b, -6, -8, -10, -17a, MIP1a) were included in the outcomes. Furthermore, as an exploratory analysis, we investigated the association of acetaminophen use during pregnancy and child metabolic profile using metabolites in cord blood. Multiple regression models were used to investigate the association between prenatal exposure to acetaminophen and the incidence of cardiometabolictraits in preschool children.

Results:16.6% of participating women reported use of acetaminophen duringgestation, with 13.3% in the first trimester and 4.6% in the 2nd trimester.Using multivariate regression models it was found that prenatal exposure to acetaminophen increased the risk for high IFN-g levels (RR:1.58; 95%CI:1.19-2.09), IL-1b (RR: 1.37; 95%CI:1.01-1.86), IL-17a (RR:1.43; 95%CI:1.07-1.90), and MIP1a (RR: 1.40;

95%CI:1.04-1.89). Moreover, acetaminophen use during pregnancy associated with an increased risk for low HDL levels (RR:1.62; 95%CI: 1.05-2.49) and high leptin levels (RR: 1.33; 95%CI: 1.01-1.75). Uterus exposure to acetaminophen during the 1st trimester of gestation associated with an increased risk for high IFN-g levels (RR: 1.45; 95%CI: 1.06, 1.98), high IL-17a levels (RR: 1.57; 95%CI: 1.17, 2.10) and high MIP1a levels (RR: 1.48; 95%CI: 1.09, 2.03), while during the 2nd trimester it associated with an increase of 2.51 in offspring's fat proportion (95%CI: 0.36, 4.65). Also, exposure to acetaminophen during the 2nd trimester associated with a two-fold increased risk for child abdominal obesity (RR:2.07; 95%CI: 0.99, 4.33) and almost two-fold increased risk for high IFN-g levels (RR: 1.79; 95%CI: 1.21, 2.65).No association was found between use ofacetaminophen and the metabolic profile of the child at birth.

Conclusions: The use of acetaminophen in pregnancy is associated with cardiometabolictraits in preschool children and an increase in the risk for high levels of inflammatory markers. The use of the substance during pregnancy may act as an endocrine disruptor, interfering with the growth and function of various hormones. To our knowledge, this is the first study that associates cardiometabolictraits in children and endometrial exposure to acetaminophen, and further studies are needed to confirm these results. Further investigation also is required to determine the biological mechanism and pathway through which acetaminophen acts during endometrial life, resulting in an increase of cardiometabolic risk factors.

Key words: acetaminophen, pregnancy, cardiometabolic traits, child, metabolic profile

1. Introduction

1.1 Acetaminophen

Acetaminophen (also known as N-acetyl-p-aminophenol or APAP) or acetaminophen (PARA) is one of the most popular analgesic and antipyretic agents worldwide. It is typically used for mild to moderate pain relief[1]. In combination with opioid pain medication, APAP is also used for more severe pain such as cancer and post-surgery pain[2]. It was discovered in 1877 and by now is the most commonly used medication for pain and fever in both the United States and Europe[3]. The World Health Organization has classified it on its List of Essential Medicines, the most effective and safe medicines needed in a health system[4].

Pharmacology of Acetaminophen

For many years, the mechanism of action of APAP was unclear. It is now known that acetaminophen blocks prostaglandin synthesis from arachidonic acid by inhibiting the enzymes cyclooxygenase (COX)-1 and -2. There are 2 sources of arachidonic acid. In most tissues, cytosolic phospholipase A_2 hydrolyzes phospholipids to yield arachidonic acid. In the brain, liver, and lung, monoacylglycerol lipase hydrolyzes the endocannabinoid 2-arachidonoylglycerol to liberate arachidonic acid. The COX enzyme, officially known as prostaglandin-endoperoxide synthase(PTGS), is an enzyme that is responsible for formation of prostanoids and its pharmaceutical inhibition provides relief from symptoms of inflammation and pain[5]. AM404 also known as N-arachidonoylaminophenol[6], is an active metabolite of acetaminophen, responsible for all or part of its analgesic action[7] and anticonvulsant effects. Chemically, it is the amide formed from 4-aminophenol and arachidonic acid.AM404 is an inhibitor of COX -1 and -2 enzymes, thus attenuating prostaglandin synthesis. Also, AM404 is thought to induce its analgesic action through its activity on the endocannabinoid, COX, and TRPV systems, all of which are present in pain and thermoregulatory pathways[8]. Therapeutic concentrations of acetaminophen inhibit COX activity when the levels of arachidonic acid and peroxide are low but have little effect when the levels of arachidonic acid or peroxide are high as seen in severe inflammatory conditions[9].

The liver, and to a lesser extent the kidney and intestine, are the major organs implicated in the metabolism of acetaminophen[10]. After a therapeutic dose, APAP

is mostly converted to pharmacologically inactive glucuronide (APAP-gluc, 52–57% of urinary metabolites) and sulfate (APAP sulfate, 30–44%) conjugates, with a minor fraction being oxidized to a reactive metabolite NAPQI (5–10%). Less than 5% of APAP is excreted unchanged[11].

Side effects in adults

Although acetaminophen is considered a safe drug with minimum side effects and is estimated that adults will make use of it at least once in their lifetime, there are no "absolutely" safe biologically active therapeutic agents. Even with therapeutic doses, acetaminophen can cause adverse drug events in certain conditions such as chronic alcohol use, malnutrition, and polypharmacy[12].

On January 13, 2011, Food and Drug Administration (FDA) Drug Safety Communication stated that "acetaminophen-containing prescription products are safe and effective when used as directed, though all medications carry some risks" but also confirmed APAP as a dose-dependent hepatotoxin. Indeed, during the past decade, acetaminophen has been identified as the leading cause of acute liver failure in the United States, and up to 50% of the cases are caused by an unintentional overdose[13].

Moreover, on January, 2014, the US FDA instructed manufacturers to add to the label of all prescription drug products that contain acetaminophen a warning highlighting the potential for allergic reactions characterized by swelling of the face, mouth and throat, difficulty breathing, itching and/or a rash, and also warns about rare cases of anaphylaxis.

Furthermore, a systematic review concluded that there is an increased risk of asthma and wheezing in both children and adults exposed to APAP[14]. The International Study of Asthma and Allergies in Childhood provides evidence that acetaminophen intake during early infancy is linked to increased risk of rhinitis, asthma, wheeze, and bronchial responsiveness in adolescents and in adults[15].

Acetaminophen use during pregnancy

It has been observed that pregnant women often use non-prescription drugs and specifically 65% of them take APAP. The use of acetaminophen is considered

relatively safe, but recent studies indicate that this may not be the case[16]. Acetaminophen has been shown to freely pass the placental barrier and in therapeutic but mainly toxic doses it can affect embryonic hepatocytes. Note that during the fetal life the liver is the main hematopoietic organ [17]. It also appears to cause endocrine disruption, inhibit prostaglandins, have antiandrogenic action and can cause cryptorchidism[18]. APAP also affects maternal thyroid hormones as well as sex hormones, which regulate brain development in the fetus. It can also interfere directly with brain development through neurotoxicity, inducing oxidative stress leading to neuronal death[19, 20]. Several studies indicate a positive association of APAP exposure in embryonic and early infancy with neurodevelopment disorders including autism and ADHD (Attention Deficit Hyperactivity Disorder)[21, 22]. Chemical endocrine disruptors such as acetaminophen have significant effects on the process of neurodevelopment because they are deposited in the fat tissues of exposed individuals, they easily pass the placenta and are expressed in breast milk. Exposure to hormonal disrupters at significant developmental phases (e.g. perinatal) may have significant effects on neurodevelopment and immune system[23].

1.2Cardiometabolic Risk

According to a Consensus Conference report published by the American Diabetes Association and the American College of Cardiology Foundation,cardiometabolic risk refers to a high lifetime risk for cardiovascular disease (CVD). Thespecific factors that can cause this increased risk include: obesity (particularly central), insulin resistance, hyperglycemia, dyslipoproteinemia, elevated low-density lipoprotein (LDL) and hypertension. The prevalence of CVD is increasing worldwide owing to the epidemic of overweight and obesity[24]. According to American Heart Association cardiometabolic risk is diagnosed by the identification of an enhanced waist circumference (above 102cm in males and 88cm in females) accompanied by the alterations in lipid profile (HDLcholesterol below 40mg/dl in males and 50mg/dl in females, and serum triglycerides above 150mg/dl). Moreover, WHO indicates that cardiovascular disease is the leading cause of morbidity and mortality worldwide with an estimate of 17.3 million deaths in 2008, and by 2030 this number could reach up to 23.3 million.

Causes and Pathogenic Mechanism

Several genome-wide scans performed in families with clustering of cardiometabolic risk factors have strongly supported an inherited component to CVD[25]. In a study of 357 children and 378 parents, children who had at least one parent with the disease, had higher levels of obesity and insulin resistance than children ofwhom neither parent had it[26]. Moreover, the Bogalusa Heart Study has shown that offspring of parents with early coronary heart disease were overweight beginning in childhood and developed an adverse cardiovascular risk factor profile at an increased rate[27].

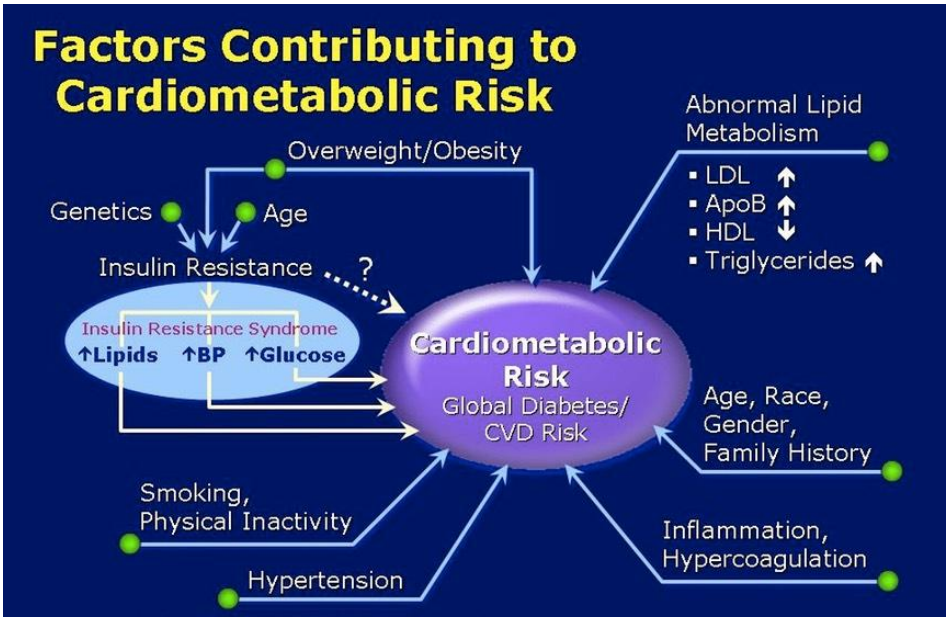
The current epidemic of overweight and obesity is mainly due to an imbalance between energy intake and energy expenditure. Low levels of physical activity, sedentary behavior and poor cardiorespiratory fitness increase the risk of CVD, type 2 diabetes, and all-cause mortality in adults[28].

Obesity is an important risk factor for cardiometabolic diseases, including diabetes, hypertension, dyslipidemia, and coronary heart disease (CHD). Several leading national and international institutions, including the World Health Organization and the National Institutes of Health, have provided guidelines for classifying weight status based on body mass index (BMI; in kg/m2)[29]. Data from epidemiologic studies demonstrate a direct correlation between BMI and the risk of medical complications and mortality rate[30]. According to WHO men and women who have a BMI $\geq$30 are considered obese and are generally at higher risk for adverse health events than are those who are considered overweight (BMI between 25.0 and 29.9) or lean (BMI between 18.5 and 24.9). Therefore, BMI has become the gold standard for identifying patients at increased risk of adiposity-related adverse health outcomes. Body fat distribution is also an important risk factor for obesity-related diseases. Excess abdominal fat (also known as central or upper-body fat) is associated with an increased risk of cardiometabolic disease. However, precise measurement of abdominal fat content requires the use of expensive radiological imaging techniques. Therefore, waist circumference (WC) is often used as a surrogate marker of abdominal fat mass, because WC correlates with abdominal fat mass (subcutaneous and intra-abdominal)[31] and is associated with cardiometabolic disease risk[32]. Men and women who have WCs 40 in (102 cm) and 35 in (88 cm), respectively, are considered to be at increased risk for cardiometabolic disease[33].

Insulin resistance, ventricular, central obesity, lipoprotein disorders and arterial hypertension and the pathological disorders used to define the cardiometabolic risk, are also considered pathogenic mechanisms[34]. The causes of cardiometabolicrisk are closely related to certain factors contributing to the development of diabetes and atherosclerosis. These factors include the production of adipocytes, proteins produced and secreted by the adipose tissue, which have both local and systemic activity[35].

It is suggested that accumulation of free fatty acids in the liver, adipocytes, skeletal muscles and the pancreas in the setting of obesity leads to impaired insulin signaling and subsequent insulin resistance. Insulin resistance in the liver leads to decrease in its effect on suppression of glucose production[36]. Additionally, hyperinsulinemia causes an increase in the transcription of genes for lipogenic enzymes in the liver, which leads to increased production of triglycerides. The increase in free fatty acids delivery to the liver is thought to result in hepatic insensitivity to the inhibitory effects of insulin on very low density lipoprotein (VLDL) secretion and overproduction of triglyceride-rich VLDL particles[37]. Elevated blood pressure is thought to be secondary to hyperinsulinemia via mechanisms such as sympathetic nervous system activity, renal sodium retention and smooth muscle growth[38].

Image 1: *Factors contributing to cardiometabolic risk*



Source: American Diabetes Association, 2006

The role of cytokines in cardiometabolic risk

The role of inflammatory cytokines in obesity is well established and studies suggest that they are crucial in the development of this pro-inflammatory state [39-41]. Based on the literature review, atherosclerosis, the underlying pathology responsible for coronary heart disease (CHD), is an inflammatory disease[42]. Inflammation, at both focal and systemic levels, plays a key role in destabilization and rupture of atherosclerotic plaques, leading to acute cardiovascular (CV) events[43, 44].In 2002, the American Heart Association and the Centers for Disease Control convened a workshop to make recommendations on how inflammatory markers should be used in conjunction with other assessments of cardiovascular risk[45].

Cytokines are a broad category of small proteins that are important in cell signaling [46]. Their release has an effect on the behavior of cells around them. It can be said that cytokines are involved in autocrine signaling, paracrine signaling and endocrine signaling as immunomodulating agents. Their definite distinction from hormones is still part of ongoing research[47]. Cytokines may include chemokines, interferons, interleukins, lymphokines, and tumour necrosis factors but generally not hormones or growth factors (despite some overlap in the terminology). Cytokines are produced by a broad range of cells, including immune cells like macrophages, B lymphocytes, T lymphocytes and mast cells, as well as endothelial cells, fibroblasts, and various stromal cells. A given cytokine may be produced by more than one type of cell[48].

Immune cells can secrete cytokines that affect the activity of adipocytes. Conversely, adipocytes can produce soluble mediators called adipocytokines (or adipokines) that not only influence energy homeostasis but also immune responses[49].Adipokines are cytokines secreted by the adipose tissue and the first adipokine to be discovered was leptin in 1994[50].

Leptin is a hormone predominantly made by adipose cells that helps to regulate energy balance by inhibiting hunger [47]. In obesity, similar to resistance of insulin in type 2 diabetes, a decreased sensitivity to leptin occurs, resulting in an inability to detect satiety despite high energy stores and high levels of leptin. Several studies suggest that the rate of leptin expression and its concentration is related to the development and the existence of inflammation, with high levels inducing pro-

inflammatory markers. The increase in fat increases leptin levels, which in turn triggers the production of pro-inflammatory agents. Thus, leptin exerts a pro-inflammatory role, resulting in high leptin levels in obese individuals[51]. Moreover, elevated concentrations of circulating leptin repeatedly have been associated with cardiometabolic risk factors, such as hypertension, obesity, insulin resistance, and type 2 diabetes[52, 53].

Adiponectin is a protein hormone which is involved in regulating glucose levels as well as fatty acid breakdown. In humans it is encoded by the ADIPOQ gene and it is produced in adipose tissue [54].The role of adiponectin in the development of inflammation is not yet clear enough, although it appears to have an anti-inflammatory effect and affects insulin resistance. It causes an increase in anti-inflammatory interleukins (IL-10) and inhibition of inflammatory activity (IL-6 and TNF-α). Studies show that, unlike leptin, in obese individuals adiponectin levels decrease while increasing in people with anorexia nervosa [55]. Several studies suggest that adiponectin is a promising cardiovascular risk marker [56]. Studies on chronic heart failure (HF) or documented cardiovascular disease (CVD) have identified hyperadiponectinemia as an independent predictor of mortality[57, 58]. Similarly, Cavusoglu et al[59] have identified a direct and independent association between adiponectin and the risk for acute myocardial infarction (AMI) and cardiovascular death in a cohort of men undergoing coronary angiography for the diagnostic investigation of chest pain.

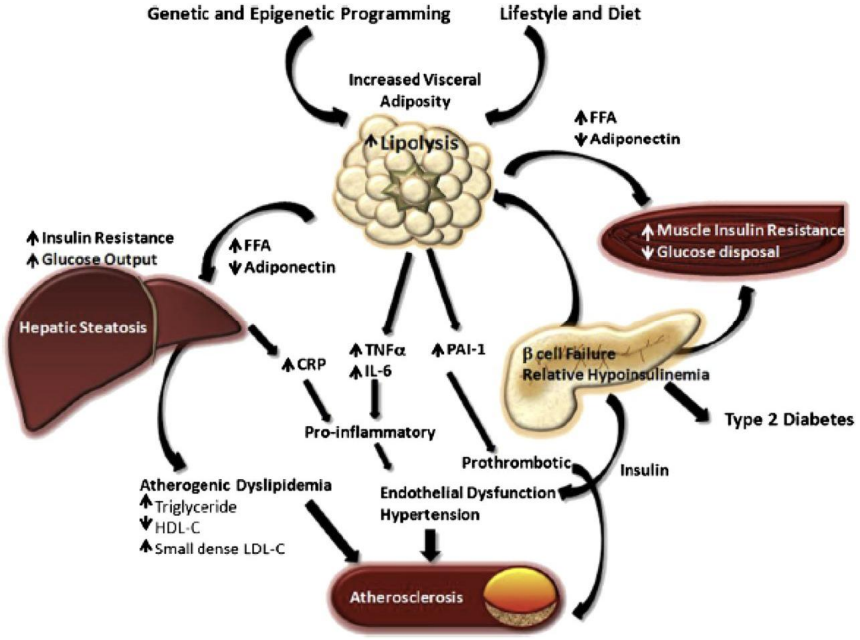
CardiometabolicRisk in childhood

Early life risk factors include epigenetic factors as well as the overnutrition of the fetus resulting in increased birth weight and low birth weight associated with rapid catch-up growth[60]. Exposure of the fetus to gestational diabetes of the mother increases the risk of CVD[61]. Cigarette smoking and excess alcohol consumption have been connected with increased cardiometabolic risk[62].

Childhood and adolescence are particularly vulnerable periods of life to the effects of cardiometabolic risk and later development of atherosclerosis, hypertension and diabetes mellitus[63]. Cardiovascular disease is the leading cause of disability and premature death worldwide, and hypertension is one of its main risk factors[64]. As the proportion of the pediatric population with obesity continues to rise, the

prevalence of cardiometabolic risk is increasing in both children and adolescents[65].Obesity is considered a global epidemic, together with an increase in cardiovascular risk factors such as hypertension.Childhood and adolescent obesity isassociated with insulin resistance, abnormal glucose metabolism, hypertension, dyslipidemia, inflammation, liver disease, and compromised vascular function[66]..The prevalence of high BP among children has risen in recent decades, and if the condition is left undiagnosed and hence untreated it may persist into adulthood[67]. This can lead to target organ damage such as left ventricular hypertrophy, increased carotid artery thickness, vascular changes in the retina, and subtle cognitive alterations[64].A better understanding of mechanisms leading to cardiometabolic risk in early life will lead to more effective prevention and intervention strategies to reduce metabolic stress in children, underlying silent CV disease and later manifest disease[68].

Image 2:*Cardiometabolic risk in childhood*



Source*: Magge SN et al. Pediatrics 2017*

1.3Association of cardiometabolicrisk factorswith the use of acetaminophen

As it is widely known, oxidative modification of low-density lipoproteins (LDL) contributes to the pathology of atherosclerosis. Recent studies in adult population have shown that acetaminophen is a potent inhibitor for Cu++-induced and cell-mediated LDL oxidation in vitro and also that myeloperoxidase (MPO)-H2O2-nitrite oxidation mediator of LDL is inhibited by acetaminophen[69, 70]. Moreover, because of concerns for negative cardiovascular effects of selective and nonselective NSAIDs (non-selective nonsteroidal anti-inflammatory drugs), acetaminophen has been suggested as a safer alternative for pain reducing and anti-inflammatory therapy in patients with cardiovascular disorders[71]. But, prospective controlled studies with acetaminophen show inconclusive results. While one study showed a 4-mm Hg increase in blood pressure, two further studies showed no change associated with the use of acetaminophen in patients with hypertension[72, 73].

There are only a few studies examining the association of acetaminophen use and cardiometabolic syndrome in adults. Most of them are inconclusive or acknowledge the fact that further research is needed. On the literature review that was conducted, it was found that there is a lack of research according the association of cardiometabolic traits in children and use of acetaminophen during pregnancy.

Possible biological mechanism

As it is already mentioned, acetaminophen is a cyclooxygenase (COX) inhibitor drug[5]. Several studies suggest that COX inhibitors may have adverse health outcomes and increase the cardiovascular risk through biological metabolic pathways[74]

Since COX discovery, many papers and reviews have been published to describe the structural bases of COX inhibition, and to debate on the therapeutic and adverse effects of worldwide clinically used nonsteroidal anti-inflammatory drugs (NSAIDs), included Cyclooxygenase-2 (COX-2) selective inhibitors (well known as Coxibs) [75].COX-2 inhibition has been widely investigated, whereas the role of COX-1 in human pathophysiology is mostly not yet well ascertained.COX-2 is a key enzyme in the production of prostaglandins, and an important anti-inflammation drug target.

Recent focus has been placed on the role of COX-2 in heart function and pathology, due to the finding that specific COX-2 inhibitors significantly increased the risk of heart disease in chronic users[76]. However, the exact role of COX-2 in cardiac physiology and disease remains controversial due to the conflicting data reported.In the cardiovascular system, COX-2 has been associated with pro-inflammatory/ pro-atherogenic stages, due to its up-regulation in monocyte-derived macrophages present in atherosclerotic lesions. 10966456 However, experimental and clinical studies suggest that COX-2 is "constitutively" expressed in some tissues, among them in the vascular endothelium[77],where COX-2-derived prostanoids, especially prostacyclin (PGI2), contribute in the maintenance of vascular homeostasis and integrity[78].

The metabolome represents the collection of all metabolites in a biological cell, tissue, organ, or organism, which are the end products of cellular processes. Metabolomics is the science that studies all chemical processes involving metabolites[79] and is the comprehensive assessment of endogenous metabolites and attempts to systematically identify metabolic pathways and quantify metabolites from a biological sample[80].

1.4Hypothesis and Objectives

Acetaminophen is one of the most common pain-relieving medications and is considered generally safe for use during all stages of pregnancy, making it the first-choice pain and fever medication for pregnant women. Given the large number of pregnant women using the drug (>50% in the United States; 50%-60% in the European Union), even a small increase in risk of adverse outcomes in the offspring can have important implications for public health[81]. Given the fact that acetaminophenhas beenassociated with hypertension and increased levels of LDL in adults, and also that is involved in metabolic pathways during pregnancy, it seems reasonable to investigate the association of prenatal exposure to acetaminophen with cardiometabolic outcomes later in childhood. To the best of our knowledge, there is no study that investigates this association. The primary objective of this study is to investigate the association between the use of acetaminophen during pregnancy, with trimester specific analysis, and cardiometabolic and adiposity outcomes as well as inflammation markers in children at the age of 4 years in a prospective cohort in Greece. Moreover, in order to explore a possible biological pathway for the

hypothesized associations, we conducted an exploratory analysis in a subgroup of the population, using metabolomics data in cord samples.

2. Methods

Study population

This study is a part of the 'Rhea' mother and child-cohort study. The Rhea study prospectively examines a population-based sample of pregnant women and their children at the prefecture of Heraklion, Crete, Greece. Methods are described in detail elsewhere[82]. Briefly, female residents (Greek and immigrants) who became pregnant during a period of one year starting in February 2007 were contacted and asked to participate in the study. The first contact was made at the time of the first comprehensive ultrasound examination (mean ± SD 11.96 ± 1.49 weeks) and several contacts followed (6th month of pregnancy, at birth, 9 months, 1st year, 4 years and 6 years after birth). To be eligible for inclusion in the study, women had to have a good understanding of the Greek language and be older than 16 years of age. Face-to-face structured questionnaires along with self-administered questionnaires and medical records were used to obtain information on several psychosocial, dietary, and environmental exposures during pregnancy and early childhood. The study was approved by the ethics committee of the UniversityHospital in Heraklion, Crete, Greece, and all participants provided written informed consent after complete description of the study.

Complete data for acetaminophen use during pregnancy and at least one cardiometabolic outcome at the age of 4 years were available for 625 mother–child pairs and thus were eligible for analysis.

Exposure

The exposure of this study is the use of acetaminophen during the 1st or 2nd or either trimesters of pregnancy. Information regarding the use of acetaminophen during pregnancy was obtained from questionnaires administered during the recruitment period (12^{th} week of gestation) and at the follow up visit of the pregnant women (30^{th} week of gestation). Women were asked to report any medication that they received along with the corresponding dose. Consequently the medications were categorized

according to their active substance with the use of the ATC (Anatomical Therapeutic Chemical categorical system) which is a system of classification proposed by the WHO. The participating children were classified as prenatally exposed to acetaminophen if their mother reported any use of the drug during pregnancy. This classification was also done separately for the 1st and 2nd trimester of gestation. These binary indicators were used as a measure of prenatal exposure to acetaminophen.

Outcomes

As outcomes we evaluatedcardiovascular diseaserelated risk factors, adiposity and inflammatory markers at the age of 4 years. Regarding child adipositymeasures, trained research assistants measuredchildren's weight, height, waist circumference andsubscapular (SS) and triceps (TR) skinfold thicknesses. For child's blood pressure and cardiometabolic biomarkers, trained research assistants measuredsystolic and diastolic blood pressure using a Dinamapautomatedoscillometric recorder. We collected blood via venipuncture and measuredlipids [total cholesterol, and high-density lipoproteincholesterol (HDL) and low-density lipoprotein cholesterol (LDL)], plasma leptin and adiponectinconcentrations and inflammatory markers concentrations in serum samplesfollowing standard protocols.

- the levels of serum lipids: total cholesterol (mg/dl) and HDL cholesterol (mg/dl), as continuous variables as well as categorical binary variables: high total cholesterol ≥200mg/dl (yes/no) and low HDL cholesterol <40mg/dl (yes/no)
- Systolic and diastolic blood pressure as continuous variables (mmHg)

- Child adiposity including measurements of: child BMI as continuous value (kg/m^2) and as categorical – classification : underweight/normal , overweight , obese- according to the guidelines of IOTF, waist circumference as continuous value (cm) and as a binary outcome (greater than the 90^{th} percentile yes/no), sum of skinfold thickness (mm), fat mass index and fat proportion as continuous variables as well as categorical binary variables(we combined 1^{st} and 2^{nd}tertile and compared it to the 3rd -high 3rdtertitle : yes/no)

- Proteins including: C-reacting protein (crp), leptin and adiponectin as continuous variables as well as categorical binary variables(we combined 1st and 2ndtertile and compared it to the 3rd -high 3rdtertitle : yes/no)
- Inflammatory markers related to childhood obesity including: IFN-g, IL-1b, IL-6, IL-8, IL-10, IL-17a, MIP1a, TNFa as continuous variables as well as categorical binary variables (we combined 1st and 2ndtertile and compared it to the 3rd -high 3rdtertitle : yes/no). The Luminex® 200 ™ platform (Luminex Corporation, USA) was used to simultaneously detect inflammatory markers in children by the immunological method. The platforms that were used (Millipore, milliplex map kit, Bilerica MA, USA) were of high sensitivity and precision and consisted of fluorescently labeled magnetic beads, thus allowing the detection of inflammation markers even in minimal sample (25μl).The quantification of the inflammation markers was determined from the reference curve.

Statistical Analysis

For the sample size calculations of main analysis, due to lack of previous knowledge, we assumed a mean expected difference in cholesterol of 8mg/dl which is clinically significant. Under a two-sided hypothesis test for a type I error of α=0.05 and 80% power the minimum required sample size is N=590.

As a first step descriptive analysis was conducted for the study population characteristics, exposure and outcomes variables. We evaluated the potential bivariate associations of all exposure and outcomes with main baseline characteristics using the Pearson's Chi-square test (χ^2)for categorical variables and either the student's t-test (normal distribution data) or Mann-Whitney test (not normal distribution data)for continuous variables. If a covariate associated with the exposure (1st trimester or 2nd trimester or both trimesters) and with at least one of outcomes with p value ≤ 0.2 it was included in the confounder set of the multivariate analyses. We concluded to include as confounders parity, maternal education, maternal pre-pregnancy BMI, maternal smoking at 30 weeks of gestation, urban residence, gestational diabetes, flu and fever during pregnancy. Moreover maternal age during pregnancy, child's gender and the exact age were included a priori, based on the literature.

In order to examine the association we conducted regression analysis. First a crude model was fitted, using as predictors only the child's sex and exact age at examination. Then a fully adjusted model was applied using all the selected confounders. Linear models for binary outcomes (with log link, Poisson distribution, and robust variance estimator) were used to estimate relative risks (RRs) and 95% confidence intervals (CIs) for the associations between prenatal exposure to APAP and the outcome variables. Linear regression models were used to examine the association of prenatal exposure to APAP with continuous outcomes (β-coef, 95% CI). As a last step, in order to assess the potential modifying effect of child's sex we included an interaction term in all models and we subsequently stratified the sample in order to investigate the associations separately in boys and in girls.

The statistical analysis was conducted using the IBM SPSS v.19, and the threshold for statistical significance was set at the 5% level for all analyses.

Exploratory Analysis

In order to explore a possible biological pathway for the associations, we conducted an exploratory analysis using metabolomics data. We used a subgroup (N=72) from our study population for whom there were available metabolomics data. Our intention was to identify possible metabolites who may mediate the association of our main analysis. We chose to use metabolomics data from cord samples, so that we can estimate the relation from an intermediate time point rather than use metabolomics data at the age of 4 years and evaluate it cross-sectionally. We had information about 183 metabolites in cord blood samples.

Based on the literature review[83, 84], we categorized the metabolites in large groups (according to their chemical structure and function) and chose to include those that have been associated with our outcomes. Finally we included 113 metabolites from 5 large groups: Carnitine-Acylcarnitine, Hydroxy- anddicarboxyacylcarnitines, Lysophosphatidylcholines, Diacylphosphatidylcholines and Acyl-alkyl-phosphatidylcholines

To identify possible metabolites associated with prenatal acetaminophen exposure, we used linear regression models with acetaminophen as the exposure and individual metabolites as the outcome. The models included the same confounders as our main

analysis. In order to detect statistically significant associations we used the false discovery rate (FDR)-adjusted p-values (i.e. q-values) according to the Simes modification of the Benjamini-Hochberg using [85] the "qqvalue" Stata command. q-value<0.05 was set as the threshold for significantly different expression.

3. Results

Maternal & Child Characteristics

Table 1 shows the characteristics of the study population.Participating mothers were predominantly Greek and had a mean (±SD) age of 29.75 (±4.85) yearsat delivery.About half of the study population had medium educational level (52%) and were multiparous (54.7%), and about athird was overweight or obese before pregnancy (36.3%) while15.5% weresmoking at30 weeks of pregnancy. Fifty eight participants (9.3%) were diagnosed withgestational diabetes. According to Table 1 acetaminophen use was more often among multiparous pregnant women (52.8% vs 47.2% for nulliparouswomen, p-value=0.045) while no other demographic characteristics were associated with acetaminophen in pregnancy.

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Fifty three ~~percent~~percentofthechildrenincludedintheanalysis were boysand the mean (±SD) child age at the time of the examination was 4.21 (±0.22) years.The majority of children (84.8%) attended nursery and approximately half of them were exposed to passive smoking.

Regarding acetaminophen use, 13.3% of women reported use during the first trimester, 4.6% during the second and 16.6% during either trimester.

Child cardiometabolic characteristics are presented in table 2. The mean (±SD) total cholesterol was 156.04 (±28.21)mg/dl and the mean (±SD) high density lipoprotein (HDL) was 49.52 (±11.21) mg/dl.At the 4-year follow-up, the mean (± SD) offspring's BMI was 16.43(±1.88) kg/m^2. In total, 94 (15%) children were classified as overweight, and an additional 46 (7.4%) were obese at 4 years of age. Mean (±SD) waist circumference was 53.61 (±4.7) cm, and a total of 75 (12%) children had a waist circumference above the90th percentile for their age.

Table 1: Maternal and Child characteristics

	Exposure (~~Paracetamol~~ Acetaminophen Total)			
	Exposed N(%) or Mean (±SD)	**Non Exposed N(%) or Mean (±SD)**	**Overall**	**p-value**
Maternal Characteristics				
Age	30.42 (4.87)	29.62 (4.85)	29.75 (4.85)	0,186
Education				0,758
Low	14 (13.5)	86 (16.5)	100 (16.0)	
Medium	54 (51.9)	271 (52.0)	325 (52.0)	
High	36 (34.6)	164 (31.5)	200 (32.0)	
Origin				0.586
Greek	97 (93.3)	492 (94.6)	589 (94.4)	
Other	7 (6.7)	28 (5.4)	35 (5.6)	
Parity				**0,045**
Nulliparous	37 (35.6)	246 (47.2)	283 (45.3)	
Multiparous	67 (64.4)	275 (52.8)	342 (54.7)	
Marital				0.732
Yes	93 (89.4)	458 (88.2)	551 (88.4)	
No	11 (10.6)	61 (11.8)	72 (11.6)	
Urban residence				0,347
Yes	83 (79.8)	404 (77.5)	487 (77.9)	
No	21 (20.2)	117 (22.5)	138 (22.1)	
Smoking (30 weeks pgn)				0,975
Yes	15 (14.4)	82 (15.7)	97 (15.5)	
No	89 (85.6)	439 (84.3)	528 (84.5)	
Pre-pregnancy BMI (kg/m²)	25.11 (5.26)	24.78 (4.96)	24,83 (5.1)	0,956
Underweight / Normal	64 (61.5)	334 (64.1)	398 (63.7)	
Overweight	24 (23.1)	115 (22.1)	139 (22.2)	
Obese	16 (15.4)	72 (13.8)	88 (14.1)	
Gestational diabetes				0,803
Yes	8 (7.7)	50 (9.6)	58 (9.3)	
No	96 (92.3)	471 (90.4)	567 (90.7)	
	Exposed N(%) or Mean (±SD)	**Non Exposed N(%) or Mean (±SD)**	**Overall**	**p-value**
Child Characteristics				
Sex				**0.027**
Male	45 (43.3)	287 (55.1)	332 (53.1)	
Female	59 (56.7)	234 (44.9)	293 (46.9)	
Exact age	4.25 (0.27)	4.21 (0.21)	4.21 (0.22)	0.447
Nursery attendance				0.579
Yes	90 (86.5)	438 (84.4)	528 (84.8)	
No	14 (13.5)	81 (15.6)	95 (15.2)	

Passive smoking at 4 years				0,98
Yes	46 (46.0)	233 (45.9)	279 (45.9)	
No	54 (54.0)	275 (54.1)	329 (54.1)	
Exposure				
Acetaminophen use	**N**	**%**		
1st trimester	83	13.3		
2nd trimester	29	4.6		
Total	104	16.6		

Table 2: Outcomes at 4 years

Outcomes at 4 years	N	% or Mean (±SD)
Cardiometabolic Characteristics		
Total cholesterol (mg/dl)	531	156.04 (28.21)
HDL cholesterol (mg/dl)	531	49.52 (11.21)
Systilic Blood Pressure (mmHg)	485	90.26 (7.61)
Diastolic Blood Pressure (mmHg)	485	53.46 (5.08)
Leptin (ng/ml)	522	2.93 (3.70)
Adiponectin (μg/ml)	524	15.14 (8.49)
Adiposity Characteristics		
Child BMI at 4 years (kg/m²)	625	16.43 (1.88)
Normal/Underweight	485	77.6
Overweight	94	15
Obese	46	7.4
Waist circumference (cm)	618	53.61 (4.75)
≥90th pct.	75	12.2
<90th pct.	618	87.8
Sum of skinfolds (mm)	578	25.27 (8.77)
Fat proportion	594	19.30 (5.80)
Inflammatory Markers	475	
C-reactiveprotein (mg/dl)	487	0.18 (0.61)
IFN-g(pg/ml)	475	29.19 (25.66)
IL-1b(pg/ml)	163	1.39 (0.94)
IL-6(pg/ml)	160	1.41 (1.21)
IL-8(pg/ml)	161	4.08 (3.00)
IL-10(pg/ml)	157	7.73 (22.58)
IL-17a(pg/ml)	162	15.39 (16.90)
MIP1a(pg/ml)	159	13.79 (6.18)
TNFa(pg/ml)	156	6.16 (2.55)

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Association between prenatal exposure to acetaminophen and child cardiometabolic traits at the age of 4 years

Crude models (using as predictors only child's age and sex) are presented in Annex Tables A1 and A2. Since the results remained the same after adjusting for various potential confounders, onlymultiple regression models are presentedbelow.

Table 3 shows the adjusted associationsbetweenprenatal exposure to acetaminophen and offspring cardiometabolic riskoutcomes, measured as continuous variables. Any prenatal exposure toacetaminophenwas positively associated with an increase of IFN-g levels by 28.25% (95%CI: 4.83%, 56.92%), IL-1b levels by 14.79% (95%CI: 4.97%, 25.44%) and IL-17a levels by 26.14% (95%CI: 4.83%, 51.78%).

In trimester-specific analysis, acetaminophen use during the 1st trimester of pregnancy was associated with increased offspring's inflammatory protein IL-17a by 27.9% (95%CI: 4.61%, 56.48%). Also, exposure to acetaminophen during the 2ndtrimester of gestation was related with 2.51%(95%CI: 0.36%, 4.65%)increased body fat proportion at the age of four. Further, second trimester exposure to acetaminophen was associated with elevated markers for inflammation, specifically with increased IFN-g levels by 56.48% (95%CI: 8.45%, 125.64%) and IL-1b levels by 24.57% (95%CI: 5.92%, 46.51%). Prenatal exposure to acetaminophen was positively but not significantly associated with sum of skinfolds (β-coeff.: 3.38, 95%CI: -0.003%, 6.77%).

Table 3: Association of acetaminophen use during pregnancy and cardiometabolic outcomes at the age of 4 years.

	Acetaminophen Total (t1 + t2)		Acetaminophen T1	Acetaminophen T2
Cardiometabolic Characteristics	**N**	**β-coeff. (95% CI)**	**β-coeff. (95% CI)**	**β-coeff. (95% CI)**
Total chol (mg/dl)	531	4.28 (-2.87 10.76)	1.98 (-4.96, 8.92)	10.34 (-2.36, 23.03)
HDL chol (mg/dl)	531	-0.36 (-2.90, 2.18)	-0.74 (-3.46, 1.99)	-1.18 (-6.18, 3.81)
SBP (mmHg)	485	1.26 (-0.62, 3.14)	1.19 (-0.91, 3.29)	0.09 (-3.06, 3.25)
DBP (mmHg)	485	0.92 (-0.33, 2.18)	1.14 (-0.27, 2.54)	-0.64 (-2.76, 1.47)
		% Change (95% CI)	**% Change (95% CI)**	**% Change (95% CI)**
Leptin	522	9.2 (-9.24, 31.52)	1.11 (-17.14, 23.24)	28.27 (-10.77, 84.41)
Adiponectin	524	-0.6 (-13.5, 14.22)	-5.64 (-18.70, 9.53)	22.51 (-6.76, 60.96)

Adiposity Characteristics	N	β-coeff. (95% CI)	β-coeff. (95% CI)	β-coeff. (95% CI)
BMI (kg/m²)	625	0.02 (-0.37, 0.41)	-0.06 (-0.50 , 0.37)	0.49 (-0.21, 1.19)
BMI z-score	625	0.05 (-0.21, 0.30)	-0.01 (-0.29, 0.28)	0.31 (-0.13, 0.76)
WC (cm)	618	-0.05 (-1.05, 0.96)	-0.35 (-1.44, 0.75)	1.22 (-0.58, 3.02)
Sum of skinfolds (mm)	578	-0.34 (-2.24, 1.57)	-1.04 (-3.13, 1.04)	3.38 (-0.003 , 6.77)
Fat proportion (%)	594	-0.32 (-1.52, 0.89)	-0.87 (-2.19, 0.45)	**2.51 (0.36, 4.65)**
Inflammatory Markers	**N**	**% Change (95% CI)**	**% Change (95% CI)**	**% Change (95% CI)**
C-reactiveprotein (mg/dl)~~Crp~~	487	-19.27 (-41.67, 11.85)	-5.07 (-32.97, 34.31)	-48.78 (-74.49, 2.84)
IFN-g(pg/ml)~~IFN-g~~	475	**28.25 (4.83, 56.92)**	19.17 (-4.34, 48.45)	**56.48 (8.45, 125.64)**
IL-1b(pg/ml)~~IL-1b~~	475	**14.79 (4.97, 25.44)**	9.73 (-0.48, 21)	**24.57 (5.92, 46.51)**
IL-6(pg/ml)~~IL-6~~	475	1.05 (-8.24, 11.27)	1.54 (-8.55, 12.74)	-4.01 (-19.45, 14.31)
IL-8(pg/ml)~~IL-8~~	475	-0.90 (-9.81, 8.90)	-3.14 (-12.58, 7.33)	1.26 (-14.74, 20.25)
IL-10(pg/ml)~~IL-10~~	475	15.43 (-2.19, 36.32)	17.85 (-1.58, 41.13)	-0.35 (-26.34, 34.91)
IL-17a(pg/ml)~~IL-17a~~	475	**26.14 (4.83, 51.78)**	**27.9 (4.61, 56.48)**	12.51 (-19.84, 57.79)
MIP1a(pg/ml)~~MIP1a~~	475	11.73 (-1.24, 26.40)	14.16 (-0.21, 3.05)	4.1 (-16.84, 30.41)
TNFa(pg/ml)~~TNFa~~	475	-1.38 (-10.25, 8.37)	-2.73 (-12.22, 7.77)	3.45 (-12.88, 22.77)

Bold indicates statistically significant associations
HDL: High density lipoprotein, SBP: systolic blood pressure, DBP: diastolic blood pressure, BMI: body mass index, WC: waist circumference
Adjusted for parity, maternal education, maternal pre-pregnancy BMI, maternal smoking at 30 weeks of gestation, urban residence, gestational diabetes, flu and fever during pregnancy

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In order to evaluate the clinical significance of these findings we also examined the associations of prenatal exposure to acetaminophen with categorical outcomes. For all outcomes without existing definitions for pathological values we used distributiontertiles. of the. We combined 1st and 2ndtertile and compared it to the 3rd. For all others we applied the existing definitions.

Table 4 presents adjusted results for prenatal exposure to acetaminophen in relation to offspring categoricalcardiometabolic risk outcomes.Acetaminophen use during any trimester of gestation wasrelated to 62%increased risk for having low levels of HDL cholesterol (<40mg/dl) (RR:1.62, 95%CI:1.05, 2.49), 33%increased risk for havinghigh levels of leptin (3rdtertile) (RR:1.33, 95%CI:1.01, 1.75), 58% increased

riskforhigh levels of IFN-g (RR:1.58, 95%CI:1.19, 2.09), 37%increased risk forhigh levels of IL-1b (RR:1.37, 95%CI:1.01, 1.86), 43%increased risk forhigh levels of IL-17a (RR:1.43, 95%CI:1.07, 1.90) and 40%increased risk for high levels of MIP1a (RR:1.40, 95%CI:1.04, 1.89) compared to nouse ofacetaminophen during pregnancy.

Moreover, based on trimester specific analysis, children whose mothers used acetaminophen during the 1st trimester hadan increased risk for having high IFN-g (RR:1.45, 95%CI:1.06, 1.98), IL-17a (RR:1.57, 95%CI:1.17, 2.10) and MIP1a levels (RR:1.48, 95%CI:1.09, 2.03) compared to those whose mothers did not use acetaminophen. For 2nd trimester, acetaminophen use was related to 120%increased risk for having low HDL cholesterol (<40 mg/dl) (RR:2.20, 95%CI:1.09, 4.44), 59%increased risk for high leptin (RR:1.59, 95%CI:1.03, 2.44), 53%increased risk for high adiponectin (RR:1.53, 95%CI:0.99, 2.38), 73%increased risk for being overweight (RR:1.73, 95%CI:1.06, 2.82), 44%increased risk for having high fat proportion (3rdtertile) (RR:1.44, 95%CI:1.02, 2.02), 79%increased risk for high IFN-g (RR:1.79, 95%CI:1.21, 2.65) and 55%increased risk for high IL-1b (RR:1.55, 95%CI:1.00, 2.40)levels. Also, prenatal exposure to acetaminophen during the 2nd trimester of gestation was positively but not significantly associated with 107% increased risk for WC ≥90thpct (RR:2.07, 95%CI:0.99, 4.33).

Table 4: Association of acetaminophen use during pregnancy and categorical cardiometabolic outcomes at the age of 4 years.

	Acetaminophen Total (t1 + t2)		Acetaminophen T1	Acetaminophen T2
Cardiometabolic Characteristics	**N**	**RR (95% CI)**	**RR (95% CI)**	**RR (95% CI)**
Total chol (≥200)	531	1.48 (0.55, 3.98)	1.02 (0.34, 3.07)	2.64 (0.63, 11.15)
HDL chol<40 mg/dl	531	**1.62 (1.05, 2.49)**	1.45 (0.91, 2.30)	**2.20 (1.09, 4.44)**
High Leptin	522	**1.33 (1.01, 1.75)**	1.19 (0.88, 1.62)	**1.59 (1.03, 2.44)**
LowAdiponectin	524	1.10(0.80, 1.51)	1.24(0.90, 1.70)	**0.66(0.27, 1.60)**
Adiposity Characteristics	**N**			
Overweight	625	1.12 (0.77, 1.64)	0.98 (0.62, 1.54)	**1.73 (1.06, 2.82)**
Obese	625	0.90 (0.40, 2.00)	1.18 (0.53, 2.64)	0.52 (0.07, 3.66)
WC>90th pct	617	1.45 (0.84, 2.51)	1.21 (0.64, 2.31)	**2.07 (0.99,4.33)**
High Sum of sk.	578	0.93 (0.67, 1.28)	0.84 (0.57, 1.24)	1.31 (0.91, 1.90)
High Fat prop	594	0.94 (0.69, 1.28)	0.76 (0,51, 1.13)	**1.44 (1.02, 2.02)**

Inflammatory Markers	N			
High crp	487	0.99 (0.69, 1.42)	1.18 (0.83, 1.68)	0.53 (0.17, 1.64)
High IFN-g	475	**1.58 (1.19, 2.09)**	**1.45 (1.06, 1.98)**	**1.79 (1.21, 2.65)**
High IL-1b	475	**1.37 (1.01, 1.86)**	1.30 (0.92, 1,84)	**1.55 (1.00, 2.40)**
High IL-6	475	1.16 (0.84, 1.61)	1.21 (0.86, 1.71)	0.83 (0.39, 1.75)
High IL-8	475	0.89 (0.60, 1.31)	0.88 (0.57, 1.34)	0.77 (0.36, 1.65)
High IL-10	475	1.28 (0.95, 1.73)	1.31 (0.94, 1.82)	1.19 (0.68, 2.10)
High IL-17a	475	**1.43 (1.07, 1.90)**	**1.57 (1.17, 2.10)**	1.06 (0.95, 1.89)
High MIP1a	475	**1.40 (1.04, 1.89)**	**1.48 (1.09, 2.03)**	1.32 (0.77, 2.26)
High TNFa	475	0.67 (0.42, 1.06)	0.62 (0.36, 1.07)	0.82 (0.38, 1.74)

Bold indicates statistically significant associations
HDL: High density lipoprotein, SBP: systolic blood pressure, DBP: diastolic blood pressure, BMI: body mass index, WC: waist circumference
Adjusted for parity, maternal education, maternal pre-pregnancy BMI, maternal smoking at 30 weeks of gestation, urban residence, gestational diabetes, flu and fever during pregnancy

Stratified analysis by child's sex

Table 5 presents the association of acetaminophen during pregnancy and continuous cardiometabolic outcomes, from a stratified analysis by child's sex.

Use of acetaminophen during either pregnancy trimester was related with a significant increase of IL-1b (% change:26%,95%CI:4%, 52%) and MIP1a levels (% change:43%,95%CI: 5%, 95%)amongboys whereas these associations were not significant in girls (p-interaction=0.028). On the other hand, prenatal exposure to acetaminophenwas associated with increasedIL-10 (% change: 49% (95%CI: 11%, 99%) only among girls(p-interaction=0.025).

Table 5: Association of acetaminophen use during pregnancy and cardiometabolic outcomes at the age of 4 years, stratified by child's sex

Acetaminophen Total (T1+T2)			
	Boys	Girls	
Cardiometabolic Characterstics	β-coeff. (95% CI)		p-interaction
Total chol (mg/dl)	8.67 (-1.50,18.85)	0.2 (-8.48,8.88)	0.232
HDL chol (mg/dl)	0.22 (-3.52,3.96)	-0.54 (-4.20,3.12)	0.812
SBP (mmHg)	1.83 (-1.19,4.86)	1.23 (-1.32,3.79)	0.531
DBP (mmHg)	1.31 (-0.68,3.31)	0.68 (-1.09,2.45)	0.612
	% Change (95% CI)		
Leptin	9 (-18,43)	6 (-19,38)	0.968

Adiponectin	-5 (-23,17)	2 (-16,25)	0.448
Adisposity Characteristics	**β-coeff. (95% CI)**		
BMI (kg/m²)	0.04 (-0.54,0.62)	-0.03 (-0.60,0.54)	0.879
BMI z-scores	0.02 (-0.27,0.31)	0.02 (-0.26,0.30)	0.975
WC (cm)	0.33 (-1.19,1.85)	-0.32 (-1.72,1.09)	0.5
Sum of skinfolds (mm)	0.27 (-2.34,2.87)	-1.34 (-4.28,1.60)	0.479
Fat proportion	-0.35 (-2.02,1.32)	-0.62 (-2.46,1.21)	0.943
Inflammatory Markers	**% Change (95% CI)**		
C-reactiveprotein (mg/dl)~~log Crp~~	-4 (-40,55)	-26 (-54,19)	0.35
IFN-g(pg/ml)~~log IFN-g~~	42 (-10,24)	45 (-3, 116)	0.92
IL-1b(pg/ml)~~log IL-1b~~	**26 (4,52)**	17 (-4,42)	0.414
IL-6(pg/ml)~~log IL-6~~	-8 (-28,16)	8 (-9,28)	0.289
IL-8(pg/ml)~~log IL-8~~	-5 (-22,17)	2 (-17 ,24)	0.875
IL-10(pg/ml)~~log IL-10~~	-12 (41,32)	**49 (11,99)**	0.025
IL-17a(pg/ml)~~log IL-17a~~	19 (-20,79)	50 (2, 122)	0.515
MIP1a(pg/ml)~~log MIP1a~~	43 (5,95)	-6 (-23,15)	0.028
TNFa(pg/ml)~~log TNFa~~	-17 (-33,3)	9 (-9,31)	0.045

HDL: High density lipoprotein, SBP: systolic blood pressure, DBP: diastolic blood pressure, BMI: body mass index, WC: waist circumference

Adjusted for parity, maternal education, maternal pre-pregnancy BMI, maternal smoking at 30 weeks of gestation, urban residence, gestational diabetes, flu and fever during pregnancy

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Exploratory Analysis – Metabolomics analysis

Associations between acetaminophen use at any time during pregnancyand child metabolic profile at birth are presented in AnnexTables A3a,b and c present. We found that the metabolite PCaaC384 from Lysophosphatidylcholines, Diacylphosphatidylcholines category was significantly associated with acetaminophen use during gestation (p-value: 0.043). However, after correction for multiple comparisons (Benjamini-Hochberg) the association did not remain statistically significant (q-value: 0.837). We found no other association between acetaminophen use during pregnancy and child metabolic profile at birth.

4. Discussion & Conclusions

In this population-based birth cohort study, in utero exposure to acetaminophen was associated with higher IFN-g, IL-1b, IL-17a, MIP1a andleptin, lower HDL cholesterol, increased fat proportion and abdominal obesity at 4 years. The trimester specific analysis regarding the prenatal exposure to acetaminophen revealed that the2ndtrimester is associated with cardiometabolic traits in more profound way than the 1sttrimester. The stratified analysis by child's sex showed that exposed boys were in greater risk for high pro-inflammatory markers than girls. To our knowledge, this is the first study examining the relationship between prenatal exposure to acetaminophen chemicals and child cardiometabolic risk factors.

In Rhea Cohort study, our analysis showed that 16.6% of pregnant women report use of acetaminophen during gestation, which islower in contrast with other European studies and with the European levels as derived from the study of Lupattelli et al. The PRINCE study, a population-based prospective pregnancy cohort study initiated in 2011 at the University Medical Center in Hamburg, Germany, reported that among the 518 enrolled women, 40% took acetaminophen as main analgesic treatment during pregnancy[86]. Furthermore,the MoBa cohort study, initiated in 2000 with more than 100.000 pregnancies, concluded that a total of 27.9% pregnant women used acetaminophen during gestation period. Moreover, the DNBC study, a cohort of pregnant females and children with long duration follow-up (about 11y), calculated that over half of all mothers reported acetaminophen use during pregnancy (56%). Last, the INMA cohort study recruited 2,644 expectant mothers among whomapproximately 42% stated that they used acetaminophen any time during gestation[87]. These variations are likely to reveal differences between the populations and the habits of pregnant women in the different countries of the survey.According to the literature review we conducted, it seems that approximately 51% of western EU females and 61% of northern EU females reported use of acetaminophen during pregnancy[88].

Regarding child cardiometabolic traits, according todata from National Health and Nutrition Examination Survey (NHANES), the prevalence of dyslipidemia among children was 23.9%, In our population we observed low prevalence of high triglycerides (4%), high total cholesterol (5%) and low LDL cholesterol (5%). Moreover, according to WHO, the prevalence of childhood obesity has increased at an

alarming rate. Globally, in 2016 the number of overweight children under the age of 5, is estimated to be over 41 million. Almost half of all overweight children under 5 lived in Asia and one quarter lived in Africa. From a pooled analysis published in Lancet, it seems that in 2016, 5.6% of girls and 7.8% of boys were obese[89]. In our population the estimations are very similar, with 15% being overweight and 7.5% obese.

The possible biological mechanism explaining these associations is based on the fact that acetaminophen acts as an endocrine disruptor, COX inhibitor and its use during gestation period could lead to many adverse pregnancy outcomes such as ADHD and asthma[3, 87]. Since we know that cardiometabolic syndrome is induced and regulated by hormones and endocrine products, our hypothesis is that acetaminophen interferes and disrupts hormonal development and expression during fetal growth, leading to an increased risk of cardiometabolic syndrome at the age of 4 years. Overall, as it was expected from the literature review, acetaminophen use during gestation period seems to increase the levels of pro-inflammatory markers at the age of 4 years, possibly by inducing a pre-state of childhood obesity.

The biological mechanism responsible for the variations observed between the two trimesters is due to the biological changes in fetal development during the first and second trimester of gestation.The second trimester is very important for the hormonal development of the fetus and makes it vulnerable to substancesthat freely cross the placenta.During the second trimester important components like red blood cells and bones are forming and the fetus develops a layer of fat, providing nourishment[90]. Moreover, during this stage, growth, sex and other fetal hormones are reaching their peak and pancreas produces glucagon. Placenta is now taking over the functions of nutrition and waste and the production of estrogen and progesterone from the corpus luteum [91, 92].

Moreover, we observed differences between the sexes regarding the levels of inflammatory markers, with boys having elevated IL-1b and MIP1a levels, and girls having elevated IL-10 and IL-17a levels. Sexual differences in adults are usually related to sexual hormone production, but in prepubescent children, levels of sex hormones are very low. However, some studies have shown that even in very young children, mean estrogen levels could be seven times higher in girls than in boys [93].

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Therefore, even in prepubescent children, hormonal differences might influence inflammatory response, modifying the level of response in girls: the potential roles of estrogens, testosterone, prolactin, and glucocorticosteroids need to be evaluated. Before puberty, prepubertal gonads are already producing sex hormones, which might play a role, not only in the control of growth hormone synthesis in early childhood, but also in the management of acute inflammation [94]. Alternatively, our observations could be explained by differences in the expression of certain genes that depend on the balance between X and Y chromosomes. Ellis [95] showed that the second X chromosome might be only partially inactivated in girls allowing the two X chromosomes to express certain genes, as well documented by Spolarics[96].

Strengths and Weaknesses

Strengths of the present study include the population-based prospective design, the relatively large sample size and the detailed childhood body fat and cardiometabolic measurements. The fairly homogenous population with regard to factors such as parity, maternal pre-pregnancy BMI,maternal smoking and socioeconomic status can reduce uncontrolled confounding. We were able to estimate acetaminophen use during both 1st and 2nd trimester of gestation. Additionallymeasures of child's cardiometabolic traits and inflammatory markers were evaluated using state-of-the-art laboratory techniques.

The weaknesses of the study include the lack of information about acetaminophen use during the 3rd trimester of gestation. Although the use of the drug was self-reported by women and may insert a potential recall bias to our study, it is logical to assume that this bias probably underestimates the relation, rather than overestimates it. Finally, although we incorporated extensive information on potential social and environmental factors that are associated with cardiometabolic risk factors, we acknowledge that residual confounding from unmeasured covariates such as parental income, social class or postnatal use of acetaminophen is still possible.

Conclusions

We found that prenatal exposure to acetaminophen was associatedwith cardiometabolic traits by increasing the risk for low HDL cholesterol, high leptin and

adiponectin, and greater offspring adiposityat the age of 4 years. Due to the fact that acetaminophen is a widely used analgesic during gestationand that the rates of obesity are increasing these findings have important public health implications.

Future Studies

We believe that this subject needs further investigation. For future studies we suggest thata larger sample could provide more ~~insightt~~insight tothe potential pathways involvedby incorporating moreobservations with available metabolomics data. Also, since theseassociations seem to be biologically plausible, it would be very interesting to investigateif theypersist in late childhood and early adolescent. Finally, it is important to assure that these associations also existin other populations with different characteristics.

5. References

1. Guggenheimer, J. and P.A. Moore, *The therapeutic applications of and risks associated with acetaminophen use: a review and update.* J Am Dent Assoc, 2011. **142**(1): p. 38-44.
2. Straube, C., et al., *Codeine, alone and with paracetamol (acetaminophen), for cancer pain.* Cochrane Database Syst Rev, 2014(9): p. CD006601.
3. Aminoshariae, A. and A. Khan, *Acetaminophen: old drug, new issues.* J Endod, 2015. **41**(5): p. 588-93.
4. *The Selection and Use of Essential Medicines.* World Health Organ Tech Rep Ser, 2015(994): p. vii-xv, 1-546.
5. Nomura, D.K., et al., *Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation.* Science, 2011. **334**(6057): p. 809-13.
6. Rogosch, T., et al., *Novel bioactive metabolites of dipyrone (metamizol).* Bioorg Med Chem, 2012. **20**(1): p. 101-7.
7. Ottani, A., et al., *The analgesic activity of paracetamol is prevented by the blockade of cannabinoid CB1 receptors.* Eur J Pharmacol, 2006. **531**(1-3): p. 280-1.
8. Hogestatt, E.D., et al., *Conversion of acetaminophen to the bioactive N-acylphenolamine AM404 via fatty acid amide hydrolase-dependent arachidonic acid conjugation in the nervous system.* J Biol Chem, 2005. **280**(36): p. 31405-12.
9. Hanel, A.M. and W.E. Lands, *Modification of anti-inflammatory drug effectiveness by ambient lipid peroxides.* Biochem Pharmacol, 1982. **31**(20): p. 3307-11.
10. Bessems, J.G. and N.P. Vermeulen, *Paracetamol (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues and protective approaches.* Crit Rev Toxicol, 2001. **31**(1): p. 55-138.
11. Prescott, L.F., *Paracetamol overdosage. Pharmacological considerations and clinical management.* Drugs, 1983. **25**(3): p. 290-314.
12. Larson, A.M., et al., *Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study.* Hepatology, 2005. **42**(6): p. 1364-72.
13. Nourjah, P., et al., *Estimates of acetaminophen (Paracetomal)-associated overdoses in the United States.* Pharmacoepidemiol Drug Saf, 2006. **15**(6): p. 398-405.
14. Etminan, M., et al., *Acetaminophen use and the risk of asthma in children and adults: a systematic review and metaanalysis.* Chest, 2009. **136**(5): p. 1316-1323.
15. Beasley, R., et al., *Association between paracetamol use in infancy and childhood, and risk of asthma, rhinoconjunctivitis, and eczema in children aged 6-7 years: analysis from Phase Three of the ISAAC programme.* Lancet, 2008. **372**(9643): p. 1039-48.
16. Chambers, C., *Over-the-counter medications: Risk and safety in pregnancy.* Semin Perinatol, 2015. **39**(7): p. 541-4.
17. Thiele, K., et al., *Prenatal acetaminophen affects maternal immune and endocrine adaptation to pregnancy, induces placental damage, and impairs fetal development in mice.* Am J Pathol, 2015. **185**(10): p. 2805-18.
18. Jensen, M.S., et al., *Maternal use of acetaminophen, ibuprofen, and acetylsalicylic acid during pregnancy and risk of cryptorchidism.* Epidemiology, 2010. **21**(6): p. 779-85.
19. Rubinow, D.R. and P.J. Schmidt, *Androgens, brain, and behavior.* Am J Psychiatry, 1996. **153**(8): p. 974-84.
20. Ghassabian, A., et al., *Maternal thyroid function during pregnancy and behavioral problems in the offspring: the generation R study.* Pediatr Res, 2011. **69**(5 Pt 1): p. 454-9.

21. Liew, Z., et al., *Acetaminophen use during pregnancy, behavioral problems, and hyperkinetic disorders.* JAMA Pediatr, 2014. **168**(4): p. 313-20.
22. Thompson, J.M., et al., *Associations between acetaminophen use during pregnancy and ADHD symptoms measured at ages 7 and 11 years.* PLoS One, 2014. **9**(9): p. e108210.
23. de Fays, L., et al., *Use of paracetamol during pregnancy and child neurological development.* Dev Med Child Neurol, 2015. **57**(8): p. 718-24.
24. Wu, Y.E., C.L. Zhang, and Q. Zhen, *Metabolic syndrome in children (Review).* Exp Ther Med, 2016. **12**(4): p. 2390-2394.
25. Teran-Garcia, M. and C. Bouchard, *Genetics of the metabolic syndrome.* Appl Physiol Nutr Metab, 2007. **32**(1): p. 89-114.
26. Pankow, J.S., et al., *Insulin resistance and cardiovascular disease risk factors in children of parents with the insulin resistance (metabolic) syndrome.* Diabetes Care, 2004. **27**(3): p. 775-80.
27. Bao, W., et al., *Longitudinal changes in cardiovascular risk from childhood to young adulthood in offspring of parents with coronary artery disease: the Bogalusa Heart Study.* JAMA, 1997. **278**(21): p. 1749-54.
28. Nocon, M., et al., *Association of physical activity with all-cause and cardiovascular mortality: a systematic review and meta-analysis.* Eur J Cardiovasc Prev Rehabil, 2008. **15**(3): p. 239-46.
29. *Obesity: preventing and managing the global epidemic. Report of a WHO consultation.* World Health Organ Tech Rep Ser, 2000. **894**: p. i-xii, 1-253.
30. Calle, E.E., et al., *Body-mass index and mortality in a prospective cohort of U.S. adults.* N Engl J Med, 1999. **341**(15): p. 1097-105.
31. Pouliot, M.C., et al., *Waist circumference and abdominal sagittal diameter: best simple anthropometric indexes of abdominal visceral adipose tissue accumulation and related cardiovascular risk in men and women.* Am J Cardiol, 1994. **73**(7): p. 460-8.
32. Kissebah, A.H., et al., *Relation of body fat distribution to metabolic complications of obesity.* J Clin Endocrinol Metab, 1982. **54**(2): p. 254-60.
33. Wang, Y., et al., *Comparison of abdominal adiposity and overall obesity in predicting risk of type 2 diabetes among men.* Am J Clin Nutr, 2005. **81**(3): p. 555-63.
34. Reynolds, K. and J. He, *Epidemiology of the metabolic syndrome.* Am J Med Sci, 2005. **330**(6): p. 273-9.
35. Handelsman, Y., *Metabolic syndrome pathophysiology and clinical presentation.* Toxicol Pathol, 2009. **37**(1): p. 18-20.
36. D'Adamo, E., N. Santoro, and S. Caprio, *Metabolic syndrome in pediatrics: old concepts revised, new concepts discussed.* Curr Probl Pediatr Adolesc Health Care, 2013. **43**(5): p. 114-23.
37. Meshkani, R. and K. Adeli, *Hepatic insulin resistance, metabolic syndrome and cardiovascular disease.* Clin Biochem, 2009. **42**(13-14): p. 1331-46.
38. Wittcopp, C. and R. Conroy, *Metabolic Syndrome in Children and Adolescents.* Pediatr Rev, 2016. **37**(5): p. 193-202.
39. Arslan, N., B. Erdur, and A. Aydin, *Hormones and cytokines in childhood obesity.* Indian Pediatr, 2010. **47**(10): p. 829-39.
40. Ledochowski, M., C. Murr, and D. Fuchs, *Obesity and cytokines.* Int J Obes Relat Metab Disord, 1999. **23**(10): p. 1104.
41. Marette, A., *Pathogenic role of inflammatory cytokines in obesity: from insulin resistance to diabetes mellitus.* Nestle Nutr Workshop Ser Clin Perform Programme, 2004. **9**: p. 141-50; discussion 151-3.

42. Madjid, M. and J.T. Willerson, *Inflammatory markers in coronary heart disease.* Br Med Bull, 2011. **100**: p. 23-38.
43. Libby, P., P.M. Ridker, and A. Maseri, *Inflammation and atherosclerosis.* Circulation, 2002. **105**(9): p. 1135-43.
44. Ross, R., *Atherosclerosis--an inflammatory disease.* N Engl J Med, 1999. **340**(2): p. 115-26.
45. Pearson, T.A., et al., *Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association.* Circulation, 2003. **107**(3): p. 499-511.
46. Zhang, J.M. and J. An, *Cytokines, inflammation, and pain.* Int Anesthesiol Clin, 2007. **45**(2): p. 27-37.
47. Stedman, T.L., *Stedman's medical dictionary*. 28th ed. 2006, Philadelphia: Lippincott Williams & Wilkins.
48. Lackie, J.M., *A dictionary of biomedicine*. 1st ed. Oxford paperback reference. 2010, Oxford: Oxford University Press. 608 p.
49. Iikuni, N., et al., *Leptin and Inflammation.* Curr Immunol Rev, 2008. **4**(2): p. 70-79.
50. Conde, J., et al., *Adipokines: biofactors from white adipose tissue. A complex hub among inflammation, metabolism, and immunity.* Biofactors, 2011. **37**(6): p. 413-20.
51. Bilir, B.E., et al., *The effects of fat distribution and some adipokines on insulin resistance.* Endokrynol Pol, 2016. **67**(3): p. 277-82.
52. Beltowski, J., *Role of leptin in blood pressure regulation and arterial hypertension.* J Hypertens, 2006. **24**(5): p. 789-801.
53. Franks, P.W., et al., *Leptin predicts a worsening of the features of the metabolic syndrome independently of obesity.* Obes Res, 2005. **13**(8): p. 1476-84.
54. Maeda, K., et al., *cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1).* Biochem Biophys Res Commun, 1996. **221**(2): p. 286-9.
55. Oh, D.K., T. Ciaraldi, and R.R. Henry, *Adiponectin in health and disease.* Diabetes Obes Metab, 2007. **9**(3): p. 282-9.
56. Oliveira, G.B., J.I. Franca, and L.S. Piegas, *Serum adiponectin and cardiometabolic risk in patients with acute coronary syndromes.* Arq Bras Cardiol, 2013. **101**(5): p. 399-409.
57. Kistorp, C., et al., *Plasma adiponectin, body mass index, and mortality in patients with chronic heart failure.* Circulation, 2005. **112**(12): p. 1756-62.
58. George, J., et al., *Circulating adiponectin concentrations in patients with congestive heart failure.* Heart, 2006. **92**(10): p. 1420-4.
59. Cavusoglu, E., et al., *Adiponectin is an independent predictor of all-cause mortality, cardiac mortality, and myocardial infarction in patients presenting with chest pain.* Eur Heart J, 2006. **27**(19): p. 2300-9.
60. Sookoian, S. and C.J. Pirola, *Epigenetics of insulin resistance: an emerging field in translational medicine.* Curr Diab Rep, 2013. **13**(2): p. 229-37.
61. Burguet, A., *Long-term outcome in children of mothers with gestational diabetes.* Diabetes Metab, 2010. **36**(6 Pt 2): p. 682-94.
62. Yu, M., et al., *Associations of cigarette smoking and alcohol consumption with metabolic syndrome in a male Chinese population: a cross-sectional study.* J Epidemiol, 2014. **24**(5): p. 361-9.
63. Tanrikulu, M.A., M. Agirbasli, and G. Berenson, *Primordial Prevention of Cardiometabolic Risk in Childhood.* Adv Exp Med Biol, 2017. **956**: p. 489-496.
64. Fuenmayor, G., et al., *Prevalence of dyslipidemia in children with congenital heart disease.* Arq Bras Cardiol, 2013. **101**(3): p. 273-6.

65. Ogden, C.L., et al., *Trends in Obesity Prevalence Among Children and Adolescents in the United States, 1988-1994 Through 2013-2014.* JAMA, 2016. **315**(21): p. 2292-9.
66. Franks, P.W., et al., *Childhood obesity, other cardiovascular risk factors, and premature death.* N Engl J Med, 2010. **362**(6): p. 485-93.
67. Lurbe, E., et al., *Determinants of Cardiometabolic Risk Factors in the First Decade of Life: A Longitudinal Study Starting at Birth.* Hypertension, 2018. **71**(3): p. 437-443.
68. Berenson, G.S. and S.R. Srnivasan, *Cardiovascular risk factors in youth with implications for aging: the Bogalusa Heart Study.* Neurobiol Aging, 2005. **26**(3): p. 303-7.
69. Nenseter, M.S., et al., *Paracetamol inhibits copper ion-induced, azo compound-initiated, and mononuclear cell-mediated oxidative modification of LDL.* Arterioscler Thromb Vasc Biol, 1995. **15**(9): p. 1338-44.
70. Chou, T.M. and P. Greenspan, *Effect of acetaminophen on the myeloperoxidase-hydrogen peroxide-nitrite mediated oxidation of LDL.* Biochim Biophys Acta, 2002. **1581**(1-2): p. 57-63.
71. Sudano, I., et al., *Nonsteroidal antiinflammatory drugs, acetaminophen, and hypertension.* Curr Hypertens Rep, 2012. **14**(4): p. 304-9.
72. Chalmers, J.P., et al., *Effects of indomethacin, sulindac, naproxen, aspirin, and paracetamol in treated hypertensive patients.* Clin Exp Hypertens A, 1984. **6**(6): p. 1077-93.
73. Radack, K.L., C.C. Deck, and S.S. Bloomfield, *Ibuprofen interferes with the efficacy of antihypertensive drugs. A randomized, double-blind, placebo-controlled trial of ibuprofen compared with acetaminophen.* Ann Intern Med, 1987. **107**(5): p. 628-35.
74. Sellers, R.S., Z.A. Radi, and N.K. Khan, *Pathophysiology of cyclooxygenases in cardiovascular homeostasis.* Vet Pathol, 2010. **47**(4): p. 601-13.
75. Perrone, M.G., et al., *Selective COX-1 inhibition: A therapeutic target to be reconsidered.* Curr Med Chem, 2010. **17**(32): p. 3769-805.
76. Fitzgerald, G.A., *Prostaglandins: modulators of inflammation and cardiovascular risk.* J Clin Rheumatol, 2004. **10**(3 Suppl): p. S12-7.
77. Stemme, V., et al., *Expression of cyclo-oxygenase-2 in human atherosclerotic carotid arteries.* Eur J Vasc Endovasc Surg, 2000. **20**(2): p. 146-52.
78. Martinez-Gonzalez, J. and L. Badimon, *Mechanisms underlying the cardiovascular effects of COX-inhibition: benefits and risks.* Curr Pharm Des, 2007. **13**(22): p. 2215-27.
79. May, C., et al., *Instruments and methods in proteomics.* Methods Mol Biol, 2011. **696**: p. 3-26.
80. Jang, C., L. Chen, and J.D. Rabinowitz, *Metabolomics and Isotope Tracing.* Cell, 2018. **173**(4): p. 822-837.
81. Stergiakouli, E., A. Thapar, and G. Davey Smith, *Association of Acetaminophen Use During Pregnancy With Behavioral Problems in Childhood: Evidence Against Confounding.* JAMA Pediatr, 2016. **170**(10): p. 964-970.
82. Chatzi, L., et al., *Cohort-Profile: The Mother-Child Cohort in Crete, Greece (Rhea Study).* Int J Epidemiol, 2017. **In press**.
83. Zhang, W., et al., *Classification of osteoarthritis phenotypes by metabolomics analysis.* BMJ Open, 2014. **4**(11): p. e006286.
84. Bachlechner, U., et al., *Associations of anthropometric markers with serum metabolites using a targeted metabolomics approach: results of the EPIC-potsdam study.* Nutr Diabetes, 2016. **6**: p. e215.
85. Li, J., Y. Shi, and A.W. Toga, *Controlling False Discovery Rate in Signal Space for Transformation-Invariant Thresholding of Statistical Maps.* Inf Process Med Imaging, 2015. **24**: p. 125-36.

86. Bremer, L., et al., *Paracetamol Medication During Pregnancy: Insights on Intake Frequencies, Dosages and Effects on Hematopoietic Stem Cell Populations in Cord Blood From a Longitudinal Prospective Pregnancy Cohort.* EBioMedicine, 2017. **26**: p. 146-151.
87. Avella-Garcia, C.B., et al., *Acetaminophen use in pregnancy and neurodevelopment: attention function and autism spectrum symptoms.* Int J Epidemiol, 2016. **45**(6): p. 1987-1996.
88. Lupattelli, A., et al., *Medication use in pregnancy: a cross-sectional, multinational web-based study.* BMJ Open, 2014. **4**(2): p. e004365.
89. *Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults.* Lancet, 2017. **390**(10113): p. 2627-2642.
90. Afif, A., et al., *Development of the human fetal insular cortex: study of the gyration from 13 to 28 gestational weeks.* Brain Struct Funct, 2007. **212**(3-4): p. 335-46.
91. Satoh, T., et al., *Ontogeny of reticular framework of white pulp and marginal zone in human spleen: immunohistochemical studies of fetal spleens from the 17th to 40th week of gestation.* Cell Tissue Res, 2009. **336**(2): p. 287-97.
92. Kadhim, H.J., J.F. Gadisseux, and P. Evrard, *Topographical and cytological evolution of the glial phase during prenatal development of the human brain: histochemical and electron microscopic study.* J Neuropathol Exp Neurol, 1988. **47**(2): p. 166-88.
93. Klein, K.O., et al., *Estrogen levels in childhood determined by an ultrasensitive recombinant cell bioassay.* J Clin Invest, 1994. **94**(6): p. 2475-80.
94. Shames, R.S., *Gender differences in the development and function of the immune system.* J Adolesc Health, 2002. **30**(4 Suppl): p. 59-70.
95. Ellis, N.A., *The war of the sex chromosomes.* Nat Genet, 1998. **20**(1): p. 9-10.
96. Spolarics, Z., *The X-files of inflammation: cellular mosaicism of X-linked polymorphic genes and the female advantage in the host response to injury and infection.* Shock, 2007. **27**(6): p. 597-604.

6. Annex

Crude models

Table A1: Association of acetaminophen use during pregnancyand continuous cardiometabolic outcomes at the age of 4 years.

	Acetaminophen Total (T1 + T2)		Acetaminophen T1	Acetaminophen T2
Cardiometabolic Characteristics	**N**	**β-coeff. (95% CI)**	**β-coeff. (95% CI)**	**β-coeff. (95% CI)**
Total chol (mg/dl)	531	4.21 (-2.19, 10.63)	2.15 (-4.73, 9,06)	10.19 (-2.38 , 22.76)
HDL chol (mg/dl)	531	-0.50 (-3.02, 2.02)	-0.78 (-3.48, 1,92)	-1.26 (-6.21, 3,68)
SBP (mmHg)	485	0.76 (-1.12, 2,65)	0.55 (-1.54, 2.65)	-0.37 (-3.51, 2.76)
DBP (mmHg)	485	0.75 (-0.51, 2.00)	0.89 (-0.51, 2,28)	-0,98 (-3.07, 1.12)
		% Change (95% CI)	**% Change (95% CI)**	**% Change (95% CI)**
log Leptin	522	8.44 (-9.97, 30.6)	0.50 (-17.72, 22.75)	19.96 (-16.72, 72.81)
log Adiponectin	524	0.40 (-12.45, 15.26)	-4.40 (-17.55, 10.74)	25.11 (-16.72, 72.81)
Adiposity Characteristics	**N**	**β-coeff. (95% CI)**	**β-coeff. (95% CI)**	**β-coeff. (95% CI)**
BMI (kg/m²)	625	0,02 (-0.38, 0.41)	-0.09 (-0.53, 0.35)	0.50 (-0.21, 1.21)
BMI z-scores	625	0.28 (-0.17,0.22)	0.03 (-0.17, 0.22)	0.30 (-0.04, 0.65)
WC (cm)	618	-0.04 (-1.05, 0.97)	-0.42 (-1.52, 0.68)	1.28 (-0.52, 3.09)
Sum of skinfolds (mm)	578	-0.42 (-2.32, 1.49)	-1.23(-3.32, 0.86)	3.25 (-0.14, 6.64)
Fat mass index	594	-0.04 (-0.34, 0.25)	-0.16 (-0.48, 0.16)	0.40(-0.12, 0.93)
Fat proportion	594	-0.32 (-1.53, 0.88)	-0.96 (-2.27, 0.36)	**2.51 (0.38, 4.65)**
Inflammatory Markers	**N**	**% Change (95% CI)**	**% Change (95% CI)**	**% Change (95% CI)**
log Crp	487	-18.37 (-41.02, 12.98)	-5.16 (-32.97, 34.18)	-49.69 (-75.04, 1.41)
log IFN-g	475	**22.77 (0.35, 50.11)**	14.39 (-8.17, 42.50)	**50 (4.17, 116.15)**
log IL-1b	475	**12.82 (3.24, 23.29)**	8.30 (-1.72, 19.33)	**20.33 (2.38, 41.32)**
log IL-6	475	-0.14 (-9.31, 10.04)	0.49 (-9.56, 11.57)	-7.21 (-22.08, 10.50)
log IL-8	475	-1.72 (-10.5, 7.92)	-3,47 (-12.82, 6.88)	-0.90 (-16.38, 17.45)
log IL-10	475	13.76 (-3.67, 34.26)	14,79 (-4,14, 37.55)	-3.54 (-28.65, 30.50)
log IL-17a	475	**21.5 (0.91, 46.31)**	**23.11 (0.63, 50.73)**	6.66 (-23.95, 49.59)
log MIP1a	475	9.96 (-2.73, 24.31)	12.58 (-1.51, 28.70)	3.45 (-17.24, 29.32)
log TNFa	475	-2.33 (-11.05, 7.25)	-3.41 (-12.70, 6.95)	1.82 (-14.02, 20.66)

With bold are appearing statistically significant relations and with asterisk (*) trending relations (p-value<0.1).

HDL: High density lipoprotein, SBP: systolic blood pressure, DBP: diastolic blood pressure, BMI: body mass index, WC: waist circumference

Adjusted for child's sex and gender

Table A2: Association of acetaminophen use during pregnancyand categorical cardiometabolic outcomes at the age of 4 years.

	Acetaminophen Total (T1 + T2)		Acetaminophen T1	Acetaminophen T2
Cardiometabolic Characteristics	**N**	**RR (95% CI)**	**RR (95% CI)**	**RR (95% CI)**
High Total chol(≥200mg/dl)	531	1.36 (0.56, 3.32)	0.96 (0.34, 2.67)	2.60 (0.64, 10.48)
Low HDL chol (<40 mg/dl)	531	**1.60 (1.05, 2.44)**	1.44 (0.90, 2.29)	**2.04 (1.08, 3.87)**
High Leptin	522	1.29 (0.99, 1.69)*	1.17 (0.86, 1.58)	1.50 (0.99, 2.27)*
High Adiponectin	524	1.09 (0.81, 1.47)	0.96 (0.69, 1.35)	**1.63 (1.08, 2.47)**
Adiposity Characteristics	**N**			
Overweight	625	1.10 (0.76, 1.60)	0.93 (0.60, 1.45)	**1.70 (1.04, 2.77)**
Obese	625	0.86 (0.39, 1.90)	1.16 (0.53, 2.52)	0.41 (0.06, 2.85)
WC ≥90th pct.	617	1.35 (0.80, 2.28)	1.10 (0.60, 2.03)	1.82 (0.86, 3.86)
High Sum of sk.	578	0.94 (0.69, 1.29)	0.83 (0.56, 1.22)	1.39 (0.96, 1.20)
High Fat mass	594	0.99 (0.72, 1.35)	0.89 (0.62, 1.28)	1.39 (0.91, 2.11)
High Fat prop	594	0.92 (0.68, 1.25)	0.74 (0.50, 1.09)	**1.42 (1.01, 1.99)**
Inflammatory Markers	**N**			
High crp	487	1.01 (0.71, 1.43)	1.17 (0.83, 1.66)	0.55 (0.19, 1.57)
High IFN-g	475	**1.61 (1.23, 2.11)**	**1.49 (1.1 , 2.02)**	**1.75 (1.19, 2.56)**
High IL-1b	475	1.34 (0.99, 1.81)*	1.27 (0.91, 1.78)	1.49 (0.97, 2.30)
High IL-6	475	1.16 (0.83, 1.60)	1.22 (0.86, 1.72)	0.76 (0.36, 1.61)
High IL-8	475	0.87 (0.59, 1.28)	0.87 (0.57, 1.33)	0.76 (0.35, 1.63)
High IL-10	475	1.26 (0.92, 1.74)	1.27 (0.90, 1.80)	1.11 (0.61, 2.00)
High IL-17a	475	**1.42 (1.07, 1.89)**	**1.55 (1.16, 2.07)**	1.04 (0.57, 1.89)
High MIP1a	475	**1.38 (1.02, 1.87)**	**1.47 (1.08, 2.01)**	1.30 (0.76, 2.21)
High TNFa	475	0.65 (0.41, 1.03)	0.59 (0.34, 1.01)	0.82 (0.39, 1.76)

With bold are appearing statistically significant relations and with asterisk (*) trending relations (p-value<0.1).

HDL: High density lipoprotein, SBP: systolic blood pressure, DBP: diastolic blood pressure, BMI: body mass index, WC: waist circumference

Adjusted for child's sex and gender

Exploratory Analysis – Metabolomics analysis

Table A3a: Association of acetaminophen use during pregnancy and child carnitines metabolites measured at birth

Carnitine, Acylcarnitine, Hydroxy- and dicarboxyacylcarnitines			
Metabolites	**β-coeff (95% CI)**	**p-value**	**q-value**
C0	0,102 (-2.003, 2.208)	0,923	0,975
C10	-0,005 (-0.016, 0.006)	0,358	0,861
C101	-0,009 (-0.028, 0.011)	0,382	0,862
C102	0 (-0.002, 0.002)	0,836	0,975

C12	-0,004 (-0.012, 0.004)	0,307	0,860
C12DC	0 (-0.002, 0.001)	0,772	0,975
C121	-0,01 (-0.035, 0.015)	0,424	0,862
C14	-0,002 (-0.007, 0.002)	0,313	0,860
C141	-0,002 (-0.006, 0.002)	0,268	0,837
C141OH	0 (-0.001, 0.001)	0,514	0,942
C142	-0,002 (-0.003, 0.000)	0,129	0,837
C142OH	0 (-0.001, 0.000)	0,722	0,975
C16	-0,017 (-0.042, 0.008)	0,175	0,837
C16OH	0 (-0.001, 0.000)	0,192	0,837
C161	-0,003 (-0.009, 0.002)	0,255	0,837
C161OH	0 (-0.001, 0.001)	0,65	0,975
C162	-0,001 (-0.001, 0.000)	0,265	0,837
C162OH	0 (-0.001, 0.000)	0,35	0,860
C18	-0,004 (-0.010, 0.002)	0,159	0,837
C181	-0,009 (-0.024, 0.005)	0,209	0,837
C181OH	0 (-0.001, 0.001)	0,778	0,975
C182	-0,005 (-0.012, 0.002)	0,173	0,837
C2	0,022 (-0.591, 0.634)	0,944	0,979
C3	-0,045 (-0.124, 0.034)	0,262	0,837
C3DCC4OH	0,001 (-0.022, 0.024)	0,903	0,975
C3OH	0 (-0.002, 0.001)	0,502	0,942
C31	0 (-0.001, 0.001)	0,483	0,941
C4	0,004 (-0.021, 0.030)	0,732	0,975
C41	0 (-0.003, 0.003)	0,807	0,975
C5	-0,004 (-0.063, 0.056)	0,902	0,975
C5DCC6OH	-0,001 (-0.004, 0.002)	0,636	0,975
C5MDC	0 (-0.004, 0.004)	0,874	0,975
C5OHC3DCM	-0,004 (-0.015, 0.006)	0,418	0,862
C51	0 (-0.001, 0.001)	0,994	0,995
C51DC	0 (-0.002, 0.001)	0,496	0,942
C6C41DC	-0,011 (-0.027, 0.004)	0,137	0,837
C61	0 (-0.002, 0.001)	0,709	0,975
C7DC	0 (-0.001, 0.001)	0,795	0,975
C8	-0,006 (-0.013, 0.000)	0,058	0,837
C9	0,001 (-0.001, 0.004)	0,234	0,837

Adjusted for parity, maternal education, maternal pre-pregnancy BMI, maternal smoking at 30 weeks of gestation, urban residence, gestational diabetes, flu and fever during pregnancy

Table A3b: Association of acetaminophen use during pregnancy and child -phosphatidylcholines metabolites measures at birth

Lysophosphatidylcholines, Diacylphosphatidylcholines			
Metabolites	**β-coeff (95% CI)**	**p-value**	**q-value**
PCaaC240	-0,003 (-0.017, 0.010)	0,604	0,975
PCaaC260	-0,003 (-0.026, 0.020)	0,82	0,975

PCaaC281	-0,033 (-0.156, 0.090)	0,59	0,975
PCaaC300	-0,214 (-0.593, 0.164)	0,262	0,837
PCaaC320	-1,426 (-3.937, 1.084)	0,26	0,837
PCaaC321	-1,252 (-3.759, 1.255)	0,322	0,860
PCaaC323	0 (-0.021, 0.021)	0,973	0,995
PCaaC341	-7,858 (-19.147, 3.430)	0,169	0,837
PCaaC342	-5,273 (-17.514, 6.967)	0,392	0,862
PCaaC343	0,001 (-0.452, 0.455)	0,995	0,995
PCaaC344	-0,003 (-0.067, 0.060)	0,923	0,975
PCaaC360	-0,269 (-0.727, 0.189)	0,244	0,837
PCaaC361	-0,982 (-4.055, 2.091)	0,525	0,942
PCaaC362	-0,784 (-9.984, 8.416)	0,865	0,975
PCaaC363	-5,121 (-15.638, 5.397)	0,334	0,860
PCaaC364	-8,427 (-17.872, 1.018)	0,079	0,837
PCaaC365	-0,084 (-0.721, 0.553)	0,792	0,975
PCaaC366	-0,006 (-0.047, 0.035)	0,769	0,975
PCaaC380	-0,132 (-0.370, 0.106)	0,271	0,837
PCaaC383	-4,36 (-9.085, 0.365)	0,07	0,837
PCaaC384	**-12,068 (-23.741, -0.394)**	**0,043**	0,837
PCaaC385	-0,959 (-5.094, 3.177)	0,645	0,975
PCaaC386	-4,354 (-13.314, 4.607)	0,335	0,860
PCaaC401	-0,024 (-0.070, 0.022)	0,297	0,860
PCaaC402	-0,028 (-0.094, 0.038)	0,397	0,862
PCaaC403	-0,014 (-0.110, 0.083)	0,781	0,975
PCaaC404	-0,028 (-0.640, 0.585)	0,928	0,975
PCaaC405	-0,02 (-1.420, 1.379)	0,977	0,995
PCaaC406	-1,723 (-5.887, 2.442)	0,411	0,862
PCaaC420	-0,06 (-0.129, 0.009)	0,088	0,837
PCaaC421	-0,032 (-0.068, 0.005)	0,085	0,837
PCaaC422	-0,009 (-0.027, 0.008)	0,295	0,860
PCaaC424	-0,008 (-0.050, 0.033)	0,694	0,975
PCaaC425	-0,004 (-0.039, 0.031)	0,834	0,975
PCaaC426	-0,003 (-0.043, 0.038)	0,895	0,975

Adjusted for parity, maternal education, maternal pre-pregnancy BMI, maternal smoking at 30 weeks of gestation, urban residence, gestational diabetes, flu and fever during pregnancy

Table A3c: Association of acetaminophen use during pregnancy and child Acyl-alkyl-phosphatidylcholines metabolites measured at birth

Acyl-alkyl-phosphatidylcholines			
Metabolites	**β-coeff (95% CI)**	**p-value**	**q-value**
PCaeC300	-0,019 (-0.051, 0.013)	0,236	0,837
PCaeC301	-0,008 (-0.036, 0.019)	0,543	0,959
PCaeC302	-0,002 (-0.007, 0.004)	0,578	0,975
PCaeC321	-0,33 (-0.705, 0.045)	0,083	0,837
PCaeC322	-0,04 (-0.100, 0.021)	0,192	0,837

PCaeC340	-0,016 (-0.209, 0.178)	0,872	0,975
PCaeC341	-0,197 (-0.889, 0.494)	0,57	0,975
PCaeC342	0,138 (-0.527, 0.804)	0,679	0,975
PCaeC343	0,035 (-0.177, 0.248)	0,739	0,975
PCaeC360	-0,003 (-0.071, 0.065)	0,932	0,975
PCaeC361	0,358 (-0.277, 0.994)	0,264	0,837
PCaeC362	0,393 (-0.236, 1.022)	0,216	0,837
PCaeC363	-0,058 (-0.462, 0.347)	0,776	0,975
PCaeC364	-1,01 (-2.295, 0.275)	0,121	0,837
PCaeC365	-0,755 (-1.690, 0.180)	0,111	0,837
PCaeC380	-0,014 (-0.135, 0.107)	0,819	0,975
PCaeC381	0,165 (-0.103, 0.434)	0,224	0,837
PCaeC382	0,166 (-0.184, 0.517)	0,345	0,860
PCaeC383	0,053 (-0.428, 0.535)	0,825	0,975
PCaeC384	-0,626 (-1.745, 0.492)	0,267	0,837
PCaeC385	-0,686 (-1.646, 0.274)	0,158	0,837
PCaeC386	-0,248 (-0.662, 0.166)	0,236	0,837
PCaeC401	-0,026 (-0.146, 0.093)	0,659	0,975
PCaeC402	0,059 (-0.089, 0.208)	0,427	0,862
PCaeC403	0,043 (-0.126, 0.213)	0,61	0,975
PCaeC404	-0,022 (-0.325, 0.281)	0,884	0,975
PCaeC405	-0,026 (-0.306, 0.255)	0,855	0,975
PCaeC406	-0,038 (-0.325, 0.249)	0,792	0,975
PCaeC420	-0,002 (-0.037, 0.033)	0,899	0,975
PCaeC421	-0,014 (-0.050, 0.022)	0,438	0,868
PCaeC422	0,006 (-0.029, 0.041)	0,736	0,975
PCaeC423	-0,004 (-0.051, 0.043)	0,869	0,975
PCaeC424	-0,037 (-0.127, 0.053)	0,416	0,862
PCaeC425	-0,05 (-0.207, 0.107)	0,523	0,942
PCaeC443	-0,005 (-0.016, 0.006)	0,389	0,862
PCaeC444	-0,023 (-0.064, 0.018)	0,274	0,837
PCaeC445	-0,092 (-0.239, 0.056)	0,218	0,837
PCaeC446	-0,059 (-0.134, 0.016)	0,119	0,837

Adjusted for parity, maternal education, maternal pre-pregnancy BMI, maternal smoking at 30 weeks of gestation, urban residence, gestational diabetes, flu and fever during pregnancy