Serum uric acid levels as an indicator for metabolically unhealthy obesity in children and adolescents

Dissertation

zur Erlangung des akademischen Grades

Dr. rer. med.

an der Medizinischen Fakultät

der Universität Leipzig

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geboren am 10.07.1984 in Recife (Brasilien)

angefertigt an: Universität Leipzig, Medizinische Fakultät, Klinik und Poliklinik für Kinder und Jugendliche, in Zusammenarbeit mit dem Leipziger Forschungszentrum für Zivilisationserkrankungen (LIFE)

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Beschluss über die Verleihung des Doktorgrades vom: 18.12.2018

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LIST OF ABBREVIATIONS

BMI	Body mass index
BP	Blood pressure
CVD	Cardiovascular disease
HDL-C	High density lipoprotein cholesterol
Hs-CRP	High sensitivity C-reactive protein
IDF	International diabetes federation
IR	Insulin resistance
KiGGS	Kinder- und Jugendgesundheitssurvey (German Health Interview and Examination Survey for Children and Adolescents)
FPG	Fasting plasma glucose
HDL-c	High density lipoprotein cholesterol
HOMA-IR	Homeostasis model assessment of insulin resistance
LIFE	Leipzig Research Center for Civilization Diseases
MS	Metabolic syndrome
MHO	Metabolically healthy obesity
MUO	Metabolically unhealthy obesity
NCEP	National Cholesterol Education Program
TG	Triglycerides
UA	Uric acid
WC	Waist circumference
WHO	World Health Organization

I. BIBLIOGRAPHISCHE BESCHREIBUNG

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Universität Leipzig, Dissertation

69 Seiten, 109 Literaturangaben, 6 Tabellen, 1 Publikationsmanuskript

Referat:

Adipositas im Kindes- und Jugendalter erhöht das Risiko für diverse chronische Krankheiten und ist aufgrund des epidemischen Anteils weltweit zu einem der größten Gesundheitsprobleme geworden. Zunehmende Evidenz zeigt, dass übergewichtige Personen, die unter "metabolisch gesunder Fettleibigkeit (MHO)" leiden, gegen Typ 2-Diabetes mellitus und kardiovaskulären Erkrankungen geschützt sind – im Gegensatz zu denen mit einer "metabolisch ungesunden Fettleibigkeit" (MUO). Jüngste Studien deuten darauf hin, dass neue Biomarker erfolgreich verwendet werden könnten, um den MHO- vom MUO-Phänotyp zu unterscheiden. Erhöte Serumharnsäurespiegel wurde als potentieller Risikofaktor für kardiometabolische Erkrankungen beschrieben, aber bisher haben nur wenige Studien deren Beziehung zu MHO und MUO innerhalb der pädiatrischen Population untersucht. In der vorliegenden Studie wurden die potenziellen klinischen und metabolischen Indikatoren, die zur Unterscheidung zwischen MHO- und MUO-Phänotypen bei Kindern und Jugendlichen beitragen können, anhand von Daten aus einer Studie in Deutschland ausgewertet, im Rahmen derer Daten von 246 übergewichtigen und adipösen und 212 normalgewichtigen Kindern und Jugendlichen erhoben wurden. Dieselben Daten wurden verwendet, um die Beziehung zwischen Serum-Harnsäure-Spiegeln und der metabolischen Gesundheit bei übergewichtigen und adipösen Kindern und Jugendlichen zu untersuchen. Eine umfassende anthropometrische, klinische und metabolische Charakterisierung des metabolischen Profils von MHO- und MUO-Phänotypen ist verfügbar. Diese Studie ergab, dass 70% der übergewichtigen und adipösen Teilnehmer einen MHO-Status hatten. Der MHO-Phänotyp war im Allgemeinen durch eine bessere Insulinsensitivität, niedrigere Triglycerid (TG)-Spiegel und einen niedrigeren High Density Lipoprotein Cholesterol-(HDL-c) im Vergleich zu ihren MUO-Peers charakterisiert. Es wurde gezeigt, dass Serumharnsäure mit Parametern verbunden ist, die zur Definition des metabolischen

Gesundheitszustands verwendet werden, wie HDL-SDS, TG-SDS, Nüchternglukose und Blutdruck. Zusätzlich schien die Serumharnsäure-Konzentration mit C-Peptid- und Cystatin C-Konzentrationen assoziiert zu sein. Das in dieser Arbeit vorgestellte Forschungsergebnis legt nahe, dass höhere Harnsäurespiegel als zusätzlicher Indikator für den MUO-Phänotyp bei übergewichtigen und adipösen Kindern und Jugendlichen verwendet werden könnten.

1 INTRODUCTION

"Corpulence is not only a disease itself, but the harbinger of others."

Hippocrates

1.1 Obesity and associated diseases, a world health threat

Obesity is associated with higher incidence of many health problems including cardiovascular diseases, type 2 diabetes and several types of cancer [1,2]. These complications contribute to a higher likelihood of morbidity and mortality in adulthood [3]. At the same time, the current obesity epidemic, which has spread from western to developing countries, is affecting even younger individuals. In fact, over the past decades, the proportion of overweight and obese children and adolescents has soared globally, rendering youth obesity a major focus of public health efforts [4]. A systematic review showed that between 1980 and 2013 the prevalence of overweight and obesity in children has increased by 47.1% worldwide [5]. Although recent studies conducted in several developed countries suggest a stabilization trend in the prevalence of obesity, especially among younger children, its prevalence in adolescents continues to increase [1,6]. In 2006, evaluating a sample of more than 17,500 young individuals (2-17 years), the German Health Interview and Examination Survey for Children and Adolescents (KiGGS) estimated that 15% of German children and adolescents were overweight and 6.1% were obese [1,7]. Globally, the prevalence of childhood obesity is particularly high in Latin America (Mexico, Brazil, Chile, Uruguay and Argentina), North Africa, the Middle East, India, and several Pacific Island and Caribbean nations [5,8].

The primary pathway linking overweight and obesity to the development of diseases is dysregulated metabolism, which is related to long-term inefficiencies in balancing intake and use of energy in the body [9]. As the prevalence of childhood and adolescence obesity increases, its health implications are becoming more evident. Obesity developed during childhood and adolescence instigates changes in metabolism, vessels and organs, thereby increasing the risk of severe cardiometabolic complications and premature death [10]. A certain combination of such abnormalities is known as metabolic syndrome (MS) and is characterized by high blood pressure, insulin resistance, unfavorable lipid profile (high levels triglycerides and/or low levels of cholesterol bound to high-density lipoprotein), liver enzymes alterations, abnormalities in fibrinolysis, subclinical inflammation and hyperuricemia. The metabolic syndrome has been used to link obesity and cardiovascular disease (CVD) risk factors [11,12]. Some studies have reported clustering of CVD risk factors among obese children and adolescents and a positive relationship between the level of overweight and the prevalence of the metabolic syndrome. A relationship between metabolic syndrome during childhood and the risk of CVD has also been observed later life.

In addition, excess body fat has been associated with increased risk of musculoskeletal conditions and obstructive sleep apnea, chronic pain, asthma and gallbladder [17].

These effects can reduce quality of life of affected individuals while also placing a substantial economic burden on health and social care systems [18]. In 2007, according to the World Health Organization, overweight and obesity were linked to about 80% of type 2 diabetes cases, 35% of ischemic heart disease cases and 55% of hypertension cases among adults in Europe. These complications result in more than 12 million life-years of health problems and 1 million deaths per year. In Germany, cardiovascular diseases were estimated to cause 39% of all deaths [19]. The treatment costs for cardiovascular diseases were 37.4 billion € in 2014 (equivalent to 1.4% of the gross domestic product GDP) and are projected to amount to 41.4 billion in 2020 [20].

Despite the substantial progress in research during the last decades, obesity is still difficult to manage. In fact, the extent of fat accumulation, its distribution within the body and the associated consequences vary considerably between individuals [21]. It is estimated that up to 30% of obese individuals do not have obesity-related comorbidities and do not show any evidence of metabolic abnormalities. Consequently, they have a reduced risk of developing type 2 diabetes mellitus and cardiovascular disease [22]. This subgroup is identified as metabolically healthy obese (MHO). However, it is unclear how healthy the individuals of this group are and whether MHO could later develop into metabolically unhealthy obesity (MUO) [23]. An improved understanding of the underlying mechanisms and the health consequences of different obese phenotypes in youth could represent the first step towards the prevention of an array of deleterious chronic diseases that commonly emerge throughout the life-course and may lead to a beneficial healthy aging process. Considering the variability of individuals within the obese population, it would be useful to allocate them into more specific groups according to their degree of metabolic abnormalities, aiming at early diagnoses and successful treatments, in order to be able to address their needs more specifically [24].

1.1.1 Definitions and classifications of overweight and obesity

Overweight and obesity are defined as an abnormal or excessive accumulation of body fat that poses a health risk [25]. The most commonly used, widely accepted, inexpensive and non-invasive indicator for the level of adiposity is the body mass index (BMI, expressed as

weight/height^2 in kg/m^2). BMI has been criticized for not being sensitive enough to detect differences in body composition (i.e. fat mass and fat-free mass) [26]. Nevertheless, it is a simple and practical method to use. Adults (≥ 18 years) with a BMI ≥ 25 kg/m² are classified as overweight and those with a BMI ≥ 30 kg/m² are classified as obese [27]. Regarding children and adolescents, however, growth and development at different ages have to be taken into account and, therefore, BMI cut-offs used for adults cannot be used. Although BMI and fat mass of adults and youths are closely correlated [26], assessing adiposity for young individuals is more complicated, as the fat mass changes with age, gender, maturation and ethnicity [28]. In addition, to be able to compare weight status between groups of children, the definition of childhood overweight and obesity is based on age and sex-specific BMI cut-off values – presented in tables or pointed out as centiles on a chart – which are generally derived from representative reference population.

1.2 A 'metabolic healthy' type of obesity

The heterogeneity of the healthy obese phenotype was first described in the 1980s by Sims [29] and has repeatedly been confirmed since then [30,31]. Analyses of epidemiological data concluded that overweight and obesity are not always associated with the onset of cardiovascular disease and increased mortality [32]. Research has focused on disentangling the underlying mechanisms that protect obese individuals from metabolic diseases [33]. In a follow-up study covering 10 years, Mongraw-Chaffin et al. [34] observed that 24% of obese individuals were metabolically healthy and did not develop metabolic syndrome. This means that 1 out of 4 individuals who have been obese over long period will not develop metabolic complications.

The main metabolic characteristics of MHO individuals have been reported to be a set of favorable metabolic profiles, such as: preserved insulin sensitivity, absence of metabolic syndrome criteria, low serum uric acid levels, low inflammatory indicators, favorable lipid, immunological and hormonal profiles, and low visceral, hepatic, and muscle fat accumulation. However, up to now, little is known about the factors that either delay the onset of metabolic disturbances or protect obese individuals from them [35]. Consequently, numerous systems have been developed to classify the clustering and the severity of metabolic risks associated with obesity.

Currently, overweight or obese individuals can be classified as metabolically healthy obese, according to three definitions: *(i)* the determination of metabolic risk factor clustering (e.g.

favorable lipid profile, normal glycemic status, and the normal blood pressure), *(ii)* a preserved insulin sensitivity, and *(iii)* the combination of the previous two definitions. Nevertheless, there is evidence that the above-mentioned definitions should be broadened by considering additional components, such as serum uric acid, inflammation markers and liver fat content [36,37]. Insulin sensitivity is predominantly assessed by using the surrogate homeostasis model assessment of insulin resistance (HOMA-Index) with subjective cut-offs. For instance, Prince et al. [38] defined MHO as the absence of insulin resistance (IR), a well-established predictor of type 2 diabetes, or as the absence of four traditional cardiovascular risk factors: high triglycerides, low HDL cholesterol, high fasting glucose and high blood pressure percentile for age, sex and height. Camhi et al. [39] define MHO as showing at least one of the following metabolic risk factors: high blood pressure, high triglycerides, high fasting glucose, low HDL cholesterol. A study that evaluated data from German obese children, defined MHO as the absence of hypertension, dyslipidemia and impaired fasting glucose [40]. Finally, two studies used metabolic syndrome criteria proposed earlier by Alberti et al. and defined MHO as waist circumference $\leq 90^{th}$ percentile together with no laboratory criterium of metabolic syndrome definition fulfilled [37,41]. MHO in children and adolescents is often defined using the pediatric International Diabetes Federation (IDF) criteria [39], the modified NCEP III criteria [42] and the age specific criteria [43] for the definition of metabolic syndrome. However, there is still no consensus regarding the number of metabolic syndrome criteria that must not be fulfilled to consider obese children and adolescent as metabolically healthy. A general overview of criteria sets that have been used to define MHO in children and adolescents is presented in **Box 1**.

Box 1. Variables and values used for the definitions of MHO in pediatric population.

Variables	Prince RL et al 2014 [38]	Camhi SM et al 2013 [39]	Reinehr T et al 2015 [40]	Mangge H et al 2013 [37]	Weghuber D et al, 2013 [41]
WC				≥ 90th percentile	≥ 90th percentile
BP	≥ 90th percentile for age, sex, and height	≥ 90th percentile for age, gender, and height	≥ 130/ 85 mmHg	≥ 130/85 mmHg	≥ 130/85 mmHg
FPG	≥ 5.6 mmol/L.	≥ 100 mg/dL	≥ 100 mg/dl	≥ 5.6 mmol/L.	≥ 5.6 mmol/L.
TG	≥ 1.25 mmol/L	≥ 110 mg/dL	150 mg/dl	≥ 1.7 mmol/L	≥ 1.7 mmol/L
HDL-cM	≤1.02 mmol/L	< 40 mg/dL	< 40mg/dl	< 1.03 mmol/L	< 1.03 mmol/L
HOMA-IR	≥ 3.16				
Criteria for MHO	None of the above	≤ 1 of the above	None of the above	None of the above	None of the above
MUO criteria	≥ 1of the above	≥2 of the above	≥1 of the above	≥ 3 of the above	≥3 of the above
BMI (percentile)					
	> 85th	> 95th	> 97th	Overweight >85th; Obese > 95th	Overweight >85th; Obese > 95th

WC: waist circumference; BP: blood pressure; FPG: fasting plasma glucose; TG: triglyceride; HDL: high density lipoprotein; HOMA-IR: homeostasis model assessment of insulin resistance; MHO: metabolically healthy obesity; MUO: metabolically unhealthy obesity; BMI: body mass index.

Although MHO in the adult population has been extensively studied, research focusing on children and adolescents is scarce. Based on diverse criteria, a recent review reported an MHO prevalence in obese children ranging from 18 to 44% [44]. The broad range was attributed to geographic distribution, age, sample size differences, different study design and different definitions used for obesity and metabolic health. Rey-Lopez et al. showed an even higher variability of MHO prevalence from 6% to 75% reported in various publications [45]. A recent study conducted in Chinese children and adolescents aged 6- to 18-years-old found prevalence of MHO was 3.9%-36.7%, using two different MHO criteria defined by insulin resistance and metabolic syndrome components respectively [22]. Data from a recently published longitudinal German study, based on a population of obese children, suggested that a substantial proportion of young obese (49.3%) displays an MHO phenotype [40]. The high variability of MHO prevalence in the aforementioned studies is due to the fact that the definition of MHO is still controversial and that no clear criteria have been widely accepted. Accumulating evidence indicates that different factors, including lifestyle, sex, age, ethnicity and specific cut-off values can largely influence the MHO prevalence [46]. Therefore, without a wide consensus on the definition of MHO individuals, the comparability between different studies and the successful interpretation of the association between MHO and long-term health effects remain difficult to achieve.

1.2.1 Distinguishing characteristics of healthy obesity

Although the underlying mechanisms leading to the transition between healthy and unhealthy obese phenotypes are poorly understood, several plausible mechanisms have been suggested in both human and animal studies [24,35,47]. Therefore, it is of great interest to identify additional biomarkers that distinguish between healthy and unhealthy overweight or obese individuals. This indicators might be useful to understand the mechanisms and the predisposing factors linking metabolic disorders to obesity [48] and could become relevant tools for identifying future diseases and to make accurate prognoses. Over the last years, several biomarkers have been proposed to distinguish different phenotypes of obesity. A biomarker of growing importance is serum uric acid: high levels of uric acid have been reported to be a clinically essential cue for inflammations, which are linked to metabolic disorders in seemingly healthy individuals [49]. Weghuber et al. [41] examined 299 overweight/obese children and adolescents and showed that serum uric acid levels were a significant predictor of metabolically unhealthy obesity (MUO) in youth populations. Other

inflammation markers, such as high-sensitivity C reactive protein (hs-CRP), may also be an important component in the cluster of metabolic risk factors [50]. Phillips and Perry [51] demonstrated that MHO individuals presented a more favorable inflammatory status than their metabolically unhealthy counterparts, including lower concentrations of hs-CRP, interleukin 6 and white blood cell count. It has been increasingly suggested to include liver fat content in the definition of MHO, because the prevalence of non-alcoholic fatty liver disease seems to be significantly lower in MHO individuals [52]. Recently, C-peptide was discovered to be a part of the immune response by regulating inflammatory cytokines and was associated with MS components [53,54]. This finding suggests that C-peptide concentrations might also be a clinically relevant indication of cardiovascular risk associated with metabolic disorders [54]. The exact attributes of additional biomarkers characterizing different obesity phenotypes have yet to be defined. There is growing scientific and medical interest in the MHO subgroup as ramifications of the health-preserving characteristics could individualize our current knowledge of chronic disease development [35].

1.3 Physiology of uric acid (UA)

Uric acid was first found in human urinary calculi by Scheele and Bergmann in 1776 [55,56]. They named the substance lithic acid and proved that it is a common urine component. In 1798 George Pearson, after isolating and characterizing uric acid from 200 urinary calculi specimens, suggested the name uric oxide [57]. The structure of uric acid was first proposed by Ludwig Medicus in 1875 [55,58]. Finally, after the pioneering works by Medicus, Liebig, and von Baeyer, the complete synthesis of uric acid was successfully performed for the first time in 1895 by the future Nobel laureate Emil Fischer [59,60].

Uric acid is a breakdown product of ingested and endogenously synthesized purines. DNA and RNA are degraded into purine nucleotides and bases, which are then metabolized to xanthine and uric acid via the action of xanthine oxidase [61]. Uric acid does not undergo any further metabolism in humans and is excreted by the kidneys and into the intestinal tract. Elevated UA levels can be determined by several causes: increased production, decreased excretion, or genetic mutations associated with renal UA transportation [62,63]. Increased UA production is associated with purine-rich diets, characterized by high consumption of meat, bean seeds, mushrooms or some types of seafood [64]. Moreover, abnormal kidney function or competition for excretion with certain medications (e.g. diuretics) can alter the excretion of UA, thereby leading to increased UA level [65]. Further causes include high-

fructose diets and excessive alcohol consumption. Namely, ethanol accelerates adenine nucleotide degradation by raising the plasma concentration of xanthine, which breaks down to produce UA. In addition, ethanol promotes dehydration by decreasing UA excretion [63].
Elevated UA levels in the blood can lead to hyperuricemia. This condition develops as a result of UA overproduction and/or altered UA secretion and is characterized by unusually high UA levels, higher that urate solubility [66]. The increase in consumption of fructose-containing drinks, food, and table sugar during the last decades has contributed to hyperuricemia by increasing the conversion of ATP to inosine, a precursor in purine metabolism [67].
A noteworthy characteristic of serum UA is showing both, pro-oxidative and antioxidative effects. In case of acute rises, serum UA acts as an antioxidant at the intravascular level. Thanks to this property, serum UA plays the essential role of the major extracellular antioxidant of human blood [68], which can benefit the endothelial function. When serum UA levels are consistently high, serum UA acts as pro-oxidant at the intracellular level. As a result, lipid oxidation increases and the synthesis of nitric oxide, which has anti-inflammatory and vasodilatory functions, decreases, potentially leading to cardiometabolic comorbidities [67,69]. The pivotal role of serum UA in the development of cardiometabolic abnormalities was also reported in other studies. For example, it was suggested that hyperuricemia may increase the risk of gouty arthritis and nephrolithiasis [70]. Therefore, it is of critical importance to understand the processes involved in augmenting serum UA levels, which might, in turn, play an active role in contributing to the development/progression of metabolic diseases.

1.3.1 Serum UA and cardiometabolic risk factors

The role of serum UA in the development of metabolic disorders has been investigated for many decades. Since the link among hyperuricemia, hyperglycemia and hypertension was first suggested by Kylin in 1923 [71], there has been a growing interest in the relationship between high serum UA levels and other metabolic abnormalities such as hyperglycemia, abdominal obesity, dyslipidemia, and hypertension [72,73]. A recent meta-analysis has demonstrated that high serum UA levels are associated with a modest increase in episodes of infarction and mortality [74]. Recent studies showed that serum UA is linked to cardiovascular dysfunction in pediatric obesity [75]. Furthermore, a recent investigation involving young individuals with hyperuricemia has suggested that a reduction in serum UA

can lead to an increase of body weight and related CVD risk factors [76]. However, it is still not entirely clear whether the cardiovascular effects associated with high UA levels can be attributed only to UA - as an independent factor - or whether they are codetermined by the presence of other factors [67]. Many studies described correlations between elevated serum UA levels, MS and several of its components [62,77,78]. Two distinct mechanisms can explain the link between hyperuricemia and Two distinct mechanisms can explain the link between hyperuricemia and MS: the first mechanism is related to hyperuricemia - induced endothelial dysfunction leading to reduced insulin-stimulated nitric oxidative - induced vasodilation in skeletal muscle, and therefore to reduced glucose intake in skeletal muscle [79]. A bidirectional causal effect between hyperuricemia and hyperinsulinemia was suggested. The second mechanism is related to the fact that uric acid induces oxidative and inflammatory alterations in adipocytes, since xanthine oxidoreductase, responsible for generating UA from xanthine, which is expressed in adipocytes and is critical to the adipogenesis process [80]. Even if the connection between hyperuricemia and MS has not been totally understood yet, many studies have described correlations between serum UA levels, MS, and several of MS components in children and adolescents [81,82]. Indeed, Cardoso et al. [83] suggested an association between high levels of uric acid and MS in adolescents. For instance, every 1 kg/m^2 increment in BMI is associated with a 5.74 μmol/L increase of serum UA levels. These results were further supported by Jones et al. [84]. The Bogalusa Heart Study demonstrated that elevated serum UA levels play a pivotal role in the pathogenesis of MS already during adolescence, suggesting that serum UA may help the early identification of high risk individuals for MS [85].

1.3.2 Serum UA and type 2 diabetes

Elevated serum UA levels seem to be involved in the development of diabetes: high serum UA levels were found to be closely associated with insulin resistance and diabetes mellitus type 2 [86]. In particular, one-quarter of diabetes cases can be attributed to high serum UA levels. This outcome most likely reflects the biochemical interaction between plasma glucose and purine metabolism, which leads to increased excretions of serum UA during hyperglycemia and glycosuria [87]. In Germany, a population-based prospective study showed that serum UA was a strong risk factor for diabetes [88]. A recent study suggested that the increase in serum UA has a positive correlation with insulin resistance in obese children and adolescents. It was found that for every increase of 1 mg/dL in serum uric acid

levels, there would be a 91% increase in the chance of insulin resistance [89]. It is worth mentioning that in prepubertal obese children, elevated levels of serum UA seem to be an early metabolic alteration associated with insulin resistance features [90]. Subsequent studies revealed that insulin resistance was very low in healthy obese individuals compared to unhealthy ones and that it increased significantly with the number of metabolic obesity comorbid disorders [41]. Low prevalence of insulin resistance was reported among obese individuals without significant comorbidities, suggesting that a preserved insulin sensitivity may be the key mechanism underlying healthy obesity [91].

1.3.3 Serum UA and hypertension

Numerous studies have demonstrated that hyperuricemia carries an increased risk for development of hypertension independently of other risk factors [62,80]. The strength of the relationship between serum UA level and hypertension decreases with increasing individual age and duration of hypertension, suggesting that serum UA levels may be more important in younger individuals with early-onset hypertension [84]. Several authors have emphasized that serum uric acid plays a role in pediatric hypertension. High serum UA levels were observed in about 90% of adolescents with recent onset hypertension and the serum UA level correlated with BP values [67]. Viazzi et al. [92] found that serum UA was directly related to hypertension independently of gender, puberty, BMI, HOMA-IR and renal function in children and adolescents. Various studies demonstrated that hypertension could lead to hyperuricemia. Hypertension can cause microvascular injury [90,93,94] which then leads to a state of tissue hypoxia and to a consequent increase in lactate production, which in turn impair the renal clearance of urate. Furthermore, tissue hypoxia also induces the production of some precursors of uric acid, such as adenosine, hypoxanthine and xanthine oxidase [95]. In addition, recent studies report that hyperuricemia is involved in the reduction of nitric oxide release and in the activation of the renin-angiotensin system. Both mechanisms cause renal vasoconstriction and, therefore, lead to increased blood pressure [96]. Serum UA's role in hypertension and cardiometabolic risk is demonstrated by the increased production of hs-CRP in endothelial and smooth muscle cells when uric acid is present [97].

1.3.4 Serum UA and kidney-related complications

Moreover, increasing evidence suggests that high serum UA levels in obese individuals may cause kidney damage [98]. A recent study described that chronic kidney disease is related to serum UA levels, MS and obesity [99]. One possibility is that underlying mechanisms, such as endothelial dysfunction and oxidative stress, might c both kidney damage and the MS [100]. Some authors show that diets with high sugar addition, which implicates excess fructose, might have a key role in development of MS and kidney disease by elevating serum UA [101]. Krishnan et al. showed that the serum UA levels are linked to diabetic retinopathy in patients with type 2 diabetes mellitus [102]. Other studies reported that uric acid is involved in the reduction of nitric oxide in renal macula densa and in the direct stimulation of the renin-angiotensin system, leading to vasoconstriction and higher blood pressure [103,104]. In addition, cystatin C, albumin and UA were found to improve the identification of risk associated with chronic kidney diseases [105,106].

1.3.5 Connection between Serum UA levels and metabolic health status

Despite the evidence that serum UA is a cardiometabolic risk factor [107], it is rarely associated in the literature with the metabolic health status in the young population. Very few studies have addressed this association directly. One example is a recent study from Mangge et al. that examined the influence of serum uric acid in the development of metabolic abnormalities in overweight/obese young individuals. The authors performed a cross-sectional investigation analyzing a group of 299 overweight and obese children aged 8-18 from the STYJOBS/EDECTA cohort, allocated into three subgroups based on their weight and the presence of metabolic abnormalities: the normal weight control group, the MHO group and the MUO group. The results indicated elevated serum UA as the best predictor of unhealthy obesity in young population [37,41]. The emergence of serum UA as possible indicator to distinguish between metabolic healthy and unhealthy phenotype is believed to have relevant implications for obesity treatment and research in the pediatric population. More research would be necessary to consolidate the position of serum UA as a clinically relevant indicator of the obesity phenotype in youth, and, perhaps, even a risk modifying therapeutic target.

THE PROJECT RESEARCH

1.4 Research question and hypotheses

As already stated in previous paragraphs, obesity is one of the most serious health issues faced by the current generation of children and adolescents and it should be prevented as early in life as possible. It is widely accepted that obese children are at high risk for developing metabolic complications that eventually may progress to type 2 diabetes and cardiovascular diseases [1]. Timely and carefully planned preventive measures are needed to reduce the risk of health-related consequences later in life. However, the concept of metabolically "healthy" obesity (MHO) was recently introduced into the field of pediatric obesity to describe obese children without any metabolic complications, in contrast to those with metabolically unhealthy obesity (MUO) [50]. Because of this, research has been focusing on potential clinical and metabolic indicators that may distinguish the MHO from MUO phenotypes and that can contribute to a better understanding of associated mechanisms. In this thesis, I focused on the distinction between MHO and MUO phenotypes and their relationship with serum uric acid in the pediatric population. Currently, there is a paucity of studies regarding these topics. Additionally, in this work I examine the possibility of using serum uric acid as an indicator of MUO phenotype in obese children and adolescents. Serum uric acid has been associated with risks of developing metabolic complications such as metabolic syndrome and type 2 diabetes mellitus in seemingly healthy obese individuals [108] and, therefore, is a promising health status biomarker for young individuals. More specifically, the following research questions are addressed:

1) What are the potential clinical and metabolic indicators that can be used to distinguish between MHO and MUO in children and adolescents?
2) Is the uric acid a potential indicator for the MUO phenotype in overweight or obese children and adolescents?
3) Is there a consistent association between serum uric acid and other cardiovascular risk factors among children and adolescents in Germany?

1.5 The LIFE-Child study

The data used in the present study were obtained from the LIFE-Child study, which assessed individuals aged 0 to 18 years and is a part of the "Leipzig Research Center for Civilization Diseases (LIFE)", a major research project of the University of Leipzig.

The LIFE-Child study was designed in 2011 with the broad aim of understanding how and through which mechanisms metabolic and environmental factors influence growth, health and development processes in newborns, children and adolescents in Germany [109]. Since its inception, LIFE-Child studies have been a source of evidence for the health inequalities in children and adolescents in Leipzig, with recent contributions focusing on age-related health conditions. Through the data provided by the LIFE-Child study it is possible to investigate complex associations between life circumstances, behavior and health outcomes. Additionally, LIFE-Child provides reference data for health-related indicators.

The instruments used for data collection were a combination of interviews and questionnaires addressing socio-demographic characteristics, personal, family history, anthropometric and laboratory measurements. The participants were recruited by the health department, by practicing pediatricians, by inviting school classes and by public relations (website, social networking, flyers and events).

Ethical approval for the study was obtained from the ethics committee of the Medical Faculty of the University of Leipzig (file reference: Reg. Nr. 264-10-19042010). Parents or legal guardians of the participants gave written informed consent for their data to be used for bona fide research purposes of data.

2 PUBLICATION MANUSCRIPT

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Submitted in: Hormone Research in Pediatrics

Accepted for publication by July 2018

Original Paper

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Running headline: Uric acid in metabolically unhealthy obesity children

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Keywords

metabolically healthy obesity, metabolically unhealthy obesity, uric acid, children, obesity

Abstract

Background: Metabolically healthy obesity (MHO) refers to those individuals who do not show cardiometabolic abnormalities. Our aim was to identify potential clinical and metabolic indicators that may help to distinguish between metabolically healthy and unhealthy individuals amongst overweight and obese (ov/ob) children and adolescents. ***Methods****:* The study involved 246 ov/ob and 212 normal weight individuals enrolled in the LIFE-Child study, aged between 6 and 18 years. Overweight/obese individuals without cardiovascular risk factors (fasting serum lipids, blood pressure and glucose) were classified as MHO. Individuals meeting one or more criteria of cardiovascular risk factors were classified as metabolically unhealthy obesity (MUO). ***Results:*** Among the 246 ov/ob individuals, 173 (70%) were MHO and 73 (30%) were MUO. The MHO individuals were younger, more likely to be male and had lower BMI-SDS. In the logistic regression models, UA SDS (OR 1.61, 95% CI 1.1 – 2.6, *p*=. 004), waist circumference SDS (OR 2.50, 95% CI 1.2 – 6.4, P=. 017), and C-peptide (OR 4.05, 95% CI 3.5 – 91, *p*=. 003) were significant indicators of MUO. ***Conclusion:*** Our results suggest that nearly one-third of the ov/ob children are already identified as MUO. Serum levels of UA can be used as an indicator of unhealthy obesity in youth where lower levels of UA indicate a lower risk and higher levels suggest a higher risk of MUO. We note that the relevance of identifying potential indicators remains the first most important step in future clinical research.

Introduction

Excessive body fat during childhood and adolescence instigates the first changes in the metabolism, vessels, and organs, which may lead to severe cardiometabolic complications, such as increased risk of cardiovascular dysfunction, hypertension, dyslipidemia and type 2 diabetes mellitus [1]. In fact, both overweight and obesity in children have increased by 47.1% since 1980 [2]. Its multifactorial nature complicates both therapy and prevention, rendering it a major problem and an important challenge for public health around the world [3]. Although the latest studies from several countries worldwide suggest a stabilization trend in the prevalence of obesity, especially among younger children, its prevalence in adolescents continues to increase [4]. Paradoxically, increasing evidence shows that a subgroup of overweight and obese individuals, reported as "metabolically healthy obesity", seems to be less prone to have the typical obesity-associated metabolic disorders, in contrast to those with metabolically unhealthy obesity [5]. The MHO has been identified by a set of favorable metabolic profiles, including preserved insulin sensitivity, the absence of criteria for metabolic syndrome, increased physical activity, low inflammatory indicators, hepatic fat content, and hormonal profile [6]. These factors might differentiate between metabolically healthy and metabolically unhealthy obese individuals. Although MHO in adult populations has been well-studied, research focused on children and adolescents is far more scarce [7]. The reported prevalence of MHO in children varies considerably, ranging from 6% to 75%, depending on the classification system used to define this condition [8,9]. Due to a lack of consensus, the definition of MHO remains controversial [10]. Therefore, it is essential to investigate underlying mechanisms of the metabolically healthy and unhealthy phenotype in overweight and obese individuals [11]. Studies aimed at identifying new biomarkers that may distinguish the different phenotypes of obesity are required since such indicators could become relevant tools for identifying future diseases, making an accurate prognosis and planning intervention strategies [12].

Over the last years, it has been suggested that further biomarkers, such as serum uric acid (UA), are associated with risks of developing metabolic syndrome, type 2 diabetes mellitus and incident cardiovascular events [13]. In fact, uric acid has been reported to be a clinically important marker of inflammation linked to metabolic disorders in seemingly healthy obese individuals [14]. Currently, there is a paucity of recent data concerning the relation among biochemical variables with MHO and MUO in the pediatric population. On the basis of these observations, this study has endeavored 1) to identify potential clinical and metabolic indicators that may help to distinguish between metabolically healthy and metabolically

unhealthy phenotypes; 2) to explore the relationship between serum uric acid levels and metabolic health in overweight and obese children and adolescents. In addition, we hypothesized that serum uric acid might be an indicator of the development of adverse cardio-metabolic outcomes in obese children and adolescents.

Methods

Research design and population

The participants' data in the present study were collected from the ongoing "Leipzig Research Centre for Civilization Diseases (LIFE)" Child study of the University of Leipzig, which initiated in 2011. The instruments used for data collection were a combination of examinations and questionnaires addressing demographic issues, personal and family history, anthropometric, and laboratory measurements. Full details have been reported previously [15,16]. In 801 children and adolescents serum uric acid levels were available (LIFE Child cohort). After excluding those who were underweight (BMI-SDS < -1.28), had missing values on data required for defining metabolic health status, had any chronic diseases, such as diabetes mellitus type 1, diagnosis of liver disease, or were taking any medication that affects glucose or lipid metabolism, 458 individuals aged 6-18 years remained (Figure 1) for analysis (239 male and 219 female). Among these, 45.2% (n = 207) were obese (BMI-SDS ≥ 1.88) and 8.5% (n = 39) were overweight (BMI-SDS between 1.28 and 1.88) and they were evaluated alongside with 46.3% (n = 212) normal weight control group (BMI-SDS between -1.28 and 1.28) [17]. Due to the fact that few participants were overweight, we combined overweight and obese for the analyses. This study was approved by the Ethical Committee of the University of Leipzig under registration No. 264-10-19042010. Written informed consent was obtained from parents or legal guardians of the participants before the children and adolescents were included in the study.

Anthropometric and clinical measurements

Anthropometric and laboratory data were collected by trained health investigators using standardized procedures. Body mass index (BMI) was calculated by weight in kilograms (kg) divided by the square height in meters (m) (kg/m^2). Age- and sex-specific BMI standard deviation scores (BMI-SDS) were calculated using the German reference data [17] as recommended by the German Working for Pediatric Obesity Consensus Guideline [18]. Waist circumference (WC), defined as midpoint between lowest rib and the upper anterior iliac spine, was measured using an inelastic tape in centimeters (cm). WC-SDS was calculated based on German references [19]. Waist-to-height ratio (WtHr) was calculated as waist circumference (cm) divided by height (cm). Pubertal development was assessed according to the definition of the Tanner stage of breast development in girls and genital stage in boys. Pubertal development was divided into three pubertal stages (Tanner stage I was classified as prepubertal; Tanner stage II, III and IV as pubertal; Tanner stage V as post pubertal) [20]. Blood pressure (BP) was measured at the right arm after a 10-minute resting

period in the supine position. BP was measured three times with a one-minute interval, and the average of all measurements was used for analyses [21]. Participants also completed questionnaires related to their demographic characteristics, medical history, alcohol consumption, and dietary habits.

Biochemical parameters

Fasting venous blood samples were collected from individuals at LIFE Child study. The measurement of laboratory parameters was performed at the Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics (ILM), University Hospital Leipzig. Total cholesterol, triglyceride, HDL-cholesterol, AST, ALT (UV tests), GGT, LDL-cholesterol, UA, alkaline phosphatase (colorimetric tests) as well as high sensitive C-reactive protein (latex-enhanced immunoturbidimetric test) were analyzed in serum samples with reagent from Roche Diagnostics using Cobas 8000 System according to the manufacturer protocol (Roche Diagnostics GmbH, Mannheim, Germany). Serum cystatin C (CysC) was analyzed using the turbidimetric immunoassay (PETIA) Tina-quant® Cystatin C (Roche Diagnostics). An oral glucose tolerance test was performed in all individuals according to the established recommendations [22]. oGTT derived laboratory data (insulin and glucose concentrations) were drawn at baseline and after 15, 30, 60, 90, 120 min. Insulin measurement was performed using the fully automated immunoassay systems of Liaison (Diasorin, Dietzenbach, Germany) and Cobas ECLIA-test on Cobas 8000 e602 (Roche, Mannheim, Germany). Both assays were adjusted for the 1.IRP WHO reference standard. Intra-assay and inter-assay coefficients were below 5.7% [23]. Insulin sensitivity we examined by the homeostasis model assessment index (HOMA-IR) [24], which was calculated using the following formula: HOMA-IR=(Insulin [mU/l]×Glucose [mmol/l])/22.5. The whole-body insulin sensitivity index (WBISI), was determined using glucose and insulin values during an oral glucose-tolerance test (OGTT) applying the Matsuda calculation [25].

Defining metabolic healthy status

For the purpose of defining metabolic status, the overweight/obese children and adolescents have been divided into two groups (i.e. metabolically healthy or metabolically unhealthy status) according to the following criteria (Supplemental Table 1): Individuals with the presence of at least one of the following cardiovascular risk factors have been classified as having MUO: dyslipidemia, defined as i) serum triglycerides (TG) > 95th percentile or ii) serum HDL cholesterol (HDL-C) < 5th percentile according to the American Academy of Pediatrics [26]; Hypertension, defined as iii) blood pressure > 95th percentile for height, sex, and age [27]; Impaired fasting glucose (IFG), defined as iv) fasting serum glucose ≥ 5.6 mmol/l. On the contrary, MHO status was based on the absence of hypertension, dyslipidemia, and IFG following the definition of Prince et al [8]. The parameters used to define metabolic healthy status were standardized because of age dependence. In addition we used the 95th percentile as the cut-off for the aforementioned cardiovascular risk factors.

Statistical analysis

Gaussian distribution of all variables was checked using the Shapiro-Wilk test in addition to graphical methods. All data are reported as mean ± standard deviation (if normally distributed), and median and interquartile range (if non-normally distributed). Categorical data are presented as number (n) and percentage (%). Differences among groups were tested using chi-squared test (x^2) for categorical variables and Mann-Whitney-Wilcoxon test or t-test for continuous variables, when appropriate, with a 95% confidence interval (95% CI) and the risk difference (RD) between the MHO and MUO groups. We used hierarchical linear regression analysis to evaluate the strength of the relationship between serum UA concentrations and cardiovascular risk factors. All statistical models were adjusted for age and sex. For overweight/obese individuals, logistic regression analysis was performed to assess the association between clinical and metabolic variables as independent variables and metabolic unhealthy status as dependent variable, controlled for age, sex, pubertal stage and BMI-SDS. Furthermore, logistic regression analyses were performed including gender, age, pubertal stage, waist circumference, UA, C-peptide, hs-CRP, and albumin in order to identify significant indicators of MUO. The regression coefficients are presented as odds ratios (ORs) with 95% confidence intervals (CI). In addition, all analyses were performed both including and excluding the overweight category; since our analyses generated similar results, all children with (BMI-SDS ≥1.28) were included in the final analyses. In order to remove the age dependence, we standardized some parameters such as fasting serum

glucose, HDL-c, triglycerides, as well as UA levels using a LMS-type method (LMSP) implemented in the package gamlss [28]. The age- and sex- specific percentiles were found preferable, especially in young children. All results with a *p* value less than 0.05 were considered to be statistically significant. Data analysis was performed using the statistical software R (version 3.1.2, R core team, Vienna, Austria) [29].

Results

Anthropometric and metabolic characteristics of the study population are displayed in table 1. The presented data consists of 246 overweight/obese and 212 normal weight children and adolescents (mean age 11.1 ± 2.8 years, 52% male). The mean BMI-SDS was -0.04 ± 0.68 in normal weight and 2.32 ± 0.52 in overweight/obese children. 41% of them were classified as prepubertal, 47% were pubertal and 12% were postpubertal. Based on the cardiovascular risk factors, the overweight/obese individuals were classified into two groups; 173 (38.0% of the total study sample) "metabolically healthy obesity" (MHO, no criteria of cardiovascular risk factors) and 73 (16.0%) "metabolically unhealthy obesity" (MUO, presence of one or more criteria of cardiometabolic risk factors). These two groups were compared to the control group (normal weight individuals, 212 [46.0%]). Among the cardiovascular risk factors in the MUO individuals, hypertriglyceridemia was the most frequent (in 54.2% of the individuals), followed by low serum HDL-C (45.8%), hypertension (19.5%) and IFG (14.7%). The MHO individuals were more often prepubertal than MUO individuals. As expected MUO group had significantly higher median BMI-SDS when compared to the MHO group (table 1). Circulating concentrations of uric acid SDS, C-peptide and hs-CRP were significantly higher in MUO compared to MHO group. Additionally, cystatin C levels were significantly higher in the controls compared to the MHO and MUO, whereas no significant difference was found between MHO and MUO groups. Markers of liver function, including ALT, AST and alkaline phosphatase, were similarly increased in MHO and MAO groups, exhibiting no differences between them, with the exception of GGT levels, which were significantly higher in MUO as compared to the MHO group. The used parameters defining the metabolic health status are summarized in table 2. MUO showed significantly higher triglycerides SDS, glucose SDS, systolic and diastolic blood pressure SDS compared to MHO individuals. As expected, glucose metabolism was altered in MUO as indicated by increased insulin levels and reduced WBISI with normal glycaemia compared to the MHO group. Anthropometric and biochemical characteristics of all participants of the study are presented in Supplemental Table 2. MUO children showed significantly increased LDL cholesterol paired with reduced HDL cholesterol SDS when compared to MHO children. Hematological indicators, including hemoglobin, red blood cell, platelet, white blood cell was increased in the MUO compared to the MHO group, although no significant differences were found between both groups. Levels of testosterone and SHBG were higher in the MHO in comparison to the MUO group; whereas levels of estradiol were significantly lower in the MHO than in the MUO group. However, after stratifying the analyses by Tanner stage to

avoid a bias caused by the different age distributions of MHO and MUO, the difference pertains only significant for Tanner 1 stage where the MHO group shows significantly lower values of estradiol. The results of the hierarchical regression analyses were incorporated to examine further the relationship between serum UA and the parameters used to define the metabolic health status. They are summarized in Table 3. Uric acid serum concentrations were associated with serum triglyceride SDS, systolic blood pressure, CysC and C-peptide. Furthermore, UA SDS was negatively associated with serum HDL SDS. However, we did not find a significant effect of glucose-SDS on UA serum levels. Additionally, a logistic regression analysis was performed to identify indicators of MUO phenotype as shown in table 4. Based on the relevant clinical and metabolic variables shown in table 1, the set of independent variables like waist circumference, UA, C-peptide, albumin and hs-CRP were included in the logistic regression models. Variables used for defining the MUO were not included in the logistic regression analysis. In conclusion, higher levels of C-peptide, waist circumference SDS, UA SDS and pubertal stage were identified as significant indicators of the MUO phenotype. However, no significant effect of sex was found. Higher levels of hs-CRP and albumin were non-significant MUO indicators when controlled for age, gender, pubertal stage and BMI-SDS (table 4).

Discussion

This study was designed to identify potential clinical and metabolic indicators that may distinguish between metabolically healthy and metabolically unhealthy phenotypes in overweight and obese children and adolescents. The results associating serum uric acid levels with the MUO status in youth are of particular interest, considering that such data were scarcely available up to now. Our study yielded several important findings:

(i) Regarding cardiometabolic characteristics, the MHO children were identified to be younger and less overweight in terms of BMI-SDS and to have lower waist circumference, lower insulin resistance index (HOMA), better insulin sensitivity and lower levels of pro-inflammatory markers. Such results which are supported by other studies [30,31], may place that a subset of overweight/obese children was at relatively low cardiometabolic risk despite possessing a high amount of body fat [32]. Interestingly, concerning the liver profile, the concentrations of ALT and AST were not significantly different between the MHO and MUO children. In contrast to our results, it has been reported that ALT and AST serum concentrations have been significantly different between MHO and MUO [33]. These findings could also be attributed to a healthier constitution, younger age, effects of the ethnicity or smaller sample size of studied participants. However, a study from Canada found a significant difference in the levels of GGT in MHO compared to the MUO group [34], as in the present work.

(ii) Another point of interest is that there were higher proportions of MHO (38.5%) in ov/ob children and adolescents than described by other authors who evaluated obesity in the pediatric age [35,36]. For instance, a recent study examining Chinese children and adolescents aged 6- to 18-years found that prevalence rates were 3.9% and 36.7% using two different MHO criteria defined respectively by insulin resistance and cardiometabolic parameters [37]. Using the same MHO criteria as in the present study, a recently published German study on the effect of pubertal status revealed that a large proportion of obese children (49.3%) exhibited a MHO status [38]. It is likely that the high variability in reported MHO prevalences could be explained by the lack of a standard definition of MHO, specific cut-off values, small sample sizes, different age ranges, as well as behavioral differences between populations [39–41].

(iii) A comprehensive logistic regression model identified C-peptide, waist circumference SDS, UA SDS, and pubertal stage as indicators of the MUO status. The current study demonstrated significantly higher levels of C-peptide and insulin in the MUO group, when compared to MHO, indicating an increased β-cell load. This result might indicate early

changes in the glucose metabolism of obese children which could be associated with the reduction of insulin sensitivity, as implied by the increased HOMA-IR and the reduced WBISI. Previously published results have shown C-peptide to be associated with insulin resistance, cardiometabolic abnormalities belonging to the metabolic syndrome, dyslipidemia, hypertension hyperuricemia and type 2 diabetes mellitus in both obese children and adults [42,43]. Cardellini et al [44] demonstrated an important role of C-peptide levels in the chemotactic effect on the inflammatory cells and suggested that it promotes the onset of atherosclerosis. To best of our knowledge, this is the first study addressing C-peptide as potential indicator of MUO in ov/ob children and adolescents. In our study, uric acid was found to be a significant indicator of MUO status after adjusting for age, sex, pubertal status and BMI-SDS. This finding is in agreement with those recently obtained by two Austrian authors, who examined overweight/obese children and adolescents and have shown that higher serum UA levels were a significant predictor of metabolically unhealthy in young populations [36,45]. In youth, serum UA increases progressively from an early age with body growth and a plateau around 15 to 17 years [46]. In our study, concentrations in overweight/obese children are significantly higher than in their normal weight peers. This may be of particular significance for overweight/obese individuals who may exhibit chronic long-term hyperuricemia. In fact, increased UA levels may contribute to endothelial dysfunction by instigating antiproliferative effects on endothelium and impairing nitric oxide production, therefore, it has been associated with the risk of cardiovascular disease [47]. In this study we found that UA was strongly associated with blood pressure. A biological explanation for this result is supported by animal models, which showed that after the onset of hyperuricemia, hypertension occurs. This indicates that the reduction of nitric oxide in the renal macula and the direct stimulation of the renin-angiotensin system are probably the mechanisms to cause vasoconstriction and, therefore, increase blood pressure [48]. According to our findings, the MUO is characterized by higher albumin and CysC levels as compared to the MHO children. In addition, CysC, albumin and UA were found to be sensitive in the identification of mild reductions in kidney function, and could potentially identify young individuals at high risk for the future development of cardiometabolic diseases [49,50]. These facts highlight that the association among UA levels and cardiometabolic disorders might be a relevant area of research, recognizing the implications of the discovery of potential new indicators and therapeutic targets in individuals with a metabolically unhealthy phenotype.

Furthermore, in our study, the pubertal children had a higher odds of being of MUO

phenotype. Reinehr et al [38] showed that entering puberty doubles the risk of switching from MHO to MUO. The difference between pubertal stage and cardiometabolic alterations may be attributable to the physiological changes of body composition and the influence of sex hormones during puberty development [51].

The major strength of this study is the description of a comprehensive clinical and metabolic analysis of MHO and MUO children and adolescents. However, despite our innovative findings, our study has the following limitations: First, the cross-sectional design of the study could not reveal any causal relationships between the metabolic health status and the investigated indicators. Further assessment of the influence of cardiovascular risk factors when obese children enter puberty is necessary. Second, it is essential to reproduce this study in a longitudinal research among different young populations. Furthermore, our study had a limited sample size, and therefore our observations need to be confirmed in a bigger cohort. Lastly, there is no standard definition of MHO. In conclusion, our study is one of the few to suggest that high uric acid levels increase the likelihood of MUO. Future longitudinal studies in larger populations are required to confirm that uric acid is an indicator of metabolic phenotypes associated with obesity, and that it may represent a useful indicator to identify children with increased cardiometabolic risk.

Financial support:

The project was supported by the LIFE-Child Research Center for Civilization Diseases funded by the European Union through the European Regional Development Fund (ERDF), the Free State of Saxony and by grant from the Coordination of Improvement of Higher Level Personnel (CAPES) from Brazil. J.S. is supported by ESPE (European Society for Pediatric Endocrinology) Research Fellowship.

Competing interests

The authors state that there is no conflicts of interest.

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Tables

Table 1. Anthropometric and metabolic characteristics of normal weight controls, MHO and MUO children

	Normal weight controls	MHO	MUO	RD (95% CI)	*P* value[a]	*P* value[b]
n (%)	212 (46)	173 (38)	73 (16)			
Baseline characteristics, anthropometry						
Age, years	11.1 ± 2.98	11.2 ± 2.64	11.4 ± 2.84	0.20 (0.03-0.98)	.610	.785
Male sex, n (%)	109 (51)	96 (55)	34 (49)		.496	.496
Pubertal status, (%)					.048	.048
Prepubertal	45.9	35.1	34.1			
Pubertal	44.8	47.3	52.2			
Postpubertal	9.3	17.6	13.7			
BMI, SDS	-0.04 ± 0.68	2.32 ± 0.52	2.57 ± 0.57	0.25 (0.09-0.38)	<.001	.001
Waist circumference, SDS	-0.2 ± 0.69	1.62 (1.33-1.89)	1.83 (1.54-2.04)	0.39 (0.05-0.27)	<.001	.000
Metabolic characteristics						
HOMA-IR	1.44 (1.00-1.91)	3.05 (2.50-3.67)	3.66 (2.61-4.70)	0.61 (1.01-2.23)	<.001	<.001
WBISI	5.31 (3.79-7.51)	4.31 (1.98-3.71)	2.00 (1.40-2.47)	0.94 (0.57-1.50)	<.001	<.001
C-peptide, nmol/L	0.58 (0.42-0.69)	0.79 (0.64-0.97)	0.97 (0.73-1.19)	0.18 (0.08-0.25)	<.001	<.001
Uric acid, SDS	-0.49 ± 0.85	0.33 (0.01-0.81)	0.69 (0.21-1.39)	0.36 (0.16-0.67)	<.001	<.001
AST, U/L	30.5 (24.7-34.8)	27.4 (22.9-34.4)	30.8 (23.2-35.3)	3.40 (1-20-3.61)	.172	.411
ALT, U/L	17.4 (14.5-21.7)	18.1 (14.5-21.7)	18.7 (15.5-23.4)	0.60 (0.21-0.84)	.209	.187
GGT, U/L	12.1 (10.3-13.9)	12.8 (10.9-15.7)	13.9 (11.5-17.5)	1.10 (0.01- 2.40)	.004	.008
hs-CRP, mg/l	0.36 (0.24-0.67)	0.48 (0.30-1.58)	0.55 (0.27-1.28)	0.07 (0.06-0.13)	.007	.048
Albumin, mg/l	44.5 (43.4-46.8)	45.0 (44.3-47.2)	46.8 (44.7-47.5)	2.30 (0.09-2.90)	.004	.030
Cystatin C, mg/dL	0.84 (0.78-0.90)	0.88 (0.85-1.00)	0.92 (0.87-1.01)	0.04 (0.01-0.04)	<.001	0.87

Data are presented as mean ± SD or median (interquartile range), item risk difference (RD) between the MHO and MUO groups, confidence interval (CI). Categorical variables were compared by chi-quadrate test; Group comparisons were analyzed by a Mann-Whitney-Wilcoxon test or t-test depending on the distribution of data. [a]*P* values for Normal weight vs. MHO vs. MUO. [b]*P* values for MHO vs. MUO. Abbreviations: MHO, metabolically healthy obesity, MUO, metabolically unhealthy obesity, BMI body mass index.

Table 2. Overview of the parameters used to define the metabolic health status

	Normal weight controls	**MHO**	**MUO**	**RD (95% CI)**	***P*value[a]**	***P* value[b]**
n (%)	212 (46.0)	173 (38.0)	73 (16.0)			
HDL, SDS	0.11 ± 0.9	-0.63 ± 0.73	-1.51 (1.03-2.01)	0.88 (0.74-1.12)	<.001	<.001
Triglycerides, SDS	-0.14 ± 0.88	0.49 (0.17-0.85)	1.78 (0.74-2.14)	1.29 (0.91-1.38)	<.001	<.001
Fasting glucose, mmol/L	4.78 (4.53-5.01)	4.87 (4.70-5.05)	4.95 (4.69-5.17)	0.08 (0.01-0.19)	.009	.047
Systolic BP, SDS	-0.02 ± 0.63	0.18 (0.17-0.64)	0.56 (0.18-1.10)	0.38 (0.07- 0.54)	<.001	.003
Diastolic BP, SDS	0.13 ± 0.47	0.44 (0.16-0.76)	0.63 (0.24-1.01)	0.19 (0.14-0.33)	<.001	.002

Data are presented as mean ± SD or median (interquartile range), item risk difference (RD) between the MHO and MUO groups, confidence interval (CI). comparisons were analyzed by a Mann-Whitney-Wilcoxon test or t-test depending on the distribution of data. [a]P values for Normal weight vs. MHO vs. MUO. [b]P values for MHO vs. MUO. Abbreviations: MHO, metabolically healthy obesity, MUO, metabolically unhealthy obesity, HDL-C high density lipoprotein cholesterol, BP blood pressure.

Table 3. Results of hierarchical regression analyses between uric acid-SDS and each parameters used to define metabolic health status

Variables	**R^2**	**B**	***P* value**
Fasting glucose, mmol/L	.538	.135	**.081**
Triglycerides, SDS	.542	.269	**<.001**
HDL, SDS	.768	-.247	**<.001**
Systolic BP, SDS	.574	2.57	**<.001**
Diastolic BP, SDS	.626	1.87	**<.001**
Cystatin, mg/dL	.441	.148	**<.001**
C-peptide, nmol/L	.559	.161	<.001

Abbreviations: β = regression coefficient, HDL, high-density lipoprotein. All calculations were adjusted for sex and age.

Table 4. Results of logistic regression models analysis of clinical and metabolic variables including MUO status as the dependent variable

Model1 (Nagelkerke R2 0.060)	**OR**	**95% CI Exp(B)**	***P* value**
Age	0.98	0.8-1.2	.777
Female gender	1.35	0.7-2.6	.214
Pubertal development stage			
Pre-pubertal	1.90	0.1-4.3	.648
Pubertal	1.40	1.1-4.4	**.010**
Waist circumference SDS	2.50	1.2-6.4	**.017**
Model 2 (Nagelkerke R2 0.353)	**OR**	**95% CI Exp (B)**	***P* value**
Uric acid SDS	1.61	1.1-2.6	**.004**
C-peptide (nmol/l)	4.05	3.5-91	**.003**
Albumin (g/l)	0.98	0.8-1.2	.829
hs-CRP (mg/l)	0.93	0.7-1.1	.414

Abbreviations OR = Odds Ratio per unit increase, CI = Confidence interval, MUO, metabolically unhealthy obesity. Male and post-pubertal stage as references. Model 1: unadjusted for confounding factors, Model 2: adjusted for age, sex, pubertal stage and BMI-SDS.

3 ZUSAMMENFASSUNG DER ARBEIT

Dissertation zur Erlangung des akademischen Grades Dr. rer. med.

Serum-Harnsäure-Spiegel als Indikator für metabolisch ungesunde Fettleibigkeit bei Kindern und Jugendlichen

eingereicht von: Edrienny Patrícia Alves Accioly Rocha

angefertigt an: Universität Leipzig, Klinik und Poliklinik für Kinder und Jugendliche der Universität Leipzig in Zusammenarbeit mit LIFE-Child, Leipziger Forschungszentrum für Zivilisationserkrankungen (LIFE)

betreut von: Prof. Dr. med. Wieland Kiess
Dr. rer. med. Mandy Vogel

Eingereicht June 2018

Übergewichtige Personen, die keine fettleibigkeitsbedingten metabolischen Komplikationen zeigen, wurden als "metabolisch gesund fettleibig" (MHO, Metabolically healthy obesity) definiert. Im Gegensatz zu metabolisch ungesunden fettleibigen (MUO, Metabolically unhealthy obesity) Individuen zeigen MHOs keine metabolischen Störungen wie Bluthochdruck, Dyslipidämie, Insulinresistenz und Entzündung [50]. Aufgrund des Mangels an allgemein akzeptierten Kriterien ist die genaue Definition des MHO-Status jedoch immer noch umstritten. Es wird allgemein angenommen, dass die MHO-Definition von der Einführung zusätzlicher Biomarker profitieren könnte, welche wiederum zur Klärung der zugrunde liegenden Mechanismen metabolischer Komplikationen herangezogen werden können [24]. Darüber hinaus hat sich die klinische Forschung hauptsächlich auf Erwachsene konzentriert, und es liegen nur wenige Studien zu MHO bei jungen Menschen vor. Daher wird die Untersuchung des MHO-Status in der jungen Bevölkerung unter Verwendung gut etablierter und potentiell neuer Indikatoren als wesentlich angesehen, um einen positiven Beitrag zur Prävention und/oder Behandlung von zukünftigen fettleibigkeitsbezogenen Krankheiten zu leisten. Unter den möglichen neuen Biomarkern wurde festgestellt, dass Serumharnsäure (Serum-UA) eine wichtige Rolle als kardiometabolischer Risikofaktor [22] für Adipositas-assoziierte Komorbiditäten bei Kindern und Jugendlichen spielt. Dennoch

haben nur wenige Studien den Zusammenhang zwischen dieser biochemischen Variablen und MHO in der jungen Bevölkerung untersucht. Der Schwerpunkt der vorliegenden Studie lag auf der Identifizierung potenzieller klinischer und metabolischer Indikatoren, die zur Unterscheidung zwischen MHO- und MUO-Phänotypen beitragen können. Die anthropometrischen, klinischen und biochemischen Merkmale von 458 Kindern und Jugendlichen wurden analysiert und diskutiert. MHO- und MUO-Individuen repräsentieren 38% bzw. 16% der dieser Grupe. Der häufigste kardiovaskuläre Risikofaktor bei MUO-Patienten war Hypertriglyceridämie (54,2%), gefolgt von niedrigem Serum-HDL-C (45,8%), Hypertonie (19,5%) und gestörter Glukosetoleranz (14,7%). Zusammenfassend deuten diese Ergebnisse darauf hin, dass eine frühzeitige Identifizierung von MUO in der Jugend möglich ist, wodurch eine frühzeitige Erkennung möglicher metabolischer Komplikationen gewährleistet ist. Verglichen mit der MUO-Gruppe zeigten MHO-Individuen niedrigere Nüchterninsulinwerte, Triglyceride, Blutdruck, Nüchternglucose und höhere Insulinsensitivität sowie niedrigere Serumharnsäure-, hs-CRP-, Albumin- und C-Peptidspiegel. Interessanterweise wurden im Gegensatz zu früheren Studien in den MHO- und MUO-Gruppen ähnlich hohe Werte für die Marker der Leberfunktion, einschließlich der zirkulierenden Konzentrationen von ALT, AST und alkalischer Phosphatase, festgestellt. Dieses Ergebnis legt nahe, dass niedrigere Leberenzyme zu dem günstigen metabolischen Profil von MHO-Individuen beitragen könnten. Darüber hinaus fördert diese Forschung ein besseres Verständnis der Wirkung potenzieller Indikatoren, die verwendet werden können, um MHO von MUO zu unterscheiden, insbesondere mit dem Fokus auf Serum-UA. Die Ergebnisse dieser Arbeit zeigen, dass Serum-UA mit mehreren kardiometabolischen Risikofaktoren assoziiert ist, die normalerweise mit Fettleibigkeit in Verbindung gebracht werden, wie Serumtriglycerid SDS, systolischer Blutdruck, C-Peptid und Cystatin C. Keine signifikante Beziehung zwischen Glukose-SDS und Serum-UA-Spiegeln wurde gefunden. Höhere Serumspiegel von UA erwiesen sich als signifikanter Indikator für den MUO-Phänotyp. Höhere C-Peptid-Spiegel, Taillenumfangs-SDS und Pubertätstadium sind mit einer höheren Wahrscheinlichkeit des MUO-Status assoziiert. Umgekehrt zeigte das Geschlecht der Person keine signifikante Wirkung. Hs-CRP und Albumin waren keine signifikanten MUO-Indikatoren, wenn sie nach Alter, Geschlecht, Pubertät und BMI-SDS kontrolliert wurden. Die in dieser Arbeit präsentierten Ergebnisse könnten für eine bessere Unterscheidung zwischen MUO- und MHO-Phänotypen nützlich sein und adipositasbedingte Komorbiditäten frühzeitig im Leben behandeln. Längsschnittstudien in größeren Kohorten mit jüngeren Individuen werden als ein vernünftiger nächster Schritt

angesehen, um das Ergebnis dieser Arbeit zu bestätigen und zu erweitern. Mögliche zukünftige Untersuchungen könnten zusätzliche Eigenschaften und Wirkungen von MHO/MUO-Indikatoren betreffen. Zum Beispiel, wie der Serum-UA-Spiegel durch Konsum zuckergesüßter Erfrischungsgetränke und Alkohol beeinfluss wird.

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II. Supplement Material

Serum uric acid levels as an indicator for metabolically unhealthy obesity in children and adolescents

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2 Tables

Table 1. Metabolic healthy status

Criteria	Cardiovascular risk factors (CRF)	MHO	MUO
		No CRF	< 2 of the CRF
	Hypertension:		
	SBP ≥ 95th percentile or		
	DBP ≥ 95th percentile		
	Dyslipidemia:		
	TG ≥ 95th percentile or HDL-C < 5th percentile		
	Impaired fasting glucose ≥ 5.6 mmol/L		

MHO - Metabolically healthy Obesity; MUO - Metabolically unhealthy Obesity; SBP - Systolic blood pressure; DBP - diastolic blood pressure; TG - Triglycerides; HDL-C High density cholesterol

Table 2. Anthropometric and biochemical characteristics of normal weight controls, MHO and MUO children

	Normal weight controls	MHO	MUO	RD (95% CI)	*P* value[a]	*P* value[b]
n (%)	212 (46.0)	173 (38.0)	73 (16.0)			
Baseline characteristics, anthropometric						
Age, years	11.1 ± 2.98	11.2 ± 2.64	11.4 ± 2.84	0.20 (0.03-0.98)	.610	.785
Male sex, n (%)	109 (51)	96 (55)	34 (49)		.496	.496
waist to height ratio, cm	0.42 ± 0.03	0.54 (0.51-0.58)	0.56 (0.53-0.61)	0.02 (0.00-0.04)	<.001	.004
Neck circumference, SDS	-0.12 ± 0.78	1.37 (0.95-1.72)	1.54 (1.04-2.10)	0.17 (0.02-0.41)	<.001	.006
Biochemical parameters						
Total-cholesterol, mmol/L	4.04 (3.68-4.52)	4.11 (3.71-4.65)	4.37 (3.88-4.99)	0.26 (0.01-0.45)	.043	.039
LDL-C, mmol/L	2.23 (1.87-2.64)	2.43 (2.05-2.84)	2.73 (2.26-3.17)	0.30 (0.08-0.46)	<.001	.004
Fasting insulin, µU/L	7.86 (5.55-10.3)	14.2 (11.0-16.8)	17.3 (13.0-20.3)	3.10 (4.42-9.64)	<.001	<.001
HbA1C, %	5.02 ± 0.31	5.08 ± 0.36	5.12 (4.91-5.30)	0.04 (0.02- 0.13)	.033	.379
Haemoglobin, g/dL	13.07 ± 0.94	12.8 ±1.02	13.1 (12.6-13.5)	0.30 (0.00-0.50)	.210	.429
Red blood cell, 10^12	4.70 ± 0.33	4.72 ± 1.56	4.76 (4.54-4.95)	0.04 (0.04-0.13)	.131	.078
Hematocrit, %	0.38 (0.36-0.39)	0.37 (0.36-0.40)	0.38 (0.37-0.40)	0.01 (0.00-0.01)	.561	.339
White blood cell, 10^9L	5.74 ± 1.55	6.45 ± 1.56	6.60 (5.80-7.60)	0.15 (0.79-0.99)	<0.001	.133
Platelet, 10^9L	279.2 ± 58.8	274 (248-315)	286 (264-331)	12.0 (2.99-32.0)	.040	.052
Neutrophils, 10^9L	2.48 (2.01-3.12)	3.22 (2.56-3.96)	3.65 (2.44-4.31)	0.43 (0.12-0.63)	.002	.173
Monocyte, 10^9L	0.49 (0.38-0.57)	0.51 (0.42-0.63)	0.50 (0.42-0.62)	0.01 (0.12-0.63)	.012	.907
Alkaline phosphatase, U/l	3.65 ± 1.46	4.01 (3.23-4.62)	4.40 (2.59-5.02)	0.39 (0.23- 0.54)	.029	.442
Total protein (g/l)	68.1 ± 4.30	70.4 ± 3.53	70.7 (68.2-72.6)	0.30 (0.30-1.40)	.002	.264
Creatine kinase, µkat/l	1.99 (1.55-2.71)	1.89 (1.49-2.45)	1.79 (1.55-2.49)	0.18 (0.10- 0.28)	.108	.864
Lpa-Lipoprotein-a, g/l	0.09 (0.04-0.29)	0.10 (0.03-0.31)	0.07 (0.03-0.20)	0.03 (0.00- 0.04)	.517	.211
Interleukin 6, pg ml	1.76 (1.50-3.07)	1.50 (1.50-2.78)	1.86 (1.50-3.01)	0.36 (0.01- 0.50)	.723	.325
Testosterone, nmol/l	0.40 (0.08-2.28)	0.39 (0.08-0.98)	0.37 (0.11-1.32)	0.02 (0.08-0.22)	.968	.931
Estradiol, pmol/l	35.8(18.4-115.5)	18.4 (17.0-94.4)	41.6 (16.4-86.8)	23.2 (0.02-21.1)	.050	.042
Shbg, nmol/l	77.5 (47.3-116)	92.1(60.5-130)	78.2 (42.2-117)	13.9 (27.2-39.0)	.123	.158

Data are presented as mean ± SD or median (interquartile range), item risk difference (RD) between the MHO and MUO groups, confidence interval (CI). Group comparisons were analyzed by a Mann-Whitney-Wilcoxon test or t-test depending on the distribution of data. [a]P values for Normal weight vs. MHO vs. MUO. [b]P values for MHO vs. MUO. Abbreviations: MHO, metabolically healthy obesity, MUO, metabolically unhealthy obesity, LDL-C low high density lipoprotein cholesterol, ALT alanine aminotransferase, AST aspartate aminotransferase, GGT gammaglutamyltransferase, HbA1c glycated hemoglobin A1c, Lp(a) Lipoprotein-a, hs-CRP high sensitivity C-reactive protein.

III. Erklärung über die eigenständige Abfassung der Arbeit

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbstständig und ohne unzulässige Hilfe oder Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Ich versichere, dass Dritte von mir weder unmittelbar noch mittelbar eine Vergütung oder geldwerte Leistungen für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen, und dass die vorgelegte Arbeit weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungs-behörde zum Zweck einer Promotion oder eines anderen Prüfungsverfahrens vorgelegt wurde. Alles aus anderen Quellen und von anderen Personen übernommene Material, das in der Arbeit verwendet wurde oder auf das direkt Bezug genommen wird, wurde als solches kenntlich gemacht. Insbesondere wurden alle Personen genannt, die direkt an der Entstehung der vorliegenden Arbeit beteiligt waren. Die aktuellen gesetzlichen Vorgaben in Bezug auf die Zulassung der klinischen Studien, die Bestimmungen des Tierschutzgesetzes, die Bestimmungen des Gentechnikgesetzes und die allgemeinen Datenschutzbestimmungen wurden eingehalten. Ich versichere, dass ich die Regelungen der Satzung der Universität Leipzig zur Sicherung guter wissenschaftlicher Praxis kenne und eingehalten habe.

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Datum	Unterschrift

IV. Curriculum Vitae

Persönliche Daten

Edrienny Patrícia Alves Accioly Rocha
Geboren am 10.07.1984 in Recife – Pernambuco - Brasilien

Ausbildung

Seit 10/2014 — Promotion in Medizin: Universität Leipzig

- Doktorvater: Prof. Wieland Kiess, Direktor der Klinik für Kinder und Jugendmedizin, Uni-Leipzig.

03/2012 - 02/2014 — Master in Krankenpflege: Universidade de Pernambuco – Pernambuco - Brasilien.

- Verbundprogram für den postgraduierten Studiengang der Krankenpflege.
- Masterarbeit: Hohes Risiko für Typ-2-Diabetes mellitus bei Kindern und Jugendlichen mit Adipositas oder Übergewicht.

02/2008 - 02/2012 — Lehrlizenz : Universidade Federal de Pernambuco – Pernambuco - Brasilien.

- Verleiht den Titel: Hochschulabsolvent - Magistererium. Erfolgreicher Abschluss zur Krankenpflege (eine Lizenz zum Lehren).

02/2007 - 02/2012 — Bachelor: Universidade Federal de Pernambuco – Pernambuco - Brasilien.

- Erfolgreicher Abschluss zur Krankenpflege.

Praxiserfahrungen

04/2013 - 05/2014 — Lehrer für die Ausbildung zur Krankenpflege (Hochschule) - Pernambuco - Brasilien.

- chirurgische Krankenpflege, psychiatrische Krankenflege, Kind und maternale Krankeflege.

05/2012 - 05/2013	Lehrer der Krankenhauspraxis von Studenten im 5. Semester des grundständigen Programms in der Abteilung der öffentlichen Krankenpflege – Universität Pernambuco - Brasilien.
09/2012 - 10/2013	Krankenschwester im Hospital IMIP – Pernambuco, Brasilien. ▪ Arbeit in der stationären Einheit mit Kinder als Patienten mit Herzerkrankungen und in der Intensivstation.
02/2012 - 12/2012	Krankenschwester im Hospital das Clinicas – Pernambuco - Brasilien. ▪ Arbeit in der Onkologie mit erwachsenen Patienten.

Stipendia

07/2017 – 08/2017	Sommerschule: "Advanced Statistik", Universität Ulm.
01/2013 – 02/2013	Public Health Collaborative Field Course, David Rockefeller Center for Latin American Studies (DRCLAS), Harvard University.
06/2012 – 07/2012	Medizinische Sommerschule, Perspektiven für die globale Gesundheit im 21. Jahrhundert – Universität Ulm. Deutschland.

Sprachkenntnisse

Portugiesisch (Muttersprache) Englisch (sehr gute Kenntnisse)

Spanisch (sehr gute Kenntnisse) Deutsch (gute Kenntnisse)

Computerkenntnisse

Microsoft Office (gute Kenntnisse)

R studio Software (Grundkenntnisse)

SPSS Software (Grundkenntnisse)

Leipzig,

V. List of publications and conference participations

Publications of the dissertation

Rocha, Edrienny P.A .A. Vogel, Mandy, Stanik, Juraj, Körner, Antje, Kiess, Wieland. **Serum uric acid levels as an indicator for metabolically unhealthy obesity in children and adolescents**. Hormone pediatric research

Other Publication

De Aquino, Camila A, Rocha, Edrienny P.A .A., Nascimento, Eloine, A. **Pilgrimage (Via Crucis) to the diagnosis of leprosy** (2015), Ver enferm UERJ, Rio de Janeiro, 2015 mar/abr; 23(2):185-190.

Posters in international conferences

E.A.A. Rocha, M. Voge, J. Stanik, A. Körner, W. Kiess: **Serum acid uric levels as an indicator for metabolically unhealthy obesity in youth: results from a population-based cohort in Germany**". Absctract und Poster, 54th EASD Annual Meeting which will be held in Berlin, from 1 - 5 October 2018.

VI. Acknowledgments

I am sincerely grateful for my supervisor Prof. Kiess, who not only accepted me as a Ph.D. student here in Germany, but also offered me the opportunity to become part of the LIFE-Child Team through my dissertation. He helped me through my professional growth during the years and taught me about scientific research. His way of encouraging me towards independent work was essential to this research.

My profound gratitude to Dr. Mandy Vogel, who made this extensive statistical project possible. Thanks to her aid, I could walk through the jungle of data and results. Not to mention that her clear view to my work improved a lot the quality of this study, as well as her support, time and encouragement. Furthermore, I would like to thank all of the LIFE Child team, without whose daily work and effort this study would not have been possible.

I also thank Dr. Juraj Stanik, Dr. Diana Pietzner, Dr. Anja Willenberg for their advices and review of the manuscripts. In addition, I would like to thank Prof. Dr. Körner for her warm and professional support.

I would also like to thank all my friends particularly the Brazilian in Germany (Fred, Rejane, Patrícia, Matheus, Murilo and Yan), nonbrazilian in Germany (Phillip, Rito, Daria, my dear Natascha, Debora, Chiara), nonbrazilian around the world (Sarina, Anna, Lydia, Tebogo), German in Germany (Charlotte, Eva, Imanuel, Selma, Anett, Lea, Josi, Conny, Mandy), and Brazilian in Brazil (Camilla, Alamo, Cássia, André, Carol, Bruno, Samantha, Juliana, Tabata, Dreyd, Gisele, Leilane) who have been very supportive in recent years and who were there to listen.

I deeply thank my friends and reviewers, Karsten Przybilla, Dr. Alexandra and Dr. Daniele for their valuable advice and constructive criticism both in the data analysis and the text that helped notably to improve my work. Yours expert advice and help have been very useful to me in many matters.

Merci beaucoup to Hugo Mahiou who has stood by me in recent years and has been a constant source of support during our studies, most recently during the Ph.D. phase, as well as for his great care, patience and unimpeded belief in me.

My deepest gratitude is to Henrique Vitorio who helped me make the accomplishment of my Ph.D. possible. Thank you so much for your love and for being my support at all times.

There are many people at the University of Leipzig and the Ph.D. group at the LIFE-Child, who have made this work possible and are not listed. My warm thanks to all of them for the support they provided me over the years. I am grateful to every participant who gave up their

time to allow this PhD to happen. The children were a pleasure to work with and I am thankful to have been allowed access into their lives.

I would especially like to thank my core family: Elódia (mom), Edvaldo (dad), Lourdes (grandmother), Edílson (uncle) and Sonia, and Elizabete (aunts) for all their love, and for all the opportunities they've given me. Of course, I thank all the others relatives, but no names here. This is could be a problem in a big family. Finally, I thank CAPES (Coordination of Improvement of Higher Level Personnel, Brazil) for funding this Ph.D. project.

III. DARSTELLUNG DES EIGENEN BEITRAGS

Die Idee zum Thema der Dissertationsarbeit habe ich gemeinsam mit meinem Doktorvater Prof. Dr. med. Wieland Kiess erarbeitet. Die Daten stammen aus der LIFE Child Studie (Quante et al., 2012, Poulain et al., 2017). Bei der Datensammlung und Datenbereinigung habe ich als studentische Hilfskraft mitgeholfen. Die Analyse der Daten und die Interpretation der Ergebnisse habe ich selbstständig durchgeführt. Dr. rer. medic. Diana Pietzner, Dr. rer. medic Mandy Vogel, Prof. Dr. med. Antje Körner, MUDr Ph.D Juraj Stanik und Dr. rer. nat. Anja Willenberg haben mich dabei freundlicherweise unterstützt. Die Formulierung des Manuskriptes habe ich selbst erarbeitet. Ideen und Kommentare der Co- Autoren habe ich im Verlauf eingearbeitet.

Prof. Dr. med. Wieland Kiess

Dr. rer. medic. Mandy Vogel

Prof. Dr. med. Antje Körner

MUDr Ph.D Juraj Stanik