Thesis

URINE-METABOLOMICS AS NON-INVASIVE MARKERS FOR ADIPOSITY IN CHILDREN

Preliminary Results of the HAPHC-Study

submitted by

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Graz, 21.01.2025 *Mechthild Aloisia Lagger, m.p.*

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Zusammenfassung

HINTERGRUND: Die vorliegende Diplomarbeit wurde im Rahmen eines größeren Projekts, des „Health and Academic Performance with Happy Children (HAPHC)"-Projekts verfasst. Diese dreijährige prospektive Kohortenstudie untersuchte den Einfluss täglich in den Unterricht integrierter körperlicher Aktivität auf die Gesundheit und kognitive Leistung von Volksschulkindern mit dem Ziel, Maßnahmen gegen Fettleibigkeit zu entwickeln. Eine standardisierte Ultraschallmethode hat sich als zuverlässig zur Ermittlung von subkutanem Fettgewebe (SAT) gezeigt. Neues Wissen über Metabolomics hat deren Potenzial zur Identifizierung von Biomarkern für bestimmte Erkrankungen, inklusive Fettleibigkeit, durch die Analyse von biologischen Flüssigkeiten gezeigt.

FRAGESTELLUNG: Diese schriftliche Arbeit untersucht, ob Veränderungen des mittels Ultraschalls gemessenen SAT nach einem Schuljahr mit Metaboliten im Harn assoziiert und ob bestimmte Urinmetaboliten bei Kindern als potenzielle prädiktive Biomarker für den Zuwachs von SAT herangezogen werden könnten.

METHODE: Die Daten von 343 Kindern im Alter von 6-11 Jahren aus drei verschiedenen öffentlichen Volksschulen in Graz, Österreich, wurden retrospektiv nach einem Schuljahr analysiert. Spontanurin-Proben der Erstmessungen wurden mittels Nuklearmagnetresonanz (NMR) -Spektroskopie analysiert. Die SAT-Werte wurden mit einer standardisierten Ultraschalltechnik bei den Erstmessungen und den Folgemessungen ermittelt. Die erhobenen Daten wurden statistisch auf Assoziationen der Veränderung des SAT und Metaboliten-Konzentrationen im Harn analysiert. Zur Korrektur der p-Werte wurde die False Discovery Rate (FDR) angewendet.

ERGEBNISSE: Siebenundfünfzig der 150 untersuchten Metaboliten wurden in den untersuchten Harnproben in mindestens 5 Proben nachgewiesen, und genauer auf Assoziationen mit SAT-Veränderungen untersucht. N-Isovaleroylglycin (IVG) wurde häufiger in den Proben von Kindern, welche ein vermindertes SAT bei der ersten Folgeuntersuchung aufwiesen ($F_{(1.341)} = 6.301$, $p = 0.013$), festgestellt, wobei diese Assoziation nach FDR nicht signifikant war ($p_{corr.} = 0.14$). Die IVG-Konzentration war signifikant mit der Gesamt-SAT-Abnahme korreliert ($r = -0.128$, $p = 0.018$). Wenn nur die Fälle mit einer SAT-Zunahme inkludiert wurden (n=205 Kinder), zeigte IVG bei den Erstmessungen, zusammen mit D-Mannose und dem Body Mass Index (BMI), einen

signifikanten Vorhersagewert für SAT-Veränderungen ($F_{(9.195)} = 7.019$, $p < 0.001$). Die Konzentration von Hippursäure war ebenso mit einer SAT-Abnahme bei der Folgeuntersuchung assoziiert ($F_{(1.341)} = 6.692$, $p = 0.01$, $p_{corr} = 0.14$), hingegen wurden Dimethylamin, Taurin, D-Mannose und -Glukose mit einer Zunahme von SAT assoziiert. Bemerkenswert ist, dass in Harnproben von 76 der insgesamt 343 Kinder (22.16%) Glukose nachgewiesen wurde. Die SAT-Dicke der Mädchen war bei der Erst- und Folgeuntersuchung höher als die der Buben. Der SAT-Zuwachs war stark mit dem Gewicht assoziiert, aber nicht mit der Körpergröße.

DISKUSSION UND SCHLUSSFOLGERUNG: Die Untersuchungen zeigten, dass die Veränderung des SAT bei der Folgeuntersuchung mit oben genannten Metaboliten assoziiert werden konnte. Die Hypothese, dass bestimmte Harnmetaboliten die SAT-Veränderungen vorhersagen können, wurde nur bedingt bestätigt. Zudem sind die Hinweise darauf, dass die in der Studie gemessenen Metaboliten (wobei deren Auswahl methodenlimitiert ist), SAT-Veränderungen vorhersagen können, schwach. Um klare Ergebnisse zu erhalten, ist es wahrscheinlich nötig, die Veränderungen des SAT bei der 2. und 3. Folgeuntersuchung abzuwarten. Wegen der Übereinstimmungen mit vorherigen Erkenntnissen sollte das Potenzial dieser Metaboliten als prognostische Biomarker weiter untersucht werden. Die in 76 Urinproben festgestellte Glukose erfordert weitere Untersuchungen bei Betroffenen. Eine Tendenz zur höheren Prävalenz von Glukosurie und höheren SAT-Dicke wurde in Mädchen verglichen mit Buben festgestellt, was mit früheren Ergebnissen übereinstimmt und weiterer Erforschung möglicher Ursachen bedarf. Diese Resultate sind relevant, da sie eine neue Möglichkeit zur Feststellung von Adipositas in früheren Stadien bei Kindern darstellen, als herkömmliche Methoden es tun. Dies wird helfen, ernsthaften Gesundheitsrisiken vorzubeugen. Es wäre ein großer Schritt für die Präventivmedizin, wenn die Analyse solcher Biomarker in Routinescreenings eingesetzt würde.

Abstract

BACKGROUND: This thesis is part of a bigger project (the "Health and Academic Performance with Happy Children (HAPHC)"-project), a 3-year controlled intervention study, that observed the impact of daily integrated physical activity on health and academic performance of primary school children, aiming at counteracting obesity (OB). Applying ultrasound (US) in a standardized protocol has been found a reliable tool for measuring the thickness of subcutaneous adipose tissue (SAT). New knowledge in the field of metabolomics has shown the potential of identifying biomarkers for certain diseases, including OB, by analysing biofluids.

AIMS AND OBJECTIVES: This thesis investigates whether changes of SAT, measured by US, after one schoolyear can be related to metabolites detected in urine and whether urinary metabolites may serve as predictive biomarkers for an increase of SAT.

METHODS: Data of 343 children aged 6-11 years from three different public primary schools in Graz, Austria, were analysed retrospectively after one schoolyear. Spot on urine samples from baseline measurements were analysed via nuclear magnetic resonance (NMR) spectroscopy and SAT-thickness was measured by the 8-sites standardised US approach at baseline and in the first follow-up (FUP-1) after one schoolyear. The retrieved data were then statistically analysed for possible associations of SAT-thickness-changes with concentrations of urinary metabolites. False discovery rate (FDR) was applied to control alpha-error inflation.

RESULTS: Fifty-seven of the 150 metabolites studied could be detected in the analysed urine samples in at least 5 samples and were further assessed for associations regarding SAT-changes. N-isovaleroylglycine (IVG) was detected at a higher frequency in the urine samples of children whose SAT had decreased at follow-up ($F_{(1.341)} = 6.301$, $p = 0.013$). However, the relation did not remain significant after FDR ($p_{corr} = 0.14$). The concentration of IVG was significantly correlated with total SAT-decrease ($r = - 0.128$, $p = 0.018$). When only including cases with an increase of SAT (n=205 children), IVG at baseline together with D-mannose and BMI significantly predicted SAT fold-changes ($F_{(9.195)} = 7.019$, $p < 0.001$). The concentration of hippuric acid (HA) was also associated with SAT-decrease at FUP ($F_{(1.341)} = 6.692$, $p = 0.01$, $p_{corr} = 0.14$), whilst dimethylamine, taurine, D-mannose and D-glucose were found to be related with SAT-increase. Notably, in urine samples of 76 out

of 343 children (22.16%) glucose was detected. SAT-thickness of girls at baseline and FUP was higher than of boys and SAT-increase was highly associated with weight, but not height.

DISCUSSION AND CONCLUSION: The results showed that change of SAT at follow-up can be associated with the above-mentioned metabolites. The hypothesis that certain metabolites may predict SAT-changes was tentatively confirmed. However, the potential of metabolites measured in this study (whereby their selection is limited methodically) to predict SAT-change is weak. To see clear results, it is probably necessary to await the SAT fold-changes of the second and third follow-up. Still, some of the results align with previous findings on these metabolites, therefore their potential as prognostic biomarkers should be further explored. The glucose detected in 76 samples, requires further testing. In girls a tendency towards higher prevalence of glucosuria and higher SAT-thickness was found compared to boys, which aligns with previous findings and demands further research.

These are relevant results because they allow for a new way of detecting adiposity in children in earlier stages than traditional methods, which will help to prevent serious health consequences. It would be a big step forward for preventive medicine to routinely implement the analysis of such biomarkers in routine urine screenings.

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List of Abbreviations

AA	Amino acid
AAA	Aromatic amino acid
AC	Acylcarnitine
ADP	Air displacement plethysmography
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
BCAA	Branched chain amino acid
BIA	Bioelectrical impedance analysis
BMI	Body mass index
B-mode	Brightness-modulation
CDC	Center of Disease Control and Prevention
CKD	Chronic kidney disease
CMP	Cardiomyopathy
CRC	Colorectal carcinoma
CRP	C-reactive protein
CT	Computed tomography
CVD	Cardiovascular disease
COSI	Childhood Obesity Surveillance Initiative
DMA	Dimethylamine
DXA	Dual-energy X-ray absorptiometry
EDC	Endocrine disrupting chemicals
FDR	False discovery rate
FFM	Fat free mass
FM	Fat mass
FT	Front thigh
FUP	Follow-up
GC-MS	Gas chromatography- mass spectroscopy
GLA	Gut-liver-axis
GLM	General linear model
HA	Hippuric acid
HAPHC	Health and Academic Performance with Happy Children
HDL	High density lipoprotein
HOMA	Homeostasis model assessment
IGF	Insulin-like growth-factor
IL-6	Interleukin 6
IOTF	International Obesity Task Force
IP	Intestinal permeability
IR	Insuline resistance
IVA	Isovaleric Academia
IVG	Isovalerylglycine
LA	Lower abdomen
LC-MS	Liquid chromatography-mass spectroscopy
LDL	Low density lipoprotein
MetS	Metabolic syndrome
MI	Mass index

MRI	Magnetic resonance imaging
NAFLD	Non-alcoholic fatty liver disease
NCD	Non-communicable diseases
NMR	Nuclear magnetic resonance
Ns	Non-significant
NW	Normal weight
OGTT	Oral glucose tolerance test
OW/OB	Overweight/Obesity
PA	Physical activity
PAAC	Physical activity across the curriculum
POCT	Point of care test
qMRI	Quantitative magnetic resonance imaging
SAT	Subcutaneous adipose tissue
SD	Standard deviation
SDOH	Social Determinants of Health
SIBO	Small intestinal bacterial overgrowth
TBW	Total body water
TG	Triglycerides
TMAO	Trimethyl aminoxide
TNF-alpha	Tumor necrosis factor-alpha
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
US	Ultrasound
WC	Waist circumference
WHO	World Health Organization
WHR	Waist-hip ratio

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1. Introduction

Child obesity (OB) is a growing burden worldwide (1) and correlates with many health issues and diseases such as hyper lipid anaemia, heart conditions, or hypertension, hyperinsulinemia, and early atherosclerotic changes, arising at later timepoints in life (2).

1.1 Definition of Overweight and Obesity by BMI

Being of major significance for public health, the development of child OB should be closely evaluated. This task seems almost impossible since there are many different definitions of OB in children and so far, no standardised definition has been agreed on (1). The World Health Organization (WHO) defines the complex disease of OB as excessive adipose tissue deposits implicating a health threat as it is strongly correlated with increased morbidity and mortality (3) (4). Hence, in the medical definition of OB, a state of elevated body fat is described, not increased body weight (5). However, in most primary care settings access to directly measure fat is limited (3), which is why it is usually obtained by anthropometrics, to get an estimate of one's body composition.

Hereby, the most often applied clinical approach to define overweight (OW) and OB in adults, adolescents and children is the body mass index (BMI) (3). It is calculated by weight/height^2. In children and adolescents BMI is scaled according to their age and sex as children grow in body size. There exist numerous definitions of OW/OB in children with differing cut-off points. Aiming to proclaim more rational and internationally derived cut-off points to facilitate comparison of prevalence rates of children and adolescents with OW/OB, Cole et al. (2000) created centile curves for each of six international surveys (1). The commonly used definition for children with OW/OB of the International Obesity Task Force's (IOTF) BMI cut-offs proposed by (Cole et al. 2000; 2007) derives from adult BMI cut-offs (6). The Center for Disease Control and Prevention (CDC) defines the OW-range in children and adolescents with values between the 85th and 94th percentile and ≥ 95th percentile as OB range (7). The WHO definition for children aged 0-5 years states, that a BMI >+2 SD is OW and a BMI >+3 SD stands for OB (8). For 5–19-year-olds OW is defined by a BMI > +1 SD and OB by a BMI >+2 SD (9) (10).

To assess prevalence and to compare numbers, a standardised method is needed for epidemiologic research. Every study published using these definitions must use the precise related terms and name the applied references (11). In the following section, the rising prevalence of OW/OB will be described, along with its disparities among the population.

1.2 Prevalence of Overweight and Obesity

According to the WHO, in 2022, one of eight individuals worldwide was living with obesity (OB). Thirty-seven million children younger than 5 years of age were overweight (OW), and more than 390 million children and adolescents between 5-19 years were affected, with 160 million living with OB. Since the 1990s, OB in adults has doubled and adolescent OB has quadrupled. From 1990 to 2022 the prevalence of OW/OB in children and adolescents between 5-19 years of age has increased significantly from 8% to 20%, equally in girls and boys (4).

Globally, health risks have shifted from former risks related to poverty like malnutrition and unclean water to risks resulting from an increased prevalence of OW/OB due to physical inactivity and other obesogenic factors to the burden of non-communicable disease (NCD) (12) (13). While OW/OB used to be regarded as an issue of high-income countries, it has been increasing in low- and middle-income countries (4).

In measurements between 2018 and 2020, the WHO Childhood Obesity Surveillance Initiative (COSI) found that in the age group of 6-to 9-year-old European children 29% were OW/OB according to the WHO-definitions. The prevalences showed large contrasts between countries. Sex differences were observed in terms of the prevalence being slightly elevated among boys, while OW prevalences increased with age in boys and girls (14).

In Austrian 8-year-olds the prevalence of OW and OB regarding the WHO-definition was at 25.3% and 9.1%, respectively. Prevalence by parental education based on the WHO-definition showed that in children with parents of low educational level the prevalence of OW/OB was higher (31.5%) than in children with parents of high educational level (15.3%) (14). This association has been described before (15). Interpreting these findings, one can conclude that for non-white children of low socio-economic backgrounds, the risk to develop OW/OB is elevated, which furthermore is aggravated by restricted availability to health services that may prevent uncontrolled weight gain and its consequences (7).

Ng et al. (2014) reported that about two in three of people with OB live in developing countries (16). Figure 1, from The Lancet (2024), presents the proportion of girls (upper row) and boys (lower row) aged 5-19 years from thin to obese globally in the years 1990 and 2022 (17). From the density plot the estimated proportions of the condition thin to obese can be read for each country, the WHO-references for OW/OB were applied.

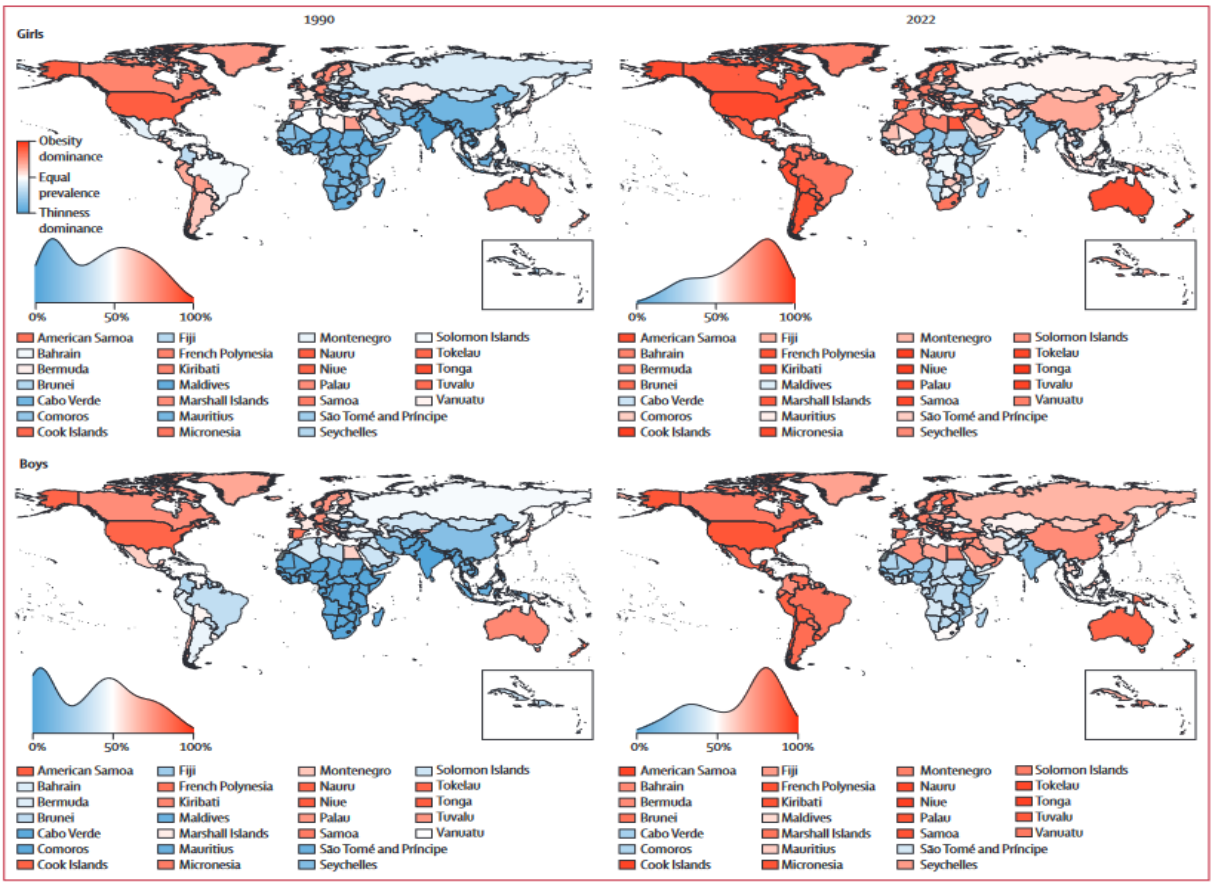


Figure 1. 'Proportion of double burden from obesity for school-aged children and adolescents (age 5–19 years)' (17)**: p. 1039 as Fig. 5 in The Lancet (2024)**

Moreover, in developing countries, OW and malnutrition may occur side by side (11). This concurrent appearance of undernutrition and OW/OB has been described as the double burden of malnutrition and has increased in the poorest low- and middle-income countries, which is due to the rising prevalence of OW. The sudden accessibility to cheap, ultra-processed drinks and food, along with a substantial decrease of pastime requiring physical activity, while there is an insufficient supply with micronutrients, are key drivers for such developments (18).

The COVID-19 pandemic has caused further increases of OB in children by coactive factors including restricted access to and lack of means to afford healthy nutritional food, declining aid in homes and constraints in physical activity (PA) (19) (20).

To recognise and prevent the development of OB early-on, this study aimed at testing for urinary metabolites correlated with changes of SAT. It is necessary to understand the multifactorial genesis of OB and its negative impacts on those affected to gain an overview of what biodynamic processes may be involved and why early diagnosis is important. Hence, this study aims to find urinary metabolites that can serve as predictive biomarkers.

1.3 Development of Obesity

While this thesis does not focus on the aetiology of obesity, this section briefly outlines some of the contributing factors to its development. The development of OW/OB happens, when calory intake is higher than demand, leading to an increase of adipose tissue mass. This imbalance is caused by obesogenic behaviours, which include reduced physical activity, while sedentary activities are enhanced, and the frequent intake of sugar sweetened drinks, low-nutrient, high saturated fat foods and reduced sleep periods (21). These behaviours are enhanced by multiple factors and their interactions. Other contributing factors include genetics, obesogenic environmental conditions, community setting (22) (4), socioeconomics, biology, behaviours and psychosocial traits (7). Biological factors influence the development of OW/OB. But just as well, OW/OB seem to influence physiological pathways by feedback-mechanisms. As cited by Russell & Russel (2019) (22), Keller and Bruce (2018) argue that food-intake and OB can influence brain structure and function, while processes in the brain determine dietary choices.
Different studies have concluded that the intestine's microbiome may contribute to the development of OB, metabolic syndrome (MetS) and non-alcoholic fatty liver disease (NAFLD) by augmenting energy intake in situations of a gut dysbiosis or small intestinal bacterial overgrowth (SIBO) (23) (24).
Sometimes single leading causes can be observed, like genetic syndromes, certain medications, diseases, or immobility. Living conditions, being obesogenic or not, are again determined by the structural setting, whether healthy affordable food is available, physical mobility is safe and accessible for all people and whether the district is well and adequately regulated (4). In addition, factors leading to OB are also often associated with preventable chronical illnesses, the death of adolescents (2), and economical burdens (25) (7).
In sum, it is difficult to account for the complexity of the development of OB, as it is caused by multiple factors and their interactions.

1.4 Related Health Problems

Children with OB have a 5 times higher risk to be obese as adults than children with normal weight (NW). A study by Simmonds et al. (2016) showed that about 55% of children with OB were remaining obese in adolescence, and that of adolescents with OB about 80% would be obese as adults, and 70% would remain obese over the age of 30 (26). Due to the pro-inflammatory effects of OB and behaviours causing the condition, physiology and metabolism are impaired, which may result in several diseases (27) and

severe physiological and psychological health impairments such as type 2 diabetes mellitus (T2DM), asthma, hypertension, cardiovascular illness, obstructive sleep apnoea, NAFLD (28), hepatocellular carcinoma, psychosocial problems, and depression. Also, a negative impact on reproductivity, bone constitution, and the evolution of certain cancers has been described. The overall quality of life, like moving or sleeping, are also affected (7) (13) (4). It has been determined that being a child or adolescent with OB (as classified by the BMI) is associated with a higher risk of cardiovascular disease, morbidity and mortality as an adult (29). To prevent these associated health burdens, interventions to fight the obesity pandemic are crucial (7). Such prevention programmes may entail great impact in primary schools, since they are implemented early and reach a lot of children regardless of their socio-economic backgrounds.

Several of these health risks associated with OB have been observed to co-occur and are collectively referred to as metabolic syndrome (MetS). MetS is an accumulation of cardiovascular and metabolic risk factors. Its four main components are elevated blood pressure, overweight, dyslipidaemia, and an impaired glucose metabolism (30). OB is generally acknowledged as a main cause for MetS, also in children (31), as it is correlated with metabolic perturbances such as insulin resistance (IR) due to the inflammatory processes activated (32). For children a general definition of MetS is still missing (33). Cook et al. (2003), one of the first publications on MetS in children, claimed that a combination of at least three of the following parameters defines paediatric MetS: waist circumference (WC) >90th percentile, RR >90th percentile, fasting glucose >110mg/dL, high density lipoprotein (HDL) cholesterol <40 mg/dL and triglycerides > 110 mg/dL (34). An adaption of these criteria was done a year after by de Ferranti et al. (2004): WC >75th percentile, triglycerides (TG) >100 mg/dL, HDL <50 mg/dL (35). Other definition criteria followed (36) (37) (31).

To date in scientific literature many different definitions of MetS in children exist (38). Reisinger et al. (2020) evaluated their differences and concluded that rather than one general definition, MetS should be defined according to sex, ethnicity or geographic zone (33). It is crucial to detect MetS early to prevent threatening outcomes. Especially in children, sex and age percentiles must be considered when assessing them for variables. However, often the metabolic changes in the peri-pubertal period, like the alternating body composition or IR are not considered (30).

MetS is a significant risk factor for cardiovascular disease (CVD) and other morbidities, increasing the risk of mortality by a factor of 1.5-2 in adults as well as in children.

Moreover, rising numbers of MetS in children with OB significantly correlate with the manifestations of metabolic diseases in adulthood (39). To avoid such developments, early recognition of a perturbed metabolic state via urinary metabolites and of adiposity via measuring SAT by US may help to prevent advanced development of the disease. In the next section, strengths and limitations of different known tools for measuring OW/OB will be shortly described. Considering these, it becomes clear that more easily applicable, cost-efficient, and reliable methods are required for a standardised approach of screening of OW/OB.

1.5 Measurement of Adiposity: Methods with their Strengths and Limitations

To prevent and treat OW/OB in children and adolescents, methods to accurately measure body fat are required. Existing specific procedures often come with a high technical expense, exposure to ionizing radiation and are restricted to institutional research. Widely used methods mostly fail in recognising abundant adipose tissue (40).

There exist numerous methods for evaluating obesity in children. Determination of body composition can be assessed molecularly and/or anatomically. However, in clinical practice the BMI and mass index (MI) are up to now the most used tools which also reflects the cut off values defining OW in children being defined and categorized by the BMI (41). Widely applied methods in the field are lacking standardization, resulting in different outcomes which is due to varying accuracy or reliability between techniques (42).

1.5.1 BMI LIMITATIONS

As a complementary body measurement for assessing a population over a period or for comparing different groups, BMI is a useful method. However, it has various disadvantages (11).

The BMI has been found to be a deficient tool to determine one's body composition, since it does not differ between lean and adipose tissue. Another problem in applying the BMI, regarding the WHO Expert Committee, is that in individuals shapes and sizes can vary from the norm, e.g. shorter or longer leg length than predicted for a certain height as stated by the WHO technical report series (1995) on Physical status: the use and interpretation of anthropometry, as cited by Kelso et al. (2020) (43). A different method for measuring relative body weight is the MI (MI=0.53m/(h·s)) which examines the sitting height and by that considers an individual's leg length (44) (45). Still, both techniques are not appropriate for assessing the amount of adipose tissue as they determine relative body weight (42) (46)

(43). Furthermore, showing a high specificity, the BMI comes with a low sensitivity, publications resulted in a 73-82% sensitivity to recognise critical obesity (47) (48) (3) as it has been revealed to not detect abundant body fat in 25-50% of children (40) (47) (3). Another limitation of the BMI is that its values are designed for mainly Caucasians and exclude the variety of ethnic groups (49). For example, Asian Indians with the same BMI show to have a higher amount of adipose tissue compared with Caucasians. As cited by Aggarwal et al. (2018) (10), Banerji et al. (1999) found that a mean BMI of 24.5 matched a mean body fat of 33% in Asian Indian men living in the US. Therefore, in Asian Indians already at a lower BMI, risks for health implications, like CVD begin to increase (50) (10). It has been shown that children or adolescents with the same BMI may largely differ in their SAT amount (>200%) indicating major body fat measurement errors when BMI is used to assess body fat in children (40). Zapata et al. (2023) found that 7% of children considered to be in the normal weight range (NW) and 62% categorised into the OW group according to BMI, by applying the definition by Cole et al., showed to have a total body fat percentage, applying Air Displacement Plethysmography (ADP), in the range of OB. Such findings imply a considerable number of misclassifications when diagnosing OW/OB in everyday clinical practice applying only BMI, leading to the underdiagnosis of children and adolescents at risk for cardiometabolic disease, which may lead to serious future consequences (3). Another effort of the present study was to underline the shortcomings of and unveil alternatives to the BMI. Hence BMI was avoided as variable, and SAT was measured and compared as parameter for adiposity. Other existing approaches for determining adiposity are listed below. However, the reasons mentioned above result in restricted applicability of them in the field.

1.5.2 TECHNIQUES FOR MEASURING OBESITY

The impact of body composition on metabolism varies between sex and age groups. To obtain measurements of body composition in children, ADP and Dual-energy X-ray Absorptiometry (DXA) have been the most broadly applied methods (51).

Below, only a short description of the methods or advantages and disadvantages will be discussed.

As a procedure to measure fat, DXA is, especially in thin people, associated with major false outcomes. Since it is unable to differentiate between three types of tissues, algorithms for calculation are needed. Such differ between institutions and result therefore in different outcomes. Another problem occurs particularly in longitudinal studies when individuals

experience a significant alteration in hydration or glycogen status between assessments (42) (52) (41).

Stable isotope dilution is another approach of measuring body composition. Hereby water, substituted with a slight amount of a stable hydrogen or oxygen isotope, is orally applied. After evenly distribution, the isotope's concentration in urine or saliva provides an estimate of total body water (TBW). Fat-free mass (FFM) results from that by applying a presumed value for FFM's hydration factor (10).

ADP is a procedure established on whole body densitometry. In infants under 6 months of age PEAPOD is used, whereas in older children and adults BODPOD comes to use (10).

Bioelectrical Impedance Analysis (BIA) is a technique by which the impedance resulting from a low voltage electric current is measured to calculate TBW which serves to determine FFM as well (10).

On the molecular level, body fat is most precisely measured by the 4-component model (42) (52). Anatomically, different methods, such as skinfold thickness measurements, anthropometrics, or imaging procedures like ultrasound (US), magnetic resonance imaging (MRI) and computed tomography (CT) are utilized. US provides the most accurate measurements for SAT (53) (54) (55) (56) because of the high image resolution up to 0.1 mm (18 MHz probe) while the pixel size in nowadays used MRI normally ranges from 1.3 mm to 2.0 mm (42) (41).

To evaluate body fat, especially subcutaneous fat tissue (SAT), the examination of skinfold is commonly practiced (43). Skinfold thickness measurements are taken at three up to five sites, resulting in various equations for that procedure to estimate fat mass (FM) (10). It is a cost and time efficient method. However, its technique offers a lot of shortcomings. On the one hand, by variation of skin elasticity, on the other hand by site-depending compression thickness (54). Furthermore, it is unreliable for obtaining SAT thickness due to the compressibility of adipose tissue and differing skin broadness depending on the site and person (54) (42) (41). Hence its accuracy and reliability are restricted (10). There is a high discrepancy between skinfold and ultrasound results (43).

Waist circumference (WC) is a predictor for the quantity of abdominal fat and is examined by applying a tight tape in a standing position at the centre between the iliac crest and lowest rib measuring after expiration as stated by the Report of a WHO Expert Committee (1995) on 'Physical status: the use and interpretation of anthropometry' as cited by Kuriyan R. (2018) (57). According to a Report of a WHO consultation (2000) on 'Obesity: preventing and managing the global epidemic' the waist-hip ratio (WHR) aims to locate

the body fat in upper or lower body, a higher WHR correlates with obesity-associated morbidities, as cited by Kuriyan R. (2018) (57).
CT provides exact data of body composition but should be carefully used in children regarding its vast radiation load (51).
Quantitative Magnetic Resonance Imaging (qMRI) proves to be a reliable technique for obtaining exact measurements of SAT, visceral adipose tissue, brown adipose tissue and lean mass (51). However, it does not measure total body adipose mass (10).
Since none of these methods are optimal for wide application for assessing adiposity, the approach of measuring SAT by US was utilized in this study. Its benefits are described in the following section.

1.5.3 SUBCUTANEOUS ADIPOSE TISSUE MEASURED BY ULTRASOUND

A promising and relatively new, yet thoroughly investigated and standardized method is measuring SAT by brightness-mode ultrasound. It is the most accurate approach to measuring thickness of SAT layers. This US technique has been standardized by Müller et al. (2016) and is done by measuring eight sites on the body to assess the pattern of SAT (55). US is highly accurate and reliable in measuring uncompressed SAT (54) and has been proven to result in the most precise values (41). It is the only technique able to measure the fat layer with and without the enclosed tissue (53) (54) (55) (58) (41).
The adipose tissue is located subcutaneously as well as viscerally, in the bone marrows and within tissues (59) (60). The proportion of SAT in the body is highly variable from a few kilograms up to half the body weight or more (56). Adipose tissue consists of adipocytes and the supporting tissue collagen and elastin fibres (59). The method of US to assess SAT has been known since the seventies (61) (62) but has only been standardized in 2016 (55).
With this US-technique, following advantages over other methods are given: no compression errors as compared to skinfold (43), the possibility to differentiate between adipose tissue and ingrained structures, non-invasiveness, and its practicability in the field (41) (40).
The US-images of the selected 8 sites show skin, SAT and the fascia of the muscle (41), as portrayed in following figure (Fig. 2). The US-images were taken at the lower abdomen in two 16-year-old girls and compared in a study by Schmid-Zalaudek et al. (2021). Having almost the same BMI (23.7 and 24.2), crucial differences (83.8, 210.1) in SAT-thickness in mm were shown between the girls, even when considering that the reference lengths were 6 cm in case 36 (left) and 8 cm in case 27 (right), the difference remained substantial (40).

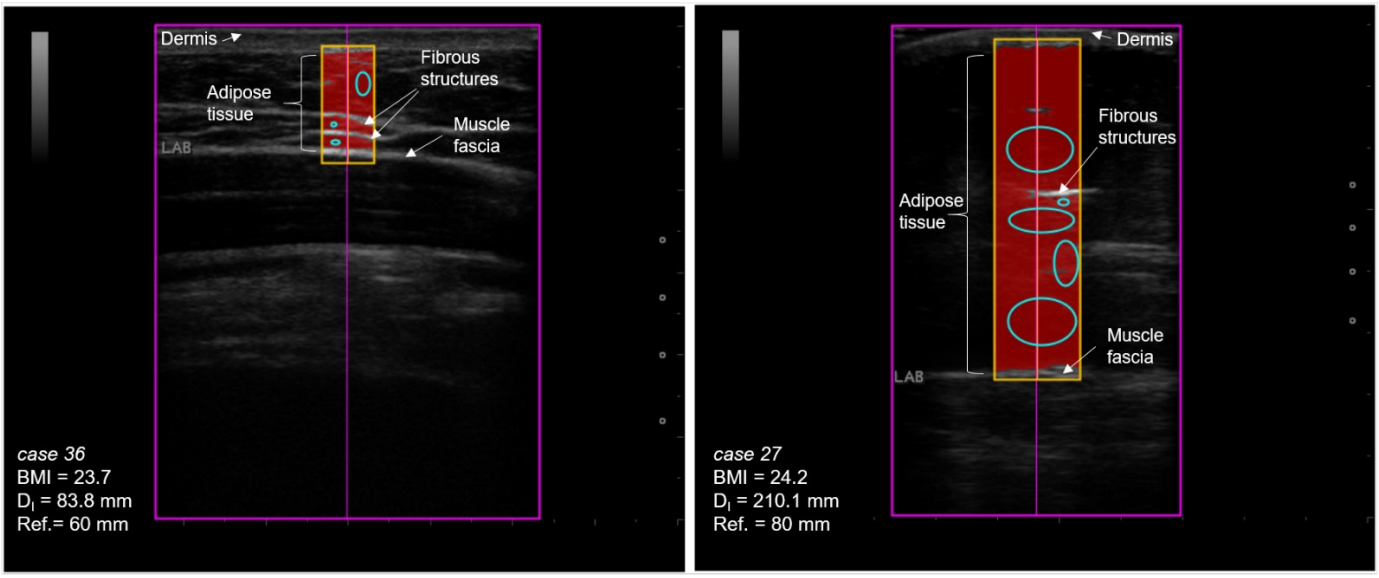


Figure 2. SAT and enclosed fibrous structures portrayed with US. Schmid-Zalaudek et al. (2021) (40)**: p. 9 as Fig. 2.**

SAT measured by US shows high reliability for precise measurement of OB and evaluating response of therapy in children and adolescents at cardio-metabolic risk (40). One limitation is that the mean thickness of SAT, which is necessary for calculating the total body fat mass, cannot be assumed by the outcomes of this procedure, as shown by Störchle et. al (2018) (41). The standardised method of US to obtain SAT-thickness was the elected approach in this study for determining potentially associated metabolic markers of OB.

1.6 Risk Markers of Obesity in Blood and Urine

As it is crucially important to recognise children at risk of OW/OB and its complications early, diagnostic tools with improved sensitivity need to be found. The present thesis attempts to discover metabolites serving as such novel tools. The next section provides an overview of existing knowledge on markers of OB and a description of the science of metabolomics and an overview of how it works.

1.6.1 BIOMARKERS ASSOCIATED WITH OBESITY

Known parameters in blood related to OB are high fasting glucose, as well as homocysteine and apolipoprotein A1. Dyslipidaemia is a condition linked to OB, where the levels of total cholesterol, low density lipoprotein (LDL) cholesterol and triglycerides (TG) are elevated, while high density lipoprotein (HDL) cholesterol is decreased. Furthermore, leptin, adiponectin and ghrelin have been associated with OB. Ghrelin has been suggested as marker for CVD-risk in children (63).

An oral glucose tolerance test (OGTT) in children with OB shows differences with a later insulin response. In subjects with OB, the expansion of adipose tissue and infiltration of macrophages result in a low-grade chronic inflammation triggering IR (64) (65) (66). Since visceral fat tissue excretes different adipokines and cytokines, OB leads to elevated levels of leptin and resistin, while adiponectin is decreased. At the same time cytokines, like tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6), are increased and cause inflammatory processes. IL-6, for example, regulates the formation of c-reactive protein (CRP) in the liver (67), leading to IR and Hyperinsulinemia. Adipokines, e.g. decreased adiponectin, also cause IR and Hyperinsulinemia, which consequently may lead to the development of CVD, cancer and T2DM (68) (69). Especially the Insulin/Insulin-like growth factor (IGF) axis and chronic low-grade inflammatory processes have been outlined as main contributing factors for obesity-derived diseases (68).
Elevated serum levels of branched-chained amino acids (BCAAs) such as, valine, leucine, and isoleucine, and lipid parameters as well as glucose and aromatic amino acids (AAAs), such as phenylalanine (70), have been found in children with OB compared to children with NW. While reduced plasma levels of glycine, asparagine and serine in children with OB have been described (71).
Measuring such markers could provide further insight into the causes of obesity-deriving chronic diseases and be used as a further tool to define OB along with anthropometrics. Nevertheless, to date too little is known about the impact of obesity-related biomarkers on its development that in clinical routine, screening of such markers is not done (68).
Markers measured in blood may be associated with parameters detected in urine, while revealing biochemical pathways associated with and thus giving way to further observation of OB.

1.6.2 METABOLOMICS IN GENERAL

To easily assess the risk for OB, biomarkers for screening would be useful (32). Recent studies have revealed the potential of using metabolomics to detect certain diseases or other conditions.
In Pschyrembel Online the term ‘metabolomics’ is defined as a science, that investigates metabolites of organisms, organs, tissues, cells or its organelles under certain physiological conditions to determine, quantify, and characterize them. Established metabolites allow direct associations to enzyme activities (72). Metabolomics and the word metabolite derive from the word, *metabolì*, which is ancient Greek and means ‘*change’* (73). Molecules with

a low molecular weight, < 1.5 kDa, implicated in cellular metabolism are referred to as metabolites (74). They play a role in many processes in the human body, among others for messaging, building of structures and in storing energy (75) (76). Metabolic profiles can be gained in a rapid and highly reliable manner with high sensitivity regarding small concentration alterations of several metabolites at once. Many advantages come along with studying metabolomics. Among others, recognising changes in metabolic dynamics and metabolite concentrations can provide precise diagnostics. Additionally, it is relatively quick, easy and heads towards a holistic picture of a subject's internal ongoings (73). There still needs to be found a standard procedure for analysing metabolomics. Promising methodologies include liquid-chromatography mass spectrometry (LC-MS) or gas chromatography coupled to mass spectrometry (GC-MS). Another commonly used method is nuclear magnetic resonance spectroscopy (^{1}H-NMR), which can provide simultaneous measurement of many metabolites in urine or serum, resulting in metabolic fingerprints associated with physiological or pathological conditions of an organism (77) (73). Yet, routine application of metabolomic measurement methods is hardly practiced (77).

1.6.3 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

The analysis of hundreds of metabolites in urine is possible with nuclear magnetic resonance (NMR) (78). Assessing concentrations via NMR is highly precise because metabolites, upon quenching enzyme activity with buffers, are quite stable and can be directly quantified. Urine metabolites show a large variety depending on several factors, one of which is sex, e.g. males tend to have higher creatinine levels in urine than women since their skeletal muscle mass is usually higher than those in women. Studies using NMR, have shown that the main urine compounds consist of urea, creatinine, hippuric acid and citric acid (78).

^{1}H-NMR spectroscopy obtains signals of protons within molecules, which are highly specific to them, enabling identification and quantification of those substances within complex biofluids (79). For the technique used in this study the magnetic characteristics of protons correlating with the chemical structure are measured, and levels of characteristic molecules in biofluids like urine can be quantified. Its advantages are minimal sample preparation and no manipulations of the samples. Metabolites can be recognised by comparison with existing data collections of NMR spectra. Data retrieval and analysis work automatically, analysed samples can be reused for different approaches since they are not damaged/altered in the process. The magnetic atomic nuclei absorb and then release

electromagnetic energy at typical frequencies. Alterations of normal reaction frequency for a specific metabolite inform about the close chemical environment and its influences (80). A disadvantage of NMR spectroscopy in comparison to MS-based methods is the lower sensitivity, which is only an issue with low concentrated metabolites (79).

NMR spectroscopy entails a lot of specific beneficial attributes for research in metabolomics (81) (82). Particularly, the high reproducibility and great dynamic range. It delivers absolute quantitation of each metabolite in the spectrum. NMR is regarded as a well-suited method in determining characteristics of unknown metabolites.

Because of the reproducibility and consistency of the NMR experiment it is fit for clinical settings. (79).

Besides the most analysed samples blood plasma/serum, tissue and urine, among others, metabolic research has also been performed on samples like cerebrospinal fluid (83), saliva (84), amniotic fluid (85), tears (86) and other biological materials.

Figure 3 shows an overview of the steps involved in analysing metabolomics, as published by Al-Sulaiti et al. (2023) (87) (note, that hereby at step 2, the MS- method is pictured in more detail, not the NMR-spectroscopy).

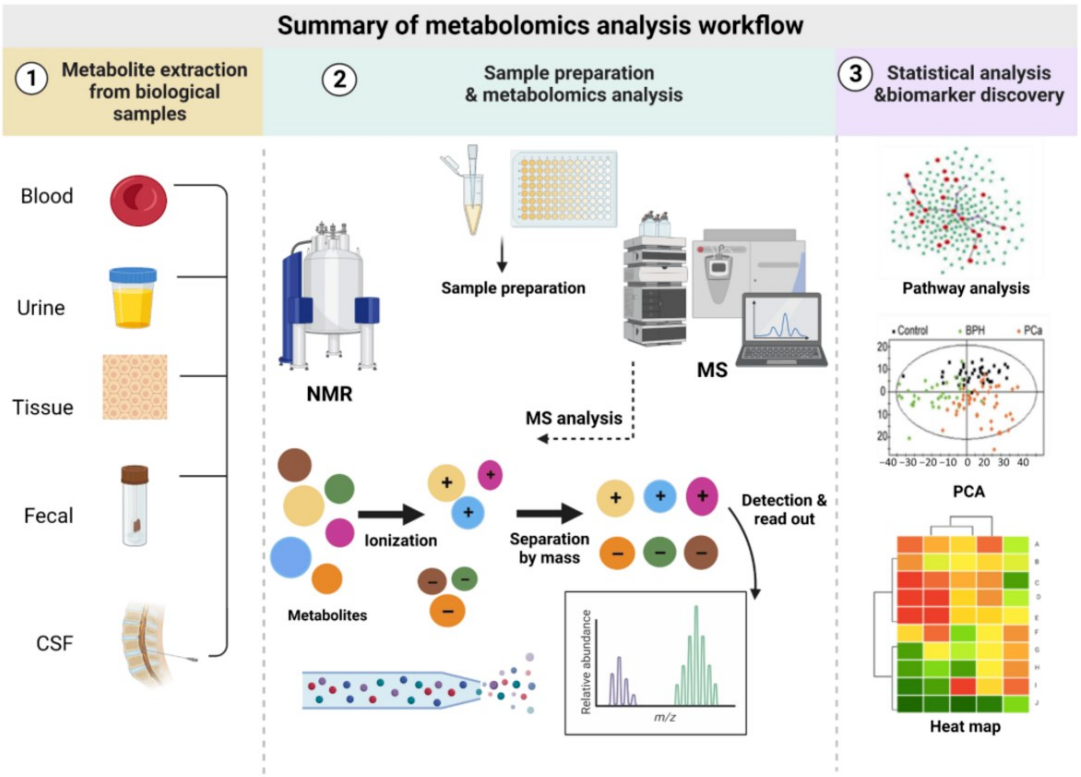


Figure 3. 'Summary of metabolomics analysis workflow'; retrieved from: https://commons.wikimedia.org/wiki/File:Summary_of_the_metabolomics_analysis_workflow.png

After determining absolute or relative concentrations of metabolites, statistical and metabolic pathway analysis is done, with emphasis on a wide range of basic and medical sciences. For analysing metabolomic data, usually uni- and multivariate statistics are applied, aiming at finding and validating assumed metabolic biomarkers (88).
The number of metabolites detected, varies between the analytical approaches. The wide coverage of databases and methods used, enables researchers to discover new urine metabolites (78). As certain compounds are NMR-invisible since they lack identifiable protons at physiological pH value, they are only traceable with other methods, e.g. mass spectrometry. Hence it makes sense to use different complementary methods (78).
Quantitation usually is either done relatively amongst different subjects or groups, which is the most applied technique, or absolutely, by measuring molar concentrations utilizing an internal or external standard (88).

1.6.4 URINE METABOLOMICS

A relatively novel approach for diagnostic use in children is the analysis of urine metabolomics, which also forms the main object of this thesis in the context of establishing biomarkers that may predict OB. The potential of urine metabolomics has not been much studied. A big advantage of urine compared to other biofluids is that it is easily obtained and contains an abundance of metabolites (89) (78), e.g. compared to serum/plasma, urine entails a substantially higher number of identifiable metabolites, with a substantial concentration range of about 10^6, presenting a powerful source of information (90) (91).
Collected data by Bouatra et al. (2013) proposes that human urine may contain >3000 identifiable compounds. The 'Human Metabolome Database' (HMDB) offers a chemical categorization tool for metabolites, revealing their huge variety. (78).
Investigating the urinary metabolites, information about intrinsic metabolites, xenobiotics, gut microbiota metabolites, as well as nutritional products can be obtained, offering a great resource of information regarding the molecular characteristics of MetS and many other diseases for instance (78). Depending on the type of analysis, the preparation steps of the urine samples necessary vary. (89) (78).
Furthermore, protein levels in urine are relatively low which minimizes macromolecular interference for metabolite analysis. The variety of salt levels and pH in human urine result in relevant variation of NMR spectra between individuals. Multiple factors, like drugs, diet, smoking, age, sex, physical activity (PA), intestinal microbiota, affect the

metabolomic profile. Hence, determining metabolites as biomarkers for diseases should be done critically (90) (91).

The promising method of urinary metabolomics has shown the potential of diagnosing various conditions in children and adults. Distinctive urinary metabolic traits have been associated with epilepsy (92), autism (93), steroid-sensitive nephrotic syndrome (94), T2DM, where it was described as valid risk stratification biomarker (95), MetS (96), dietary impacts and food quality (97), parameters for pesticide exposure (98), determining sex differences (99), as biomarker for measuring response to treatment of NAFLD (100), prepuberty (101), bisphenol-A interferences especially with the amino acid metabolism and the steroidogenesis pathway in preadolescent girls were observed (102), elevated mono-n-butyl-phthalate levels were found in the urine of children with OW/OB showing a positive correlation with obesity signs (103). Urinary metabolites have been proven a potential accurate diagnostic tool to correctly identify illnesses, such as primary aldosteronism and KCNJ5-mutated aldosterone-producing adenomas (104).

Elevated urinary isoleucine and tyrosine levels have been found to be significantly associated with MetS (105).

Previously found blood parameters to be related to MetS are BCAAs (valine, isoleucine) amino acids (histidine and glycine), elevated glucose-levels indicating IR (106), dyslipidaemia and OB were characterised by elevated steroid lipids (107) (108) and several other metabolites have been associated with OB (109), such as altered levels of trimethylamine-N-oxide (110) (111) or salicylic acid (112). Bruzzone et al. (2021) found relations for other dysregulated parameters to MetS, such as maltitol, trigonelline, and nicotinuric acid (96).

High urinary/blood levels of aromatic +/- branched chain amino acids (BCAA) have been found to correlate with IR and the risk of developing MetS (113) (114) (100) (24).

As the sampling of urine is non-invasive it is easier to access and shows better patient compliance, especially in children (77).

The metabolic profile could be used to distinguish intermediate states between MetS and metabolic healthy subjects. E.g. reduced urinary levels of imidazole and histidine may be related to a perturbation in the concentration of endogenous ligands of the a2-adrenogenic and imidazoline receptor, resulting in episodes of elevated blood pressure (115) (116).

Considering above-described attributes of urinary metabolites, their diagnostic value for OB becomes clear, which is why they merit further research.

2. Research Questions

The HAPHC-project has been initiated with the intent to fight the health burden of OW/OB by implementing interventions in the setting of schools to achieve a whole systemic approach. As OB has become a substantial health threat and is nowadays affecting more children and adolescents than ever, it is crucial to investigate measures for preventing and tackling this global phenomenon. This study aimed to assess, whether the occurrence of specific urinary metabolites, detected by NMR spectroscopy, in children can be associated with SAT-change, measured by brightness-mode (B-mode) ultrasound, after one school year.

Research Question: Which urine metabolites (measured at the begin of the study) are relevant and can predict the increase of SAT within one year (measured at the begin and the FUP)?

Can specific metabolites be found that may be applied as biomarkers to predict SAT change and can previously associated metabolites with SAT, such as allantoin and succinic acid (117), serve as predictive markers?

With the aim of finding novel early biomarkers for OB in children, we hypothesize that:

1. Certain urinary metabolites are associated with SAT-changes and predict such.
2. The metabolites allantoin and succinic acid, that were associated with higher SAT-thickness at baseline, predict SAT-increase.

The further goal is to provide governmental and public institutions with the findings to help, implementing measures for preventing the development of OB.

3. Methods and Materials

3.1 Ethical Approval

All steps of this study were in accordance with the ethical guidelines as confirmed by the Research Ethics Committee of the Medical University of Graz, Austria (33-488 ex 20/21). The study got registered in the ClinicalTrials.gov (NCT04956003). The children were recruited to participate in the HAPHC project on a voluntary basis. Furthermore, written informed consents from their legal guardians were obtained which was a requirement for participating, and can be found in the annex of Huberts' thesis (117). Refusal to take part in measurements and opting out of the study in general were possible options at any given moment.

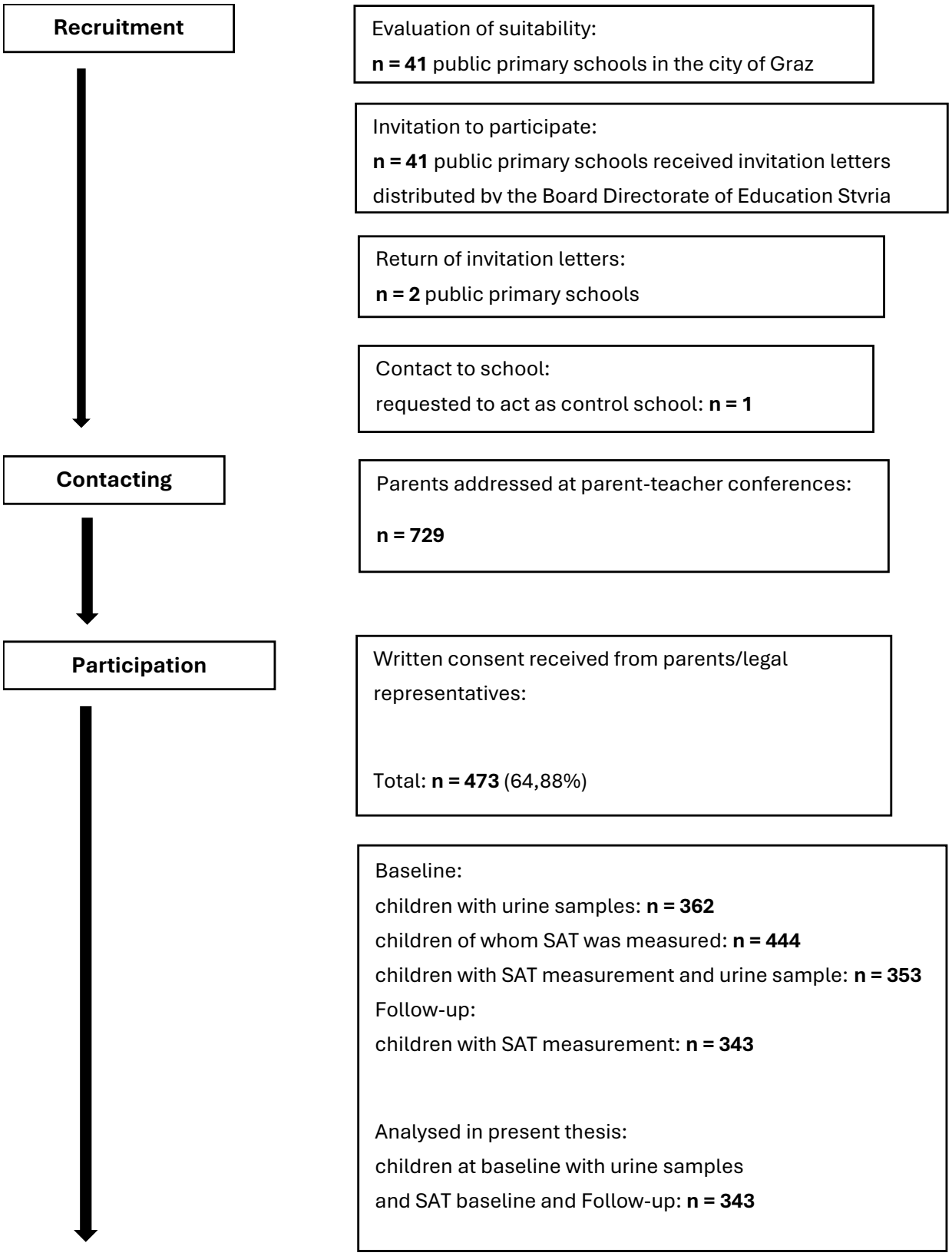
3.2 Participants and Procedure

The three-year non-randomized controlled intervention study was performed in Graz, Austria, and included two intervention schools and one control school. It started in September 2021 and was finalized in June 2024. The participants were in the age range from 6 to 11 years and measured heights from 112 to 158 cm at baseline with a mean of 133 cm. The children weighted 14.2 to 73.1 kg and the BMI ranged from 8.8 to 31.5 kg/m^2.

In the final days of February 2021, invitation letters for participating in the HAPHC project were mailed to each of the 41 public elementary schools in Graz by the Board Directorate of Education Styria. Three primary schools agreed to take part in the program, and two schools were chosen as intervention schools, while one was the designated control school. The number of enrolled children was 353 (from grades 1, 2, 3 and 4). The final number of children of whose parents' written consent had been given at the baseline measurements was 460 out of 471 children. The missing children were either absent at baseline assessment or withdrew from the study at site. At baseline, SAT was measured in 444 children and urine samples were obtained from a total of 362 children. In the first follow-up (FUP-1), SAT was measured in only 343 children due to similar reasons as mentioned above. All these 343 children also had their urine samples analysed at baseline.

Inclusion criteria: All the children who agreed to take part in the study and who's legal guardian's written consents had been given were included in the study.

Exclusion criteria: There were no primary exclusion criteria from participating in the study's activities. Nevertheless, known present diseases or disabilities were documented and reflected upon, when interpreting the outcoming dataset (118). Children with special educational needs were included in all activities and measures but excluded from most analyses.


Recruitment
Evaluation of suitability:
n = 41 public primary schools in the city of Graz
Invitation to participate:
n = 41 public primary schools received invitation letters
distributed by the Board Directorate of Education Styria
Return of invitation letters:
n = 2 public primary schools
Contact to school:
requested to act as control school: n = 1
Contacting
Parents addressed at parent-teacher conferences:
n = 729
Participation
Written consent received from parents/legal
representatives:
Total: n = 473 (64,88%)
Baseline:
children with urine samples: n = 362
children of whom SAT was measured: n = 444
children with SAT measurement and urine sample: n = 353
Follow-up:
children with SAT measurement: n = 343
Analysed in present thesis:
children at baseline with urine samples
and SAT baseline and Follow-up: n = 343

Before starting with the interventional procedures, the enrolled children were measured at baseline in September 2021. In the final weeks of each school year, the follow-up (FUP) examinations were run, this being the FUP-1 in June 2022, FUP-2 in June 2023 and the last and third FUP in June 2024.

In the study, several variables of interest were assessed. Besides the SAT and urine samples, other analyses were run such as among others, cognition, physical performance, and cardiovascular parameters. Only the relevant measurements of the study regarding the present thesis are further described, namely the analysis of urinary metabolomics at baseline via NMR spectroscopy and the examination of SAT via US at baseline and follow-up-1. The baseline and follow-up-1 (FUP-1) data of the children from the three primary schools were combined. The performed tests and measurements were non-invasive and enacted according to protocol. See Goswami et al. (2022) (118) for more information. During school days the measurements took place in the buildings of each participating school. Mainly the fitting rooms and gymnasiums were used as examination sites. Children in groups of 4-6 were measured each in one turn between 8 am and 2 pm. It was intended to collect the urine samples in the mornings, they were then stored at -80°C and analysed with NMR spectroscopy by the team of the laboratory of the division of Medical Chemistry of the Medical University of Graz within 4 months. The results were then statistically analysed and interpreted by the same researchers, who also provided the graphics.

The SAT measurements were taken by experienced researchers of the division of Physiology and Pathophysiology. While measurements were taken, no child was present alone with only one examiner. The resulting images were then analysed with a software (further described below) by the researchers as well. The statistical analyses were supported by the supervisors to this thesis.

3.3 Measurements

3.3.1 ULTRASOUND MEASUREMENT OF SUBCUTANEOUS ADIPOSE TISSUE

Ultrasound (US) Brightness mode (B-mode) was applied for assessing the SAT thickness by use of the US system, Esaote laptop color Doppler system, SMT, Germany, for taking the measurements.

In search for exact measurement methods of an athlete's body composition in the field, Müller et al. (2016) developed a standardised approach for assessing SAT with ultrasound.

Eight sites for measuring were determined, namely upper and lower abdomen (UA; LA), erector spinae (ES), distal triceps (DT), brachioradialis (BR), lateral thigh (LT), front thigh (FT) and medial calf (MC) (55). This 8- sites standardised approach was used in the current study.

US imaging functions by a signal emitted, that penetrates tissue with the speed of sound (c), depending on the kind of tissue, an echo is received, that portrays as the image. Normally c= 1540 m/s is applied for measuring the distance from the probe's surface to the borders between two tissues in diagnostic US schemata. However, in adipose tissue the speed of sound with 1450 m/s is slower than in other soft tissues, according to El-Brawany, Nassiri, Terhaar et al. (2009) as cited by Müller et al. (2016) (55). In the applied B-mode images are created by series of US emits entering the tissue to generate an image in which the screen-brightness is analogue to the echo concentration in the scan's layer. Because of the squeezable SAT, the transducer must be placed without any pressure, which can be achieved by applying a 3-5 mm thick US gel layer on the ultrasonic probe. The protocol for the certain method requires measured subjects to be in a supine, prone or rotated lying position, depending on the site measured. The GE Logic e US system using a linear probe at 18 MHz was applied for generating the SAT images in the publication of Müller et al. (2016) with an axial resolution of approximately 0.10 - 0.15 mm. Usually, frequencies of 3 to 22 MHz are applied in diagnostics, according to soft tissue's wavelength of 0.5-0.07 mm. When applying named recommendations for accurate thickness measuring, only the variable elasticity of tissues and wrinkled borders remain as challenges for accuracy.

On both sides of the SAT the US resolution limit causes a border resolution error that lies by 0.1 mm, when using 18 MHz, the measured length is the product of multiplying the speed of sound by half the echo time. Diverging applied speeds of sound applied by the US system from the tissue's real sound speed deteriorates the distance calculating inaccuracy (55).

For subsequently estimating the amount of total SAT, anthropometrics need to be measured as well. The relevant data includes body weight (w), body and sitting height (h, s), leg length, the hip's, waist's, bicep's, and thigh's circumferences and body mass (m). The height was measured with a precision of up to 0.1 cm by using a stadiometer and instructing the children to stand and put off their shoes.

An electronic scale, Tanita MC980MA, Tokyo, Japan, was used for measuring body weight. The children were wearing shorts, a T-shirt and no socks when being weighed. The accuracy of the body weight measurements was up to 0.1 kg. For worn clothes 0.4 kg were

subtracted to acquire the actual body weight. The circumferences of the hip, the waist, the biceps, the thigh, the leg length, and sitting height were examined using a tape measure with an accuracy of 0.1 cm. Waist measurements were taken at the narrowest point of the waist in mid-breath position, while the hip was determined at the widest point of the buttocks. With the arm flexed, the biceps was measured at its thickest point. The thigh's measurements were taken at the US-measurement site for FT (see Figure 4). Sitting height was assessed in an upright position, sitting on a table with the feet placed on a box. In a standing position, the distance from the floor to the spina iliaca anterior superior was measured for obtaining the leg length (117).

For the US imaging procedure to measure the SAT, the eight sites were marked according to protocol. The precise location of these sites is crucial for resulting in high accuracy of the data. To determine such, Figure 4 shows an overview, the instructions in detail are reported in Müller et al. (2016) (55).

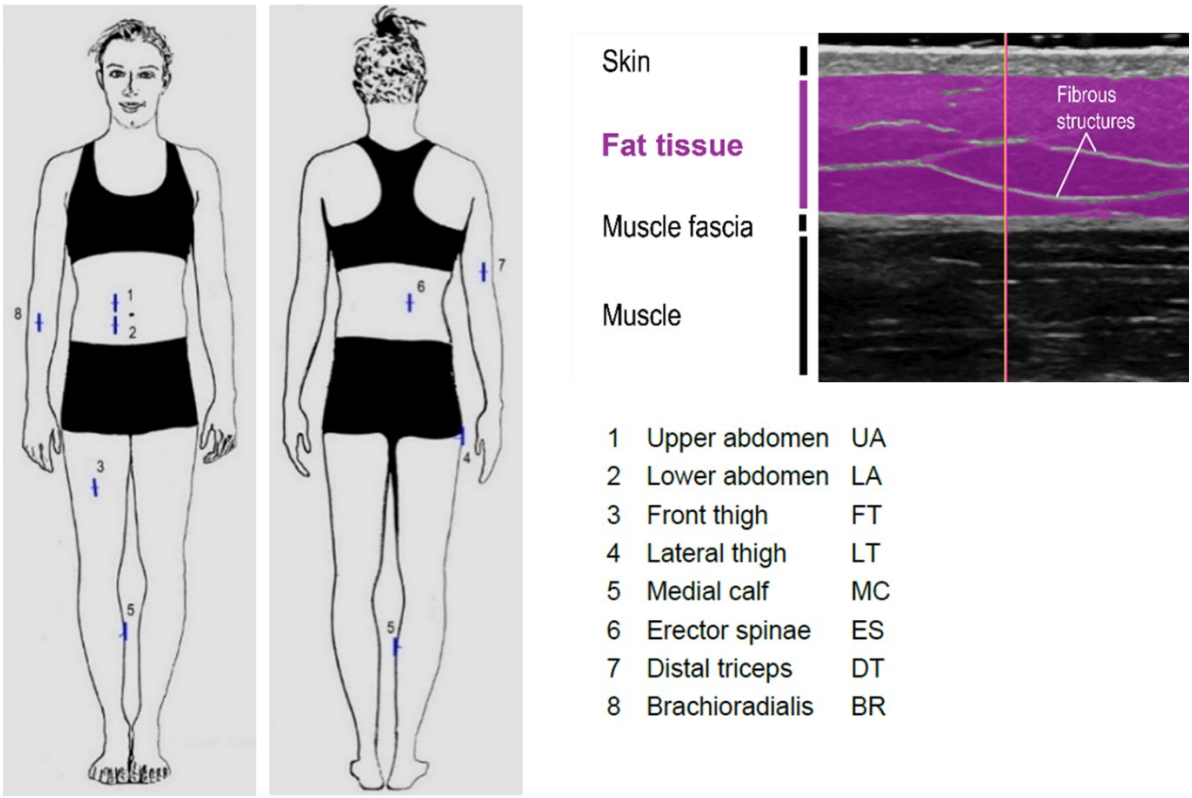


Figure 4. Eight sites for the standardised US-measurement of SAT (Müller et al. (2016) (55))

An interactive image segmentation was used for determining the outline of SAT on each image. Thickness for every US-image of all eight measuring points was automatically determined by the software (NISOS-BCA-F4.2; rotosport.at) (40).

The SAT-thickness from each of the eight measurement sites was added together including the embedded fibrous structures for calculating the sum of the subcutaneous tissue. It was

then considered for classifying the groups regarding their amount of SAT at baseline and follow-up (FUP), calculating SAT-fold change, since the measured value alone cannot reveal other information or be used for further calculations. The embedded fasciae were included in the measurements since already minimal tilting of the ultrasound probe has shown to result in varying amounts of fibrous structures in the US-images. Which lead to the inclusion of the embedded fascia into the thickness of the SAT measurements (DI). Below (Fig. 5), two US images of the SAT measurements at the LA-position of the present study are shown.

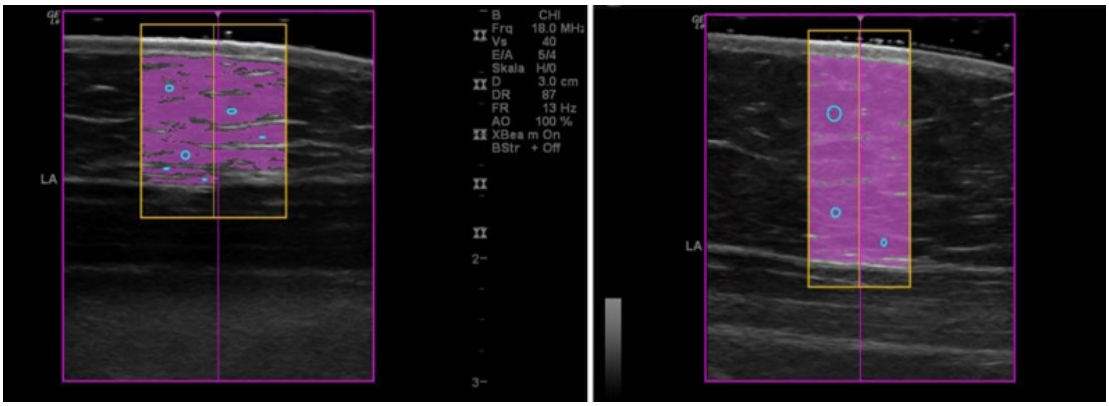


Figure 5. US-images of SAT of the site "Lower Abdomen" at baseline (left) and follow-up (right) in a boy participating in this study; SAT is marked purple

3.3.2 NMR SPECTROSCOPY MEASUREMENT OF URINE METABOLOMICS

In the present study, NMR spectroscopy was the method chosen for analysing the urine samples. Non-fasting spot-on urine was mostly collected in the morning hours on the days of the measurements. The participating children were asked to pee into labelled cups. Then urine samples were pipetted into small plastic tubes and stored at -80°C.

Analysis was run by researchers of the Division of Medical Chemistry at the Medical University of Graz. Following procedure was performed: After thawing the urine samples, 60 ul of IVDr buffer was mixed with 540 ul of urine sample and filled into 5 mm 4-inch NMR glass tubes. Subsequently, samples were analysed directly in patches of 96 samples at 300 K on a Bruker Avance Neo NMR spectrometer (Bruker Biospin, Rheinstetten, Germany) at 600 MHz with a TXI 600 probe head. Corresponding to the manufacture's guidelines all samples were recorded with NOESY (noesygppr1d, NS = 32, TD = 65536, SI = 131072) and 2D-JRES (jresgpprqf, NS = 2, TD = 8192/40, and SI = 16384/256 for F2/F1, respectively) spectra. B.I.Quant-UR ne^{TM} (Bruker), a specific server-based algorithm, was employed to quantify up to 150 metabolites per urine sample to determine

pertinent information from sample spectra. To outline the extracted data, an Excel sheet was created by the researchers, showing all metabolites in each sample as free concentrations in mmol/L and concentrations normalized on creatinine in mmol/mol CREA, which is commonly practiced in this procedure due to differing fluid uptake. It is also argued that as in quantification and analysis of urine metabolites a high variability of metabolites in different samples exists, identifying co-occurrence of several compounds may help to observe patterns and hence simplify the detection of changes in these (117).

3.3.3 STATISTICAL ANALYSIS

For calculation of descriptive statistics IBM SPSS (statistics for Windows, Version 29.0. Armonk, NY: IBM Corp) was used. To avoid alpha-inflation, p-values, conventionally contemplated as significant with a value of $p < 0.05$, false discovery rate (FDR) was utilized as correctional tool.

To test the hypothesis, whether metabolites could predict SAT-fold-change within a year, multivariate statistical analysis was performed by the same researchers performing the NMR analysis applying the open available server-hosted statistical software MetaboAnalyst (https://www.metaboanalyst.ca/) (119) (120) to detect the most significant metabolites. First, T1/T0 (follow-up/baseline) fold values of SAT were calculated from 343 children and then the terciles of them were calculated. One-way ANOVA using terciles of fold values of SAT as independent factor was performed on all metabolites with an occurrence >5 (out of a total number of n= 343 samples). Occurrence in this sense simply means a measured concentration above the limit of the detection of the method (which otherwise is denoted as ‘0’ in the raw data). ANOVA were hence calculated on 57 metabolites and those resulting in significant differences between terciles were further analysed. Furthermore, the statistical software Rstudio/R (v 4.3), using packages rstatix (for calculating multiple ANOVA) and a generalized linear model (GLM) for implementing most relevant parameters into one model were calculated in R.

4. Results

4.1 Descriptive Statistics

For the purposes of this thesis, statistics were calculated with data of the children, who had provided a urine sample at baseline and who had their SAT measured at baseline and follow-up (FUP-1), which in total were 343 children. The age range lay between 6-11

years with a mean age of 7.88 years and a standard deviation (SD) of 1.29. Body height was between 112.0- 157.5 cm with an overall mean of 133.34 ± 9.6 cm SD.
In total, data of 161 (46.9%) girls and 182 (53.1%) boys were included for the present thesis. Across the school grades sex was equally distributed ($\chi^2_{(3)} = 4.054$, p= 0.256, Table 1) and in each of the participating schools no sex difference was found ($\chi^2_{(2)} = .048$, p= 0.786).
No significant difference was found between girls and boys considering their mean age ($t_{(341)} = -1.071$, p= 0.285). Girls (n=161) were on average 7.80 ± 1.30 years old and boys (n=182); 7.95 ± 1.28 years old.

Table 1. Sex distribution across grades (n in absolute numbers)

	Sex		
Grade	Girls	Boys	Total
1	39	39	78
2	44	39	83
3	36	57	93
4	42	47	89
	161	182	343

Table 2. Anthropometrics at baseline and follow-up

	Mean ± SD (min.-max.)	
	Baseline	Follow-up
Age (years)	7.88 ±1.29 (6-11)	
Height (cm)	133.31 ± (112.0-157.5)	137.18 ± 9.74 (115.50-163.10)
Weight (kg)	31.81 ± (17.8-73.1)	34.59 ± 10.59 (18.00-81.70)
BMI (kg/m^2)	17.58 ±3.45 (8.80-31.55)	18.05 ± 3.58 (12.16- 32.85)
BMI-SDS	.58 ±1.30 (-3.42-5.04)	.63 ± 1.28 (-2.70-4.56)

Girls and boys differed significantly regarding their height at baseline ($t_{(341)}$= - 2.320, p = 0.021) and follow-up ($t_{(341)}$= -2.044, p = 0.042), but did not differ relevantly regarding their weight, neither at baseline ($t_{(341)}$= - 0.894, p = 0.372) nor at follow-up ($t_{(341)}$= - 0.601, p = 0.548, see Table 3). Similarly, the increase of height ($t_{(341)}$= 1.516, p = 0.130) and change of weight ($t_{(341)}$= 1.078, p = 0.282) did not differ between girls and boys as reflected in the

BMI-SDS, which also did not differ significantly (BMI-SDS baseline: $t_{(341)}$= -1.187, p = 0.236; BMI-SDS follow-up: ($t_{(341)}$= - 0.898, p = 0.370, see also Table 3).

Table 3. Changes of mean body height and weight from baseline to follow-up by sex; n: Girls= 161, Boys= 181

		Baseline	Follow-up
		Mean ± SD	Mean ± SD
Height (cm)	Girls	132.04 ± 10.14	136.04 ± 10.35
	Boys	134.42 ± 8.86	138.19 ± 9.07
Weight (kg)	Girls	31.31 ± 10.42	34.23 ± 11.33
	Boys	32.24 ± 8.88	34.92 ± 9.90
Change height (cm)	Girls	4.00 ± 1.26	
	Boys	3.80 ± 1.13	
Change weight (kg)	Girls	2.92 ± 2.22	
	Boys	2.67 ± 1.96	
BMI-SDS	Girls	0.49 ±1.3	0.57 ±1.25
	Boys	0.66 ±1.29	0.69 ±1.31

4.2 SAT Changes from Baseline to Follow-up

Table 4 gives an overview of the changes in the total SAT, as measured as the sum of SAT at the eight standardized sites (D_I). Notably, at baseline ($t_{(341)}$= 3.152, p = 0.002) as well as at follow-up ($t_{(341)}$= 3.358, p < 0.001) girls differed significantly in their amount of SAT which was higher than in boys at both timepoints. A sex difference which could not be shown by the BMI.

Table 4. SAT (mean ±SD) at baseline and follow-up in girls and boys; SAT in mm

		SAT	
		Baseline	Follow-Up
SAT	Girls	78.94 ± 41.93	83.21 ± 45.32
	Boys	64.84 ± 40.83	67.12 ± 43.34
SAT	total	71.46 ± 41.89	74.68 ± 44.94
T_1T_0-fold	n = 343	1.04 ± .18	
T_1T_0-diff	n = 343	3.21 ± 13.74	

The total amount of SAT at baseline was highly correlated to the amount of SAT at follow-up ($r = 0.952$, $p < 0.001$), as shown in Figure 6, indicating not much change of the relative SAT over the observation period of most individuals (with some obvious deviations).

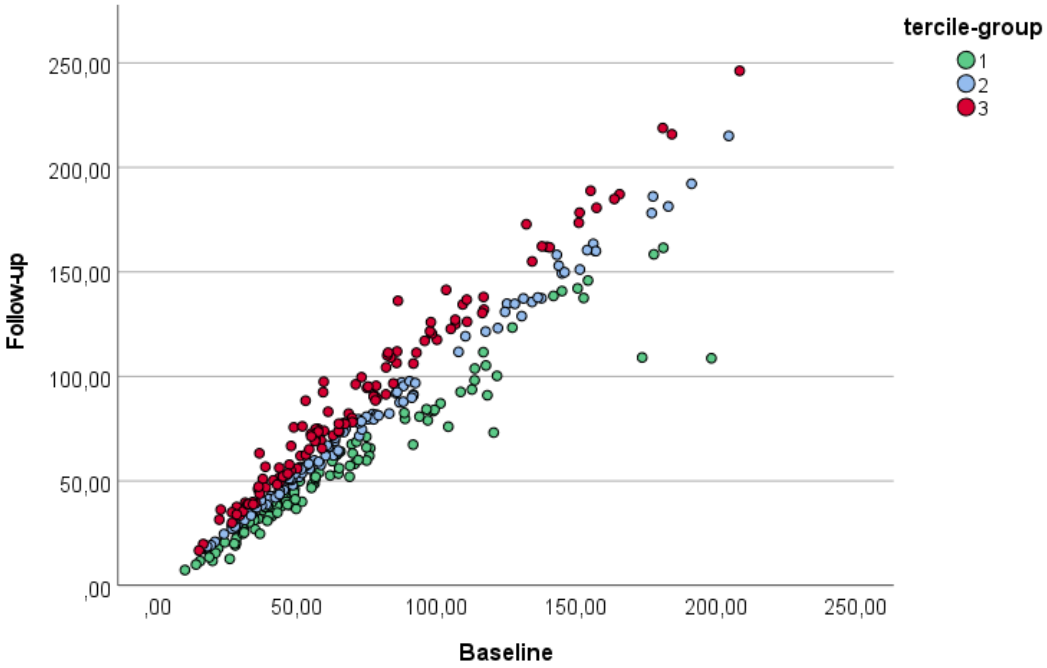


Figure 6. Total SAT sum at baseline and follow-up in each tercile-group

For the classification in tercile based groups, the change in the total sum of SAT from baseline to follow-up was used for calculating the fold change values (T_1T_0-fold = SAT T1/T0). Accordingly, children with SAT values below the 33.33 percentile and thus a SAT fold change of .98 and below were assigned to tercile group 1, while children with a cut-off value of T1/T0- fold change above 1.11 and thus over the 66.66th percentile were assigned to tercile group 3. As given by Figure 6, SAT in children of the first tercile showed a decrease of SAT, while in both other groups the amount of SAT increased. Figure 6 gives the SAT total sum at baseline and follow-up for the three groups assigned according to their fold change.

In 59.8% of the children the total amount of SAT increased within one school year, whereby the proportion in girls (67.1% of 161) was higher than in boys (53.3% of 182; $\chi^2_{(1)} = 6.7505$, $p = 0.009$).

Table 5. SAT in the three tercile groups; note that the groups are based on the relative (fold) SAT-change

	SAT (in *mm*)			
	Baseline	Follow-up	Mean	
Tercile group	mean ± SD	mean ± SD	Fold Change	n
1	65.43 ± 40.55	56.02 ± 34.50	0.86 ± 0.10	114
2	74.76 ± 44.37	77.43 ± 45.61	1.04 ± 0.04	115
3	74.17 ± 40.31	90.55 ± 47.01	1.24 ± 0.13	114

Both, the absolute change of SAT and the fold change, showed a significant correlation to the increase of body weight ($r = 0.463$, $p < 0.001$; $r = 0.422$, $p < 0.001$; respectively). As shown in Figure 7, the SAT fold change was mainly driven by the increase of weight ($F_{(2, 339)} = 41.31$, $p < 0.001$), while no difference was found regarding the change in height from baseline to follow-up ($F_{(2, 339)} = 0.244$, $p = 0.784$).

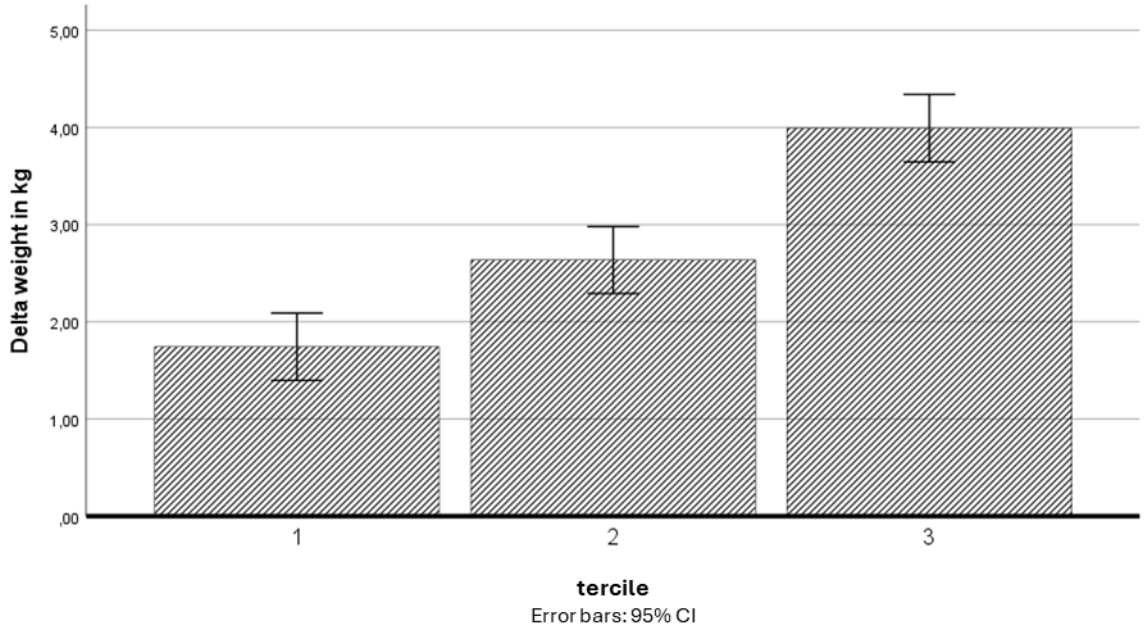


Figure 7. Change of weight in the three SAT fold-change based tercile- groups

Notably, according to the ANOVA, the relative amount of SAT (fold change) increased less in children of the intervention schools (mean fold change = 1.03 ± 0.18; $F_{(1, 343)} = 7.485$, $p = 0.007$) as compared to the control school (mean fold change = 1.10 ± 0.18*),* though this is not part of the present thesis.

The fold change in SAT was found to be independent of the school grade ($F_{(3, 339)} = 0.470$, $p = 0.704$). Similarly, also the two-way ANOVA performed on the raw values of SAT at baseline and follow-up revealed a significant main effect for pre-/post measurement ($F_{(3, 339)}=18.247$, $p < 0.001$) and a grade ($F_{(3, 339)}= 8.585$, $p < 0.001$), but no interaction ($F_{(3, 339)}= 0.412$, $p = 0.744$), meaning that the increase is similar at all ages, but the total amount is higher at higher ages (see Figure 8).

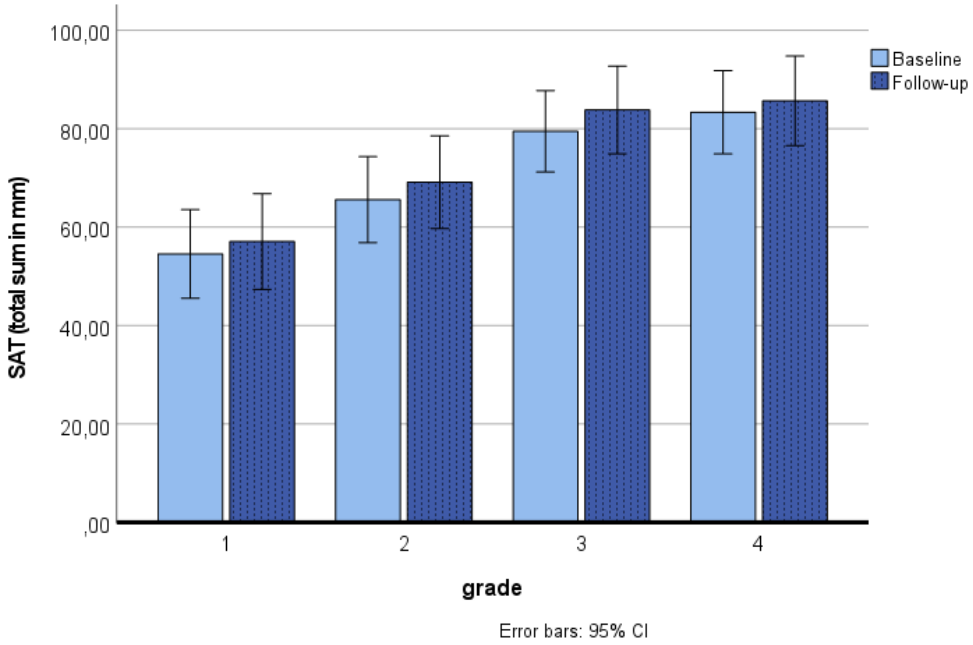


Figure 8. SAT-changes among school grades at baseline and follow-up

4.3 Metabolomics Associated with SAT Fold-Change

Urine metabolomics were analysed in all 343 children. Fifty-seven out of 150 metabolites were detected (with an occurrence >5) and pre-analysed by univariate one-way ANOVA, comparing the SAT fold change tercile based groups. With those metabolites revealing significant group differences, further analyses were performed. False-discovery rate (FDR) was applied to control alpha error inflation.

Dimethylamine at baseline, which was detected in almost all samples, was higher in children who revealed an SAT -increase at follow-up ($F_{(1, 341)} = 4.037$, $p = 0.045$, $p_{corr.} = 0.33$, see Figure 9 and Table 6), although the result was non-significant (ns) after correction. Notably, dimethylamine was only related to the relative SAT increase, but not to the absolute amount as determined by the difference between baseline and follow-up ($r = 0.08$, $p = 0.143$). Dimethylamine was also negatively related to sex, which means higher

concentrations in girls (r = - 0.147, p = 0.007), BMI (r = - 0.153, p < 0.005), and age (r = - 0.226, p < 0.001).

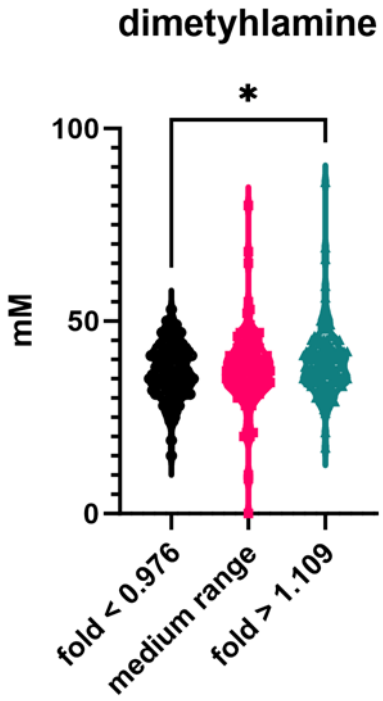


Table 6. Dimethylamine in mmol/mol in the tercile groups

	Dimethylamine			
Terzile_fold	Mean	SD	n	Occurrence(%)
1	36.99	± 6.89	115	33.63
2	37.78	± 9.43	113	33.04
3	39.3	± 8.87	114	33.33
Total	38.02	± 8.49	342	100

Figure 9. Dimethylamine concentrations in mmol/mol in the tercile groups

N-isovaleroylglycine only occurred in a limited number of cases (165 out of 343 = 48.10 %) but was detected at a higher frequency in children whose SAT had decreased at follow-up ($F_{(1, 341)}$ = 6.301, p = 0.013, $p_{corr.}$= 0.14). This result was also found when only samples containing the metabolite were compared ($F_{(2, 162)}$= 4.056, p= 0.019; occurrence 36.36%). As shown in Figure 10 and Table 7, the concentration of N-isovaleroylglycine was also higher in children with SAT decrease, also reflected by a low, but significant inverse correlation with the total amount of SAT increase (r = - 0.128, p = 0.018).

In contrast, the occurrence of hippuric acid was the same in all tercile groups (33%) and it was found in 320 out of the 343 samples (occurrence = 93.29 %). The concentration of hippuric acid though was also highest in subjects with low fold values ($F_{(1, 341)}$ = 6.692, p = 0.01, $p_{corr.}$= 0.14, see Figure 11 and Table 8). When including only children in whose urine samples the metabolite was detectable, hippuric acid concentration was higher in children whose SAT decreased within one school year ($F_{(2, 317)}$ = 4.932, p = 0.008). The absolute SAT change was however not related to the metabolite's concentration (r = - 0.078, p = 0.151).

Table 7. N-Isovaleroylglycine concentrations in mmol/mol in the tercile groups

N-Isovaleroylglycine				
Terzile_fold	Mean	SD	n	Occurrence (%)
1	3.45	1.51	60	36.36
2	3.32	1.31	56	33.94
3	2.78	0.90	49	29.70
Total	3.21	1.31	165	100

Table 8. Hippuric acid concentrations in mmol/mol in the tercile groups

Hippuric acid				
Terzile_fold	Mean	SD	n	Occurrence (%)
1	362.98	492.79	107	33.44
2	246.24	158.30	106	33.13
3	246.23	163.16	107	33.44
Total	285.27	317.54	320	100

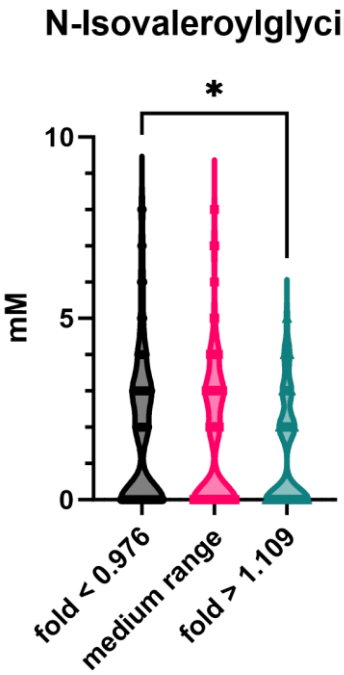


Figure 10. N-Isovaleroylglycine concentrations in mmol/mol in the tercile groups

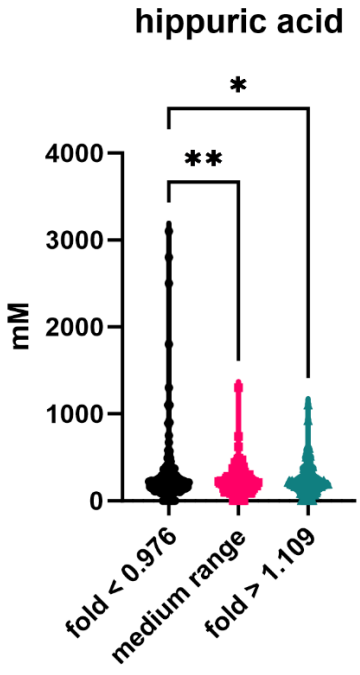


Figure 11. Hippuric acid concentrations in mmol/mol in the tercile groups

In contrast, taurine, which was only rarely present in samples, was detected more often in children's urine samples with an increased SAT at follow-up ($F_{(1, 341)} = 6.398$, $p = 0.012$, $p_{corr} = 0.14$, see Table 9 and Figure 12). Including only cases with detectable metabolites though, revealed no significant difference in the concentrations ($F_{(2, 17)} = 0.0849$, $p = 0.445$), though was slightly related to the absolute increase of SAT ($r = 0.149$, $p = 0.006$).

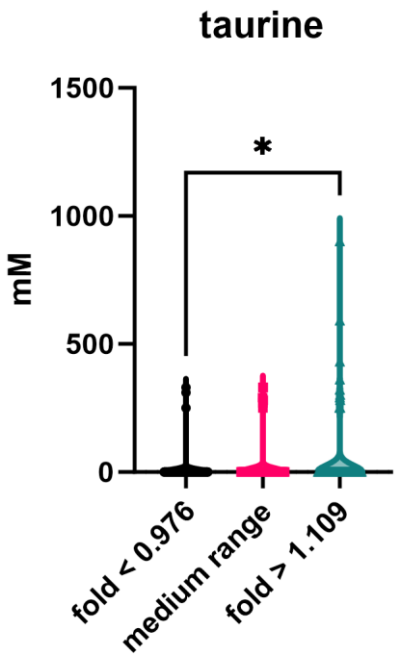


Figure 12. Taurine concentrations in mmol/mol in the tercile groups

Table 9. Taurine concentrations in mmol/mol in the tercile groups

	Taurine			
Terzile_fold	Mean	SD	n	Occurrence (%)
1	296.67	41.63	3	15
2	291.67	32.51	6	30
3	383.64	199.01	11	55
Total	343	153.08	20	100

Remarkably, in urine samples of 76 out of 343 children (22.16%) glucose could be detected. This finding will be further discussed below. Primarily the differences of elevated glucose and mannose levels observed between children whose SAT increased within one school year compared to those whose SAT did not increase were significant (glucose: $F_{(1, 341)} = 3.999$, $p = 0.046$, $p_{corr.} = 0.32$, D-mannose: $F_{(1, 341)} = 4.944$, $p = 0.027$, $p_{corr.} = 0.25$, see Figures 13 and 14 and Tables 10 and 11), however it was not significant after FDR correction anymore. Furthermore, mannose was only found in very few samples (5 children). Even though the results were non-significant, when including only samples with detectable metabolites, both, glucose and mannose, were more frequently observed in tercile group 3, hence in children whose SAT had increased. While the negative correlation of glucose and sex ($r = - 0.177$, $p = 0.001$) indicated higher concentrations in girls as compared to boys, D-mannose was related to the absolute increase of SAT ($r = 0.231$, $p < 0.001$).

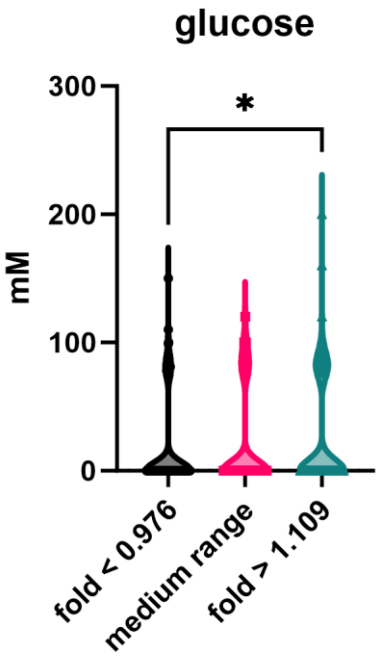


Figure 13. Glucose concentrations in mmol/mol in the tercile groups

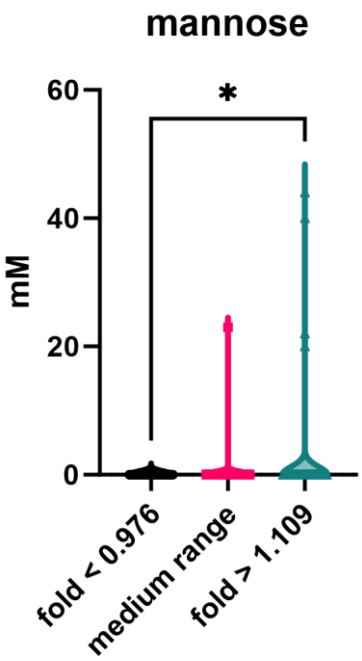


Figure 14. Mannose concentrations in mmol/mol in the tercile groups

Table 10. Glucose concentrations in mmol/mol in the tercile groups

D-Glucose				
Terzile_fold	Mean	SD	n	Occurrence (%)
1	88.32	17.43	19	25
2	89.5	12.14	26	34.21
3	90.87	25.88	31	40.79
Total	89.76	19.77	76	100

Table 11. Mannose concentrations in mmol/mol in the tercile groups

D-Mannose				
Terzile_fold	Mean	SD	n	Occurrence (%)
1	0	0	0	0
2	23		1	20
3	31.5	12.26	4	80
Total	29.8	11.28	5	100

To compare this study's results with previous results (117), succinic acid and allantoin were also studied in detail, though for succinic acid, results revealed no significant difference between the SAT tercile groups ($F_{(2, 168)} = 0.941$, $p = 0.392$). There was, however, also a tendency for higher succinic acid concentration in children's urine samples whose SAT decreased (see Table 12), which corresponds to the results of Huberts (2024).

Succinic acid was also negatively correlated to sex (r = - 0.125, p = 0.020), indicating a higher concentration in girls compared to boys, BMI (r = - 0.256, p < 0.001) and age (r = - 0.281, p < 0.001). For allantoin, including only samples where the metabolite was detectable, the result shortly failed significance ($F_{(2, 158)} = 2.756$, p = 0.067). Allantoin was present in more samples in tercile group 1, though higher concentrations were found in tercile group 3 (see Table 13). Allantoin was also negatively correlated to BMI (r = - 0.196, p < 0.001) and age (r = - 0.226, p < 0.001), which corresponds to the previous findings. Similarly, for glycine a weak, but non-significant tendency towards a higher concentration in children whose SAT increased was found ($F_{(2.321)} = 1.493$, p = 0.226, see Table 14). Glycine was only found to be negatively related to sex (r = - 0.180, p < 0.001), meaning higher concentrations in girls.

Table 12. Succinic acid concentrations in mmol/mol in the tercile groups

	Succinic acid			
Terzile_fold	Mean	SD	n	Occurrence (%)
1	13.16	4.77	64	37.43
2	14.02	5.34	56	32.75
3	12.76	4.45	51	29.82
Total	13.32	4.87	171	100

Table 13. Allantoin concentrations in mmol/mol in the tercile groups

	Allantoin			
Terzile_fold	Mean	SD	n	Occurrence (%)
1	30.62	10.34	60	37.27
2	30.63	9.80	54	33.54
3	35.49	15.60	47	29.19
Total	32.04	12.09	161	100

Table 14. Glycine concentrations in mmol/mol in the tercile groups

	Glycine			
Terzile_fold	Mean	SD	n	Occurrence (%)
1	147.05	93.29	107	33.02
2	140.81	64.06	112	34.57
3	160.21	92.5	105	32.41
Total	149.16	84.18	324	100

4.4 Prediction of SAT-Increase by the Metabolomic Profile

Together with the variables sex, age and BMI, the previously identified significant metabolites were included in a multiple linear regression model to predict the SAT fold change ranging from 0.488 to 1.721 (-51% to +72%) within the first year of investigation. N-Isovaleroylglycine and D-mannose (in combination) were able to significantly predict SAT fold change ($F_{(9,333)} = 2.498$, $p = 0.009$), however with a low overall R^2 of 0.063 ($R^2_{adj.} = 0.038$, see Table 15) indicating a weak goodness-of-fit (121).

Table 15. Prediction of the fold change of SAT (T1/T0)

	Coefficients B	SE	*Beta*	t-value	p	$R^2_{adj.}$
(constante)	0.996	0.095		10.453	<.001	**0.038**
Sex	-0.015	0.02	-0.042	-0.772	0.441	
BMI	0.001	0.003	0.022	0.376	0.707	
Age	0.001	0.008	0.009	0.159	0.874	
Dimethylamine	0.001	0.001	0.05	0.844	0.399	
N-Isovaleroylglycine	-0.011	0.005	-0.109	-2.004	**0.046**	
Taurine	0.0002	0.000	0.104	1.935	0.054	
Hippuric acid	-0.0004	0.000	-0.078	-1.436	0.152	
D-Glucose	0.0003	0.000	0.066	1.168	0.244	
D-Mannose	0.006	0.003	0.126	2.282	**0.023**	

A generalized linear model was performed in R (Table 16) to overcome the assumptions of the linear regression. Here, N-isovaleroylglycine at T0 substantially contributed to the prediction of SAT fold-changes, yet other parameters were nearly significant as well.

Table 16. Generalized linear model

	Estimate	SE	t value	p
(Intercept)	0.9645	0.0993	9.717	$< 2e^{-16***}$
Sex	-0.0167	0.02	-0.834	0.4051
BMI	0.0024	0.0030	0.806	0.4207
Age	0.0016	0.0082	0.193	0.8473
Dimethylamine	0.0017	0.0012	1.416	0.1578
Isovaleroylglycine	-0.0107	0.0054	-1.995	**0.0468***
Taurine	0.0002	0.0001	1.921	0.0556
Hippurate	-0.0000	0.0000	-1.343	0.1803
Glucose	0.0003	0.0003	1.085	0.2788
Mannose	0.0000	0.0001	0.258	0.7962

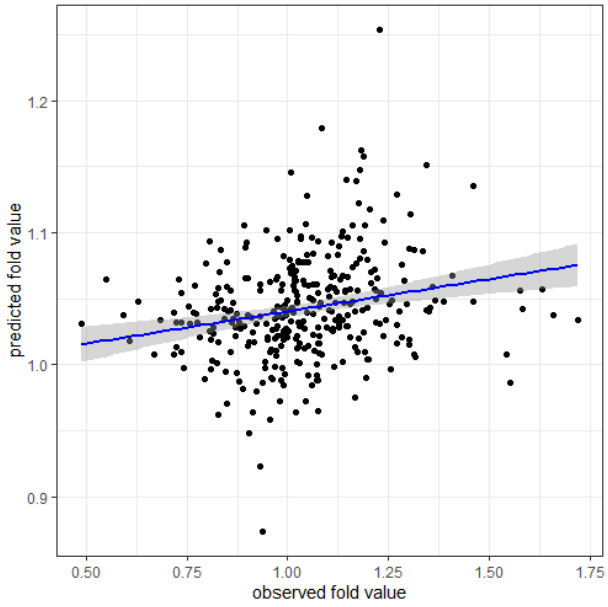


Figure 15. Comparing predicted vs. observed SAT fold-changes

On the 1st view there was not much correlation (r = 0.22) between predicted and observed fold values (Figure 15). Though, due to n = 343 samples, $p < 1 \times 10^{-16}$ is highly significant. Including only cases with an increase of SAT (n=205 children) at follow-up and predicting the absolute SAT increase revealed a similar result with N-isovaleroylglycine, D-mannose and BMI as significant predictors for the SAT change ($F_{(9, 195)} = 7.019$, $p < 0.001$) and an R^2 of 0.245 ($R^2_{adj.} = 0.21$, Table 17) for the overall model and thus moderate goodness-of-fit.

Table 17. Model to predict the absolute SAT-increase, only including cases with an increased SAT

	Coefficients B	SE	*Beta*	t-value	p	$\mathbf{R^2_{adj.}}$
(constante)	-15.548	6.185		-2.514	0.013	**0.21**
Sex	-0.306	1.244	-0.016	-0.246	0.806	
BMI	0.906	0.194	0.329	4.68	**< 0.001**	
Age	0.612	0.535	0.079	1.144	0.254	
Dimethylamine	0.108	0.072	0.106	1.498	0.136	
N-Isovaleroylglycine	-0.743	0.381	-0.126	-1.95	**0.053**	
Taurine	0.01	0.006	0.107	1.679	0.095	
Hippuric acid	0.008	0.004	0.119	1.845	0.066	
D-Glucose	-0.008	0.016	-0.032	-0.489	0.625	
D-Mannose	0.442	0.133	0.221	3.322	**0.001**	

As shown in a previous study, SAT at baseline was significantly associated with succinic acid, allantoine (low SAT), glycine (high SAT) and ethylmalonic acid concentrations (117).

Interestingly, among the highly abundant metabolites at baseline with an occurrence >10% were dimethylamine (0.997), hippuric acid (0.931), N-isovaleroylglycine (0.467) and D-glucose (0.218). Taurine (0.058) and D-mannose (0.014) were among the scarcely occurring metabolites (<10%). Moreover, co-occurrence of allantoin, which was negatively associated with SAT, with N-isovaeroylglycine (3.8 %) was determined among others (117).

5. Discussion

5.1 Summary of the Results

The hypothesis of the thesis, namely that urinary markers are associated with SAT-changes and may predict SAT fold-change, was tentatively confirmed. In the evaluated urine samples dimethylamine, N-isovaleroylglycine, hippuric acid, taurine, D-glucose, and D-mannose were notably differing between the tercile-based groups. Hippuric acid and N-isovaleroylglycine were detected in higher concentrations in children with decreased SAT at follow-up, whilst higher concentrations of dimethylamine, taurine, D-glucose and -mannose were related with SAT-increase at follow-up. N-isovaleroylglycine and D-mannose showed a significant predictive value of SAT fold-change. Thus, whether a combination of these markers may to some degree, predict SAT change, was tested.

Notably, at baseline glycine concentrations in urine were found to be significantly higher in individuals with higher SAT compared to those with lowest SAT as described by Huberts (2024) (117). In this study however, only a weak non-significant link was observed between urinary glycine concentrations at baseline and subjects with an increase of SAT at follow-up. Regarding the other metabolites significantly correlated with SAT at baseline, like succinic acid, allantoin and ethylmalonic acid, in this study, no notable link to the SAT-fold-change was found. Hence, this shows that metabolites associated with higher SAT-thickness at baseline do not entail prognostic value for predicting SAT fold-change. This means that probably different biomarkers need to be identified for present state of SAT, and for predicting an increase of SAT.

It is also worth noting that, in girls compared to boys a tendency towards higher prevalence of glucosuria and higher SAT-thickness was found in present study, which is according to previous findings. Moreover, a study analysing serum metabolic profiles for OW/OB in

young children with the focus on early differences between boys and girls found that in girls more often a serum metabolic profile for OW/OB was present (122). Such results require further research for possible causes.

5.2 Metabolites Associated with SAT Fold-Change at Follow-Up

5.2.1 N-ISOVALEROYLGLYCINE

The different statistical approaches all showed a relation between SAT-changes and N-isovaleroylglycine (IVG) concentration in samples and IVG showed a significant predictive value for SAT-change. Higher concentrations of IVG were found in individuals whose SAT had decreased at follow-up. Lower urinary levels of IVG have been observed in subjects with OB (in adolescents: Concepcion et al. 2020 (123), adults: Tan et al. (2024) (124)) compared to individuals with normal weight before.

N-isovaleroylglycine is categorised to the class of organic compounds n-acyl-alpha amino acids. It belongs to the group of acyl glycines that are trivial derivates of fatty and branched-chain amino acids (BCAAs). It has been found that in certain malfunctions related to mitochondrial fatty-acid beta oxidation, specific acyl glycines are excreted at a higher rate, which is measurable in biofluids. N-acyltransferase generates acyl glycines by transferring the acyl moiety of acyl-CoA to glycine to form CoA-SH (free coenzyme A) + N-acylglycine (125). Considering previous findings of decreased glycine concentrations in serum of individuals with OB (124) (32), it is reasonable that the pathway of acyl glycine synthesis is limited at the step where glycine becomes necessary, hence resulting in a reduction of acyl glycine excretion (124). Acyl glycines have been described as serum markers for OB in previous studies (124) (122) (123) (71). Due to the significant relation of acyl glycines with SAT-change in this study, a possible explanation was attempted by looking at the upstream processes of their formation that involve glycine. It was recently detected, that in subjects with OB, plasma levels of glycine might be decreased due to the longer time of de novo synthesis of glycine (126) but it may also be caused by glycine being incorporated into gluconeogenesis, which is activated in the presence of elevated levels of fatty acids, relocating pyruvate to Krebs cycle (127), which may be a co-actor for higher levels of blood glucose (70). A further focus in other studies looking at the association of Glycine with OB, could be why its de novo synthesis is decreased due to IR in individuals with OB. Hypoglycinaemia has shown to elevate risk of T2DM in prospective studies. In slim children of parents with T2DM (128) and in in subjects before

the onset of clinical features of T2DM (129), plasma glycine levels were reduced compared to healthy individuals (128). Glycine in plasma has shown a positive association with Insulin sensitivity and a negative one with IR (129). On pancreatic beta-cells, receptors for glycine have been identified, however, the role of glycine on insulin secretion is not known (130). Furthermore, an inverse correlation of glycine concentrations in plasma with risk of acute myocardial infarction has been detected. Elevated levels have shown an association with reduced prevalence of OB and DM and an improved lipid profile (131).

Other diseases, which are related to IVG are among others, an autosomal recessive disorder, called Isovaleric Academia (IVA), where an increase of IVG happens due to mutations in the isovaleryl-CoA dehydrogenase gene (132), and colorectal carcinoma (CRC). IVG-concentration in CRC is significantly higher in the cecum and ascending tumors' samples compared to normal mucosa (133).

In this study elevated IVG was associated with SAT-decrease at follow-up, which is in accordance with previous studies that measured decreased levels of IVG in urine of subjects with OB. In adolescents aged 13-19 years, a strong correlation of urinary IVG with insulin resistance (IR) as measured by Homeostasis Model Assessment (HOMA)-IR was observed. Furthermore, a significant decrease of acyl glycines, including IVG, was measured in adolescents with T2D or OB (123). Considering this, IVG and the role of further N-acyl glycines in OB should be further evaluated. In children aged 6-11 years however, the association of lower IVG with SAT-increase appears to be a novel finding.

5.2.2 HIPPURIC ACID

As with IVG, higher levels of hippuric acid (HA) were found to be related to SAT-decrease in children at follow-up. Like IVG, HA belongs to the group of acyl glycines and therefore is also a product of the enzymatic reaction catalysed by N-acyltransferases as described above (125). Reduced concentrations of HA, among other gut microbiota-derived metabolites, were detected in urine of adolescents with OB in a study by Cho et al. (2017) (32), which corresponds to our findings. Decreased levels of benzoate derivates and HA in urine have been steadily found as a biomarker for OB (134) (135).

In 1841, the identification of HA was described the first time (136). Due to the fact, that the metabolite was primarily extracted from horse urine, the name 'hippuric', deriving from the Greek word for horse, 'hippos', was designated for the acid (137). It has been

observed that phytochemicals occurring in a variety of fruits, coffee, green and black tea and wine are metabolised by gut microbiota to benzoic acid, which is further conjugated in the liver with glycine to HA, hence HA also may reflect alterations in liver function (138). The metabolite is then extracted from circulation in the kidneys and removed from the body with urine (137). Recent studies have determined HA as a suitable biomarker for fruit and vegetable intake, because a significant association has been measured (139) (140). Moreover, the metabolite serves as an unspecific marker for exposure to toluene (141) (125). Increased urinary HA concentrations have been detected in correlation with the consumption of acetylsalicylic acid and sodium benzoate (138) (142). In the clinical setting, levels of urinary HA have been assessed after sodium benzoate treatment of liver disorders of, i.e. inborn error of urea synthesis (143). According to a study by R.R. Pero (2010), as cited by Bhattacharyya et al. (2023) (144), HA also may be applied for evaluating intestinal microbiome health, especially for the microbiota shikimate pathway, which besides producing HA from quinic acid, generates essential amino acids (AAs), like phenylalanine and tryptophane, and vitamins. Hence HA can indicate alterations in gut microbiota activity (145). Xu et al. (2024) detected how HA influences the Alistipes indistincturs, an intestinal bacterium, for lowering uric acid (146). Interestingly, uric acid has been found to potentially be the catalysator of inflammatory reaction processes in OB (66). As a uremic toxin HA causes malfunctions in protein binding of drugs (125). Sarcopenia, cognitive deficiency, and frailty, all impact urinary and plasma concentrations of HA, which is why it might serve as a biomarker for age-related conditions. Generally, HA-levels get higher with increasing age, however, in individuals with physical frailty, decreased levels in both urine and plasma have been detected (147) (145). Reduced HA acid levels in urine have been measured in patients with Crohn's disease in a study by Williams et al. (2009) as cited by Bhattacharyya et al. (2023) (144), Schizophrenia (148) and OB (32) which is supposedly caused by a change in gut microbiota function (144). A recent study detected HA in urine, with among others glutamate, as strong biomarkers for colorectal carcinoma (CRC). Point of care tests using urine for CRC discovery could be helpful, especially in countries with restricted resources (149).

With the advance in technologies, different tools for easy detection of HA in urine the clinical setting have been developed, such as primarily Cd(ii)/Zn(ii)-based luminescent sensors (150), molecular-imprinted electrochemical sensor (151), hydrophilic "turn-on"-type molecularly imprinted polymer (MIP) microparticles (152), and a colorimetric assay (144). Such approaches may provide improved cost-efficiency, point-of care clinical tools

for a simpler, quicker, and more precise detection of urinary HA. The colorimetric assay e.g. was found to be qualified for CRC-screening (144).
A significant inverse correlation at urinary HA levels over 0.76 mM with the risk of metabolic disease was determined using fluorescein. Thus, implying the impact of dietary choices, specifically the intake of tea, fruits and coffee on metabolism. Fast determination of urinary HA for assessing health status could be reached by application of this method (153). This technique could be a great tool for screening of OB as well, if further studies confirm the results of the present thesis. HA and its derivates have been described as promising drug candidates, e.g. antiretroviral compounds. Furthermore, the potential of HA derivates for anti-bacterial, antifungal and antitumor usage has been determined (154). Among other factors, higher levels of HA may be caused by Mediterranean diet. Additionally, as this dietary choice is healthier, it may have led to a decrease of SAT in present study (155). In children between 7-18 years with a mean age of about 10 years, urinary hippuric acid concentrations were inversely correlated with height and weight in a lifestyle intervention study (156). This is in accordance with our findings of higher urinary HA in children with decreased SAT at follow-up. Also, the significant associations of the acyl glycines, HA and IVG, with SAT fold-change, are aligning with previous findings on these metabolites related to OB. However, the novelty of this study is due to the method of measuring SAT and not anthropometrics and that these associations also are relevant in children aged 6 to 11 years.

5.2.3 TAURINE

Higher urinary levels of taurine in children with increased SAT at follow-up were found. This result may be explained by dietary differences among the groups, as taurine occurs in various kinds of food, which are presumably a significant contributing factor to different SAT-changes. After an intervention program together with weight loss, urinary taurine and glycine were found to be significantly reduced in adolescents (157). Decreased taurine plasma levels have been measured in individuals with T2DM compared to controls (158). Protective effects of taurine on OB and T2DM, due to its antioxidative, anti-inflammatory properties as well as taurine promoting insulin secretion and sensitivity, have been found. It was observed to activate factors that play a role in fatty acid oxidation to enhance energy spending, lower the amount of energy applied for synthesis of glycogen and lipogenesis, and decrease oxidation of glucose after food intake to reduce lipogenesis (159). Several

studies' findings imply that in OB and diabetes there is a deficiency of taurine. This may be the result of reduced intestinal absorption of taurine and lower synthesis rates in adipocytes, while there is an increased excretion of taurine with the urine (160). The higher urinary excretion of taurine in states of OB corresponds to the higher concentrations of taurine associated with SAT-increase in this study. However, a global study described a substantial decreased risk of CVD in individuals with higher 24-hour urinary excretion of taurine and magnesium compared to the average, leading to the conclusion that since taurine and magnesium are biomarkers for vegetables, nuts, soy, etc., dietary recommendations of these foods could be made for prevention of CVD (161). Also, another study showed an inverse association of urinary taurine levels with BMI (162), which is contrary to our findings. However, direct comparability is not given due to different methods used BMI, SAT and a different cohort observed. Decreased plasma concentrations of taurine have been detected in patients with different diseases such as hypertension, obesity, depression, gout, or hypothyroidism. Sources with high concentrations of taurine are different vegetables, fish and animal products (125). Taurine is frequently occurring in energy drinks (163). There is a large quantity of taurine found e.g. in the brain, the retina, the kidneys and the gallbladder, where it impacts physiological and pathological conditions. Taurine acts as stabilizing factor in cell membranes, as a neurotransmitter in the brain, and assists transport of ions like potassium, sodium, magnesium, and calcium. Maple syrup urine disease, an inborn defect of metabolism, has been described to reveal increased concentrations of taurine (125). Taurine concentration in the heart, is about 100 times higher than in plasma. It acts as regulator of ion shifts intracellularly, controls calcium, and has an antioxidative effect in the heart. Taurine deficiency has been associated with cardiomyopathy (164). Short-term application of taurine leads to an intracellular increase of sodium, subsequently calcium increases, causing a positive inotropic impact on the heart (163). Oral supplementation with taurine stopped retinal degeneration and corrected cardiomyopathy (CMP) in a genetically caused taurine transporter deficiency (165). Taurine acts as an important inhibitory neurotransmitter in the brain besides gamma-aminobutyric acid (GABA). Due to the inhibitory characteristics, it has anxiolytic and anticonvulsant effects and a significant reduction of taurine in the brain has been described in patients with depression. Taurine is involved in multiple metabolic processes, such as bile acid biosynthesis, sulphur metabolism and inner membrane transport. Supplementary taurine may provoke the excretion of insulin and prolactin. Bilirubin and cholesterol excretion is increased in bile

under the impact of taurine. As 5-L-Glutamyl-taurine is produced by the parathyroid gland, which is glutamic acid-taurine, it also being involved in endocrinologic pathways is shown (125).

The link of higher taurine concentrations in urine with SAT-increase at follow-up corresponds to a previous study, describing weight loss in adolescents associated with decreased urinary taurine levels. However, in children the finding of associating taurine with SAT-increase seems to be new.

5.2.4 DIMETHYLAMINE

In this study, subjects with higher dimethylamine levels revealed a tendency towards a SAT-increase at follow-up. The organic secondary amine, dimethylamine (DMA), is highly occurrent in human urine. Asymmetric dimethylarginine (ADMA), an inhibitor of nitric oxide production, and trimethylamine N-oxide, frequently found in foods, are common sources of urinary DMA. Significantly elevated urinary DMA levels have been measured in subjects after eating fish and seafoods (166). It is a metabolite of bacteria Micrococcus and Arthrobacter and present in plants. DMA furthermore is listed as a uremic toxin (125). Industrially, it is applied as a fungicide in agriculture, de-hairing agent in tanning, and it is found in cleaning supplies and dyes (125).

Also, DMA has been studied in the context of air pollution (167). It is involved in citalopram metabolism. In acid milieu in the body, e.g. gastric, DMA goes through nitrosation resulting in dimethylnitrosamine (NDMA), which is an oncogenic substance (125). Drugs containing Metformin have shown to cause detectable concentrations of N-dimethylnitrosamine (NDMA). A daily intake of NDMA higher than 96 ng would be problematic when applied long-term (168). Higher urinary DMA concentrations were observed in children with inflammatory diseases compared to children with attention deficit hyperactivity disorder (ADHD) (169).A direct association of BMI with urinary DMA has been described in adults (162). Lower urinary levels and higher plasma levels of DMA and trimethylamine-N-oxide (TMAO) were measured in children with advanced stages of chronic kidney disease (CKD) (G2-G4) compared to children with CKD in an early stage (G1). Furthermore, DMA concentrations in plasma and cardiovascular risk, were revealed to be inversely related. (170). In a study analysing differences of urinary metabolic profiles between neonates that were breast- or formula fed, urinary excretion of DMA showed to be the discriminative factor of the breastfed neonates (171). At baseline, along with succinic acid and allantoin, dimethylamine ($r=-0.261$, $p< 0.001$, occurrence

48%) was revealing the strongest correlation with weight, BMI, and body height as described by Huberts (2024) (117). This may align with the mild association of DMA concentrations in children with an increase of SAT at follow-up in the present study.

5.2.5 D-GLUCOSE

Glucose was detected in 76 (22.16 %) of the 343 urine samples, of which in various samples the physiological concentrations of glucose in urine of <0.83 mMol/L (<15 mg/dL; note that this is an adult reference value, as none for children exist to date (172)) were surpassed, which is referred to as glucosuria. Normoglycemic glucosuria occurs, when the renal threshold is reduced, e.g. due to tubulointerstitial nephritis, and hyperglycaemic glucosuria happens, when the glucose concentration in serum surpasses the renal threshold, which for instance is the case in a manifest diabetes mellitus (173). There was no substantial link between urinary glucose and the SAT-increase.

In a school screening programme for glucosuria in a Korean province, 110 children with previously unknown diabetes were detected. Glucosuria was higher present in children above elementary school grades and among girls. Individuals with newly detected diabetes by urine did not exhibit any symptoms. The researchers concluded that urinary glucose screening in schools offers a practicable and easy tool to early diagnosis of asymptomatic T2DM (174), which was also found in other studies (175). Type 1 diabetes mellitus (T1DM) is the most common type among children, though in various reports a continuously increase of young individuals affected by T2DM has been described (176) (177), also in Austria, whereas other types of diabetes were found to occur twice as much in the age group up to <15 years (178)**.** This echoes the childhood obesity epidemy and poses a challenge for public health. Due to its period of being asymptomatic and the severe possible health consequences, T2DM screening in children was agreed on by the American Diabetes Association and the American Academy of Paediatrics (179). Glucosuria screening at schools has been conducted since 1974 in different countries and in Japan OGTT is performed in children whose second urine samples after two weeks also result positive for glucose. Japanese studies revealed a high incidence of discovery of childhood T2DM by screening for urinary glucosuria in schools, and over 80% of these children had OB (180) (181) (174). Cost-efficient, easy-applicable techniques are required especially in communities with low socioeconomic resource for detecting diabetes. In the last years, technology developments resulted in new approaches of analysing glucosuria, such as

applying natural enzymes, metal or graphene. Especially several types of biosensors based on nanozymes, which are cost-efficient, stable and produced in high amounts, and portable devices like smartphones or analytical tools, based on microfluidic paper, have simplified real time precise monitoring of urinary glucose (182).

Not all 76 samples in which glucose was detected (in total 22.16%) can simply be classified samples from prediabetic or even diabetic children, but rather reflect the high sensitivity of NMR spectroscopy, which can detect concentrations from 0.63 mMol/L in the applied method used. Since glucose is filtrated in the glomeruli and actively re-absorbed in the proximal tubule there is always some glucose in urine, <0.1% (<0.25 mg/ml= < 1.39 mMol/L) (183). Hence, low concentrations might rather reflect physiological concentrations. In the most applied urine test strips, based on the glucose oxidase method, glucose concentration in urine is considered negative <2 mMol/L (184). The low sensitivity of such test strips is the reason why screening for diabetes with those is obsolete (172). In the study by Kim et al. (2017) urine was regarded as positive with concentrations of glucose above 5.6 mmol/L (174), which is a substantially higher value for positivity than it was in this study (0.83 mMol/L). Still, the urine samples with glucose, especially the samples with glucosuria, need to be followed up upon. Probably more than one long-term follow-up study is needed to define a clear cut-off value for urinary glucose for diagnosis of pre-diabetes or diabetes (confirming test needed like OGTT) in children with this method. In Austria, in recent years, a significant increase of diabetic ketoacidosis was observed at first manifestation of T1DM, showing that often, in almost every second child, diagnosis is delayed (185). The highest standardised incidence of T1DM in Austria was observed in 2021, timely co-occurring with the Covid-19 pandemic (178). In children and adolescents with missing symptoms but detected hyperglycaemia and/or glycosuria, which may transiently occur in the course of an infection, further clarification should be carried out in a paediatric diabetology centre (178). To differentiate between possible underlying causes, blood glucose levels should be measured, when glucosuria is present and to prevent major health consequences and not overlook a developing T2DM, in affected children of the present study, follow-up testing of their urinary glucose levels and an OGTT, would be advised aligning with the protocol followed in Japan, as mentioned above. The mild association of urinary glucose and SAT-increase and the higher occurrence of glucosuria among girls aligns this study with the findings of the study by Kim et al. (2017) (174).

5.2.6 D-MANNOSE

D-mannose showed an association with SAT-increase at follow-up in this study and showed together with IVG a significant predictive value for SAT-increase.

The isomer of glucose is classified as an aldohexose. It occurs in two distinctively sized rings, the furanose and pyranose form. It can be synthesized in humans from glucose or transformed into it (125). D-mannose is about five times more active than glucose in non-enzymatic glycation, which might be the reason that during evolution it did not become the primary energy source (186). The aldohexose can be found in animals, microbes and plants. Digestion of polysaccharides also results in mannose. High-mannose-typed oligosaccharides have a substantial function in the control of protein quality. For the innate immune system mannose-binding lectin is essential, since it connects to sugar structures on microbes subsequently leading to the activation of the complement system due to a certain protease.

Associations of D-mannose and diseases like galactosemia, fructosuria, and fructose intolerance have been described. For the prevention of urinary tract infections, D-mannose is supplemented as it blocks the binding of bacteria to urothelial cells by saturation of the bacterial film (125). The liver is mainly responsible for mannose-processing. It has been suggested that the metabolite might contribute to the evolution of IR since it exhibits malfunctioning glycosylation, that may impact insulin receptors located in liver tissue (187) (188). Together with fasting glucose and α-hydroxybutyrate, mannose is a recognised early biomarker for T2DM (106). Elevated Mannose levels have been detected in plasma samples of children with OB (71) and serum samples of adults with OB (189), which is in line with our findings. Although mannose was only weakly related to SAT-increase in the present study, when looking at known correlations to OB and T2DM, further studies assessing the diagnostic value of urinary metabolites to detect OB in children should include D-mannose.

5.3 Urinary Metabolites Previously Associated with OW/OB in Children

As OB is related with metabolic dysfunctions and in metabolomics small alterations in metabolic pathways can be recognised, metabolites present as useful biomarkers for predicting metabolic disturbances and increasing the understanding of OB. Cho et al. (2017) found a significant difference in the metabolic profiles between children and adolescents with and without OB (32). They identified distinguishing urinary metabolites,

which revealed that insulin resistance triggered by inflammation, oxidative stress, ammonia toxicity as well as dyslipidaemia and elevated urinary levels of nucleic acid oxidation biomarkers (190), could constitute important metabolomic signatures in adolescents with OB. Such findings align with the results of studies indicating inflammation as a substantial factor of T2DM and OB (32). Moreover, at a high specificity and sensitivity, metabolites such as docosanoic acid or 12-oxo-20-carboxy-leukotriene B4, were correlated with inflammation, increased aspartate aminotransferase (AST), alanine aminotransferase (ALT), BMI, and Cholesterol. Their significantly elevated levels in young subjects with OB indicated that they may serve as markers to classify adolescents being at increased cardiovascular risk along with OB (32). An increase of adipose tissue has also shown to be related to a decrease of urine pH (191). Metabolite classes involved in the determined metabolic features of OB in assessed studies by Rangel-Huerta et al. (2019) were sexual steroids, protein metabolism, acylcarnitines (AC), lipids, carbohydrates, amino acids (AAs), and other ungrouped molecules (188).

In a systematic review on metabolic traits of OB in children, four studies assessing urinary metabolic profiles were found, in which 71 correlations of metabolites with OB were described. Overall, often a consistency of correlations found in urine with those described in blood was noticed. However, two studies looking at both (123) (192), suggested that biological explanation of metabolites detected in urine and blood may vary (193).

In children with OB and NAFLD, small intestinal bacterial overgrowth (SIBO) and intestinal permeability (IP) were found to be higher in all children with OB. BCAAs and their metabolites showed associations with an abundance of visceral fat centimetres (oxo-valerate/ leucine) as well as with a stronger disturbance of IP and SIBO (valine metabolites). The study's results showed that urine metabolomics may present a tool to define OB in children via a metabolic signature in determining pathways typically representing food preferences and gut-liver-axis dysfunctions (24).

Comparing metabolites measured in blood and urine, in urinary metabolites substantially more associations to diet and microbial-derivates have been detected. Therefore, more studies measuring blood and urinary markers could further enlighten the influence of these features in metabolic characteristics associated with OB. Analysing metabolites in urine is becoming a more commonly used tool to gain objective insights in diet (194) and may offer a simpler approach for determining gut microbiota than metagenomic assessment of faecal probes (195) (193).

Numerous connections between BMI and urinary compounds, with among others succinate and citrate (both tricarboxylic acid cycle intermediates) have been described (162), corresponding to the results by Huberts et al. (2024) where a correlation of succinate and SAT was described (117).
Detecting metabolic characteristics related to age could help efficient prevention therapy for young individuals with OB. Body composition has been advocated to determine the risk of OB, dyslipidaemia and CVD (196). Also, puberty and sex hormones have been described to promote the development of OB (197). A correlation of DHEA-S serum concentrations with BMI and adiposity in children with OB was found by Butte et al. (2015) (71) (188). Already in 5-year-olds differences regarding sex became visible in more noticeable serum metabolic profiles for OW/adiposity in girls (122).
A reliable characteristic of paediatric OB metabolomics, when looking at studies including blood and urine has been observed. This included amino acids, especially BCAAs and aromatic amino acids (AAAs), lipids, steroids and carnitines. These profiles seemed widely concurring with those found in adults (193). Also, it was argued that metabolic associations with OB from results in studies on adults may be applied to children (198). The difficulty lies in differing pathological changes from physiological variations. Advancing individual therapy and enhancing individual nutrition could be achieved with metabolomics. Furthermore, disease-associated changes of one's phenotype may be determined as well as the efficacy of drugs (199). Identification of specific biomarkers for certain conditions is a promising approach for future diagnostics. In the case of the present study, to apply a tool measuring certain urinary metabolites associated with OB would be the further goal, once the relevant metabolites have been established.

5.4 Height and Weight Development

Children show drastic changes in growth and weight development between the age of 6 to 11. The weight increase in this study was independent from the BMI at baseline. Each child presented with a relatively similar weight gain. Children who had more weight in the beginning also weighed more at follow-up.
The change of weight's mean in girls was 2.92 kg (± 2.22) and in boys 2.67 kg (± 1.96) reflecting a higher gain of weight in girls compared to boys, while the height's changes did not differ as much between the sexes. Though a significant difference of height ($p < 0.042$) between girls and boys was found at baseline with boys being taller than girls. The

absolute and relative change of SAT was not correlated with height but significantly with weight ($p < 0.001$).

5.5 SAT

The standardized 8-sites approach of measuring SAT via US has multiple times showed high inter- and intra- observer reliability in different samples of adults and good intra-observer reliability in children (200) (43) (201). However, in a study by Störchle et al. (2018) in ten subjects the SAT means of eight sites were compared to the mean of 216 random sites, resulting in an amplified estimate of the 8 sites for the overall SAT compared to the estimate of the 216 sites (41). This shows the restricted potential of assuming total SAT from the 8-sites approach. Moreover, besides this matter, more research is necessary to recognise what amount of SAT-increase is clinically relevant, and a clear definition and/or classification of OW/OB according to SAT-thickness reference values is still missing. By utilization of terciles for categorising SAT fold-change in this study, this limitation was addressed.

Notable differences between the sexes regarding SAT was shown (Table 4). Generally, it becomes visible that girls tend to have a significantly ($p < 0.001$; girls' mean at follow-up (FUP): 83.21 mm and boys' mean at FUP: 67.12 mm) higher SAT than boys, which has been described in literature before. For example, examining 274 German preschool children resulted in significant differences of SAT thickness between girls and boys, while BMI, WC and anthropometrics like body height and mass did not differ relevantly (200), another study also resulted in higher SAT in girls (40) and in a study by Störchle et al. (2017) the LT-position was applied the first time and SAT-thickness there showed significant differences between women and men (56). In these studies, SAT was described as higher in females. The differences revealed in SAT measurements between girls and boys, namely higher SAT-thickness in girls, did not show via BMI nor weight.
Notably, although not many studies measuring SAT by US have been conducted so far, the children of the present study showed to have a higher mean SAT-thickness in mm (baseline: 71.46 ± 41.89; FUP: 74.68 ± 44.94) than in other studies. For instance, in a study by Schmid-Zalaudek et al. (2021) in 7–10-year-old South African children the mean SAT in mm was 47.65 ± 21.7 (40) and in German preschool children the mean SAT in mm was 46.5 ± 16.3 (200). Yet in the German study it can be argued that the lower mean of SAT

may be due to the younger age of the examined children compared to those in this study. In present study it was also shown that the total SAT amount is higher at increased age (see above, Figure 8).

5.6 Dietary Factors

As described above, the metabolites significantly correlating with SAT-change are present in a variety of foods and therefore elevated urinary levels of such may be influenced by the children's dietary habits. However, diet has not been closely evaluated/ considered in the present study, although it had been assessed in questionnaires. Nevertheless, dietary choice has an impact on body composition and therefore, if a metabolite indicating a certain diet associated with an increase or decrease of fat, this could be a correlating factor to presumed associations and weaken significance. Since via metabolomics the diet, derivates of microbiota metabolism and physiological alterations, can be assessed, future research should look at these factors' associations and OB in children with metabolomics (202) (193).

Metabolomics may serve as early markers of dietary choices for recognising risk factors of poor nutritional health, leading to interventions and recommendations. For instance, HA, N- methylnicotinic acid, urea, and sucrose together have been correlated with Mediterranean diet (155). A demonstration of how dietary factors alone are also influenced by various factors: While vegan diet has been associated with reduced risks of MetS and CVD, a risk of nutritional lack may be given. However, direct comparability is constrained since individuals with vegan diets more commonly are from higher socioeconomic backgrounds than non-vegetarians making a lifestyle focused on health more affordable, e.g. by supplementation of essential nutrients like vitamin B12 etc (203).

5.7 Limitations

First, as mentioned above, no unified standardised definition for OW/OB in children exists up to now, hence studies having investigated whether a biomarker for OW/OB could be found, may have applied different definitions. Furthermore, several studies on metabolomics and OW/OB exist, however some of them analysed serum, plasma, or urine, or both. In this study, urinary metabolites were analysed regarding SAT-change in children, which up to date does not represent a reference value for OW/OB and therefore direct comparability is not given. However, this fact also accounts for the unique character

of the present thesis, being the first one comparing these certain variables besides Huberts (2024) (117).
The concentration differences for detected metabolites correlating with SAT-change were barely significant or non-significant anymore after post hoc adjustment for multiple comparison. For prediction of SAT fold-change in an individual pupil by applying metabolomics presumably more research is still needed. A closer evaluation of confounding factors and assessing them prior to taking samples or being more restrictive with whom to include is necessary. Interestingly, confounding factors which were included into the generalized-linear model (GLM) to predict SAT fold-change, and which were expected to somehow contribute to the model like sex, age, and BMI, did not significantly contribute to it.

Most metabolites were only present in a few samples, therefore no prediction from these metabolite concentrations or occurrences and no identification of biomarkers for OW/OB in children is possible to date. Larger scaled studies are necessary to gain more insight into metabolic profiles of OW/OB. Hereby, also a closer evaluation of dietary influences, as well as differentiation of known diseases should be done.
In existing studies, methods vary widely as does the coverage of metabolites and the accuracy and quantification showing challenges in discovering patterns of metabolic profiles due to inconsistency of correlations (193). Even when comparing this thesis with Huberts´ thesis (2024), with similar methods and participants, but other research questions, different associations of urinary metabolites with SAT became visible, requiring the need for distinctive markers for each outcome.

Assessing puberty-stages with the Tanner-Test to control pubertal metabolic impacts was not done, due to limited capacities and to avoid reservations of parents and children towards participating in the study. Future studies should account for puberty status and related metabolic changes. Urinary metabolites, such as glycine or serine, associated with early puberty have been identified in a cohort of 7 to 12-year-olds (101), hence some effect may not be excluded. Still, the oldest children being 11, no substantial pubertal metabolic changes would be expected, although early onset of puberty is increasing**.**

The time of collection of the urine samples differed, even though it was aimed to obtaining morning urine. Moreover, food or fluid intake prior to urinating had not been evaluated. As

described above, certain metabolites are highly associated with the intake of certain foods, e.g. HA with fruits (139), which may confound in present results as this variable has not been considered. This implies another reason to critically look at present results. However, regarding fluid intake the normalisation via creatinine was done to overcome the variance in concentrations. Anyhow, as outlined above, more precise inclusion criteria would help to avoid confounding errors and determine correlation more clearly. Further studies are needed to confirm the results.

5.8 Conclusion

The aim of this study was to test, whether certain urinary metabolites may predict SAT-change in children and therefore may be applicable in the field to prevent the development of OB. This was done via US to measure SAT and avoid the BMI. The hypothesis, that certain metabolites may predict SAT-changes could be tentatively confirmed. It was also observed that:

- SAT-increase was highly associated with weight, but not height. Children in tercile group 1 showed a decrease of SAT at FUP while both other groups had an increase of SAT. In 59.8% (n=205) of the children, the total SAT- amount increased within one schoolyear, with a higher proportion of girls. The differences of SAT between girls and boys found, would not have been detected via BMI nor weight and are aligning with previous findings.
- Fifty-seven of 150 metabolites showed an occurrence >5 in the urine samples. These metabolites were statistically analysed regarding associations with SAT fold-change.
- Elevated levels of N-isovaleroylglycine (IVG) and hippuric acid (HA), both of which are acyl glycines, were related with SAT-decrease at FUP, which is in accordance with prior findings of associating lower IVG and HA with OB. Hence, their potential as such markers should be further explored.
- IVG and D-mannose, showed a significant predictive value for SAT-change.
- Taurine, dimethylamine, D-mannose and -glucose were associated with SAT-increase.
- Glucose was detected in 76 samples, which requires further testing.

- In girls compared to boys a tendency towards higher prevalence of glucosuria and higher SAT-thickness was found, which is according to previous findings. Such results require further research for possible causes.
- This study's results did not align with the results of the associations of SAT-thickness and metabolites at baseline. However, the findings indicate that a prediction of SAT fold-change may be possible when grouping metabolites.

To assess the reliability of this result and to determine whether these metabolites are fit to be regarded as biomarkers, further studies are required, as well as exploring the role of these metabolites in the development of obesity. However, these are relevant results because they allow for a new way of detecting adiposity in children, which may detect earlier stages of OB than traditional methods and therefore prevent serious health consequences. Additionally, they could replace/complement the usage of BMI, a measurement instrument that has proven to be problematic in many aspects. It would be a big step forward for preventive medicine to routinely implement the analysis of such biomarkers in routine urine screenings.

5.9 Outlook

For now, it is known that certain metabolites can be linked to SAT-thickness, namely glycine, succinic acid, and allantoin (Huberts, Phillip (2024) (117)). In this study particularly N-isovaleroylglycine (IVG) and D-mannose revealed a relevant predictive value for SAT fold-change. Nevertheless, from these findings no exact prognosis for SAT fold-change can be made. Further studies are required to measure and observe longer time periods and developments. Also, a clear definition and/or classification of OW/OB according to SAT-thickness reference values needs to be established.

OB does not affect all population groups the same way (204). It has been found that Social Determinants of Health (SDoHs), i.e. racism (205) or harming environmental conditions, have a major impact on the development of OB (206). Such SDoHs may trigger changes in metabolism, immunology and epigenetics (207) (208) (209). Recently more focus is put on socioeconomic factors and inequity promoting the development of OB, leading to a shift from the idea of individual's responsibility to recognising the structural settings' impacts and holding governments and bigger companies accountable (210). An overview of 9

levels of responsibility for OB-development and possible targets for intervention has been promoted. It is listing the national, food production, educational, health care, public health, local policy makers, societal, parental and individual level, as detailed described in Berry (2020) (211). Also, DiCiaula et al. (2024) criticized in their study that the impact of environmental aspects, such as unsustainable food manufacture, pollution of the environment, largely spread endocrine disrupting chemicals (EDC), packaging and marketing and the climate crisis on increasing OB-rates worldwide is commonly ignored by policy makers and professionals in the health care settings (212). Governmental initiatives are crucial to develop both prevention and treatment programmes on the ground, such as setting obesity funds and nutritional health standards in schools and improving regulation of behavioural trends. While there is mostly no training for diagnosing and treating OB in children for medical staff, affected families often lack the adequate support and instructions. Funding educational programmes for medical staff and community health workers would offer a huge advantage to address the problem in the field. For programmes to be effective they should be set long term and responsibilities on measuring them be set (213). Accountability systems to decrease NCDs should be promoted (19). In Europe different public programmes have already been started to evaluate, prevent and reduce OB, such as the 'COSI' (Childhood Obesity Surveillance Initiative), 'CHOP' (EU Childhood Obesity Programme), 'Identification and prevention of Dietary- and lifestyle-induced health EFfects In Children and infantS' (IDEFICS, Germany) and 'Enorm in Form' (ÖGK Wien) as well as the HAPHC-project, with varying methods and targeted children. Findings of such programmes need to be passed on to governmental institutions and the public to develop strategies to prevent the development of OW/OB in children. The Austrian Obesity Alliance has been initiated in 2022 to advocate novel strategies in managing OB and to recognize it as a disease. Moreover, the necessity to incorporate treatment and prevention of OB in the universal health care system has been emphasised (214). For treatment, like health and lifestyle interventions, including families as well as health professionals and public institutions, like schools, and the community are required (215) (216).

The technique of metabolic profiling comes with a lot of advantages and could become the key to precisely analyse individual metabolic processes as response to pathophysiological stimuli like changes in the environment, lifestyle, drugs, epigenetic factors and various diseases. With advances in the field of metabolomics it could become a tool for early

diagnosis of diseases before onset of clinically relevant symptoms, as well as monitoring progress of diseases, in finding the most suitable therapeutic intervention, and serve as a predictor to responsiveness to therapy and to evade overtreatments. Another possible field of application could be nutritional evaluation, as well as observing children's growth (73). Once there exist urinary biomarkers for OW/OB in children, POCT applicable in clinical practice for screening for such, should be developed. Studying metabolomics opens a highly relevant medical, socio-economic, interdisciplinary field, because it enables early recognition of diseases and the chance for early intervention for healthy happy children with a healthy happy future!

To add the molecular aspect into the definition of MetS or a molecular definition of the condition as such has already been proposed in Bruzzone et al. (2021) (96). The same could apply to defining OW/OB in children. In primary school children, like up to now the BMI, devices for OB-screening, like urine sticks (73), could be applied in a large scale.

6. References

1. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Papers Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ. 2000;320(7244):1240-3. doi:10.1136/bmj.320.7244.1240.
2. Friedemann C, Heneghan C, Mahtani K, Thompson M, Perera R, Ward AM. Cardiovascular disease risk in healthy children and its association with body mass index: Systematic review and meta-analysis. BMJ. 2012;345(7876):1-16. doi:10.1136/bmj.e4759.
3. Zapata JK, Azcona-Sanjulian MC, Catalán V, Ramírez B, Silva C, Rodríguez A, et al. BMI-based obesity classification misses children and adolescents with raised cardiometabolic risk due to increased adiposity. Cardiovascular Diabetology. 2023;22(1):1-10. doi:10.1186/s12933-023-01972-8.
4. World Health Organization. Obesity and overweight. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight [Accessed 11th December].
5. Yárnoz-Esquiroz P, Olazarán L, Aguas-Ayesa M, Perdomo CM, García-Goñi M, Silva C, et al. 'Obesities': Position statement on a complex disease entity with multifaceted drivers. Eur J Clin Invest. 2022;52(7):1-6. doi:10.1111/eci.13811.
6. Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. Pediatric Obesity. 2012;7(4):284–94. doi:10.1111/j.2047-6310.2012.00064.x.
7. Smith JD, Fu E, Kobayashi MA. Prevention and Management of Childhood Obesity and Its Psychological and Health Comorbidities. Annual Review of Clinical Psychology. 2020;16:351–78. doi:10.1146/annurev-clinpsy-100219-060201.
8. De Onis M. WHO Child Growth Standards based on length/height, weight and age. Acta Paediatrica, International Journal of Paediatrics. 2006;95(Suppl. 450):76–85. doi:10.1080/08035320500495548.
9. De Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. Bulletin of the World Health Organization. 2007;85(9):660–67. doi:10.2471/BLT.07.043497.
10. Aggarwal B, Jain V. Obesity in Children: Definition, Etiology and Approach. Indian Journal of Pediatrics. 2018;85(6):463–71. doi:10.1007/s12098-017-2531-x.
11. Rolland-Cachera MF. Childhood obesity: Current definitions and recommendations for their use. International Journal of Pediatric Obesity. 2011;6:325–31. doi:10.3109/17477166.2011.607458.
12. World Health Organization. Global health risks: mortality and burden of disease attributable to selected major risks. Geneva: WHO; 2009. https://www.who.int/publications/i/item/9789241563871.

13. Muthuri SK, Francis CE, Wachira LJM, LeBlanc AG, Sampson M, Onywera VO, et al. Evidence of an Overweight/Obesity Transition among School-Aged Children and Youth in Sub-Saharan Africa: A Systematic Review. PLoS ONE. 2014;9(3): e92846. doi:10.1371/journal.pone.0092846.
14. World Health Organization. Regional Office for Europe. Report on the fifth round of data collection, 2018–2020: WHO European Childhood Obesity Surveillance Initiative (COSI). Copenhagen: WHO; 2022. https://apps.who.int/iris/handle/10665/363950.
15. Ogden CL, Carroll MD, Lawman HG, Fryar CD, Kruszon-Moran D, Kit BK, et al. Trends in Obesity Prevalence Among Children and Adolescents in the United States, 1988-1994 Through 2013-2014. Journal of the American Medical Association. 2016;315(21):2292–99. doi:10.1001/jama.2016.6361.
16. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: A systematic analysis for the Global Burden of Disease Study 2013. The Lancet. 2014;384(9945):766–81. doi:10.1016/S0140-6736(14)60460-8.
17. Phelps NH, Singleton RK, Zhou B, Heap RA, Mishra A, Bennett JE, et al. Worldwide trends in underweight and obesity from 1990 to 2022: a pooled analysis of 3663 population-representative studies with 222 million children, adolescents, and adults. The Lancet. 2024;403(10431):1027–50. doi:10.1016/S0140-6736(23)02750-2.
18. Popkin BM, Corvalan C, Grummer-Strawn LM. Dynamics of the double burden of malnutrition and the changing nutrition reality. The Lancet. 2020;395(10217):65–74. doi:10.1016/S0140-6736(19)32497-3.
19. Chrissini MM, Panagiotakos DB. Public health interventions tackling childhood obesity at European level: A literature review. Preventive Medicine Reports. 2022;30:102068. doi:10.1016/j.pmedr.2022.102068.
20. Cuschieri S, Grech S. COVID-19: a one-way ticket to a global childhood obesity crisis? Journal of Diabetes & Metabolic Disorders. 2020;19:2027-30. doi:10.1007/s40200-020-00682-2.
21. Sisson SB, Krampe M, Anundson K, Castle S. Obesity prevention and obesogenic behavior interventions in child care: A systematic review. Preventive Medicine. 2016;87:57–69. doi:10.1016/j.ypmed.2016.02.016.
22. Russell CG, Russell A. A biopsychosocial approach to processes and pathways in the development of overweight and obesity in childhood: Insights from developmental theory and research. Obesity Reviews. 2019;20(5):725–49. doi:10.1111/obr.12838.

23. Guercio Nuzio S, Di Stasi M, Pierri L, Troisi J, Poeta M, Bisogno A, et al. Multiple gut–liver axis abnormalities in children with obesity with and without hepatic involvement. Pediatric Obesity. 2017;12(6):446–52. doi:10.1111/ijpo.12164.
24. Troisi J, Pierri L, Landolfi A, Marciano F, Bisogno A, Belmonte F, et al. Urinary Metabolomics in Pediatric Obesity and NAFLD Identifies Metabolic Pathways/Metabolites Related to Dietary Habits and Gut-Liver Axis Perturbations. Nutrients. 2017;9(485):1-15. doi:10.3390/nu9050485.
25. Hamilton D, Dee A, Perry IJ. The lifetime costs of overweight and obesity in childhood and adolescence: a systematic review. Obesity Reviews. 2018;19:452–63. doi: 10.1111/obr.12649.
26. Simmonds M, Llewellyn A, Owen CG, Woolacott N. Predicting adult obesity from childhood obesity: a systematic review and meta-analysis. Obesity Reviews. 2016;17(2):95–107. doi: 10.1111/obr.12334.
27. González-Muniesa P, Mártinez-González MA, Hu FB, Després JP, Matsuzawa Y, Loos RJF, et al. Obesity. Nature Reviews Disease Primers. 2017;3(17034). doi:10.1038/nrdp.2017.34.
28. Nobili V, Alkhouri N, Alisi A, Della Corte C, Fitzpatrick E, Raponi M, et al. Nonalcoholic fatty liver disease: a challenge for pediatricians. JAMA Pediatrics. 2015;169(2):170–6. doi:10.1001/jamapediatrics.2014.2702.
29. Sommer A, Twig G. The Impact of Childhood and Adolescent Obesity on Cardiovascular Risk in Adulthood: a Systematic Review. Current Diabetes Reports. 2018;18(91):1-6. doi:10.1007/s11892-018-1062-9.
30. Wasniewska M, Pepe G, Aversa T, Bellone S, de Sanctis L, Di Bonito P, et al. Skeptical Look at the Clinical Implication of Metabolic Syndrome in Childhood Obesity. Children. 2023;10(735):1-31. doi:10.3390/children10040735.
31. Christian Flemming GM, Bussler S, Körner A, Kiess W. Definition and early diagnosis of metabolic syndrome in children. Journal of Pediatric Endocrinology and Metabolism. 2020;33(7):821–33. doi:10.1515/jpem-2019-0552.
32. Cho K, Moon JS, Kang JH, Jang HB, Lee HJ, Park SI, et al. Combined untargeted and targeted metabolomic profiling reveals urinary biomarkers for discriminating obese from normal-weight adolescents. Pediatric Obesity. 2017;12(2):93–101. doi:10.1111/ijpo.12114.
33. Reisinger C, Nkeh-Chungag BN, Fredriksen PM, Goswami N. The prevalence of pediatric metabolic syndrome - a critical look on the discrepancies between definitions and its clinical importance. International Journal of Obesity. 2021;45:12–24. doi:10.1038/s41366-020-00713-1.
34. Cook S, Weitzman M, Auinger P, Nguyen M, Dietz WH. Prevalence of a Metabolic Syndrome Phenotype in Adolescents Findings from the Third National Health and

Nutrition Examination Survey, 1988-1994. Arch Pediatr Adolesc Med. 2003;157(8):821-7. doi:10.1001/archpedi.157.8.821.

35. De Ferranti SD, Gauvreau K, Ludwig DS, Neufeld EJ, Newburger JW, Rifai N. Prevalence of the metabolic syndrome in American adolescents: Findings from the Third National Health and Nutrition Examination Survey. Circulation. 2004;110(16):2494–97. doi:10.1161/01.CIR.0000145117.40114.C7.
36. Sinha R, Fisch G, Teague B, Tamborlane WV, Banyas B, Allen K et al. Prevalence of Impaired Glucose Tolerance Among Children and Adolescents With Marked Obesity. The New England Journal of Medicine. 2002;346(11):802-10. doi:10.1056/NEJMoa012578.
37. Olza J, Gil-Campos M, Leis R, Bueno G, Aguilera CM, Valle M, et al. Presence of the metabolic syndrome in obese children at prepubertal age. Ann Nutr Metab. 2011;58(4):343–50. doi:10.1159/000331996.
38. Tropeano A, Corica D, Pomi AL, Pepe G, Morabito LA, Curatola SL, et al. The metabolic syndrome in pediatrics: Do we have a reliable definition? A systematic review. European Journal of Endocrinology. 2021;185(2):265–78. doi:10.1530/EJE-21-0238.
39. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet. 2005;365(9468):1415–28. doi:10.1016/S0140-6736(05)66378-7.
40. Schmid-Zalaudek K, Brix B, Sengeis M, Jantscher A, Fürhapter-Rieger A, Müller W, et al. Subcutaneous adipose tissue measured by b-mode ultrasound to assess and monitor obesity and cardio–metabolic risk in children and adolescents. Biology (Basel). 2021;10(449):1-14. doi:10.3390/biology10050449.
41. Störchle P, Müller W, Sengeis M, Lackner S, Holasek S, Fürhapter-Rieger A. Measurement of mean subcutaneous fat thickness: eight standardised ultrasound sites compared to 216 randomly selected sites. Sci Rep. 2018;8(1):16268. doi:10.1038/s41598-018-34213-0.
42. Ackland TR, Lohman TG, Sundgot-Borgen J, Maughan RJ, Meyer NL, Stewart AD, et al. Current Status of Body Composition Assessment in Sport: review and position statement on behalf of the ad hoc research working group on body composition health and performance, under the auspices of the I.O.C. Medical Commission. Sports Med. 2012;42(3):227-49. doi:10.2165/11597140-000000000-00000.
43. Kelso A, Müller W, Fürhapter-Rieger A, Sengeis M, Ahammer H, Steinacker JM. High inter-observer reliability in standardized ultrasound measurements of subcutaneous adipose tissue in children aged three to six years. BMC Pediatr. 2020;20(1):145. doi: 10.1186/s12887-020-02044-6.
44. Müller W, Fürhapter-Rieger A, Ahammer H, Lohman TG, Meyer NL, Sardinha LB, et al. Relative Body Weight and Standardised Brightness-Mode Ultrasound Measurement of Subcutaneous Fat in Athletes: An International Multicentre

Reliability Study, Under the Auspices of the IOC Medical Commission. Sports Medicine. 2020;50(3):597–614. doi:10.1007/s40279-019-01192-9.
45. Müller W. Determinants of ski-jump performance and implications for health, safety and fairness. Sports Med. 2009;39(2):85-106. doi:10.2165/00007256-200939020-00001.
46. Lackner S, Mörkl S, Müller W, Fürhapter-Rieger A, Oberascher A, Lehofer M, et al. Novel approaches for the assessment of relative body weight and body fat in diagnosis and treatment of anorexia nervosa: A cross-sectional study. Clinical Nutrition. 2019;38(6):2913–21. doi:10.1016/j.clnu.2018.12.031.
47. Javed A, Jumean M, Murad MH, Okorodudu D, Kumar S, Somers VK, et al. Diagnostic performance of body mass index to identify obesity as defined by body adiposity in children and adolescents: A systematic review and meta-analysis. Pediatric Obesity. 2015;10(3):234–44. doi:10.1111/ijpo.242.
48. Simmonds M, Llewellyn A, Owen CG, Woolacott N. Simple tests for the diagnosis of childhood obesity: a systematic review and meta-analysis. Obesity Reviews. 2016;17(12):1301–15. doi:10.1111/obr.12462.
49. Nishida C, Barba C, Cavalli-Sforza T, Cutter J, Deurenberg P, Darnton-Hill I, et al. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. The Lancet. 2004;363(9403):157–63. doi:10.1016/S0140-6736(03)15268-3.
50. Misra A, Chowbey P, Makkar BM, Vikram NK, Wasir JS, Chadha D, et al. Consensus Statement for Diagnosis of Obesity, Abdominal Obesity and the Metabolic Syndrome for Asian Indians and Recommendations for Physical Activity, Medical and Surgical Management. J Assoc Physicians India. 2009;57:163-70. www.japi.org163.
51. Simoni P, Guglielmi R, Gómez MPA. Imaging of body composition in children. Quantitative Imaging in Medicine and Surgery. 2020;10(8):1661–71. https://qims.amegroups.org/article/view/42022.
52. Aragon AA, Schoenfeld BJ, Wildman R, Kleiner S, VanDusseldorp T, Taylor L, et al. International society of sports nutrition position stand: Diets and body composition. Journal of the International Society of Sports Nutrition. 2017;14:16. doi:10.1186/s12970-017-0174-y.
53. Müller W, Horn M, Fürhapter-Rieger A, Kainz P, Kröpfl JM, Ackland TR, et al. Body composition in sport: Interobserver reliability of a novel ultrasound measure of subcutaneous fat tissue. Br J Sports Med. 2013;47(16):1036–43. doi:10.1136/bjsports-2013-092233.
54. Müller W, Horn M, Fürhapter-Rieger A, Kainz P, Kröpfl JM, Maughan RJ, et al. Body composition in sport: A comparison of a novel ultrasound imaging technique

to measure subcutaneous fat tissue compared with skinfold measurement. Br J Sports Med. 2013;47(16):1028–35. doi:10.1136/bjsports-2013-092232.
55. Müller W, Lohman TG, Stewart AD, Maughan RJ, Meyer NL, Sardinha LB, et al. Subcutaneous fat patterning in athletes: Selection of appropriate sites and standardisation of a novel ultrasound measurement technique: ad hoc working group on body composition, health and performance, under the auspices of the IOC Medical Commission. Br J Sports Med. 2016;50(1):45–54. doi:10.1136/bjsports-2015-095641.
56. Störchle P, Müller W, Sengeis M, Ahammer H, Fürhapter-Rieger A, Bachl N, et al. Standardized Ultrasound Measurement of Subcutaneous Fat Patterning: High Reliability and Accuracy in Groups Ranging from Lean to Obese. Ultrasound Med Biol. 2017;43(2):427–38. doi:10.1016/j.ultrasmedbio.2016.09.014.
57. Kuriyan R. Body composition techniques. Indian Journal of Medical Research. 2018;148(5):648–58. doi:10.4103/ijmr.IJMR_1777_18.
58. Müller W, Maughan RJ. The need for a novel approach to measure body composition: is ultrasound an answer? British Journal of Sports Medicine. 2013;47(16):1001–2. doi:10.1136/bjsports-2013-092882.
59. Eston R, Reilly T. (eds.) Kinanthropometry and exercise physiology laboratory manual: tests, procedures, and data. 3rd ed. London: Routledge; 2009. doi:10.4324/9780203868744.
60. Hattori K, Numata N, Ikoma M, Matsuzaka A, Danielson RR. Sex Differences in the Distribution of Subcutaneous and Internal Fat. Human Biology. 1991;63(1):53-63. https://www.jstor.org/stable/41464142.
61. Booth RAD, Goddard BA, Paton A. Measurement of fat thickness in man: a comparison of ultrasound, Harpenden calipers and electrical conductivity. British Journal of Nutrition. 1966;20(4):719–25. doi:10.1079/bjn19660073.
62. Bullen BA, Quaade F, Olesen E, Lund SA. Ultrasonic Reflections Used for Measuring Subcutaneous Fat in Humans. Biology. 1965;37(4):375-384. https://www.jstor.org/stable/41448748.
63. Varda NM, Medved M, Ojsteršek L. The associations between some biological markers, obesity, and cardiovascular risk in Slovenian children and adolescents. BMC Pediatr. 2020;20(1):81. doi:10.1186/s12887-020-1978-5.
64. Gállego-Suárez C, Bulan A, Hirschfeld E, Wachowiak P, Abrishami S, Griffin C, et al. Enhanced Myeloid Leukocytes in Obese Children and Adolescents at Risk for Metabolic Impairment. Front Endocrinol (Lausanne). 2020;11:327. doi: 10.3389/fendo.2020.00327.
65. Yoshimura A, Ohnishi S, Orito C, Kawahara Y, Takasaki H, Takeda H, et al. Association of peripheral total and differential leukocyte counts with obesity-related

complications in young adults. Obes Facts. 2015;8(1):1–16. doi:10.1159/000373881.
66. González-Domínguez Á, Belmonte T, Domínguez-Riscart J, Ruiz-Ocaña P, Muela-Zarzuela I, Saez-Benito A, et al. Altered insulin secretion dynamics relate to oxidative stress and inflammasome activation in children with obesity and insulin resistance. J Transl Med. 2023;21(1):559. doi:10.1186/s12967-023-04337-7.
67. Salamt N, Muhajir M, Aminuddin A, Ugusman A. The effects of exercise on vascular markers and C-reactive protein among obese children and adolescents: An evidence-based review. Bosnian Journal of Basic Medical Sciences. Association of Basic Medical Sciences of FBIH; 2020;20(2):149–56. doi:10.17305/bjbms.2019.4345.
68. Nimptsch K, Konigorski S, Pischon T. Diagnosis of obesity and use of obesity biomarkers in science and clinical medicine. Metabolism. 2019;92:61–70. doi:10.1016/j.metabol.2018.12.006.
69. He J, Stryjecki C, Reddon H, Peralta-Romero J, Karam-Araujo R, Suarez F, et al. Adiponectin is associated with cardio-metabolic traits in Mexican children. Sci Rep. 2019;9(1):3048. doi:10.1038/s41598-019-39801-2.
70. Chavira-Suárez E, Rosel-Pech C, Polo-Oteyza E, Ancira-Moreno M, Ibarra-González I, Vela-Amieva M, et al. Simultaneous evaluation of metabolomic and inflammatory biomarkers in children with different body mass index (BMI) and waist-to-height ratio (WHtR). PLoS One. 2020;15(8):e0237917. doi:10.1371/journal.pone.0237917.
71. Butte NF, Liu Y, Zakeri IF, Mohney RP, Mehta N, Voruganti VS, et al. Global metabolomic profiling targeting childhood obesity in the Hispanic population. American Journal of Clinical Nutrition. 2015;102(2):256–67. doi:10.3945/ajcn.115.111872.
72. Justenhoven C. Metabolomics. In: Pschyrembel. https://www-1pschyrembel-1de-10013b4w401b2.han.medunigraz.at/metabolomics/H0G94/doc/ [Accessed 11th December 2024].
73. Bardanzellu F, Fanos V. How could metabolomics change pediatric health? Italian Journal of Pediatrics. 2020;46(1):37. doi:10.1186/s13052-020-0807-7.
74. Patti GJ, Yanes O, Siuzdak G. Innovation: Metabolomics: the apogee of the omics trilogy. Nature Reviews Molecular Cell Biology. 2012;13(4):263–9. doi:10.1038/nrm3314.
75. Dunn WB, Broadhurst DI, Atherton HJ, Goodacre R, Griffin JL. Systems level studies of mammalian metabolomes: The roles of mass spectrometry and nuclear magnetic resonance spectroscopy. Chem Soc Rev. 2011;40(1):387–426. doi:10.1039/b906712b.

76. Hagenbeek FA, van Dongen J, Pool R, Harms AC, Roetman PJ, Fanos V, et al. Heritability of Urinary Amines, Organic Acids, and Steroid Hormones in Children. Metabolites. 2022;12(6):474. doi:10.3390/metabo12060474.
77. Zhang A, Sun H, Wu X, Wang X. Urine metabolomics. Clinica Chimica Acta. 2012;414:65–9. doi:10.1016/j.cca.2012.08.016.
78. Bouatra S, Aziat F, Mandal R, Guo AC, Wilson MR, Knox C, et al. The Human Urine Metabolome. PLoS One. 2013;8(9):e73076. doi:10.1371/journal.pone.0073076.
79. Stryeck S, Horvath A, Leber B, Stadlbauer V, Madl T. NMR spectroscopy enables simultaneous quantification of carbohydrates for diagnosis of intestinal and gastric permeability. Sci Rep. 2018;8(14650). doi:10.1038/s41598-018-33104-8.
80. Bruker. NMR 101. https://www.bruker.com/en/resources/library/application-notes-mr/nmr-101.html [Accessed 11th December 2024].
81. Edison AS, Colonna M, Gouveia GJ, Holderman NR, Judge MT, Shen X, et al. NMR: Unique Strengths That Enhance Modern Metabolomics Research. Analytical Chemistry. 2021;93(1):478–99. doi:10.1021/acs.analchem.0c04414.
82. Wishart DS. NMR metabolomics: A look ahead. Journal of Magnetic Resonance. 2019;306:155–61. doi:10.1016/j.jmr.2019.07.013.
83. Albrecht B, Voronina E, Schipke C, Peters O, Parr MK, Díaz-Hernández MD, et al. Pursuing experimental reproducibility: An Efficient Protocol for the Preparation of Cerebrospinal Fluid Samples for NMR-based Metabolomics and Analysis of Sample Degradation. Metabolites. 2020;10(6):251. doi:10.3390/metabo10060251.
84. Lohavanichbutr P, Zhang Y, Wang P, Gu H, Nagana Gowda GA, Djukovic D, et al. Salivary metabolite profiling distinguishes patients with oral cavity squamous cell carcinoma from normal controls. PLoS One. 2018;13(9):e0204249. doi:10.1371/journal.pone.0204249.
85. Orczyk-Pawilowicz M, Jawien E, Deja S, Hirnle L, Zabek A, Mlynarz P. Metabolomics of Human Amniotic Fluid and Maternal Plasma during Normal Pregnancy. PLoS One. 2016;11(4):e0152740. doi:10.1371/journal.pone.0152740.
86. Yazdani M, Elgstøen KBP, Rootwelt H, Shahdadfar A, Utheim ØA, Utheim TP. Tear Metabolomics in Dry Eye Disease: A Review. International Journal of Molecular Sciences. 2019;20(15):3755. doi:10.3390/ijms20153755.
87. Al-Sulaiti H, Almaliti J, Naman CB, Al Thani AA, Yassine HM. Metabolomics Approaches for the Diagnosis, Treatment, and Better Disease Management of Viral Infections. Metabolites. 2023;13(8):948. doi:10.3390/metabo13080948.
88. Nagana Gowda GA, Raftery D. Quantitative NMR Methods in Metabolomics. In: Handbook of Experimental Pharmacology. 2023;277:143–64. doi:10.1007/164_2022_612.

89. Khamis MM, Adamko DJ, El-Aneed A. Mass spectrometric based approaches in urine metabolomics and biomarker discovery. Mass Spectrometry Reviews. 2017;36(2):115–34. doi:10.1002/mas.21455.
90. Emwas AH, Luchinat C, Turano P, Tenori L, Roy R, Salek RM, et al. Standardizing the experimental conditions for using urine in NMR-based metabolomic studies with a particular focus on diagnostic studies: a review. Metabolomics. 2015;11(4):872–94. doi:10.1007/s11306-014-0746-7.
91. Emwas AH, Roy R, McKay RT, Ryan D, Brennan L, Tenori L, et al. Recommendations and Standardization of Biomarker Quantification Using NMR-Based Metabolomics with Particular Focus on Urinary Analysis. Journal of Proteome Research. 2016;15(2):360–73. doi:10.1021/acs.jproteome.5b00885.
92. Akiyama T, Saigusa D, Inoue T, Tokorodani C, Akiyama M, Michiue R, et al. Exploration of urine metabolic biomarkers for new-onset, untreated pediatric epilepsy: A gas and liquid chromatography mass spectrometry-based metabolomics study. Brain Dev. 2024;46(4):180–6. doi:10.1016/j.braindev.2023.12.004.
93. Gevi F, Belardo A, Zolla L. A metabolomics approach to investigate urine levels of neurotransmitters and related metabolites in autistic children. Biochim Biophys Acta Mol Basis Dis. 2020;1866(10):165859. doi:10.1016/j.bbadis.2020.165859.
94. Hu L, Lin L, Huang G, Xie Y, Peng Z, Liu F, et al. Metabolomic profiles in serum and urine uncover novel biomarkers in children with nephrotic syndrome. Eur J Clin Invest. 2023;53(7):e13978. doi:10.1111/eci.13978.
95. Salihovic S, Broeckling CD, Ganna A, Prenni JE, Sundström J, Berne C, et al. Non-targeted urine metabolomics and associations with prevalent and incident type 2 diabetes. Sci Rep. 2020;10(1):16474. doi:10.1038/s41598-020-72456-y.
96. Bruzzone C, Gil-Redondo R, Seco M, Barragán R, de la Cruz L, Cannet C, et al. A molecular signature for the metabolic syndrome by urine metabolomics. Cardiovasc Diabetol. 2021;20(1):155. doi:10.1186/s12933-021-01349-9.
97. Andueza N, Muñoz-Prieto D, Romo-Hualde A, Cuervo M, Navas-Carretero S. Changes in urinary metabolomic profile show the effectiveness of a nutritional intervention in children 6–12 years old: The ALINFA study. Food Sci Nutr. 2024;12(8):5663-5676. doi:10.1002/fsn3.4226.
98. Konstantinou C, Gaengler S, Oikonomou S, Delplancke T, Charisiadis P, Makris KC. Use of metabolomics in refining the effect of an organic food intervention on biomarkers of exposure to pesticides and biomarkers of oxidative damage in primary school children in Cyprus: A cluster-randomized cross-over trial. Environ Int. 2022;158:107008. doi:10.1016/j.envint.2021.107008.
99. Caterino M, Ruoppolo M, Villani GRD, Marchese E, Costanzo M, Sotgiu G, et al. Influence of Sex on Urinary Organic Acids: A Cross-Sectional Study in Children. Int J Mol Sci. 2020;21(2):582. doi:10.3390/ijms21020582.

100. Miccheli A, Capuani G, Marini F, Tomassini A, Praticò G, Ceccarelli S, et al. Urinary (1)H-NMR-based metabolic profiling of children with NAFLD undergoing VSL#3 treatment. Int J Obes. 2015;39(7):1118–25. doi:10.1038/ijo.2015.40.
101. Wang S, Fang J, Li J, Wang S, Su P, Wan Y, et al. Identification of urine biomarkers associated with early puberty in children: An untargeted metabolomics analysis. Physiol Behav. 2023;270:114305. doi:10.1016/j.physbeh.2023.114305.
102. Khan A, Park H, Lee HA, Park B, Gwak HS, Lee HR, et al. Elevated Metabolites of Steroidogenesis and Amino Acid Metabolism in Preadolescent Female Children With High Urinary Bisphenol A Levels: A High- Resolution Metabolomics Study. Toxicological Sciences. 2017;160(2):371–85. doi:10.1093/toxsci/kfx189.
103. Xia B, Zhu Q, Zhao Y, Ge W, Zhao Y, Song Q, et al. Phthalate exposure and childhood overweight and obesity: Urinary metabolomic evidence. Environ Int. 2018;121(Pt 1):159–68. doi:10.1016/j.envint.2018.09.001.
104. Prete A, Lang K, Pavlov D, Rhayem Y, Sitch AJ, Franke AS, et al. Urine steroid metabolomics as a diagnostic tool in primary aldosteronism. Journal of Steroid Biochemistry and Molecular Biology. 2024;237:106445. doi:10.1016/j.jsbmb.2023.106445.
105. Reddy P, Leong J, Jialal I. Amino acid levels in nascent metabolic syndrome: A contributor to the pro-inflammatory burden. J Diabetes Complications. 2018;32(5):465–9. doi:10.1016/j.jdiacomp.2018.02.005.
106. Peddinti G, Cobb J, Yengo L, Froguel P, Kravić J, Balkau B, et al. Early metabolic markers identify potential targets for the prevention of type 2 diabetes. Diabetologia. 2017;60(9):1740–50. doi:10.1007/s00125-017-4325-0.
107. Olszanecka A, Kawecka-Jaszcz K, Czarnecka D. Association of free testosterone and sex hormone binding globulin with metabolic syndrome and subclinical atherosclerosis but not blood pressure in hypertensive perimenopausal women. Archives of Medical Science. 2016;12(3):521–8. doi:10.5114/aoms.2016.59925.
108. Marchand GB, Carreau AM, Weisnagel SJ, Bergeron J, Labrie F, Lemieux S, et al. Increased body fat mass explains the positive association between circulating estradiol and insulin resistance in postmenopausal women. Am J Physiol Endocrinol Metab. 2018;314(5):E448–E456. doi:10.1152/ajpendo.00293.2017.
109. Ho JE, Larson MG, Ghorbani A, Cheng S, Chen MH, Keyes M, et al. Metabolomic Profiles of Body Mass Index in the Framingham Heart Study Reveal Distinct Cardiometabolic Phenotypes. PLoS One. 2016;11(2):e0148361. doi:10.1371/journal.pone.0148361.
110. Barrea L, Annunziata G, Muscogiuri G, Di Somma C, Laudisio D, Maisto M, et al. Trimethylamine-N-oxide (TMAO) as Novel Potential Biomarker of Early Predictors of Metabolic Syndrome. Nutrients. 2018;10(12):1971. doi:10.3390/nu10121971.

111. Gao X, Tian Y, Randell E, Zhou H, Sun G. Unfavorable Associations Between Serum Trimethylamine N-Oxide and L-Carnitine Levels With Components of Metabolic Syndrome in the Newfoundland Population. Front Endocrinol (Lausanne). 2019;10:168. doi:10.3389/fendo.2019.00168.
112. Vizzari G, Sommariva MC, Cas MD, Bertoli S, Vizzuso S, Radaelli G, et al. Circulating Salicylic Acid and Metabolic Profile after 1-Year Nutritional–Behavioral Intervention in Children with Obesity. Nutrients. 2019;11(5):1091. doi:10.3390/nu11051091.
113. Würtz P, Mäkinen VP, Soininen P, Kangas AJ, Tukiainen T, Kettunen J, et al. Metabolic signatures of insulin resistance in 7,098 young adults. Diabetes. 2012;61(6):1372–80. doi:10.2337/db11-1355.
114. Cheng S, Wiklund PK, Pekkala S, Autio R, Munukka E, Xu L, et al. Serum metabolic profiles in overweight and obese women with and without metabolic syndrome. Diabetol Metab Syndr. 2014;6(1):40. doi:10.1186/1758-5996-6-40.
115. Schäfer SG, Kaan EC, Christen MO, Löw-Kröger A, Mest H -J, Molderings G -J. Why Imidazoline Receptor Modulator in the Treatment of Hypertension? Ann N Y Acad Sci. 1995;763(1):659–72. doi:10.1111/j.1749-6632.1995.tb32460.x.
116. Bousquet P, Hudson A, García-Sevilla JA, Li JX. Imidazoline Receptor System: The Past, the Present, and the Future. Pharmacol Rev. 2020;72(1):50–79. doi:10.1124/pr.118.016311.
117. Huberts P. Association between subcutaneous adipose tissue and urine metabolites in elementary school children. [Diplomarbeit] Graz: Medical University of Graz; 2024.
118. Goswami N, Hansen D, Gumze G, Brix B, Schmid-Zalaudek K, Fredriksen PM. Health and Academic Performance With Happy Children: A Controlled Longitudinal Study Based on the HOPP Project. Front Cardiovasc Med. 2022;9:820827. doi:10.3389/fcvm.2022.820827.
119. Pang Z, Lu Y, Zhou G, Hui F, Xu L, Viau C, et al. MetaboAnalyst 6.0: towards a unified platform for metabolomics data processing, analysis and interpretation. Nucleic Acids Res. 2024;52(W1):W398–406. doi:10.1093/nar/gkae253.
120. Chong J, Soufan O, Li C, Caraus I, Li S, Bourque G, et al. MetaboAnalyst 4.0: Towards more transparent and integrative metabolomics analysis. Nucleic Acids Res. 2018;46(W1):W486–94. doi:10.1093/nar/gky310.
121. Cohen J. Statistical Power Analysis for the Behavioral Sciences. 2nd ed. New York: Routledge. 2013. doi:10.4324/9780203771587.
122. Azab SM, Shanmuganathan M, de Souza RJ, Kroezen Z, Desai D, Williams NC, et al. Early sex-dependent differences in metabolic profiles of overweight and adiposity in young children: a cross-sectional analysis. BMC Med. 2023;21(1):176. doi:10.1186/s12916-023-02886-8.

123. Concepcion J, Concepcion J, Chen K, Saito R, Gangoiti J, Mendez E, et al. Identification of pathognomonic purine synthesis biomarkers by metabolomic profiling of adolescents with obesity and type 2 diabetes. PLoS One. 2020;15(6):e0234970. doi:10.1371/journal.pone.0234970.
124. Tan HC, Hsu JW, Tai ES, Chacko S, Kovalik JP, Jahoor F. The impact of obesity-associated glycine deficiency on the elimination of endogenous and exogenous metabolites via the glycine conjugation pathway. Front Endocrinol (Lausanne). 2024;15:1343738. doi:10.3389/fendo.2024.1343738.
125. Wishart DS, Guo AC, Oler E, Wang F, Anjum A, Peters H, et al. HMDB 5.0: The Human Metabolome Database for 2022. Nucleic Acids Res. 2022;50(D1):D622–31. doi:10.1093/nar/gkab1062.
126. Tan HC, Hsu JW, Tai ES, Chacko S, Wu V, Lee CF, et al. De Novo Glycine Synthesis Is Reduced in Adults With Morbid Obesity and Increases Following Bariatric Surgery. Front Endocrinol (Lausanne). 2022;13: 900343. doi:10.3389/fendo.2022.900343.
127. Yan-Do R, MacDonald PE. Impaired "Glycine"-mia in Type 2 Diabetes and Potential Mechanisms Contributing to Glucose Homeostasis. Endocrinology. 2017;158(5): 1064–73. doi:10.1210/en.2017-00148.
128. Perseghin G, Ghosh S, Gerow K, Shulman GI. Metabolic Defects in Lean Nondiabetic Offspring of NIDDM Parents: A Cross-Sectional Study. Diabetes. 1997;46(6):1001-9. doi: 10.2337/diab.46.6.1001.
129. Takashina C, Tsujino I, Watanabe T, Sakaue S, Ikeda D, Yamada A, et al. Associations among the plasma amino acid profile, obesity, and glucose metabolism in Japanese adults with normal glucose tolerance. Nutr Metab (Lond). 2016;13:5. doi:10.1186/s12986-015-0059-5.
130. Yan-Do R, Duong E, Manning Fox JE, Dai X, Suzuki K, Khan S, et al. A glycine-insulin autocrine feedback loop enhances insulin secretion from human β-cells and is impaired in type 2 diabetes. Diabetes. 2016;65(8):2311–21. doi:10.2337/db15-1272.
131. Ding Y, Svingen GFT, Pedersen ER, Gregory JF, Ueland PM, Tell GS, et al. Plasma Glycine and Risk of Acute Myocardial Infarction in Patients With Suspected Stable Angina Pectoris. J Am Heart Assoc. 2016;5(1):e002621. doi:10.1161/JAHA.115.002621.
132. Mütze U, Reischl-Hajiabadi A, Kölker S. Classic Isovaleric Acidemia. 2024. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024.
133. Jain A, Morris MT, Berardi D, Arora T, Domingo-Almenara X, Paty PB, et al. Charting the metabolic biogeography of the colorectum in cancer: challenging the

right sided versus left sided classification. Molecular cancer. 2024;23(1):211. doi:10.1186/s12943-024-02133-5.
134. Lord RS, Bralley JA. Clinical Applications of Urinary Organic Acids. Part 2. Dysbiosis Markers. Alternative Medicine Review. 2008;13(4):292-306. https://altmedrev.com/wp-content/uploads/2019/02/v13-4-292.pdf.
135. Calvani R, Miccheli A, Capuani G, Tomassini Miccheli A, Puccetti C, Delfini M, et al. Gut microbiome-derived metabolites characterize a peculiar obese urinary metabotype. Int J Obes. 2010;34(6):1095–8. doi:10.1038/ijo.2010.44.
136. Ure A. On gouty concretions, with a new method of treatment. Medico-chirurgical transactions. 1841;24:30-5. doi:10.1177/095952874102400105.
137. Lees HJ, Swann JR, Wilson ID, Nicholson JK, Holmes E. Hippurate: The natural history of a mammalian-microbial cometabolite. J Proteome Res. 2013;12(4):1527–46. doi:10.1021/pr300900b.
138. Toromanović J, Kovac-Besović E, Sapcanin A, Tahirović I, Rimpapa Z, Kroyer G, et al. Urinary hippuric acid after ingestion of edible fruits. Bosn J Basic Med Sci. 2008;8(1):38-43. doi:10.17305/bjbms.2008.2994.
139. Guerra A, Folesani G, Mena P, Ticinesi A, Allegri F, Nouvenne A, et al. Hippuric acid in 24 h urine collections as a biomarker of fruits and vegetables intake in kidney stone formers. Int J Food Sci Nutr. 2014;65(8):1033–8. doi:10.3109/09637486.2014.950210.
140. Krupp D, Doberstein N, Shi L, Remer T. Hippuric acid in 24-hour urine collections is a potential biomarker for fruit and vegetable consumption in healthy children and adolescents. Journal of Nutrition. 2012;142(7):1314–20. doi:10.3945/jn.112.159319.
141. Pruneda-Alvarez LG, Ruíz-Vera T, Ochoa-Martínez AC, Pérez-Maldonado IN. Urinary trans-trans muconic acid (exposure biomarker to benzene) and hippuric acid (exposure biomarker to toluene) concentrations in Mexican women living in high-risk scenarios of air pollution. Arch Environ Occup Health. 2017;72(6):351–8. doi:10.1080/19338244.2016.1272539.
142. Bridges JW, French MR, Smith RL, Williams RT. The Fate of Benzoic Acid in Various Species. Biochem. J. 1970;118(1):47-51. doi:10.1042/bj1180047.
143. Feoli-Fonseca JC, Lambert M, Mitchell G, Melançon SB, Dallaire L, Millington DS, et al. Chronic Sodium Benzoate Therapy in Children with Inborn Errors of Urea Synthesis: Effect on Carnitine Metabolism and Ammonia Nitrogen Removal. Biochem Mol Med. 1996;57(1):31-6. doi:10.1006/bmme.1996.0006.
144. Bhattacharyya D, LeVatte MA, Wishart DS. A fast and accurate colorimetric assay for quantifying hippuric acid in human urine. Anal Biochem. 2023;680. doi:10.1016/j.ab.2023.115303.

145. De Simone G, Balducci C, Forloni G, Pastorelli R, Brunelli L. Hippuric acid: Could became a barometer for frailty and geriatric syndromes? Ageing Research Reviews. 2021;72:101466. doi:10.1016/j.arr.2021.101466.
146. Xu YX, Liu L Di, Zhu JY, Zhu SS, Ye BQ, Yang JL, et al. Alistipes indistinctus-derived hippuric acid promotes intestinal urate excretion to alleviate hyperuricemia. Cell Host Microbe. 2024;32(3):366-381.e9. doi:10.1016/j.chom.2024.02.001.
147. Ticinesi A, Guerra A, Nouvenne A, Meschi T, Maggi S. Disentangling the Complexity of Nutrition, Frailty and Gut Microbial Pathways during Aging: A Focus on Hippuric Acid. Nutrients. 2023;15(5):1138. doi:10.3390/nu15051138.
148. Cai HL, Li H De, Yan XZ, Sun B, Zhang Q, Yan M, et al. Metabolomic analysis of biochemical changes in the plasma and urine of first-episode neuroleptic-naïve schizophrenia patients after treatment with risperidone. J Proteome Res. 2012;11(8):4338–50. doi:10.1021/pr300459d.
149. Zhang L, Zheng J, Ismond KP, MacKay S, Levatte M, Constable J, et al. Identification of urinary biomarkers of colorectal cancer: Towards the development of a colorectal screening test in limited resource settings. Cancer Biomarkers. 2023;36(1):17–30. doi:10.3233/CBM-220034.
150. Zhao JJ, Zhang L, Liu PY, Chen WZ, Liu ZL, Wang YQ. Water-stable Cd(ii)/Zn(ii) coordination polymers as recyclable luminescent sensors for detecting hippuric acid in simulated urine for indexing toluene exposure with high selectivity, sensitivity and fast response. Dalton Transactions. 2021;50(2):553–61. doi:10.1039/D0DT03648J.
151. Gao H, Chai J, Jin C, Tian M. Molecularly imprinted electrochemical sensor based on CoNi-MOF/RGO nanocomposites for sensitive detection of the hippuric acid. Anal Chim Acta. 2024;1296:342307. doi:10.1016/j.aca.2024.342307.
152. Zhang W, Li Q, Zhang H. Efficient Optosensing of Hippuric Acid in the Undiluted Human Urine with Hydrophilic “Turn-On”-Type Fluorescent Hollow Molecularly Imprinted Polymer Microparticles. Molecules. 2023;28(3):1077. doi:10.3390/molecules28031077.
153. Li W, Dong H, Niu K, Wang HY, Cheng W, Song H, et al. Analyzing urinary hippuric acid as a metabolic health biomarker through a supramolecular architecture. Talanta. 2024;278:126480. doi:10.1016/j.talanta.2024.126480.
154. Tahir T, Ashfaq M, Asghar H, Shahzad MI, Tabassum R, Ashfaq A. Medicinal Importance of Azo and Hippuric Acid Derivatives. Mini-Reviews in Medicinal Chemistry. 2019;19(9):708–19. doi:10.2174/1389557518666180727162018.
155. Stratakis N, Siskos AP, Papadopoulou E, Nguyen AN, Zhao Y, Margetaki K, et al. Urinary metabolic biomarkers of diet quality in European children are associated with metabolic health. Elife. 2022;11:e71332. doi:10.7554/eLife.71332.

156. Lee Y, Cho JY, Cho KY. Serum, Urine, and Fecal Metabolome Alterations in the Gut Microbiota in Response to Lifestyle Interventions in Pediatric Obesity: A Non-Randomized Clinical Trial. Nutrients. 2023;15(9):2184. doi:10.3390/nu15092184.
157. Pathmasiri W, Pratt KJ, Collier DN, Lutes LD, McRitchie S, Sumner SCJ. Integrating metabolomic signatures and psychosocial parameters in responsivity to an immersion treatment model for adolescent obesity. Metabolomics. 2012;8(6):1037–51. doi:10.1007/s11306-012-0404-x.
158. De Luca G, Calpona PR, Caponetti A, Romano G, Di Benedetto A, Cucinotta D, et al. Taurine and osmoregulation: Platelet taurine content, uptake, and release in type 2 diabetic patients. Metabolism. 2001;50(1):60–4. doi:10.1053/meta.2001.19432.
159. Ahmed K, Choi HN, Yim JE. The Impact of Taurine on Obesity-Induced Diabetes Mellitus: Mechanisms Underlying Its Effect. Vol. 38, Endocrinology and Metabolism (Seoul). 2023;38(5):482–92. doi:10.3803/EnM.2023.1776.
160. Murakami S. Role of taurine in the pathogenesis of obesity. Molecular Nutrition and Food Research. 2015;59(7):1353–63. doi:10.1002/mnfr.201500067.
161. Yamori Y, Taguchi T, Mori H, Mori M. Low cardiovascular risks in the middle aged males and females excreting greater 24-hour urinary taurine and magnesium in 41 WHO-CARDIAC study populations in the world. In: Journal of Biomedical Science. 2010;17 Suppl 1(Suppl 1):S21. doi:10.1186/1423-0127-17-S1-S21.
162. Elliott P, Posma JM, Chan Q, Garcia-Perez I, Wijeyesekera A, Bictash M, et al. Urinary metabolic signatures of human adiposity. Sci Transl Med. 2015;7(285):285ra62. doi:10.1126/scitranslmed.aaa5680.
163. Kaur A, Yousuf H, Ramgobin-Marshall D, Jain R, Jain R. Energy drink consumption: a rising public health issue. Reviews in Cardiovascular Medicine. 2022;23(3):83. doi:10.31083/j.rcm2303083.
164. Ito T, Murakami S. Taurine deficiency associated with dilated cardiomyopathy and aging. J Pharmacol Sci. 2024;154(3):175–81. doi:10.1016/j.jphs.2023.12.006.
165. Ansar M, Ranza E, Shetty M, Paracha SA, Azam M, Kern I, et al. Taurine treatment of retinal degeneration and cardiomyopathy in a consanguineous family with SLC6A6 taurine transporter deficiency. Hum Mol Genet. 2020;29(4):618–23. doi:10.1093/hmg/ddz303.
166. Mitchell SC, Zhang AQ, Smith RL. Dimethylamine and diet. Food and Chemical Toxicology. 2008;46(5):1734–8. doi:10.1016/j.fct.2008.01.010.
167. Liu L, Yu F, Du L, Yang Z, Francisco JS, Zhang X. Rapid sulfuric acid-dimethylamine nucleation enhanced by nitric acid in polluted regions. Proc Natl Acad Sci U S A. 2021;118(35):e2108384118. doi:10.1073/pnas.2108384118.
168. Schlingemann J, Boucley C, Hickert S, Bourasseau L, Walker M, Celdran C, et al. Avoiding N-nitrosodimethylamine formation in metformin pharmaceuticals by

limiting dimethylamine and nitrite. Int J Pharm. 2022;620(9):121740. doi:10.1016/j.ijpharm.2022.121740.
169. Hanusch B, Sinningen K, Brinkmann F, Dillenhöfer S, Frank M, Jöckel KH, et al. Characterization of the L-Arginine/Nitric Oxide Pathway and Oxidative Stress in Pediatric Patients with Atopic Diseases. Int J Mol Sci. 2022;23(4):2136. doi:10.3390/ijms23042136.
170. Hsu CN, Chang-Chien GP, Lin S, Hou CY, Lu PC, Tain YL. Association of Trimethylamine, Trimethylamine N-Oxide, and Dimethylamine with Cardiovascular Risk in Children with Chronic Kidney Disease. J Clin Med. 2020;9(2):336. doi:10.3390/jcm9020336.
171. Falaina V, Fotakis C, Boutsikou T, Tsiaka T, Moros G, Ouzounis S, et al. Urine Metabolomic Profile of Breast- versus Formula-Fed Neonates Using a Synbiotic-Enriched Formula. Int J Mol Sci. 2022;23(18):10476. doi:10.3390/ijms231810476.
172. Lackner KJ, Peetz D. Glukose im Urin. In: Gressner AM, Arndt T. (eds.) Lexikon der Medizinischen Laboratoriumsdiagnostik. Springer Reference Medizin. 3rd ed. Berlin: Springer; 2019. p.981. doi:10.1007/978-3-662-48986-4.
173. Amboss. Urindiagnostik. https://next.amboss.com/de/article/o600NS?q=glukosurie#Z491da78e735aafb624b9e78529376173 [Accessed 11th December 2024].
174. Kim MS, Lee DY. Urinary Glucose Screening for Early Detection of Asymptomatic Type 2 Diabetes in Jeonbuk Province Korean Schoolchildren. J Korean Med Sci. 2017;32(6):985–91. doi:10.3346/jkms.2017.32.6.985.
175. Chen J, Guo H, Yuan S, Qu C, Mao T, Qiu S, et al. Efficacy of urinary glucose for diabetes screening: a reconsideration. Acta Diabetol. 2019;56(1):45–53. doi:10.1007/s00592-018-1212-1.
176. Harron KL, Feltbower RG, McKinney PA, Bodansky HJ, Campbell FM, Parslow RC. Rising rates of all types of diabetes in south Asian and non-south Asian children and young people aged 0-29 years in West Yorkshire, U.K., 1991-2006. Diabetes Care. 2011;34(3):652–4. doi:10.2337/dc10-1512.
177. Valaiyapathi B, Gower B, Ashraf AP. Pathophysiology of Type 2 Diabetes in Children and Adolescents. Curr Diabetes Rev. 2019;16(3):220–9. doi:10.2174/1573399814666180608074510.
178. Rami-Merhar B, Fröhlich-Reiterer E, Hofer SE, Fritsch M. Diabetes mellitus in childhood and adolescence (Update 2023). Wien Klin Wochenschr. 2023;135(Suppl 1):106–14. doi:10.1007/s00508-023-02169-5.
179. Copeland KC, Silverstein J, Moore KR, Prazar GE, Raymer T, Shiffman RN, et al. Management of newly diagnosed type 2 Diabetes Mellitus (T2DM) in children and adolescents. Pediatrics. 2013;131(2):364–82. doi:10.1542/peds.2012-3494.

180. Urakami T, Kubota S, Nitadori Y, Harada K, Owada M, Kitagawa T. Annual Incidence and Clinical Characteristics of Type 2 Diabetes in Children as Detected by Urine Glucose Screening in the Tokyo Metropolitan Area. Diabetes Care. 2005;28(8): 1876-1881. doi:10.2337/diacare.28.8.1876.
181. Urakami T, Morimoto S, Nitadori Y, Harada K, Owada M, Kitagawa T. Urine glucose screening program at schools in Japan to detect children with diabetes and its outcome-incidence and clinical characteristics of childhood type 2 diabetes in Japan. Pediatric Research. 2007;61(2):141–5. doi:10.1203/pdr.0b013e31802d8a69.
182. Wang TT, Huang XF, Huang H, Luo P, Qing LS. Nanomaterial-based optical- and electrochemical-biosensors for urine glucose detection: A comprehensive review. Advanced Sensor and Energy Materials. 2022;1(21):100016. doi:10.1016/j.asems.2022.100016.
183. Liman M, Jialal I. Physiology, Glycosuria. StatPearls. Treasure Island (FL): StatPearls Publishing; 2024. https://www.ncbi.nlm.nih.gov/books/NBK557441/.
184. Deutsche Gesellschaft für Nephrologie, Deutsche Gesellschaft für Klinische Chemie und Laboratoriumsmedizin e.V. S2k-Leitlinie Rationelle Labordiagnostik zur Abklärung Akuter Nierenschädigungen und Progredienter Nierenerkrankungen. Berlin: Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften e. V.; 2021. https://register.awmf.org/assets/guidelines/115-001l_S2k_Rationelle_Labordiagnostik_Abkl%C3%A4rung_Nierensch%C3%A4digungen_Ni erenerkrankungen_2021-09_01.pdf.
185. Nagl K, Waldhör T, Hofer SE, Fritsch M, Meraner D, Prchla C, et al. Alarming Increase of Ketoacidosis Prevalence at Type 1 Diabetes-Onset in Austria—Results From a Nationwide Registry. Front Pediatr. 2022;10:820156. doi:10.3389/fped.2022.820156.
186. Sharma V, Ichikawa M, Freeze HH. Mannose metabolism: More than meets the eye. Biochemical and Biophysical Research Communications. 2014;453(2):220–8. doi:10.1016/j.bbrc.2014.06.021.
187. Lee S, Zhang C, Kilicarslan M, Piening BD, Bjornson E, Hallström BM, et al. Integrated Network Analysis Reveals an Association between Plasma Mannose Levels and Insulin Resistance. Cell Metab. 2016;24(1):172–84. doi:10.1016/j.cmet.2016.05.026.
188. Rangel-Huerta OD, Pastor-Villaescusa B, Gil A. Are we close to defining a metabolomic signature of human obesity? A systematic review of metabolomics studies. Metabolomics. 2019;15(6):93. doi:10.1007/s11306-019-1553-y.
189. Gogna N, Krishna M, Oommen AM, Dorai K. Investigating correlations in the altered metabolic profiles of obese and diabetic subjects in a South Indian Asian population using an NMR-based metabolomic approach. Mol Biosyst. 2015;11(2):595–606. doi:10.1039/c4mb00507d.

190. Jørs A, Lund MAV, Jespersen T, Hansen T, Poulsen HE, Holm JC. Urinary markers of nucleic acid oxidation increase with age, obesity and insulin resistance in Danish children and adolescents. Free Radic Biol Med. 2020;155:81–6. doi:10.1016/j.freeradbiomed.2020.05.009.
191. Hua Y, Krupp D, Esche J, Remer T. Increased body fatness adversely relates to 24-hour urine pH during childhood and adolescence: Evidence of an adipo-renal axis. American Journal of Clinical Nutrition. 2019;109(5):1279–87. doi:10.1093/ajcn/nqy379.
192. Lau CHE, Siskos AP, Maitre L, Robinson O, Athersuch TJ, Want EJ, et al. Determinants of the urinary and serum metabolome in children from six European populations. BMC Med. 2018;16(1):202. doi:10.1186/s12916-018-1190-8.
193. Handakas E, Lau CH, Alfano R, Chatzi VL, Plusquin M, Vineis P, et al. A systematic review of metabolomic studies of childhood obesity: State of the evidence for metabolic determinants and consequences. Obesity Reviews. 2022;23 Suppl 1:e13384. doi:10.1111/obr.13384.
194. Garcia-Perez I, Posma JM, Gibson R, Chambers ES, Hansen TH, Vestergaard H, et al. Objective assessment of dietary patterns by use of metabolic phenotyping: a randomised, controlled, crossover trial. Lancet Diabetes Endocrinol. 2017;5(3):184–95. doi:10.1016/S2213-8587(16)30419-3.
195. López-Contreras BE, Morán-Ramos S, Villarruel-Vázquez R, Macías-Kauffer L, Villamil-Ramírez H, León-Mimila P, et al. Composition of gut microbiota in obese and normal-weight Mexican school-age children and its association with metabolic traits. Pediatr Obes. 2018;13(6):381–8. doi:10.1111/ijpo.12262.
196. Baker JL, Olsen LW, Sørensen TIA. Childhood Body-Mass Index and the Risk of Coronary Heart Disease in Adulthood. New England Journal of Medicine. 2007;357(23):2329–37. doi:10.1056/NEJMoa072515.
197. Zhai L, Liu J, Zhao J, Liu J, Bai Y, Jia L, et al. Association of obesity with onset of puberty and sex hormones in Chinese girls: A 4-year longitudinal study. PLoS One. 2015;10(8):e0134656. doi:10.1371/journal.pone.0134656.
198. Szczerbinski L, Wojciechowska G, Olichwier A, Taylor MA, Puchta U, Konopka P, et al. Untargeted Metabolomics Analysis of the Serum Metabolic Signature of Childhood Obesity. Nutrients. 2022;14(1):214. doi:10.3390/nu14010214.
199. Assfalg M, Bertini I, Colangiuli D, Luchinat C, Schäfer H, Schütz B, et al. Evidence of different metabolic phenotypes in humans. Proc Natl Acad Sci U S A. 2008;105(5):1420-1424. doi:10.1073/pnas.0705685105.
200. Kelso A, Vogel K, Steinacker JM. Ultrasound measurements of subcutaneous adipose tissue thickness show sexual dimorphism in children of three to five years of age. Acta Paediatrica. 2019;108(3):514–21. doi:10.1111/apa.14496.

201. Müller W, Fürhapter-Rieger A, Ahammer H, Lohman TG, Meyer NL, Sardinha LB, et al. Relative Body Weight and Standardised Brightness-Mode Ultrasound Measurement of Subcutaneous Fat in Athletes: An International Multicentre Reliability Study, Under the Auspices of the IOC Medical Commission. Sports Med. 2020;50(3):597–614. doi:10.1007/s40279-019-01192-9.
202. Chang K, Khandpur N, Neri D, Touvier M, Huybrechts I, Millett C, et al. Association between Childhood Consumption of Ultraprocessed Food and Adiposity Trajectories in the Avon Longitudinal Study of Parents and Children Birth Cohort. JAMA Pediatr. 2021;175(9):e211573. doi:10.1001/jamapediatrics.2021.1573.
203. Bali A, Naik R. The Impact of a Vegan Diet on Many Aspects of Health: The Overlooked Side of Veganism. Cureus. 2023;15(2):e35148. doi:10.7759/cureus.35148.
204. Byrd AS, Toth AT, Stanford FC. Racial Disparities in Obesity Treatment. Current obesity reports. 2018;7(2):130–8. doi:10.1007/s13679-018-0301-3.
205. Browne NT, Hodges EA, Small L, Snethen JA, Frenn M, Irving SY, et al. Childhood obesity within the lens of racism. Pediatric Obesity. 2022;17(5):e12878. doi:10.1111/ijpo.12878.
206. Rossen LM, Talih M. Social determinants of disparities in weight among US children and adolescents. Ann Epidemiol. 2014;24(10):705-713.e2. doi:10.1016/j.annepidem.2014.07.010.
207. Timper K, Brüning JC. Hypothalamic circuits regulating appetite and energy homeostasis: Pathways to obesity. Disease Models and Mechanisms. 2017;10(6):679–89. doi:10.1242/dmm.026609.
208. Baumer Y, Powell-Wiley TM. Interdisciplinary approaches are fundamental to decode the biology of adversity. Cell. 2021;184(11):2797–801. doi:10.1016/j.cell.2021.04.010.
209. Gao W, Liu JL, Lu X, Yang Q. Epigenetic regulation of energy metabolism in obesity. Journal of Molecular Cell Biology. 2021;13(7):480–99. doi:10.1093/jmcb/mjab043.
210. Kumanyika SK. A Framework for Increasing Equity Impact in Obesity Prevention. Am J Public Health. 2019;109(10):1350–7. doi:10.2105/AJPH.2019.305221.
211. Berry EM. The Obesity Pandemic—Whose Responsibility? No Blame, No Shame, Not More of the Same. Front Nutr. 2020;7:2. doi:10.3389/fnut.2020.00002.
212. Di Ciaula A, Portincasa P. Contrasting obesity: is something missing here? Intern Emerg Med. 2024;19(2):265–9. doi:10.1007/s11739-024-03559-x.
213. Pryke R. Child obesity: Who is measuring who? and what? Perspectives in Public Health. 2014;134(2):76–7. doi:10.1177/1757913914521931.
214. World Health Organization. Time to accept that obesity is a disease – Austria is building a national system to treat it. https://www.who.int/europe/news-room/08-09-

2022-time-to-accept-that-obesity-is-a-disease---austria-is-building-a-national-system-to-treat-it [Accessed 11th December 2024].

215. Raphael JL, Carl Cooley W, Vega A, Kowalkowski MA, Tran X, Treadwell J, et al. Outcomes for Children with Chronic Conditions Associated with Parent- and Provider- reported Measures of the Medical Home. J Health Care Poor Underserved. 2015;26(2):358–76. doi:10.1353/hpu.2015.0051.

216. Hampl SE, Hassink SG, Skinner AC, Armstrong SC, Barlow SE, Bolling CF, et al. Clinical Practice Guideline for the Evaluation and Treatment of Children and Adolescents With Obesity. Pediatrics. 2023;151(2):1-100. doi:10.1542/peds.2022-060640.