Thesis

Subcutaneous adipose tissue measured by ultrasound: reference values for primary school children

submitted by

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Graz, 07.08.2025 *Philipp Hönes m.p.*

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Zusammenfassung

Hintergrund: In den letzten Jahren haben Übergewichtig und Adipositas weltweit, aber vor allem auch bei Kindern, stark zugenommen. Verschieden Entwicklungen tragen zu diesem Phänomen bei. Zum einen verändern sich die Essgewohnheiten, es wird deutlich zu viel Zucker aber auch fettreiche Nahrung konsumiert. Gleichzeitig nimmt die körperliche Aktivität stark ab, da die Kinder viel Zeit vor dem Smartphone oder anderen technischen Geräten verbringen. Aktuell wird weltweit der Body-Mass-Index (BMI) als Standard zur Quantifizierung von Übergewicht und Adipositas benutzt. Leider misst dieser weder das Fett, noch kann er Informationen bezüglich der Verteilung oder Menge dessen geben.
Diese Thesis ist ein Teil der Studie „Health and Academic Performance with Happy Children (HAPHC)", die sich mit den Auswirkungen von Fettleibigkeit bei Kindern im Grundschulalter auf verschiedene Aspekte des täglichen Lebens beschäftigt. Dabei sollte versucht werden, den Grad der Fettleibigkeit einfach und exakt zu quantifizieren und Methoden erprobt werden, wie man diesem Phänomen entgegnen kann.

Zielsetzung: Diese Thesis beschäftigt sich mit Übergewicht und Adipositas bei Kindern bestimmt anhand des mittels Ultraschall gemessenen Fettgewebes. Um den Grad der Fettleibigkeit bei Kindern zu bestimmen, wurde bei der Studie das subkutane Fett der Kinder anhand von mittels Ultraschall gemessenen Fettschichten bestimmt. Das Ziel der These war die Erstellung von Referenzwerten für die Verteilung des subkutanen Fettgewebes bei Kindern im Alter von 6 bis 11 Jahren. Außerdem sollte ein Teil der Thesis auch die Methode der Messung, in diesem Falle mittels B-Mode Ultraschall, beleuchten und die Schwächen sowie die Stärken dieser Messung hervorgehoben werden.

Methoden: Für die aktuelle Thesis wurden die Daten von 404 Kindern eingeschlossen. Das Alter der Kinder lag zwischen 6 und 11 Jahren. Insgesamt haben drei Grundschulen aus Graz an der Studie teilgenommen. Das subkutane Fett wurde dafür an acht standardisierten Stellen mmittels B-mode Ultraschall gemessen. Anschließend wurden die Ergebnisse nach Altersklassen und Geschlecht getrennt, analysiert und dargestellt. Zusätzlich wurden die Ergebnisse der SAT-Messungen für die acht Stellen, aber auch für die Gesamtsumme in Perzentilen dargestellt. Somit soll eine einfache Einordnung der Menge an subkutanem Fett in Relation zur einer Referenzstichprobe ermöglicht und verschiedene Stichproben miteinander verglichen werden.

Ergebnisse: Für die nach Alter und Geschlecht getrennt analysierten Daten der 404 Kinder wurden die Ergebnisse, analog zum BMI, in Perzentilen dargestellt. Dabei wurde nicht nur die jeweils gemessene Stelle, sondern auch die Summe aller acht Messpunkte (D_I) als Indikator für die (Gesamt-)Fettleibigkeit in Perzentile eingeteilt. Der Durchschnitt der Gesamtsumme der acht Messstellen für alle Altersklassen lag für die Jungen bei D_I = 65,14 mm ± 41,97 mm und für die Mädchen bei D_I = 76,78 mm ± 39,34 mm.
Zusätzlich konnte gezeigt werden an welchen der acht Messstellen sich das meiste subkutane Fett ansiedelt und welche am besten mit dem BMI korrelieren. Die höchste BMI-Korrelation hatte dabei der Messpunkt upper abdomen (UA, $r = 0{,}902$, $p < 0.05$) und lower abdomen (LA, $r = 0{,}896$, $p < 0.05$). Dahinter folgen front thigh (FT, $r = 0{,}853$, $p < 0.05$), distal triceps (DT, $r = 0{,}853$, $p < 0.05$), erector spinae (ES, $r = 0{,}836$, $p < 0.05$), medial calf (MC, $r = 0{,}814$, $p < 0.05$), brachioradialis (BR, $r = 0{,}788$, $p < 0.05$), lateral thigh (LT, $r = 0{,}722$, $p < 0.05$). Es wurde auch gezeigt, dass Mädchen über alle Altersklassen hinweg mehr SAT aufwiesen als Jungen. Außerdem zeigte sich, dass das Ausmaß an SAT mit zunehmendem Alter ansteigt. Im Gegensatz zur Messung des SAT, welches sich in Abhängigkeit des Geschlechts signifikant unterschied, zeigten die anthropometrischen Maße und Kennwerte keinen Unterschied in der untersuchten Altersklasse.

Schlussfolgerung: Die vorliegende Diplomarbeit ergab in Übereinstimmung mit der Literatur deutlich Hinweise darauf, dass der subkutane Fettanteil bei Frauen (Mädchen) bereits im Kindesalter deutlich höher ist und mittels Ultraschalles nachgewiesen werden kann. Die Zusammenfassung der Daten, getrennt nach Alter und Geschlecht, liefert zudem die Grundlage zur Bewertung des Fettanteils bei Kindern für acht standardisierte Positionen sowie für die Gesamtmenge an subkutanem Fett. Um die Problematik/Unzuverlässigkeit des BMI mittels Ultraschallmessung des Subkutanfetts zu umgehen, bedarf es Referenzwerten zur Bewertung einzelner gemessenen Daten. Anhand der Ergebnisse ist es möglich Referenzwerte für Kinder in der oben genannten Altersklasse zu bestimmen und somit auch mit zukünftigen Ergebnissen oder Studien zu vergleichen. Die Messmethode mittels Ultraschalles zeigte sich als ausgezeichnete Methode in Bezug auf Zuverlässigkeit und Reproduzierbarkeit. Die inter-rater-reliability war mit einer ICC von 0.998 (95% CI 0.980 – 0.999) sehr hoch. Ebenso die intra-rater-reliability, mit Werten der ICC von 0.994 (95% CI 0.983 – 0.998). Es ist außerdem möglich mit Ultraschall Gewebegrenzen von 0.1 – 0.2 mm sichtbar zu machen, was die extrem gute Genauigkeit der Methode unterstreicht. Dadurch,

dass sie außerdem günstig, portabel und schnell durchzuführen ist, hat die Methode viel Potenzial bei der Bestimmung der Körperzusammensetzung und der Messung des subkutanen Fettes. Jedoch werden die hohen Messwerte nur erreicht, wenn geschultes Personal die Messungen vornehmen. Außerdem ist eine einheitliche Software und Messprotokoll notwendig um vergleichbare Ergebnisse zu erhalten. Da anderen Methoden, wie der BMI noch schneller und leichter zu handhaben sind, ist es schwer vorstellbar, dass sich diese Methode in den nächsten Jahren flächendeckend etablieren wird. Auf bestimmten Gebieten, wie im Spitzensport oder für Ärzte ist sie aber sicher eine sehr gute Alternative.

Abstract

Background: In recent years, obesity has been on the rise worldwide in general, but especially among children. Various developments are contributing to this phenomenon. On the one hand, eating habits are changing with far too much sugar and high-fat food being consumed. At the same time, physical activity is decreasing sharply as children spend a lot of time in front of their smartphones or other technical devices.
This thesis is part of the study "Health and Academic Performance with Happy Children (HAPHC)". This study deals with the effects of a school-based physical activity intervention on measures of health and academic achievements in primary school children. It also aimed to quantify the degree of obesity by direct measures of subcutaneous adipose tissue (SAT) by ultrasound to generate reference values for children aged 6-11 years-

Aims and Objectives: This thesis deals with the measurement of overweight and obesity in children, more specifically with the amount and distribution of subcutaneous adipose tissue (SAT).. The aim of the thesis was to establish reference values for subcutaneous fat in children aged 6 to 11 years measured by ultrasound. In addition, the thesis describes the method of measurement using bright (B)-mode ultrasound, and highlight the weaknesses and strengths of this measurement in comparison to other body indices.

Methods: For the current thesis, data were collected in three elementary schools in Graz. The age of the children was between 6 and 11 years. Subcutaneous fat was measured at eight standardized sites. Bright-mode ultrasound was used to measure the thicknesses of the subcutaneous fat layers at these eight sites. The results were then broken down by age group and sex. In addition, the results of the SAT measurements were presented for the eight sites and for the total sum in percentiles. This should enable a simple classification of the amount of subcutaneous fat and to compare different samples with each other.

Results: Subcutaneous adipose tissue was measured in 404 children. and was then broken down by age and sex. For comparisons, the results were presented in percentiles. Using these percentiles, the reference values for children between the ages of 6 and 11 years can now be determined for the first time. Not only the respective measured point, but also the sum of all 8 measured points (DI) is presented in percentiles. The average of the total sum of the 8 measurement points for all age groups was DI = 65.14 mm ± 41.97 mm for boys and DI =

76.78 mm ± 39.34 mm for girls. Girls were shown to have a significantly higher amount of SAT and the thickness of the measured SAT increased with increasing age.
In addition, it could be shown at which of the 8 measuring points the most subcutaneous fat is located and which sites correlate best with the BMI. The highest BMI correlation was found for the upper abdomen (UA) ($r = 0.902$, $p < 0.05$) and lower abdomen (LA, $r = 0.896$, $p < 0.05$). These were followed by front thigh (FT, $r = 0.853$, $p < 0.05$), distal triceps (DT, $r = 0.853$, $p < 0.05$), erector spinae (ES, $r = 0.836$, $p < 0. 05$), medial calf (MC, $r = 0.814$, $p < 0.05$), brachioradialis (BR, $r = 0.788$, $p < 0.05$), lateral thigh (LT, $r = 0.722$, $p < 0.05$).
While the SAT measures differed significantly between girls and boys, no differences were found in anthropometric measures and body indices.

Conclusion: This diploma thesis shows a detailed breakdown of subcutaneous fat values measured at eight different sites in children aged six to ten years. Based on the results, it is possible to provide reference values for children in the above-mentioned age groups and thus to compare them with future results or studies. The ultrasound measurement method proved to be an excellent method in terms of reliability and reproducibility. The inter-rater reliability was very high with an ICC of 0.998 (95% CI 0.980 - 0.999). Intra-rater reliability was also very high, with an ICC of 0.994 (95% CI 0.983 - 0.998). It is also possible to visualize tissue boundaries of 0.1 - 0.2 mm with ultrasound, which underlines the extremely good accuracy of the method. Because it is also inexpensive, portable and quick to perform, the method has great potential for determining body composition and measuring subcutaneous fat. However, the high measurement values can only be achieved if trained personnel carry out the measurements. In addition, a standardized software and measurement protocol is necessary to obtain comparable results. As other methods, such as the BMI, are even faster and easier to use, it is difficult to imagine that this method will become established across the board in the next few years. However, it is certainly a very good alternative in certain areas, such as top-class sport or for doctors.

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List of Abbreviations

B	circumferences of the biceps
BIA	bioelectrical impedance analysis
BMI	body-mass-index
BMC	bone mineral content
B-mode	brightness-mode
BR	brachioradialis
BRI	body roundness index
CS	control school
CT	computer tomography
CV	coefficient of variation
D_I	subcutaneous adipose tissue including the fibrous structures
DT	distal triceps
DXA	Dual x-ray absorptiometry
EO	external oblique
ES	erector spinae
FM	fat mass
FT	front thigh
h	body height
H	circumferences of the hip
HAPHC	Health and Academic Performance with Happy Children
ICC	interclass correlation coefficient
IS1	intervention school 1
IS2	intervention school 2
ISAK	International Society for the Advancement of Kinanthropometry
l	leg length
LA	lower abdomen
LM	lean mass
LT	lateral thigh
m	body mass
Max	maximum
MC	medial calf

Min	minimum
MRI	magnetic resonance imaging
S	sitting height
SAT	subcutaneous adipose tissue
SD	standard deviation
STEPS	STEPwise approach to Surveillance
T	circumferences of the thigh
TBF	total body fat
TBW	total body water
WC	waist circumference
WHO	World Health Organization
WHR	waist-to-hip ratio
WHtR	waist-to-height ratio

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1. Introduction

Overweight and obesity in children is reaching alarming dimensions in many countries all over the world (1). The highest increase can be observed in low- and middle-income countries and in the minority ethnic groups in these countries. Especially in Africa and Asia live about 75% of the affected children aged under 5 years. Among others, lifestyle changes and therewith the increase in screen hours, changes in food intake related to quality as well as quantity and a decrease in physical activity led to this global problem (2,3).

Childhood obesity and overweight are known as major risk factors for several diseases, above all to the three major non-communicable of them: cardiovascular disease, type 2 diabetes and cancer (4). There are also several other negative effects on the body of the children like high blood pressure, cardiac dysfunction, endothelial dysfunction, dyslipidemia, inflammation, non-alcoholic liver diseases or respiratory and orthopedic disorders (5). Four of them (insulin resistance, obesity, hypertension, dyslipidemia) can be summarized as metabolic syndrome. Studies have shown that children or adolescents who have such risk factors are more likely to develop a chronic disease in adulthood (6,7).

Childhood obesity in particular is very often carried over into adulthood (8).

In addition to the physical consequences of being overweight, there are also psychological effects. Overweight children are bullied or teased at school much more often than children of normal weight (9).

Therefore, this is one of the most important challenges of the public health and the society in the next decades. The two main factors to face overweight and obesity is a healthy diet and adequate physical activity (10).

1.1 Anatomy of fat

There are basically two types of adipose tissue, white and brown adipose tissue. Both types of tissue are formed by adipocytes but have different functions in the body. In the human body the white adipose tissue represents the largest proportion of the total adipose tissue.

White adipose tissue consists of adipocytes. These contain a single lipid droplet that takes up the majority of the cell. The other cell components, such as the nucleus and the cell organelles are displaced to the plasmalemma.

The fat is absorbed into the cell either via the bloodstream or via new synthesis directly in the cell from glucose. The white adipose tissue primarily serves as a storage fat, which is released as an energy source when there is a calorie deficit. If the body needs energy, the fat is broken down into glycerol and fatty acids and supplied to the body via the bloodstream.
The white fat tissue also forms building fat, which is found on the soles of the hands and feet. Another characteristic is its ability to retain water, which is therefore very important for the body's water and salt balance.
Several adipocytes are joined together to form fat lobules. These in turn are surrounded by collagen fibers that allow deformation under mechanical stress but then return to their original shape. This tissue therefore has an additional cushioning function (11,12).

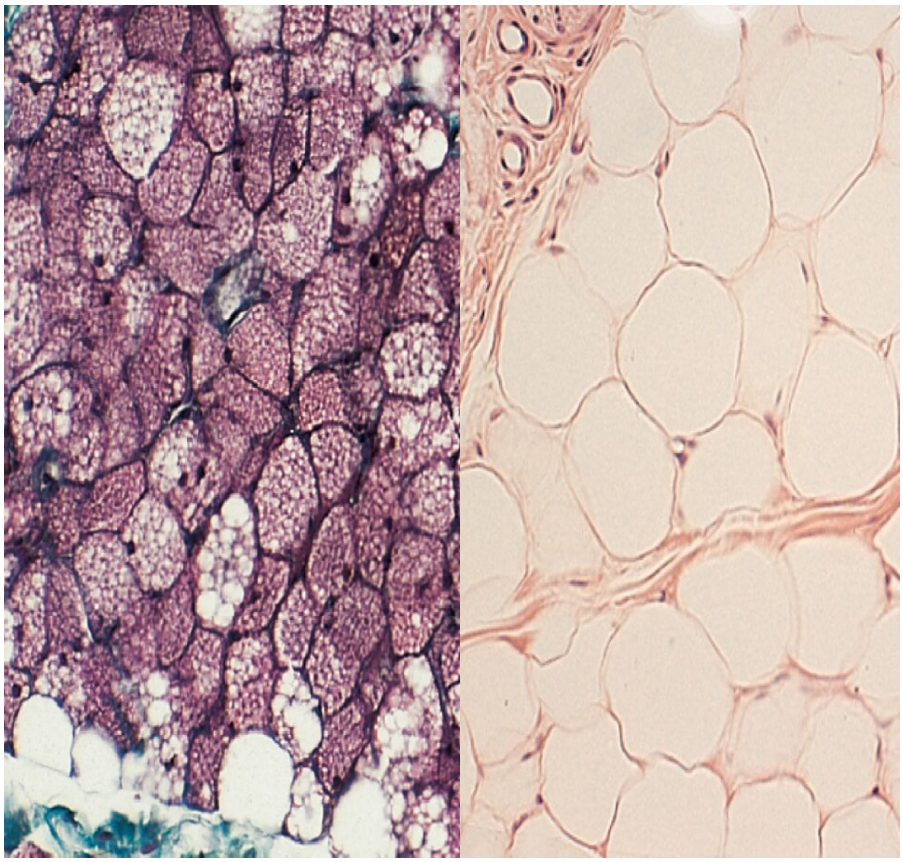

Figure 1: Histological image of brown (left) and white (right) adipose tissue (13)

There are also other lipids such as phospholipids, which play an important role in cell membranes, and isoprenoid derivatives, which are essential for vitamin and cholesterol metabolism.

However, the majority of human fat consists of triglycerides, including storage fat. These triglycerides consist of a trivalent alcohol, glycerol, to which three fatty acid residues are attached. For each unit of mass, the triglycerides provide the most amount of energy for the body, namely 39kJ/mol. In contrast to carbohydrates and proteins with 17kJ/mol (14,15).

The white fat can also be divided into 2 different parts, the subcutaneous fat and the visceral fat. The study situation is not entirely clear regarding the distribution of the two. However, it can be assumed that subcutaneous fat accounts for approximately 80-90% of total body fat. The 10-20% of the visceral fat is mostly represented in the abdomen, but also in gonadal, epicardial, retroperitoneal and perirenal depots (16).

The two tissues differ not only in their localization, but also in their composition. Visceral fat contains more immune and inflammatory cells. It is also better vascularized and innervated and therefore metabolically more active than subcutaneous fat. Subcutaneous fat, on the other hand, is more sensitive to insulin and has a higher affinity for free fatty acids and triglycerides uptake (17).

In contrast to white adipose tissue, brown adipose tissue consists of many small droplets of fat that surround the cell nucleus. Brown adipose tissue is significantly more innervated and vascularized. It is mainly used by newborns and infants for thermoregulation by providing energy quickly (11).

1.2 Childhood Obesity – How to measure it and why?

Compared to adults, the study situation about body composition and the measurement of it is still limited in children and adolescents (18).

The current most used parameter in relation to obesity in children is the body-mass-index (BMI). The World Health Organization (WHO), and most other institutions and studies use this parameter. The WHO developed BMI percentiles that are adapted to age and sex. These percentiles make it easy to determine whether a child is obese or not. They have therefore become established in everyday life and are used first and foremost. As the BMI has some weaknesses, there are additional methods to quantify obesity and include fat distribution. The waist-circumference (WC) and the waist-to-height ratio (WHtR) should be mentioned here in particular (19,20).

Other studies used caliper to measure children´s body fat (21,22), however with low accuracy.

There are also various technologies that can be used to measure body fat. These techniques are dual-energy x-ray-absorptiometry (DEXA), computer tomography (CT), magnetic resonance imaging (MRI) and measurements by ultrasound (18,23).

These techniques have outperformed anthropometric measurements in recent years. They provide a more precise overview of the body composition and can also distinguish between different tissues (18).

However, all of those techniques got their own advantages and disadvantages and they also vary especially in their accuracy and precision. Especially for working with children, just some of those are applicable (24,25).

Before getting into more detail, some basic aspects should be discussed

In recent years, several studies have shown that it is useful to measure the SAT. There has been a clear correlation between increased SAT values at the waist and an increased risk of cardiometabolic diseases, especially in children.

A Chinese study with children aged 6 to 18, which measured the SAT by DEXA, showed that higher amounts of SAT are linked to heightened levels of triglycerides and total cholesterol and high levels of low-density and low levels of high-density lipids cholesterol (26–31).

These findings emphasize the important role of SAT assessment in determining an increased cardiometabolic risk (2).

1.3 Body Composition

The composition of the human body provides a lot of information and has relevance for many different areas of life. In recent years, these information has been especially important for athletes and their coaches (32). Nowadays, however, this information is also becoming increasingly valuable for the public. Particularly in relation to the problem of obesity, which is increasingly affecting young and old and is responsible for many chronic diseases (33).

There are many different methods for measuring body composition however no universal method has been established. All methods have their strengths and limitations in terms of what they are able to measure. One method divides the body into four compartments. The simple principle is, that the information from one compartment can be used to determine that of the other.

The simplest method splits the body into two compartments, fat mass (FM) and fat-free mass (FFM). This is e.g., the principle of the skinfold measurement. The three-compartment model also contains bone mineral content (BMC), which ca be measured by dual-energy X-ray absorptiometry (DEXA). To split the body into four compartments, the total body water (TBW), which is divided into extracellular- and intracellular fluid, must also be measured. This is possible with the bioelectrical impedance analysis (BIA).

Some of those methods are called direct methods because they use a quantitative relationship to estimate the amount of body fat. On the other hand, there are doubly indirect methods, which use a regression equation against an indirect method to determine body fat. For a better understanding of the compartment model and it´s methods a visual representation is illustrated in Figure 2.

To find suitable measuring instruments, one need to know which parameters you want to measure and which of them are on a high level in terms of reliability and validity (34). Beyond these points, the costs, the portability and the time needed to get the results, in total the applicability in practice, is also very important and has to be considered (2).

In the following paragraph, the various current methods will be outlined and their respective validity and reliability assessed.

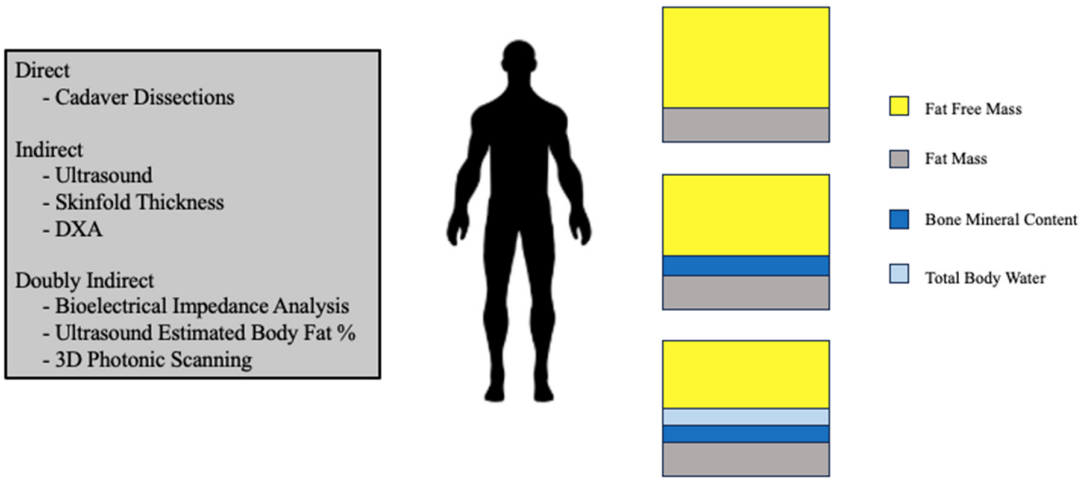

Figure 2: Compartment Modell and Measurement Techniques

1.4 Measurement Methods

1.4.1 Skinfold Thickness

The first theoretical documented mentioning of skinfold measurements was in 1890, but the first precision instrument for such measurements was invented in 1953 and called “skinfold caliper” (35).

This instrument creates a skinfold, which is nothing more than two parallel layers composed of skin and subcutis. To measure the depth of the SAT of the individual it is necessary to do this on various sites all over the body (32,36).

Special skinfold equations than calculate the amount of the total body fat in % (TBF) based on the foundation of the body density which is determined by regression analysis (36,37).

Skinfold thickness measurement is one of the most inexpensive techniques, the only thing it requires is a calibrated caliper and a measuring tape to determine the anthropometrics. This allows this technology to be used in a very wide range of field-based settings. It was also shown that this technique is the least affected, related to hydration status, meal intake or everyday activities (32).

But there are several technical limitations with this method. At first, there is the assumption that the thickness and the compressibility of the skin are constant over the measuring sites. Furthermore, it is not only affected by the strength of the grip and the pressure performed, but also by the skin temperature, sex and the age of the participant.

Studies have also shown that already measurement differences of 1 cm at the specific sites can significantly affect the outcome. Therefore, the best option for this technique is that the same person takes all the measurements, which is of course a large limitation regarding big studies or measurements in different countries or settings.

As mentioned above, there are special equations to estimate values for the total body fat in %. Currently there are more than hundred such formulae, though the equations haven´t been validated to detect regular changes in the composition of an individual? (32).

After a particular education program, the intra-rater reliability of the skinfold thickness via caliper was shown to be good to excellent, with values ranging from 0.926-0.998 (95% CI). The interrater reliability resulted in slightly lower values with studies showeding ranges from 0.62-0.999 (95% CI). One explanation for the strong fluctuations is the different number of measurement points in the various studies and the strong differences in fat distribution between women and men (36).

1.4.2 Body-Mass-Index - BMI

The body mass index (BMI), which is defined as the ratio of weight in kilograms (kg) to height in square meters (m^2), is widely used to determine the weight status in individuals (25,34,38). Over time, a classification with different reference values was created by the WHO. So, a BMI <18.5 kg/m^2 is classified as underweight, the normal range is set between 18.5-24.9 kg/m^2, the status overweight begins at BMI >25.0 kg/m^2. But overweight is additionally divided into preobese (BMI <29.9 kg/m^2), obese class one (<34.9 kg/m^2), obese class two (<39.9 kg/m^2) and obese class three (>40.0 kg/m^2). These values are independent of the age and sex of the person (38). However, these values only apply to adults. Special percentiles have been developed for children so that they can be better compared with their peers (39).

BMI values below the ≤5th percentile is defined as underweight. Values between the ≥85th and ≤95th percentile are considered overweight and values above the ≥95th percentile are considered obese (40). For children there is also an additional BMI assessment, the body mass index standard deviation score (BMI-SDS). The BMI SDS measures the number of the SDs from the mean BMI for children, matched for sex and age (41). The WHO defined the cut-off values for the BMI SDS as follows: overweight >+1SD, obesity >+2SD, thinness <-2SD, severe thinness <3-SD. Especially for children with severe obesity the BMI SDS is very useful. For example, to assess the therapeutic success of a BMI change of 40 kg/m^2 to 35 kg/m^2, you would see the 99.9 percentile on both percentile curves. The BMI SDS values, however, decrease from 3.5 to 3.0. Furthermore, it is possible to compare the individual value of a child with its own age group (42).

It is extremely simple to get the anthropometrics and therefore it is very inexpensive and commonly used.

But with the BMI, it is not possible to get information about the amount of total body fat or the distribution of it, and you also cannot distinguish between fat and fat-free mass.

Also, for a specific BMI the percentage of the body fat changes with age. This change of the body fat is again dependent on ethnicity, sex and individual differences.

Especially children showed high inter-individual differences regarding the speed of the growth of their different tissues, which makes it very hard to make statements about the body composition with BMI (25,34,43).

1.4.3 Body roundness index - BRI

The body roundness index was first implemented in 2013. It was the first attempt to predict both, the percentage of the total body fat and the percentage of the VAT only using anthropometric measurements. For this method, the human body is modelled as an ellipse respectively an oval. An ellipse captures the body height in relation to the body girth which provides the body roundness. The roundness of an ellipse is described by a dimensionless value called eccentricity. The eccentricity measures the extent of an ellipse´s deviation from a perfect circle, with values between 0 and 1, where 0 indicates a perfect circle and 1 represents a vertical line.

The following formula was developed in a study by Diana Thomas.

$$\mathrm{BRI} = 364.2 - 365.5 \times \sqrt{1 - \left(\frac{\left(\frac{\mathrm{WC}}{2\pi}\right)^2}{(0.5\ \mathrm{height})^2}\right)}$$

Figure 3: Formula for Body roundness index (BRI)(44)

It has been shown that the BRI provides a better assessment of the risk of cardiovascular disease, kidney disease and cancer than conventional anthropometric indicators.

In addition, a high BRI value is associated with a higher probability of cardiovascular disease specific mortality and all cause mortality.

For the estimation of total body fat in percent and the percentage of the VAT, the BRI outperformed the BMI and the waist circumference. But especially for children there is a lack of data and it is not possible to give an estimation of the SAT, which is a big limitation (44–46).

1.4.4 Waist Circumference - WC

Waist circumference is another indicator of abdominal obesity. (47) To measure the waist circumference, it requires just a non-stretchable measuring tape, which makes it a very inexpensive and quick method and therefore it is commonly used (25). There are several measuring points described in the literature, the WHO established a standardized procedure with the so-called STEPS (STEPwise approach to Surveillance) which defines the measuring point in the midpoint between the iliac crest and the last rib which can be palpated. The WHO also defined cut-off points above which an increased probability of certain diseases

occurs. For men the value is set >102 cm and for women >88 cm (48). It is very difficult to verify the reliability and accuracy of this method because the literature is rather ambiguous. Some studies showed a higher sensitivity and specificity compared to BMI or waist-to-height ratio (WHtR), others in turn showed opposite results (49).

There are also some important facts, which can be seen as limitations, to be considered before taking the measurements. The tightness of the tape should be on a similar level for each measuring, it should not constrict the tissue underneath. To minimize this risk, the STEPS protocol recommends a specific tape which provides a consistent level of tension. In- and expiration also influences the accuracy of the measurement. Therefore, each measure should be taken at the end of exhalation.

The contents of the gastrointestinal tract e.g., gas, water, food, and the strain of the abdominal cover also play a major role and increase the probability of measurement errors. These are some of the reasons why there is a intra-measurer error of 1.31 cm and an inter-measurer error of 1.56 cm (48).

1.4.5 Waist-to-height ratio - WHtR

The waist-to-height-ratio WHtR, which is the waist circumference in cm divided by the height in cm, is an easy and simple method to detect central obesity. Central obesity also provides better information about the likelihood of getting certain diseases than total body fat. Compared to BMI and WC, studies found out, that WHtR is cheaper, more sensitive and easier to determine. It is also adapted to the body size compared to the WC. In the literature, a cut-off point of 0.5 for increased risk of several diseases, has therefore emerged in recent years, which can be used for women and men and many ethnicities, but is also limited to adults. In children a decrease in WHtR from 0.69 to 0.48 from birth up to five years of age can be seen. This continues until the early teenage years up to 0.40 and levels off at around 0.43 by the age of 18 (50,51). This is mainly due to the fact that the children are still growing, and their body proportions are still changing. These factors are also dependent on age, sex and ethnicity.

Therefore, it is hard to implement a specific cut-off during the childhood. New studies show that it would be important to find cut-offs that are adapted to age, sex and ethnicity (52).

1.4.6 Dual x-ray absorptiometry - DXA

Dual x-ray absorptiometry was originally developed to measure the bone density. With the last years it has also been recognized as a good method to measure body composition. Because of its ability to detect and differentiate between the three tissues fat, lean mass and bone content, it has become the gold standard for measuring body composition.

The physical principle of DXA is to measure the x-ray transmission through the human body at two, a high and a low level of energy. The x-rays are attenuated differently by the tissues of the body. It depends on the density and thickness of the tissue and the intensity of the energy. A higher energy level of the photons decreases the attenuation of the x-ray beam. Therefore, in tissues with low-density, a lot more photons are passing through compared to tissues with higher density, for example bone tissue. DXA provides the R-value, which is the ratio of the attenuation of the low and high energy levels of the x ray, which means that each type of tissue got its own characteristic R-value (53).

The DXA only measures two compartments, bone and lean tissue. To make statements about the body composition, one needs to get information about the composition of the lean tissue, especially the amount of fat and fat-free soft tissue. In order to differentiate between fat and fat-free mass, it is necessary to measure the attenuation in areas where there is no bone. If no bone is present, the ratio of the attenuation is approximately linear to the fat mass in the tissue. With the information of the attenuation in areas with bone and soft tissue and also in regions with only soft tissue, the three compartments (lean tissue, fat mass and bone mineral mass) can be separated (54).

Beside the fact that DXA can differentiate between those three compartments, there are several other advantages of this method. One of them is that this method can be performed at the whole body and also in single body regions. It is also a non-invasive method with fast scan times, a whole-body scan requires approximately only five minutes.

There are also some limitations coming with this method. The high costs of an DXA device and it´s non portability limits the use to the clinical settings. For field measurements it is therefore not usable. It also requires specific skills, especially technical skills. Such devices got a variety of hard- and software versions and different instrument calibration procedures among manufacturers. Kasper et al. showed that the same DXA device with different software versions achieved significantly different results. The total body mass of the participants was the same, but there was an increase of 18% in fat-mass (FM) and a decrease

of 4% in lean mass (LM), questioning its reliability. In addition, this method cannot distinguish between the three types of fat (subcutaneous, visceral and intramuscular) (32,53). Furthermore, there is the problem with ionizing radiation, which should be considered when using this method for children. However, one study showed that a typical DXA scan for an infant was at 0.00893 milliSieverts (mSv), which is less than the amount of one day daily background radiation. Still, in studies on children one might rather choose a method with no radiation exposure at all (34).

There are no specific data for reliability or validity for children of the age from 6 to 11 years, but some studies analyzed those parameters for adults and infants. The interclass correlation coefficient (ICC) ranged from 0.94 to 0.99. Three studies analyzed the validity of the DXA measurements, comparing the results to direct carcass analysis of piglets. The results showed high numbers for validity ranging from 0.94 to 0.99. Other studies provide also excellent repeatability for measuring body fat with this method, with ranges of the coefficient of variation (CV) of <1% to 5.9% (34,43).

1.4.7 Bioelectrical Impedance Analysis (BIA)

The human body consists of different kind of cells and tissues. These cells contain specific structures, which are stored in the intracellular fluid (ICF) and are surrounded by a membrane. The whole cell is again stored in extracellular fluid (ECF). If an alternating electric signal is now applied to these cells, the cells generate a complex electrical impedance, which is the bioelectric impedance. This impedance is dependent of the frequency of the ac signal and the composition of the cell and the tissue. The frequency response is therefore strongly dependent on the physiological and physiochemical composition of the respective tissue and varies from person to person (55).

The BIA devices do not directly measure the body composition, they use the bodies different electrical properties to estimate the total body water (TBW). The TBW can be estimated by measuring the impedance (Z), which is given by the reactance (Xc) and resistance (R). The relationship between those is: $Z^2 = R^2 + Xc^2$. R is the resistance of the body to a given alternating current and reactance measures the electrical charge which is stored in the membranes of the cells. With this information the devices estimates values for TBW, intracellular fluid, extracellular fluid, FM, FFM and BMI (56,57).

There are two different types of BIA devices, one works with single frequency (50 kHz) and the other with multifrequency (1-1000kHz). The literature has shown that the multifrequency devices are superior to the single in terms of precision (58).

There are also differences in the number of electrodes used, two or four, whereby the method with 4 electrodes is slightly superior to the method with 2 electrodes.

These electrodes are placed on the feet and hands of the participant. Most manufacturers have their own specifications for fitting these unless there are specific protocols that specify a particular location. The measurement should take place while the participant is lying on a flat surface, e.g. an examination table or a sports mat, with minimal clothing. You also need to know some biographics of the participant before measuring, including age, sex and ethnicity. A complete body composition measurement using BIA therefore takes about 15 minutes (57).

Besides the time needed per measurement, this method is very inexpensive. It is portable, which makes it very useful for measurements outside the clinical setting. It is a safe, non-invasive method with no risk potential, which makes it particularly beneficial when working with children. In addition, data is obtained not only on fat and fat-free mass, but also on total body water, intracellular and extracellular fluid (55).

As with any method, there are limitations that need to be considered before taking the measurements. Since the BIA measures total body water, the hydration status is essential for the measurement. This means that you can get different results just because of the amount the participant drank or ate before. The sensitivity of the conductive surface and the positioning of the electrodes also play an important role. Hair in particular can minimize contact with the skin and distort the results. Studies showed that physical exercises can have an impact on body composition estimates measured with BIA, due to a higher cutaneous blood flow and therefore an accumulation of the skin electrolytes. It is therefore extremely important to use standardized protocols if you want to have comparable data. However, there are many different protocols, which makes it difficult to compare the results, especially for large studies or measurements with different devices (32,53,57).

Regarding to reliability and accuracy of the measurement method, differences are often reported between the single and multifrequency devices. The multifrequency devices usually delivere better results.

Various studies have shown that the ICC of the BIA devices is very high in most cases. One study with 88 children aged between 10 to 12 years, showed nearly perfect associations for

the ICC with values from 0.98-1.00 for fat mass in %, body mass in kg und muscle mass in kg (59).

When it comes to fat mass in %, the BIA method usually underestimates the value compared with the gold standard DXA. A Finnish study concludes that the BIA underestimates FM by 2-6% (60). Another study with 3655 participants showed a similar result. It compared the measurements from DXA to BIA to a given BMI level. Between a BMI range from 18.5 to >40 kg/m^2 the BIA device underestimates the fat mass from 2.51 – 5.67 kg. For the fat free mass, it showed a reversed outcome, it overestimated the FFM between a range from 3.38 – 8.25 kg. However, these were data from adults (61).

The study mentioned above, which was performed with children, showed similar results compared to the studies with adults. BIA underestimates the amount of the FM by 2.6 kg for boys and 2.33 kg for girls (59).

Another study that compared the single body segments came to the same conclusion. There were strong differences between the measurements with DXA and BIA in all body segments. FM was particularly underestimated in the arm and leg (mean difference arm: 0.42kg, leg: 1.94 kg). FFM on the other hand was overestimated, especially on the arm and trunk (mean difference arm: 0.97 kg, trunk: 5.58 kg) (62).

1.4.8 Computed Tomography – CT

CT and Magnetic resonance imaging (MRI) are now considered to be the most accurate methods when it comes to assessing body composition. They have also provided valuable scientific insight into the relationship between body composition and disease risk and progression (63). The technique of CT is based on the attenuation of X-rays. Each tissue attenuates the X-rays to a different extent and the CT device creates an image which consists out of pixels. From these images, different compartments of the body can be recognized due to their different densities which are expressed in so-called houndsfield units (HU). Each pixel of the CT image shows HU as a measure of the attenuation of the body tissue compared to water. The HU of water is 0 and the HU of air is fixed at 1000. With this information the device creates a two-dimensional image of the human body which consists of cross-sections (53).

To analyze these cross-section images, the user has to delineate manually the compartment of interest, which is then extrapolated from this one region to the whole body. Studies have shown that the L3 region provides the most accurate results for extrapolation (64).

One of the biggest advantages of this method is its versatility. The CT scan makes it possible to view many different compartments. These include bone mass, visceral and subcutaneous fat, connective tissue, vessels, organs and skeletal muscles. Studies have also shown high validity and reliability. It is also a very quick and painless method to gain the data about body composition. (57).

The biggest limitation of this method is surely the radiation exposure. The effective radiation dose of a CT scan is between 5000 to 15000 µSv. The US Administration of Foods and Drugs estimates for this amount of radiation a 1-in-2000 lifetime risk of trigger a fatal cancer. This is an extremely high value, compared to a radiation dose of approximately 0.2 µSv for a whole-body DXA scan. This fact himself makes the determination of body composition by CT unusable, especially for measurements on children and repeated measurement protocols (57).

Besides that, there are limitations regarding to very high costs and its limited availability and portability, respectively (65).

1.4.9 Magnetic Resonance Imaging – MRI

Together with CT, MRI is the most accurate way to measure body composition (66). The MRI device uses a magnetic field to align protons in the tissue of the human body. These protons are mostly stored in the hydrogen molecules. This alignment requires a very strong magnetic field, 15.000 – 30.000 times stronger than that of the earth. In the next step, a short radio-frequency wave is needed to activate the protons. The protons absorb the wave for a short time until it is interrupted again, if this happens the protons release energy. The various kinds of tissues in the human body include different amounts of water and thus also hydrogen. A software detects and digitize the alignment, the density of the protons and the relaxation time. With this information a greyscale image will be generated. This image can then be analyzed to quantify the different body tissues. Like the CT, the MRI is able to show the different kinds of tissues, including subcutaneous and visceral adipose tissue, bone, vessels, connective tissue, skeletal muscles and organs. (57). Moreover, MRI is the only method to detect ectopic fat structures. The biggest advantage compared to the CT is that MRI works without any harmful ionizing radiation. Therefore it can be used with indefinite repeatability and especially in studies on children (67). It is also known as a method with a high validity and repeatability.

However, one of the biggest limitations is the costs of the MRI. Compared to CT or DXA, the MRI scan is much more expensive. Due to the size of an MR device, it is difficult to transport and therefore the availability is limited, which makes it inconvenient for the use in the field. In addition, highly trained personnel are needed for execution of the procedure(57). In terms of accuracy for the measurement of SAT thickness, the image resolution for the MRI devices is typically between 1.3 to 2.0 mm compared to 0.1 mm for US devices (68).

1.4.10 Ultrasound – US Brightness mode

As described above, there are a several methods to determine body composition and the amount of SAT. All of them have their limitations and prospects. For the happy children study, which was executed in the schools, the SAT measurements via brightness mode (B-mode) Ultrasound was used. In the following paragraph, the method will be explained in detail and why it is currently believed to be the best of the methods mentioned before

In recent years, it has become increasingly clear that measuring body composition using ultrasound could become much more important (69).
The US device consists of a transducer, which produces the ultrasound waves. This conducer is able to emit and detect the ultrasound echoes reflected back. When using such a device, the transducer sends out a jet of soundwaves in the body of the participant. Tissue boundaries cause the sound waves to be reflected to the transducer. These reflections create electrical signals which then are detected by the ultrasound scanner. With the speed of the waves and the time the different echoes need to return, the US device calculates the distance between the transducer and the tissue boundary. This information is then used to create a two-dimensional image of organs and tissues (70).
Using a special image segmentation algorithm, the SAT contour can be detected and a proprietary software automatically performs measurements of the SAT thickness (2). Ultrasound measurement also makes it possible to visualize embedded fibrous structures. This makes it possible to calculate and compare the entire SAT with and without these structures (71).

The first measurements of fat thickness using ultrasound were carried out in 1965. In 1966, the first study comparing US and caliper measurements was published. This was also the start of the interest in fat measurements using ultrasound. But it took another 50 years until

a standardized US method to measure subcutaneous adipose tissue was implemented in 2016 (68,72).

Initially, the method was mainly used for competitive sports. Especially for sports where the weight of the athlete is important, such as ski jumping or long-distance running. At first there was no standardization and no measurement sites for this method. Therefore, one study of the Medical University of Graz tried to implement appropriate sites and a standardization for the US measurements. Initially, an attempt was made to use the same sites as for the Caliper measurement, which are defined by the International Society for the Advancement of Kinanthropometry (ISAK). But it showed out that those sites aren´t transferable to measurements by US as they do not provide clear images. Moreover, there is specific knowledge of anatomy needed to mark these sites. This motivated to search for new locations that are easy to find and therewith reproducible, and which can be marked with a high degree of precision in less than 1 hour of training. The new sites all provide clear US images due to a clearly visible fascia which overlies each of the eight sites. This makes it easy to identify the different anatomical structures. All distances needed are relative to the persons height and the thickness of the SAT does not change much in the surrounding area (71). Figure 4 show a typical US image of one of the standardized sites.

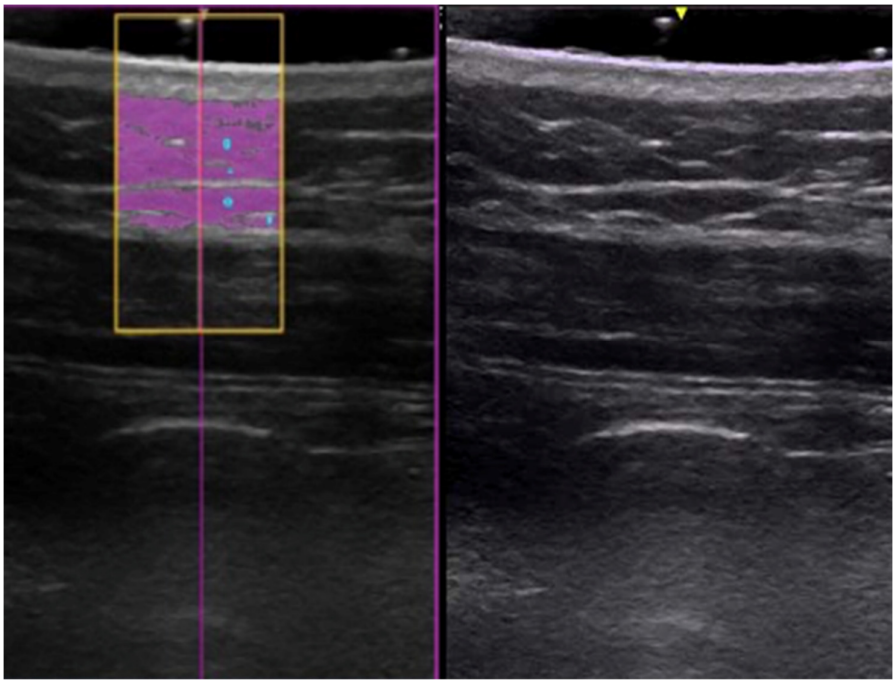

Figure 4: Ultrasound image of SAT (13)

An overview of the 8 sites and the instructions to mark these can be found in Table 1.

One problem with the 8 sites is that they do not provide information about the mean SAT thickness all over the body. That information was obtained by measurements of 216 random sites. The study found out that the 8 sites overestimated the mean SAT obtained from the 216 sites. For the 8 sites the mean ranged from 2.9 to 10.2 mm and for the 216 sites the range was 1.9 to 6.1 mm. A calibration is therefore necessary (68).

As with all other measurement methods, accuracy and reliability play a very important role. A Study by Kelso et al. showed very high values in terms of inter-observer reliability. Three experienced observers took independent measurements (on the 8 sites) on 20 children aged three to six years. These measurements resulted in a standard error of the estimate (SEE) of 1.1 mm, and the 95% limits of agreement were within ±2.1 mm. With an ICC of 0.998 (95% CI 0.980-0.999) and a median difference in the mean SAT thickness of 0.8 mm (about 1.9% of the mean SAT thickness), the results show high reliability. Two other studies on adults showed similar results. The absolute values of observer differences were just 0.3 mm in both studies and values for the 95% limits of agreement were within ±1.0 mm and ±1.2 mm (73). Another study by Kelso showed similar results for intra-rater reliability. The measurements were taken on 16 children aged three to five years on two different days. The ICC (0.994, $p < 0.01$) and 95% CI (0.983-0.998) showed excellent intra-observer reliability. The mean difference in the sum of SAT thickness at all measured sites was 0.79 mm with 95% of differences ranged from -0.44 to 2.03 mm.

Two other studies with adults showed a median difference in SAT thickness ranging from 0.4 to 0.9 mm with 95% of differences ranging from -2.2 to 1.9 mm. These results illustrate that these standardized measurements can provide very reliable data regardless of size, age and SAT thickness in children and adults with normal and overweight (74).

When it comes to accuracy, several studies by Müller et al. showed high levels of accuracy. With the B-Mode US it is possible to determine tissue borders to 0.1 to 0.2 mm. These values cannot be achieved by any of the other methods (71,73,75).

One thing to bear in mind is that these high values can only be achieved by experienced examiners. In terms of intra-rater reliability, inexperienced examiners showed three times higher deviations and inter-rater reliability was twice as high.

It is therefore essential to train the examiners before SAT measurements using ultrasound. (71). Another advantage, especially compared to other technical methods, is the lack of radiation. Ultrasound does not require any ionizing radiation and is therefore suitable for use in children and pregnant women (76).

2. Aims and Objectives

As previously noted, the increasing prevalence of overweight and obesity among children represents a critical public health issue. A broad spectrum of associated comorbidities has been identified, including cardiovascular, musculoskeletal, dental, and psychological conditions. The etiology of this upward trend in pediatric obesity is multifactorial and remains difficult to fully delineate. Nonetheless, poor nutritional habits and insufficient physical activity are recognized as key contributing factors. Despite extensive efforts by numerous research groups, childhood obesity remains an unresolved challenge, highlighting the ongoing need for effective preventive and therapeutic strategies.

This thesis focuses on the amount and pattern of subcutaneous adipose tissue in children.
Most known methods, especially the widely used BMI, have significant weaknesses. Therefore, the need for new methods is growing. The measurement of subcutaneous fat by B-mode ultrasound is a relatively new non-invasive method, using a standardizes protocol. Several studies have been performed, however for children only a few studies have gained data.

The main aim of this study was to define reference values for the amount and pattern of subcutaneous fat in primary school children, aged six to ten years, and therewith provide a database for comparison in future studies.
The reference values obtained refer to the standardized eight body sites and the total amount of these sites.

The data used in this thesis were collected as part of the larger Happy Children study. More information about the mentioned study can be found in Goswami et al. (3).

3. Methods

3.1 Study design

This thesis was a part of a larger study called "Optimizing cardiometabolic Health, Cognition and Academic Performance with Happy Children (HAPHC) using school-based physical activity", short Happy Children. The aim of this study was to optimize the physical and mental health, psycho-social aspects, wellbeing and the academic performance of elementary school children by implementing a daily based session of physical activity across the curriculum. In addition to the physical education and sports lessons provided for in the curriculum, learning content from various subjects was practiced in movement, so that each child achieved the recommended average of 45 minutes of moderate to intensive physical activity per day.

The aim of this implementation was to counteract the development of obesity and to improve or maintain cardiometabolic health (e.g. vascular function, metabolism, heart function).

In addition, the effect of daily physical activity on mental health, well-being, social aspects and cognitive performance parameters was assessed.

Twelve elementary schools in three European countries (Austria, Slovenia, Belgium) were taking part in the Happy Children study, three of them in Graz, Austria . Some of the schools were assigned to intervention schools, others served as control schools without the daily based intervention.

The intervention schools received teaching equipment and their teachers received a special training based on the Norwegian Health Oriented Pedagogical Project (HOPP) study,.

The teachers were asked to track the number of lessons they conducted each week. They were also required to adjust their teaching methods and learning materials during the implementation of active learning, in order to tailor them to the local curriculum and any other specific needs or requirements.

The aim of this thesis was to provide reference values for the amount of SAT measured by B-mode ultrasound in elementary school children, aged 6 to 10 years for girls and boys.

Therefore, the thesis included only baseline data collected at the beginning of the study and implementation of the daily intervention. The baseline data were collected in fall 2021. After the data were collected, two of the schools were assigned to the intervention group and the other school served as the control school. A total of 473 children were enrolled in the study and examined for research purposes.

3.2 Ethical approval

The Ethics Committee of the Medical University of Graz, Austria (33-488 ex 20/21 approved this study. It is also registered in the ClinicalTrials.gov (NCT04956003). All participants in the study took part voluntarily, and a signed consent from the parents or the legal representatives as wel as of each child was required. All children in the intervention schools participated in the program, but only those with written consent from their parents or legal representatives were included in the measurements and tested for the study purposes. Every child had the right to decline any individual measurement or to withdraw from the entire testing process at any point.

3.3 Recruitment

At the end of February 2021, the Board Directorate of Education Styria send out an invitation letter for participation in the Health and Academic Performance with Happy Children study to each of the 42 public elementary schools in Graz. As the implementation of the physical activity intervention as part of regular school lessons requires a high level of motivation on the part of the teachers involved, explicit attention was paid to the voluntary participation of the schools during the recruitment process.

Two of these schools voluntarily agreed to act as intervention schools. Another one agreed to act as a control school.

A total of 404 children aged 6-11 years (grades 1 to 4), whose subcutaneous fat was measured, were included in the analyses of the present thesis.

Children with different cultural contexts, socioeconomic backgrounds, male and female took part in the study. No child was excluded in advance. However, if a child had a particular illness or pre-existing disability, this was noted and taken into account when considering the results.

The group of 11-year-olds was excluded for the collection of the reference values split by age groups, as there were only 15 children in this group. However, the 11-year-olds were included for the presentation of the total sample. This means that reference values for each age group was based on 389 children in total, with 192 girls and 197 boys.

For the following thesis, the baseline data of the SAT for all three schools were summarized. Depending on the amount of the SAT, the children were divided into different percentile ranks.

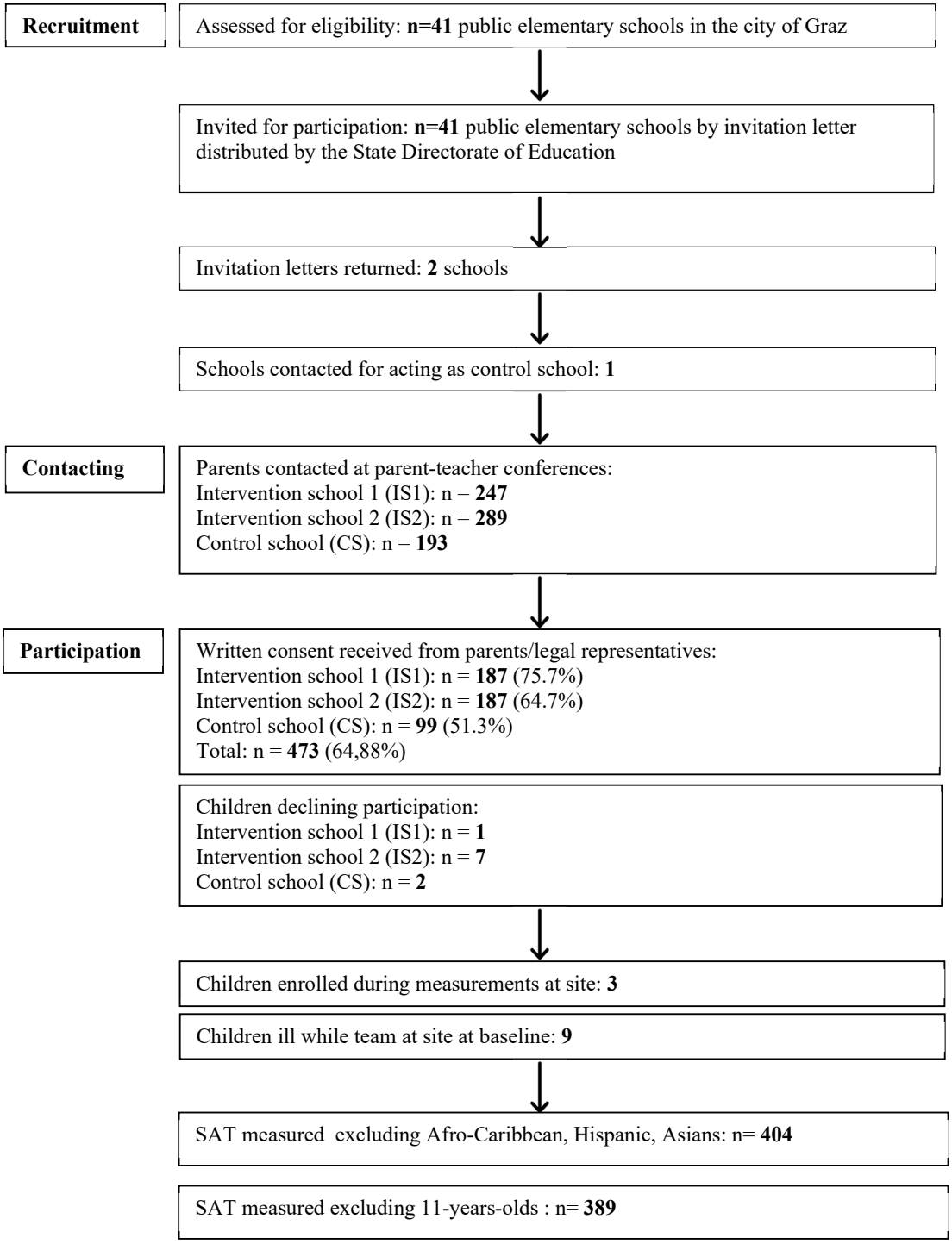

Figure 5: Recruitment process of the study participants: n = number of children

3.4 Protocol and Setting

The baseline measurements were carried out before the start of the intervention, in all three schools in September 2021. At the end of each school year, the follow-up measurements were then taken, starting in June 2022. The second and third follow-up measurements were then carried out in June 2023 and June 2024.
Along with the children's subcutaneous adipose tissue which was measured during these on-site checks, many other tests such as physical fitness, cognitive performance, urine samples and other parameters were also measured as part of the Happy children study.

In all schools the measurements took place in the gymnasium and the locker rooms between 8 am and 2 pm. The children were therefore divided into small groups of 4-6 pupils. During the examinations, it was ensured that no child was ever alone in a room with an examiner.

3.5 SAT-Measurements

For this thesis the subcutaneous adipose tissue was measured by a B-Mode ultrasound device (Esaote laptop color Doppler system, SMT, Germany). A linear transducer with 18 MHz (harmonic mode, axial resolution: 0.10-0.15) was used. The measurements were taken by experienced examiners from the Department of Physiology and Pathophysiology.
To avoid compression of the adipose tissue, the probe was placed over the measurement points with no pressure, and with an ultrasound gel layer of 3-5mm (2,5).

As it is not possible to measure the total subcutaneous body fat, anthropometric data have to be collected to estimate the total SAT from the results of the eight sites. These anthropometric measurements contain height (h), sitting height (h), leg lengths (l), body mass (m) and the circumferences of hip (H), biceps (B), thigh (T) and the waist (W).
Height was measured to the nearest 0.1 cm while standing without shoes using a stadiometer. The body weight was measured barefoot and in light clothing (shorts and t-shirt) with an electronic scale (Tanita MC-980MA, Tokyo, Japan) to the nearest 0.1 kg. To compensate for the effect of the weight of the clothing, an amount of 0.4 kg was subtracted from the weight.
Circumference of the waist was also measured to the nearest 0.1 cm at full exhalation and at the level of the navel. The hip circumference was measured at the largest part of the bum.

Leg length was determined in a standing position, measured from the floor to the spina iliaca anterior superior. For the sitting height, the participant had to be in an upright position while sitting at a table with the feed positioned on a box. All circumferences and lengths were measured to the nearest 0.1cm with a non-elastic tape (2,13).

The measurement points for the study were the same 8 which were standardized by another study of the Medical University of Graz. For the trunk it is the upper abdomen (UA), lower abdomen (LA) and erector spinae (ES), for the upper limb brachioradialis (BR) and distal triceps (DT) and the lower limb front thigh (FT), lateral thigh (LT) and medial calf (MC).
For exact and comparable results and it is very important to accurately mark these sites over the body of the participant (2,5).
The following Table 1 and Figure 6 provide an overview of the 8 positions and their exact marking on each participant:

Table 1: Description of ultrasound (US) sites and measurement procedure (77)

Site name	**Description of the sites** **Marking is done in a standing or sitting position, on the right side of the body; see Figure 6** **All distances (d) are percentages of body height (h)**	**All US measurements are taken in a lying position!** **Always use a thick layer of US gel (at least 3–5 mm)**
UA **Upper abdomen**	1. Mark a vertical line at a distance d=0.02 h (2% of body height h) lateral to the center of the umbilicus (omphalion) 2. Project vertically and mark a horizontal line at d=0.02 h superior to the omphalion (In case this site is above a tendinous inscription of the rectus abdominis (where subcutaneous adipose tissue (SAT) is thicker), move the probe some mm to the end of this inscription and measure the thickness there)	Lying in a supine position. Have the participant stop breathing at mid-tidal expiration and then capture the image
LA **Lower abdomen**	1. The same line as for the upper abdomen 2. Project vertically and mark a horizontal line at d=0.02 h inferior to the omphalion	Lying in a supine position. Have the participant stop breathing at mid-tidal expiration and then capture the image.
FT **Front thigh**	1. Put the foot on the anthropometric box which is placed in front of a wall so that the thigh is horizontal and the big toe and the knee touch the wall. 2. Mark the site at a horizontal distance d=0.14 h from the wall	Lying in a supine position.

LT **Lateral thigh**	1. Place the foot on the anthropometric box so that the thigh is horizontal and the leg is vertical. 2. Mark the site at d=0.18 h above the surface at the most medial aspect (use a ruler to determine the most medial aspect when looking vertically down	Lying in a rotated position. The participant rolls onto the left side with both knees at a 90° angle, with the right leg over the left leg.
MC **Medial calf**	1. Place the foot on the anthropometric box so that the thigh is horizontal and the leg vertical 2. Mark the site at d=0.18 h above the surface at the most medial aspect (use a ruler to determine the most medial aspect when looking vertically down	Lying in a rotated position. The participant rolls onto the right side with the right knee at a 90° angle so that the lateral aspect of the right leg is supported.
ES **Erector Spinae**	1. Mark a transverse line at d=0.14 h above the solid surface (table) on which the person is sitting in a stretched upper body position with thighs horizontal and legs unsupported. 2. Mark the site at d=0.02 h lateral to the spinous process of the vertebra	Lying in a prone position.
DT **Distal Triceps**	1. Put the lower arm on a support surface (table) with the hand in the mid-prone position. Mark a vertical line on the most posterior aspect of the arm. 2. Mark the site on the vertical line at a distance from the surface of d=0.05 h	Lying in a prone position. Capture the image with the dorsal surface of the hand on the table. Make sure the probe orientation is perpendicular to the skin.
BR **Brachioradialis**	1. The participant puts the forearm with the hand in the mid-prone (‘shake-hands’) position on a support table and contracts the brachioradialis (Bild 2. Draw a longitudinal line on the most anterior surface of the brachioradialis muscle. 3. Mark a transverse line at a distance d=0.02 h distally from the anterior surface of the biceps brachii tendon (press the end of the meter rod onto the stretched tendon). Project this line transversely to intersect with the longitudinal line	Lying in a supine position. Take the image with the arm in a mid-prone position and in contact with the thigh (muscles of the arm are relaxed). Avoid imaging the vein in case there is one in the vicinity.

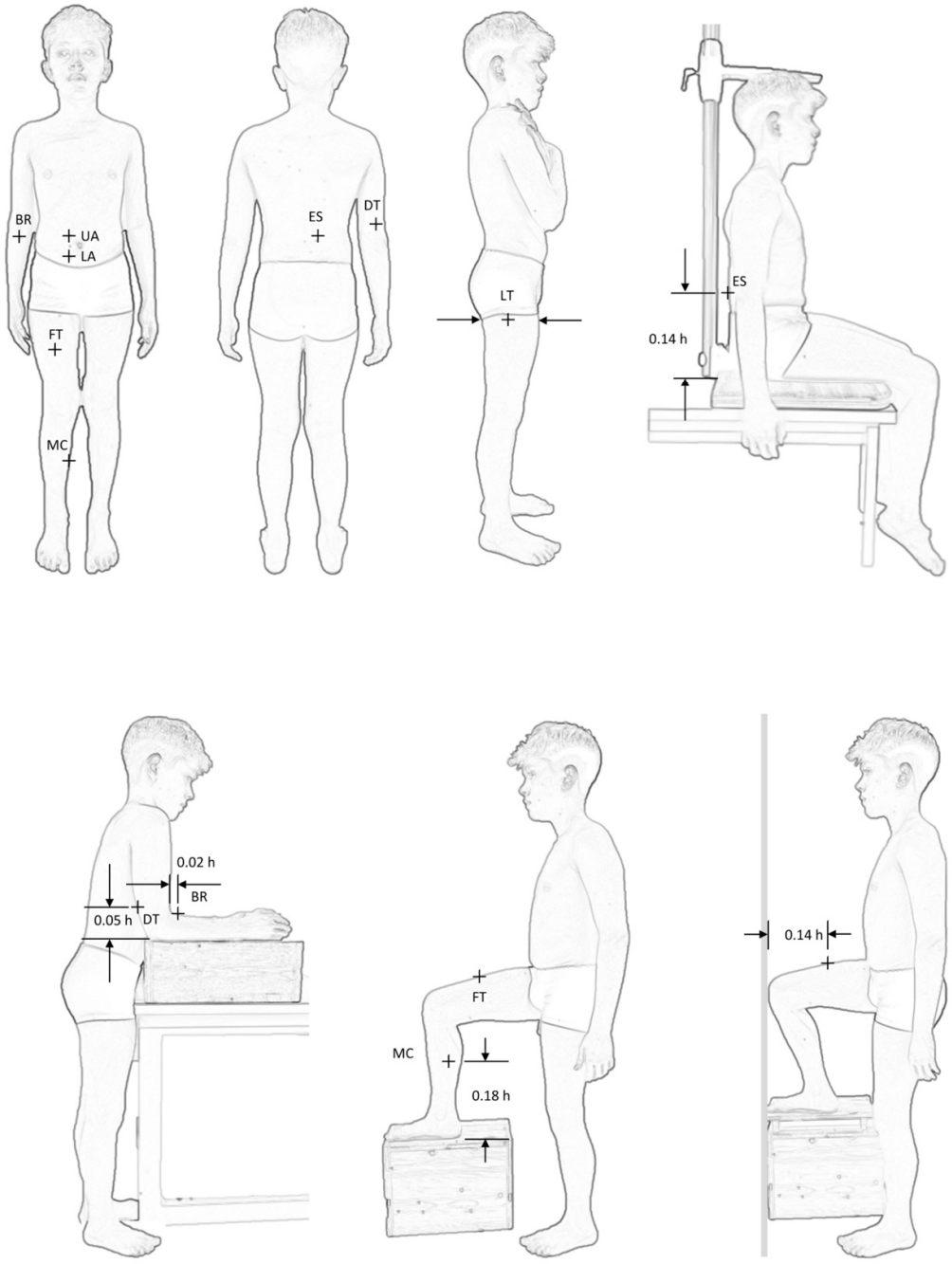

Figure 6: Overview of the ultrasound sites on the children's body

A typical ultrasound image shows a gel layer at the very top of the image. Below that the epidermis and dermis are seen. Below theses, the subcutaneous fat can be seen.

Within the subcutaneous fat are fibrous structures, which can also be seen on the image. The end of the subcutaneous fat layer is marked by the muscle fascia which lies above the muscle. To exactly determine where the muscle fascia is located, the tissue can be compressed with the transducer. Fat is much easier to compress than muscle fascia, which can also be seen on the US image (77).
Figure 7 show a typical US image with all the layers and the embedded fibrous structures.

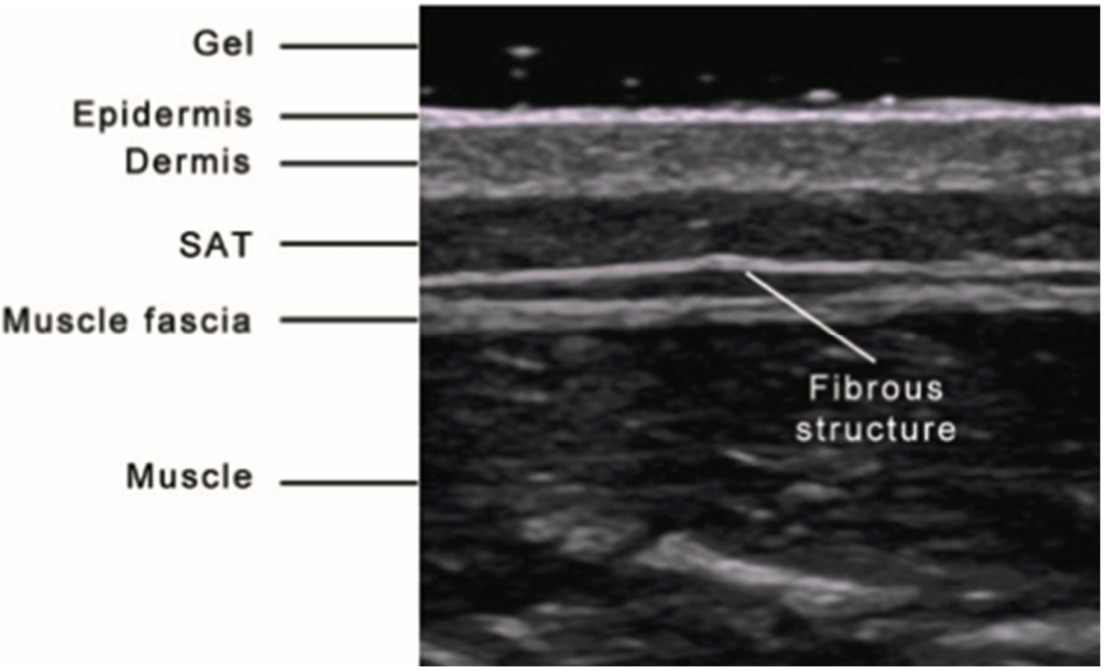

Figure 7: Typical picture of an ultrasound image (77)

An interactive image segmentation algorithm was used to identify the SAT contour in the ultrasound images A special software (NISOS-BCA-F4.2; rotosport.at) then automatically performed thickness measurements for each of the eight images. The limit of agreement for the sums of the eight measurement sites was shown to be excellent, with values of 1 to 2 mm (2).

For the SAT measurements in this study, the fibrous structures embedded in the subcutaneous fat, are included in the measurements.

3.6 Statistical Analysis

For the descriptive statistics including calculation of percentiles as well as group and site comparisons IBM SPSS (statistics for Windows, Version 29.0. Armonk, NY: IBM Corp) was used. Analyses of group (sex) differences were performed by independent t-tests, while the eight standardized sites were compared by multivariate analysis of variances (ANOVA) using sex as the between subjects factor. All data were checked for outliers prior to analyses and validated by two independent researchers. Descriptive statistics included the calculation of mean ± SD (standard deviation) of body circumferences and anthropometrics, the Cormic index, the Mass Index, the BMI, the BMI-SDS, WHtR and the percentiles corresponding to the WHO percentiles of BMI for children.

WHtR was calculated by dividing the waist circumference in cm by the person's height in cm. The BMI is the ratio of body weight in kg to height squared in meters. The BMI-SDS was calculated by subtracting the average BMI for that age and sex from the person's BMI, then dividing by the standard deviation of BMI in that group. The Cormic Index was calculated by dividing the sitting height by the total body height.

4. Results

In total 473 children aged 6-11 years were enrolled in Happy Children study. Of the 473 children, 444 took part in the SAT measurement. Of the 444 children, 404 were Caucasian, 34 were Afro-Caribbean, 4 Asian and 2 had Hispanic origin.

A Comparison of anthropometry between Afro-Caribbean and all other children (White, Asian, Arabic) revealed significant differences with regard to body height ($t_{(442)}$= 2.951, p = 0.003) and leg length ($t_{(442)}$ = 4.021, p < 0.001). On average African-Caribbean children were taller and had longer legs. For calculations of the reference values, Afro-Caribbean, Asian and Hispanic children were excluded, resulting in a total n = 404 Caucasian children.

For analysis of the total sample (n=404), the group of the 11-years old children (n=15) were included (n = 404). For the analysis of the SAT by age-groups, the 11-years old were excluded, due to an undersized sample (n = 15).
Of the 389 children, 192 were female and 197 male which represents a very balanced sample.

4.1 Total sample

The following tables and figures provide an overview of the entire sample. The next chapter 4.2 will break down the results by age group and sex.

Table 2: Number of children per age-group and sex

		sex		
		Female	male	total
age	6	20	15	35
	7	49	38	87
	8	47	53	100
	9	46	50	96
	10	30	41	71
	11	6	9	15
total		198	206	404

Since in particular children with overweight tended to refuse measurement of SAT, the number of children with overweight and obesity was lower (22.28%) compared to the total study sample of 473 children (23.48%). No difference was found in the number of children

with normal weight, overweight or obesity depending with regard to sex ($\chi^2_{(2)} = 0.003$, p = 0.999). In girls 44 (22.2%) of 198 and in boys 46 (22.3%) of 206 were classified as overweight/obese according to the WHO BMI cut-offs (see p. 7).

Table 3: Frequency of girls and boys classified as normal weight, overweight or obese according to the WHO-criteria.

sex		normal weight	overweight	obese	total n
girls	N	154	27	17	198
	%	77,8%	13,6%	8,6%	100%
boys	N	160	28	18	206
	%	77,7%	13,6%	8,7%	100%
Total		314	55	35	404

Abbreviations: n = number of children

4.1.1 Anthropometric data

Descriptive statistics for anthropometric data and body indices split by sex are summarized in Table 4. Significant differences between girls and boys were only seen in age ($t_{(402)} = -2.024$, p = 0.044), height ($t_{(402)} = -2.973$, p = 0.003), the cormic-index ($t_{(402)} = -2.876$, p = 0.004) and waist circumference ($t_{(402)} = -2.265$, p = 0.024), but no other bodily index such as BMI, BMI-SDSs or WHtR.

Table 4: Descriptive statistics for anthropometric data and body indices split by sex

	girls					**boys**				
	mean	**SD**	**min.**	**max.**	**n**	**mean**	**SD**	**min.**	**max.**	**n**
age	8,18	1,32	6,00	11,00	198	8,44	1,31	6,00	11,00	206
body-height [m]	1,31	0,10	1,08	1,58	198	1,34	0,09	1,12	1,57	206
body-mass [kg]	30,61	9,51	16,90	65,60	198	32,07	9,13	18,80	71,60	206
leg-length [m]	0,74	0,06	0,59	0,90	198	0,75	0,06	0,62	0,90	206
sitting-height [m]	0,70	0,05	0,56	0,83	198	0,71	0,04	0,59	0,81	206
waist [m]	0,59	0,09	0,44	0,94	198	0,61	0,09	0,47	0,93	206
hip [m]	0,71	0,10	0,54	1,02	198	0,71	0,09	0,55	1,02	206
biceps [m]	0,22	0,03	0,17	0,31	198	0,22	0,03	0,17	0,36	206
thigh [m]	0,57	2,83	0,26	40,20	198	0,38	0,06	0,28	0,59	206

BMI	17,43	3,35	12,04	31,55	198	17,57	3,30	13,01	31,83	206
BMI-SDS	0,49	1,25	-3,42	3,29	198	0,63	1,35	-2,10	5,34	206
MI	17,51	3,44	12,13	31,10	198	17,51	3,32	12,68	31,68	206
cormic-index	0,54	0,01	0,49	0,57	198	0,53	0,01	0,50	0,57	206
ws-ratio	0,84	0,09	0,63	1,19	198	0,85	0,10	0,68	1,23	206
WHtR	0,45	0,05	0,33	0,65	198	0,45	0,05	0,37	0,68	206

Abbreviations: BMI = body-mass-index, BMI-SDS = body-mass-index standard deviation score, MI = mass index, ws = waist-to-standing height ratio, WHtR = waist-to-height-ratio, SD = standard deviation, min = minimum, max = maximum, n = number of children

4.1.2 SAT in the total sample

Table 5 and Table 6 show the results of the SAT measurements in the total sample split by sex. The sum of the 8 measuring points is given as D_I. For the total amount of SAT (D_I) a significant difference was found ($t_{(402)} = 2.874$, $p = 0.004$), indicating that girls aged 6-11 years have a higher total amount of SAT as compared to same-aged boys (see Table 6 first row). According to the multivariate ANOVA conducted to compare the eight standardized sites, the overall effect was significant ($F_{(8, 395)} = 5.317$, $p < 0.001$), indicating more SAT in girls as compared to boys at the upper abdomen ($p = 0.015$), lower abdomen ($p = 0.004$), front thigh ($p = 0.003$), lateral thigh ($p = 0.010$), medial calf ($p = 0.009$) and erector spinae ($p < 0.001$) whilst no sex difference was found at the distal triceps ($p = 0.179$) and brachioradialis ($p = 0.216$).

Table 5: SAT in girls

Girls (6-11y)

[n=198]	Percentiles [SAT mm]										
	1st	**3rd**	**5th**	**15th**	**25th**	**50th**	**75th**	**85th**	**95th**	**97th**	**99th**
D_I	18,01	28,05	29,70	39,54	47,26	65,74	98,83	122,53	157,28	177,29	184,50
	min.					*mean*	*SD*				max.
	17,82					76,78	39,34				**185,55**
BMI	*15,16*	*13,19*	*15,39*	*13,51*	*16,47*	*16,14*	*17,16*	*21,91*	*22,16*	*25,54*	*25,64*
UA	0,61	1,11	1,49	2,50	3,22	6,80	14,49	23,23	30,01	32,84	38,66
	min.					*mean*	*SD*				*max.*
	0,56					10,31	9,39				41,84
LA	1,28	3,32	3,71	5,83	7,47	13,02	22,03	29,51	36,11	39,17	42,93
	min.					*mean*	*SD*				*max.*

	0,92					15,88	10,71				54,22
FT	3,35	4,10	4,56	5,91	6,83	8,66	11,53	13,12	17,01	18,92	21,16
	min.					*mean*	*SD*				*max.*
	2,95					9,44	3,78				25,15
LT	2,89	5,64	6,12	8,28	11,07	14,81	19,40	23,08	25,91	30,18	32,21
	min.					*mean*	*SD*				*max.*
	1,55					15,48	6,46				36,00
MC	2,34	2,60	2,84	3,68	4,14	5,59	7,65	9,13	12,36	13,99	15,24
	min.					*mean*	*SD*				*max.*
	2,29					6,31	2,90				18,78
ES	0,86	1,45	1,61	2,72	3,21	4,94	7,80	9,64	14,34	16,61	18,88
	min.					*mean*	*SD*				*max.*
	0,60					6,07	3,96				21,42
DT	2,62	4,03	4,45	5,60	6,56	8,03	10,69	11,93	15,92	17,71	19,41
	min.					*mean*	*SD*				*max.*
	0,91					8,76	3,45				21,84
BR	1,36	2,03	2,24	2,92	3,31	4,38	5,62	6,34	8,05	8,80	9,38
	min.					*mean*	*SD*				*max.*
	1,32					4,60	1,73				10,41

Abbreviations: D_I = sum of SAT, BMI = body-mass-index, min = minimum, max = maximum, SD = standard deviation, n = number of children, UA = upper abdomen, LA = lower abdomen, FT = front thigh, LT = lateral thigh, MC = medial calf, ES = erector spinae, DT = distal triceps, BR = brachioradialis

Table 6: SAT in boys

Boys (6-11y)

[n=206]	Percentiles [SAT mm]										
	1st	**3rd**	**5th**	**15th**	**25th**	**50th**	**75th**	**85th**	**95th**	**97th**	**99th**
D_I	18,67	20,59	22,34	29,32	**34,43**	**50,89**	**83,10**	112,84	152,29	175,78	204,41
	min.					*mean*	*SD*				*max*
	15,10					65,14	41,97				**219,62**
BMI	*15,02*	*15,52*	*14,38*	*17,34*	*16,12*	*17,22*	*16,48*	*20,00*	*24,47*	*25,91*	*31,02*
UA	0,53	0,62	0,72	1,23	1,84	4,07	10,08	16,21	27,23	31,37	43,03
	min.					*mean*	*SD*				*max.*
	0,48					8,01	9,17				51,17
LA	0,74	1,89	2,11	3,40	4,45	8,69	17,09	24,59	35,00	36,46	48,51
	min.					*mean*	*SD*				*max.*
	0,38					12,74	10,80				55,15
FT	3,14	3,44	3,74	4,40	5,07	6,94	9,99	12,77	17,76	20,02	22,96
	min.					*mean*	*SD*				*max.*
	2,17					8,19	4,38				27,44
LT	1,97	3,72	4,47	6,37	7,49	12,04	17,12	22,24	29,05	32,10	41,48
	min.					*mean*	*SD*				*max.*

	1,44					13,60	7,85				42,19
MC	1,49	2,00	2,26	2,90	3,38	4,59	6,95	8,40	12,06	13,82	16,17
	min.					*mean*	*SD*				*max.*
	1,35					5,51	3,06				16,73
ES	0,64	0,85	0,93	1,55	1,94	3,27	6,04	8,27	11,15	12,32	18,19
	min.					*mean*	*SD*				*max.*
	0,49					4,51	3,47				20,07
DT	3,29	3,93	4,34	5,06	5,79	7,19	9,83	11,70	16,08	18,12	21,09
	min.					*mean*	*SD*				*max.*
	2,74					8,25	3,58				21,96
BR	1,34	1,78	1,96	2,44	2,81	3,71	5,22	6,60	8,95	9,51	11,92
	min.					*mean*	*SD*				*max.*
	1,18					4,36	2,12				12,48

Abbreviations: D_I = sum of SAT, BMI = body-mass-index, min = minimum, max = maximum, SD = standard deviation, n = number of children, UA = upper abdomen, LA = lower abdomen, FT = front thigh, LT = lateral thigh, MC = medial calf, ES = erector spinae, DT = distal triceps, BR = brachioradialis

The next two figures (Figure 8 and Figure 9) show a box plot version of the SAT measurements. This type of figure clearly shows the minimum, maximum, median and upper and lower quartiles.

The boys have a higher maximum and more outliers than the girls. Nevertheless, the total amount of SAT in all places was lower except in distal triceps and brachioradialis.

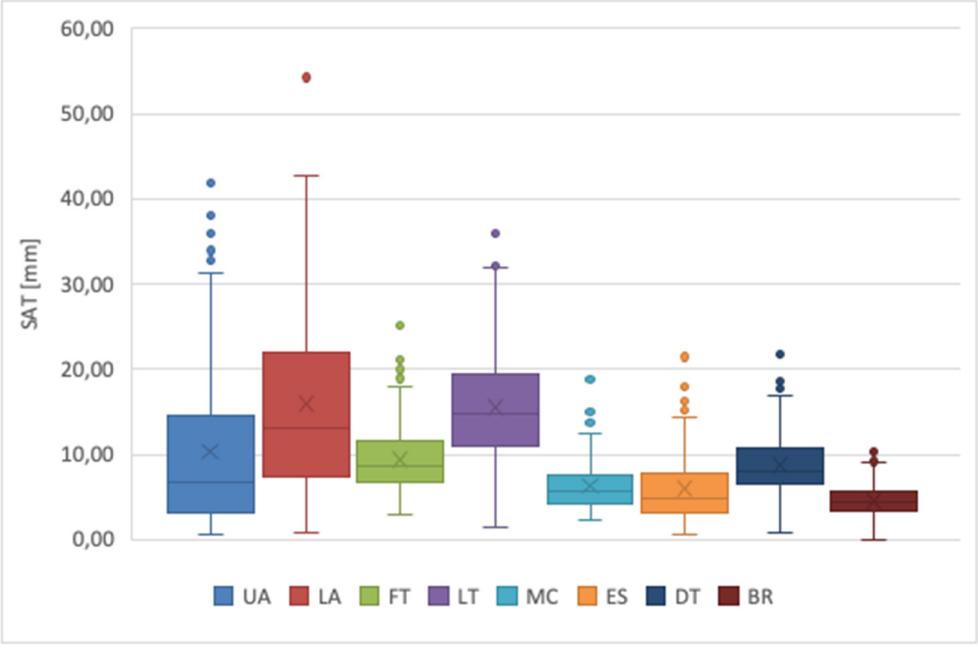

Figure 8: SAT at the eight standardized sites in girls

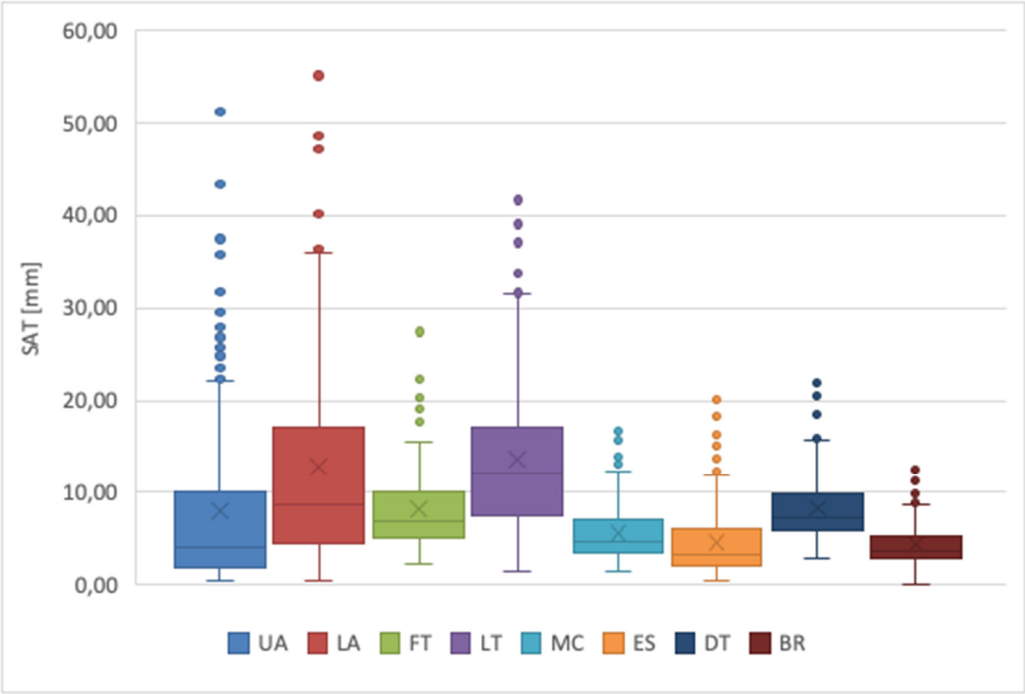

Figure 9: SAT at the eight standardized sites in boys

All eight sites showed highly significant intercorrelations, with upper and lower abdomen showing highest correlation. Furthermore, due to the relatively higher number of children with overweight/obesity, the total amount of SAT and the BMI (as well as the BMI-SDS) correlated highly significant ($r = 0.911$, $p < 0.001$). In particular, SAT at the upper and lower abdomen were higher correlated to the BMI (see Table 7).

Table 7: Correlation of SAT and BMI

		BMI	BMI_SDS	UA	LA	FT	LT	MC	ES	DT	BR
BMI	r	1	,934**	**,902****	**,896****	,853**	,772**	,814**	,836**	,853**	,788**
	p		0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	n	404	404	404	404	404	404	404	404	404	399
BMI-SDS	r	,934**	1	,813**	,831**	,809**	,762**	,774**	,768**	,815**	,783**
	p	0,000		0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	n	404	404	404	404	404	404	404	404	404	399
UA	r	,902**	,813**	1	**,967****	,864**	,802**	,818**	,871**	,844**	,796**

	p	0,000	0,000		0,000	0,000	0,000	0,000	0,000	0,000	0,000
	n	404	404	404	404	404	404	404	404	404	399
LA	r	,896**	,831**	,967**	1	,877**	,842**	,834**	,876**	,853**	,817**
	p	0,000	0,000	0,000		0,000	0,000	0,000	0,000	0,000	0,000
	n	404	404	404	404	404	404	404	404	404	399
FT	r	,853**	,809**	,864**	,877**	1	,864**	,865**	,862**	,875**	,819**
	p	0,000	0,000	0,000	0,000		0,000	0,000	0,000	0,000	0,000
	n	404	404	404	404	404	404	404	404	404	399
LT	r	,772**	,762**	,802**	,842**	,864**	1	,836**	,817**	,821**	,772**
	p	0,000	0,000	0,000	0,000	0,000		0,000	0,000	0,000	0,000
	n	404	404	404	404	404	404	404	404	404	399
MC	r	,814**	,774**	,818**	,834**	,865**	,836**	1	,817**	,850**	,803**
	p	0,000	0,000	0,000	0,000	0,000	0,000		0,000	0,000	0,000
	n	404	404	404	404	404	404	404	404	404	399
ES	r	,836**	,768**	,871**	,876**	,862**	,817**	,817**	1	,824**	,759**
	p	0,000	0,000	0,000	0,000	0,000	0,000	0,000		0,000	0,000
	n	404	404	404	404	404	404	404	404	404	399
DT	r	,853**	,815**	,844**	,853**	,875**	,821**	,850**	,824**	1	,808**
	p	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000		0,000
	n	404	404	404	404	404	404	404	404	404	399
BR	r	,788**	,783**	,796**	,817**	,819**	,772**	,803**	,759**	,808**	1
	p	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	
	n	399	399	399	399	399	399	399	399	399	399

** p < 0.001, * p < 0.05

Abbreviations: BMI = body-mass-index, BMI-SDS = body-mass-index standard deviation score, UA = upper abdomen, LA = lower abdomen, FT = front thigh, LT = lateral thigh, MC = medial calf, ES = erector spinae, DT = distal triceps, BR = brachioradialis, r = correlation coefficient, n = number of children

For reliability analysis, Cronbach's alpha was calculated to assess the internal consistency of the total amount of SAT assessed by the eight standardized sites. The internal consistency was shown to be excellent, with Cronbach's alpha for the total sum of SAT of $\alpha = 0.93$.

Table 8: Scale statistics for internal consistency analysis

Site	mean	variance, if removed	corrected item scale correlation	squared multiple correlation	Cronbachs Alpha
UA	61,63	1043,77	0,936	0,941	0,911
LA	56,52	942,95	0,953	0,95	0,922
FT	61,97	1396,81	0,923	0,871	0,917
LT	56,27	1207,03	0,872	0,805	0,912
MC	64,87	1485,19	0,884	0,813	0,925

ES	65,49	1425,83	0,902	0,819	0,920
DT	62,25	1446,77	0,897	0,827	0,921
BR	66,28	1565,98	0,847	0,738	0,934

Abbreviations: UA = upper abdomen, LA = lower abdomen, FT = front thigh, LT = lateral thigh, MC = medial calf, ES = erector spinae, DT = distal triceps, BR = brachioradialis

4.2 Reference values for age groups

This chapter presents the reference values of the SAT measurements broken down by sex and age group. There is one table for girls and one for boys. These tables show the percentiles of the SAT values at the 8 points measured, as well as the mean value, the standard deviation and the minimum and maximum.

In addition, another table shows the anthropometric data of the girls and boys in their respective age groups. The results are also shown here with the mean value, standard deviation and minimum and maximum.

4.2.1 SAT in 6-year-old girls and boys

For the group of six-year-old girls (n=20) and boys (n=15) no significant difference was found in the total amount of SAT ($t_{(33)} = 0.577$, $p = 0.568$). Similarly, also the multivariate ANOVA revealed no significant sex difference for any of the eight sites ($F_{(8, 26)} = 0.419$, p 0 0.899). Therefore, tables were only produced for the pooled sample of 6-year-old girls and boys.

Table 9: SAT in 6-year-old girls and boys (pooled)

6-years

Percentiles [SAT mm] [n=35]

	min.	**1st**	**3rd**	**5th**	**15th**	**25th**	**50th**	**75th**	**85th**	**95th**	**97th**	**99th**	*max.*	*mean*	*SD*
D_I	**24,1**	24,1	24,3	26,4	30,3	**39,3**	**61,1**	**91,3**	105,5	144,8	152,1		**152,9**	66,8	33,2
UA	0,7	0,7	0,8	1,0	1,5	2,0	5,0	12,8	16,6	22,5	23,4		23,5	7,4	6,7
LA	2,7	2,7	2,7	2,9	3,6	4,3	10,3	20,6	22,5	31,4	34,2		34,5	12,4	8,8
FT	3,8	3,8	3,8	3,9	4,6	5,6	8,1	11,2	13,1	15,8	20,0		20,5	8,9	3,8
LT	4,6	4,6	4,7	5,5	6,8	8,5	12,2	18,2	20,3	26,9	31,2		31,6	14,1	6,3
MC	2,9	2,9	2,9	3,0	3,3	3,8	5,3	7,5	8,0	10,2	11,1		11,2	5,7	2,2
ES	0,9	0,9	0,9	1,0	1,9	2,3	5,0	6,1	8,5	12,9	13,1		13,2	5,2	3,2
DT	0,9	0,9	1,2	3,5	5,1	6,6	8,4	10,1	11,5	16,2	16,7		16,8	8,4	3,2
BR	1,5	1,5	1,6	2,0	2,4	3,1	4,3	5,9	7,2	8,4	9,2		9,3	4,6	2,0

Abbreviations: D_I = sum of SAT, min = minimum, max = maximum, SD = standard deviation, n = number of children, UA = upper abdomen, LA = lower abdomen, FT = front thigh, LT = lateral thigh, MC = medial calf, ES = erector spinae, DT = distal triceps, BR = brachioradialis

Table 10: Anthropometry and body indices in 6-year-old girls and boys

	girls					boys				
	mean	SD	min.	max.	n	mean	SD	min.	max.	n
body-height [m]	1,19	0,04	1,12	1,27	20	1,23	0,04	1,17	1,32	15
body-mass [kg]	23,48	4,49	17,90	35,80	20	26,57	5,21	20,80	40,70	15
waist [m]	0,55	0,05	0,48	0,66	20	0,59	0,07	0,52	0,79	15
hip [m]	0,65	0,06	0,57	0,80	20	0,67	0,06	0,60	0,84	15
BMI	16,47	2,52	13,05	22,55	20	17,41	2,28	14,09	23,36	15
BMI_SDS	0,48	1,29	-1,68	2,92	20	1,18	1,22	-1,01	3,96	15
MI	16,17	2,55	12,96	23,13	20	16,94	2,09	13,87	22,14	15
WHtR	0,46	0,04	0,39	0,53	20	0,47	0,04	0,42	0,59	15

Abbreviations: min = minimum, max = maximum, SD = standard deviation, n = number of children, UA = upper abdomen, LA = lower abdomen, FT = front thigh, LT = lateral thigh, MC = medial calf, ES = erector spinae, DT = distal triceps, BR = brachioradialis

Girls and boys at the age of six years differed significantly only with regard to body-height ($t_{(33)} = -2.689$, $p = 0.011$), which was higher in boys, however, in none of the other body indices measured.

4.2.2 SAT in 7-year-old girls and boys

The total sum of SAT differed significantly between girls and boys ($t_{(85)} = 2.504$, $p = 0.014$), indicating that girls have more SAT as boys already at the age of seven years. Accordingly, also the multivariate analysis for the eight sites revealed a significant sex difference ($F_{(8, 77)} = 2.628$, $p = 0.013$), which was due to the higher amount of SAT in girls at all sites (for all $p < 0.05$) but the distal triceps ($p = 0.451$). Table 11 and Table 12 give the percentiles for the SAT measures split by sex.

Table 11: SAT in 7-year-old girls and boys

7-years

girls	Percentiles [SAT mm]												[n=49]		
	min.	**1st**	**3rd**	**5th**	**15th**	**25th**	**50th**	**75th**	**85th**	**95th**	**97th**	**99th**	max.	*mean*	*SD*
DI	**19,4**	19,4	23,9	28,5	36,4	**42,0**	**52,0**	**69,3**	87,4	130,3	136,9		**142,1**	61,7	28,8
UA	1,0	1,0	1,2	1,4	2,1	2,5	4,5	9,0	11,9	25,0	27,4		28,9	7,0	6,9
LA	0,9	0,9	1,5	2,7	4,8	6,0	8,8	14,3	18,0	33,8	35,4		36,0	11,8	8,7
FT	3,4	3,4	3,6	3,9	5,0	6,2	7,6	9,3	11,2	12,5	14,5		16,3	8,0	2,6

LT	2,9	2,9	4,3	5,7	7,8	9,7	12,5	16,0	19,5	23,9	25,8		26,2	13,4	5,3
MC	2,3	2,3	2,5	2,6	3,2	3,9	5,3	6,4	7,5	8,5	10,3		11,9	5,3	1,9
ES	1,3	1,3	1,4	1,5	2,3	2,9	4,0	5,7	7,2	10,9	12,8		14,0	4,5	2,6
DT	4,0	4,0	4,3	4,7	5,5	5,9	7,1	8,8	9,6	12,0	12,3		12,6	7,6	2,1
BR	1,9	1,9	2,0	2,2	2,8	3,2	3,9	5,0	5,8	7,3	8,6		9,2	4,2	1,5
						Percentiles [SAT mm]									[n=38]
boys	min.	**1st**	**3rd**	**5th**	**15th**	**25th**	**50th**	**75th**	**85th**	**95th**	**97th**	**99th**	max.	*mean*	*SD*
DI	**22,3**	22,3	22,5	23,3	28,2	**32,8**	**41,5**	**52,9**	76,7	98,1	114,7		**118,3**	47,6	22,3
UA	0,5	0,5	0,6	0,7	1,0	1,3	2,4	6,6	9,3	12,5	15,5		16,2	4,1	3,8
LA	1,5	1,5	1,6	1,9	3,5	4,1	6,8	9,5	14,6	17,9	22,9		23,9	8,0	5,2
FT	3,7	3,7	3,7	3,8	4,1	4,5	6,0	7,3	9,8	14,6	15,3		15,5	6,6	2,9
LT	4,8	4,8	4,9	5,5	6,1	6,6	8,9	12,8	16,2	23,3	23,8		23,9	10,6	5,0
MC	1,9	1,9	2,0	2,1	2,8	3,1	4,2	5,2	5,8	8,4	9,4		9,6	4,4	1,7
ES	0,7	0,7	0,7	0,9	1,4	1,6	2,4	3,5	4,5	8,3	10,0		10,3	2,9	2,0
DT	4,5	4,5	4,6	4,7	5,3	5,8	6,8	8,1	9,3	12,2	12,4		12,4	7,2	2,1
BR	1,9	1,9	1,9	2,2	2,5	2,8	3,3	4,1	4,5	6,3	8,5		9,0	3,6	1,3

Abbreviations: D_I = sum of SAT, min = minimum, max = maximum, SD = standard deviation, n = number of children, UA = upper abdomen, LA = lower abdomen, FT = front thigh, LT = lateral thigh, MC = medial calf, ES = erector spinae, DT = distal triceps, BR = brachioradialis

In contrast, none of the other anthropometric measures (BMI, MI, WHtR) indicated any sex difference in 7-year-old boys and girls (see Table 12).

Table 12: Anthropometry and body indices in 7-year-old girls and boys

	girls					**boys**				
	mean	**SD**	**min.**	**max.**	**n**	**mean**	**SD**	**min.**	**max.**	**n**
body-height [m]	1,23	0,05	1,08	1,33	49	1,24	0,06	1,12	1,36	38
body-mass [kg]	24,74	4,62	16,90	37,70	49	25,11	4,68	18,80	42,00	38
waist [m]	0,55	0,06	0,45	0,72	49	0,56	0,05	0,47	0,72	38
hip [m]	0,66	0,06	0,54	0,87	49	0,65	0,05	0,57	0,82	38
BMI	16,20	2,50	12,05	23,90	49	16,11	1,98	13,01	22,71	38
BMI_SDS	0,24	1,24	-2,71	3,14	49	0,23	1,15	-2,10	3,28	38
MI	16,16	2,41	12,13	23,82	49	15,85	2,08	12,68	23,82	38
WHtR	0,45	0,04	0,37	0,58	49	0,45	0,03	0,38	0,53	38

Abbreviations: BMI = body-mass-index, BMI-SDS = body-mass-index standard deviation score, MI = mass index, ws = waist-to-standing height ratio, WHtR = waist-to-height-ratio, SD = standard deviation, min = minimum, max = maximum, n = number of children

4.2.3 SAT in 8-year-old girls and boys

In the group of 8-year old girls and boys no significant difference was found in the total SAT ($t_{(98)}$ = 0.828, p = 0.409), although the multivariate approach for the eight sites was significant ($F_{(8, 91)}$ = 2.685, p = 0.011).

Table 13: SAT in 8-year-old girls and boys

8-years

girls						**Percentiles SAT [mm]**									[n = 47]
	min.	**1st**	**3rd**	**5th**	**15th**	**25th**	**50th**	**75th**	**85th**	**95th**	**97th**	**99th**	*max.*	*mean*	*SD*
DI	**17,8**	17,8	22,4	30,7	40,2	**46,9**	**63,8**	**88,7**	106,6	147,6	161,6		**165,8**	72,0	33,4
UA	0,6	0,6	0,8	1,1	2,6	3,2	6,0	11,1	16,6	24,6	28,1		30,1	8,3	7,0
LA	1,3	1,3	2,0	3,3	5,8	8,1	12,4	18,2	25,3	32,8	34,6		35,7	14,0	8,7
FT	3,0	3,0	3,7	4,7	5,7	6,9	9,0	11,3	11,6	16,8	18,7		20,0	9,3	3,4
LT	4,5	4,5	5,2	6,2	10,3	11,5	14,5	19,2	23,0	25,1	31,2		36,0	15,5	6,2
MC	2,5	2,5	2,5	2,7	4,0	4,2	5,6	7,3	8,8	12,5	13,3		14,0	6,2	2,7
ES	0,9	0,9	1,1	1,4	2,7	3,3	5,0	8,1	9,0	13,2	13,8		14,3	5,7	3,3
DT	2,6	2,6	2,9	3,6	5,8	6,8	8,3	9,9	11,7	16,4	18,3		18,6	8,6	3,2
BR	1,8	1,8	1,9	2,0	2,6	3,0	4,3	5,4	5,7	8,2	8,7		8,8	4,4	1,7
boys						**Percentiles SAT [mm]**									[n = 53]
	min.	**1st**	**3rd**	**5th**	**15th**	**25th**	**50th**	**75th**	**85th**	**95th**	**97th**	**99th**	*max.*	*mean*	*SD*
DI	**15,1**	15,1	17,4	19,2	24,6	**29,3**	**53,3**	**83,8**	111,0	181,8	197,0		**219,6**	65,2	46,8
UA	0,7	0,7	0,7	0,8	1,1	1,5	4,4	11,8	15,9	31,9	42,7		51,2	8,3	10,3
LA	0,7	0,7	1,5	2,1	3,1	4,1	9,3	18,4	26,1	36,1	40,6		47,2	12,6	10,9
FT	2,2	2,2	2,8	3,3	3,9	4,5	6,5	10,9	12,1	22,5	24,7		27,4	8,1	5,1
LT	1,4	1,4	1,8	2,6	4,8	6,5	12,4	17,9	23,5	32,2	36,8		41,7	13,3	8,9
MC	1,3	1,3	1,4	1,7	2,6	3,0	4,7	6,7	8,4	15,8	16,3		16,7	5,6	3,6
ES	0,6	0,6	0,7	0,8	1,3	1,9	3,3	6,5	8,2	12,4	15,5		18,3	4,6	3,7
DT	2,7	2,7	3,1	3,7	4,7	5,3	7,1	9,8	11,7	19,8	21,4		22,0	8,1	4,2
BR	1,3	1,3	1,6	1,8	2,4	2,7	4,1	5,6	6,7	10,5	12,2		12,5	4,6	2,5

Abbreviations: D_I = sum of SAT, min = minimum, max = maximum, SD = standard deviation, n = number of children, UA = upper abdomen, LA = lower abdomen, FT = front thigh, LT = lateral thigh, MC = medial calf, ES = erector spinae, DT = distal triceps, BR = brachioradialis

In the group of 8-years old children, only WHtR ($t_{(98)}$ = -2.086, p = 0.04) and waist circumference ($t_{(98)}$ = -2.358, p = 0.02) differed significantly indicating that girls had a higher WHtR but a lower waist circumference than boys. Furthermore, a tendency towards slightly higher body height ($t_{(98)}$ = -1.721, p = 0.088) in boys was shown whilst none of the other anthropometric showed a significant difference.

Table 14: Anthropometry and body indices in 8-year-old girls and boys

	girls					boys				
	mean	**SD**	**min.**	**max.**	**n**	**mean**	**SD**	**min.**	**max.**	**n**
body-height [m]	1,30	0,05	1,14	1,39	47	1,32	0,06	1,21	1,45	53
body-mass [kg]	28,40	5,16	18,30	41,50	47	31,09	8,37	19,90	65,00	53
waist [m]	0,57	0,06	0,47	0,74	47	0,61	0,09	0,51	0,91	53
hip [m]	0,70	0,07	0,58	0,86	47	0,71	0,09	0,55	1,01	53
BMI	16,65	2,30	12,79	24,04	47	17,61	3,60	13,57	31,83	53
BMI_SDS	0,35	1,03	-2,16	2,88	47	0,75	1,47	-1,71	5,34	53
MI	16,67	2,32	13,54	23,67	47	17,42	3,48	13,36	30,75	53
WHtR	0,44	0,04	0,39	0,56	47	0,46	0,05	0,40	0,64	53

Abbreviations: BMI = body-mass-index, BMI-SDS = body-mass-index standard deviation score, MI = mass index, ws = waist-to-standing height ratio, WHtR = waist-to-height-ratio, SD = standard deviation, min = minimum, max = maximum, n = number of children

4.2.4 SAT in 9-year-old girls and boys

For children aged 9-years a significant sex difference was found in the total amount of SAT ($t_{(94)} = 2.467$, $p = 0.008$) with remarkably more SAT in girls as compared to boys. Although the multivariate ANOVA shortly failed significance ($F_{(8, 86)} = 1.926$, $p = 0.066$), univariate comparisons indicated significant differences between girls and boys at the upper ($p = 0.013$) and lower abdomen ($p = 0.011$), front thigh ($p = 0.029$), medial calf ($p = 0.049$) and erector spinae ($p = 0.005$), where more SAT was detected throughout in girls.

Table 15: SAT in 9-year-old girls and boys

9-years

girls	Percentiles SAT [mm]														[n = 46]
	min.	**1st**	**3rd**	**5th**	**15th**	**25th**	**50th**	**75th**	**85th**	**95th**	**97th**	**99th**	*max.*	*mean*	*SD*
DI	18,0	18,0	21,7	29,5	40,0	**49,4**	**77,4**	**122,0**	150,9	176,6	181,8		184,5	89,0	45,7
UA	0,6	0,6	0,7	1,0	1,9	4,3	9,8	23,0	26,6	32,6	36,7		38,6	13,1	10,5
LA	2,3	2,3	2,8	3,6	5,2	9,6	17,0	30,0	33,7	38,3	40,9		42,7	19,3	11,8
FT	3,5	3,5	3,8	4,1	6,4	6,8	9,0	13,5	15,1	18,6	19,6		20,1	10,2	4,3
LT	1,6	1,6	3,4	6,2	8,3	11,9	16,2	23,6	25,8	31,6	32,0		32,2	17,5	7,7
MC	2,3	2,3	2,4	2,6	3,9	4,6	5,9	9,9	10,6	14,7	17,3		18,8	7,1	3,5
ES	0,6	0,6	1,0	1,7	2,8	3,2	6,1	9,5	12,7	17,7	20,1		21,4	7,1	4,8
DT	3,7	3,7	4,0	4,4	6,4	6,9	8,3	12,4	14,9	18,9	20,8		21,8	10,0	4,2
BR	1,3	1,3	1,3	1,7	3,0	3,4	4,5	6,1	7,1	8,5	8,8		9,0	4,8	1,9
boys	Percentiles SAT [mm]														[n = 50]
	min.	**1st**	**3rd**	**5th**	**15th**	**25th**	**50th**	**75th**	**85th**	**95th**	**97th**	**99th**	*max.*	*mean*	*SD*
DI	18,7	18,7	20,4	22,2	32,2	**38,4**	**54,6**	**73,3**	115,2	171,1	197,8		204,8	66,7	42,8
UA	0,5	0,5	0,6	0,8	1,8	2,3	3,8	8,7	20,3	29,3	33,9		35,7	7,9	8,8
LA	0,4	0,4	1,0	1,9	3,8	6,3	8,8	15,6	26,8	40,4	44,5		48,6	13,0	11,3

FT	3,1	3,1	3,6	4,0	4,7	5,3	7,5	9,3	11,6	18,3	19,7	20,3	8,2	4,0
LT	2,3	2,3	3,4	5,1	7,6	9,2	12,7	16,7	21,5	38,0	40,5	42,2	14,4	8,3
MC	2,1	2,1	2,2	2,3	3,2	3,4	4,4	7,2	8,9	13,6	15,0	16,2	5,7	3,3
ES	0,5	0,5	0,7	0,9	1,8	2,3	3,4	5,8	8,9	12,5	15,6	16,3	4,6	3,5
DT	4,3	4,3	4,4	4,6	5,6	6,0	7,0	9,8	12,9	16,7	18,5	20,5	8,5	3,7
BR	1,7	1,7	1,8	2,1	2,4	3,0	3,8	5,2	6,3	9,2	10,3	11,3	4,4	2,1

Abbreviations: D_I = sum of SAT, min = minimum, max = maximum, SD = standard deviation, n = number of children, UA = upper abdomen, LA = lower abdomen, FT = front thigh, LT = lateral thigh, MC = medial calf, ES = erector spinae, DT = distal triceps, BR = brachioradialis

Notably, in none of the other anthropometric measurements and body indices any significant sex difference was found (see Table 16)

Table 16: Anthropometry and body indices in 9-year-old girls and boys

	girls					boys				
	mean	**SD**	**min.**	**max.**	**n**	**mean**	**SD**	**min.**	**max.**	**n**
body-height [m]	1,37	0,07	1,20	1,55	46	1,37	0,06	1,27	1,52	50
body-mass [kg]	35,57	9,16	22,40	57,30	46	33,99	8,67	24,30	64,30	50
waist [m]	0,63	0,09	0,44	0,84	46	0,62	0,09	0,50	0,91	50
hip [m]	0,76	0,08	0,64	0,97	46	0,74	0,08	0,63	0,98	50
BMI	18,72	3,37	14,11	25,64	46	18,02	3,59	14,27	31,02	50
BMI_SDS	0,85	1,16	-1,13	2,86	46	0,75	1,36	-1,33	5,04	50
MI	18,87	3,43	13,94	26,57	46	18,02	3,52	13,96	29,72	50
WHtR	0,46	0,05	0,33	0,56	46	0,46	0,06	0,38	0,68	50

Abbreviations: BMI = body-mass-index, BMI-SDS = body-mass-index standard deviation score, MI = mass index, ws = waist-to-standing height ratio, WHtR = waist-to-height-ratio, SD = standard deviation, min = minimum, max = maximum, n = number of children

4.2.5 SAT in 10-year-old girls and boys

Although also in 10- year old children a clear tendency towards a higher amount of total SAT in girls was observed, the test was non-significant ($t_{(69)} = 1.233$, $p = 0.226$) due to the high variance in the data. The multivariate approach however was significant ($F_{(8,59)} = 2.392$, $p = 0.026$) and indicated significantly more SAT in girls, in particular at the erector spinae ($p = 0.021$), whilst other sites didn't reach significance.

Table 17: SAT in 10-year-old girls and boys

10-years

girls	Percentiles SAT [mm]												[n = 30]	
	min.	**1st**	**3rd**	**5th**	**15th**	**25th**	**50th**	**75th**	**85th**	**95th**	**97th**	**99th**	**max.**	**mean**	**SD**
DI	33,5	33,5	33,5	35,5	47,9	**55,9**	**77,4**	**121,0**	**142,7**	177,5			177,7	90,5	41,7
UA	2,8	2,8	2,8	3,1	3,9	5,1	11,0	25,0	29,5	34,2			35,9	14,8	10,7
LA	6,3	6,3	6,3	6,3	8,5	10,9	18,7	29,6	34,5	42,3			42,8	20,5	11,1
FT	4,9	4,9	4,9	5,3	6,8	7,7	10,4	13,1	14,4	21,9			25,1	10,8	4,3
LT	4,0	4,0	4,0	4,9	10,5	12,0	16,0	19,2	22,5	27,6			30,0	16,2	5,7

MC	3,0	3,0	3,0	3,1	3,8	4,1	6,4	7,9	9,1	13,6		15,0	6,6	2,8
ES	2,7	2,7	2,7	2,7	3,3	3,8	6,4	9,5	13,1	18,4		18,9	7,5	4,7
DT	3,4	3,4	3,4	4,1	5,2	6,5	9,0	11,5	13,8	15,7		16,5	9,2	3,4
BR	2,2	2,2	2,2	2,6	3,3	3,4	4,6	6,1	7,0	9,6		10,4	5,0	1,9

boys						**Percentiles SAT [mm]**								[n = 41]
	min.	**1st**	**3rd**	**5th**	**15th**	**25th**	**50th**	**75th**	**85th**	**95th**	**97th**	**99th**	**max.**	**mean**	**SD**
DI	18,8	18,8	19,2	21,1	29,3	**34,4**	**71,5**	**119,4**	135,8	154,8	187,4		198,7	77,3	46,8
UA	0,5	0,5	0,5	0,6	1,2	1,8	6,9	23,2	25,6	31,6	40,4		43,4	11,6	11,3
LA	1,9	1,9	1,9	2,0	3,4	4,9	15,5	29,5	33,5	35,0	49,9		55,1	17,0	12,9
FT	3,4	3,4	3,4	3,5	4,4	5,4	8,4	12,6	14,5	18,0	18,8		19,1	9,1	4,4
LT	4,3	4,3	4,4	4,5	6,8	8,2	13,7	22,3	24,6	29,4	30,1		30,3	15,2	7,9
MC	1,9	1,9	1,9	2,2	2,8	3,7	5,3	8,9	9,9	12,2	12,8		13,0	6,1	3,1
ES	0,8	0,8	0,9	1,2	1,7	2,0	4,7	8,4	9,0	11,8	12,2		12,3	5,2	3,4
DT	3,7	3,7	3,7	3,8	5,0	5,8	7,6	10,7	13,2	16,3	18,0		18,5	8,7	3,6
BR	1,2	1,2	1,3	1,8	2,4	2,7	5,0	6,6	7,3	8,7	9,5		9,7	4,7	2,2

Abbreviations: D_I = sum of SAT, min = minimum, max = maximum, SD = standard deviation, n = number of children, UA = upper abdomen, LA = lower abdomen, FT = front thigh, LT = lateral thigh, MC = medial calf, ES = erector spinae, DT = distal triceps, BR = brachioradialis

Also for the group of 10-year old children, none of the body circumferences or indices indicated any significant sex difference, underlining the high sensitivity of the SAT method.

Table 18: Anthropometry and body indices in 10-year-old girls and boys

	girls					**boys**				
	mean	**SD**	**min.**	**max.**	**n**	**mean**	**SD**	**min.**	**max.**	**n**
body-height [m]	1,42	0,07	1,26	1,55	30	1,44	0,06	1,29	1,57	41
body-mass [kg]	37,80	10,77	21,60	61,60	30	37,76	9,27	26,00	71,60	41
waist [m]	0,64	0,10	0,51	0,83	30	0,64	0,09	0,53	0,93	41
hip [m]	0,78	0,11	0,63	0,98	30	0,76	0,08	0,65	1,02	41
BMI	18,63	4,22	12,04	28,16	30	18,19	3,54	14,03	30,59	41
BMI_SDS	0,48	1,52	-3,42	3,01	30	0,54	1,40	-1,66	3,82	41
MI	19,10	4,31	13,14	28,52	30	18,45	3,63	14,33	31,68	41
WHtR	0,45	0,06	0,38	0,59	30	0,45	0,06	0,37	0,62	41

Abbreviations: BMI = body-mass-index, BMI-SDS = body-mass-index standard deviation score, MI = mass index, ws = waist-to-standing height ratio, WHtR = waist-to-height-ratio, SD = standard deviation, min = minimum, max = maximum, n = number of children

4.2.6 Age differences in SAT amount and distribution

Univariate ANOVA comparing the age groups 6, 7, 8, 9 and 10 with regard to the total SAT (sum mean incl.) revealed a significant main effect for the factors age ($F_{(4,379)} = 6.516$, $p <$

0.001) and sex ($F_{(1,379)} = 8.530$, $p = 0.004$) but no interaction ($F_{(4,379)} = 0.550$, $p = 0.699$). Post-hoc comparisons indicated a significant increase in SAT from the age of 7 years to 9 years ($p = 0.002$) and 10 years ($p < 0.001$). As shown in the figure below (Figure 10), the total SAT in girls and boys decreased from age 6 to 7, thereafter increased steadily with age, which was more pronounced in girls.

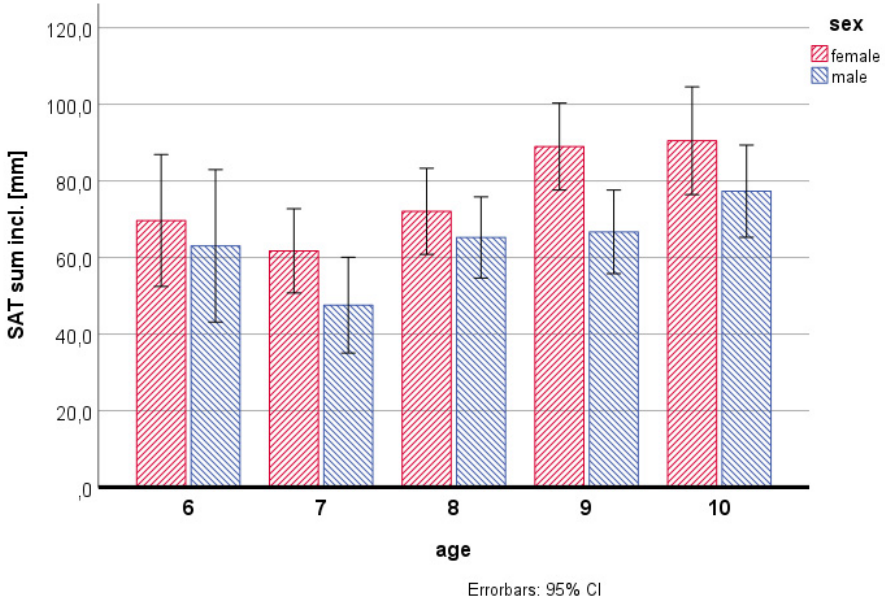

Figure 10: Total SAT (sum mean included) in girls and boys split by age

Similarly, the multivariate ANOAVA for all eight sites compared by age and sex revealed a significant main effect for age ($F_{(32,1355)} = 2.599$, $p < 0.001$) and sex ($F_{(8,367)} = 5.374$, $p < 0.001$), but no interaction ($F_{(32,\ 1355)} = 1.117$, $p < 0.300$). Respective univariate tests indicated a significant higher amount of SAT at all eight sites (all $p < 0.05$) with increasing age and sex differences at most sites (all $p < 0.05$), except the distal triceps and brachioradialis. Summarizing, the results show that older children have more SAT and girls have more SAT than boys, independent of age. Note that the graph shows different children at different ages and not a change over time/years (see Figure 11 and Figure 12)

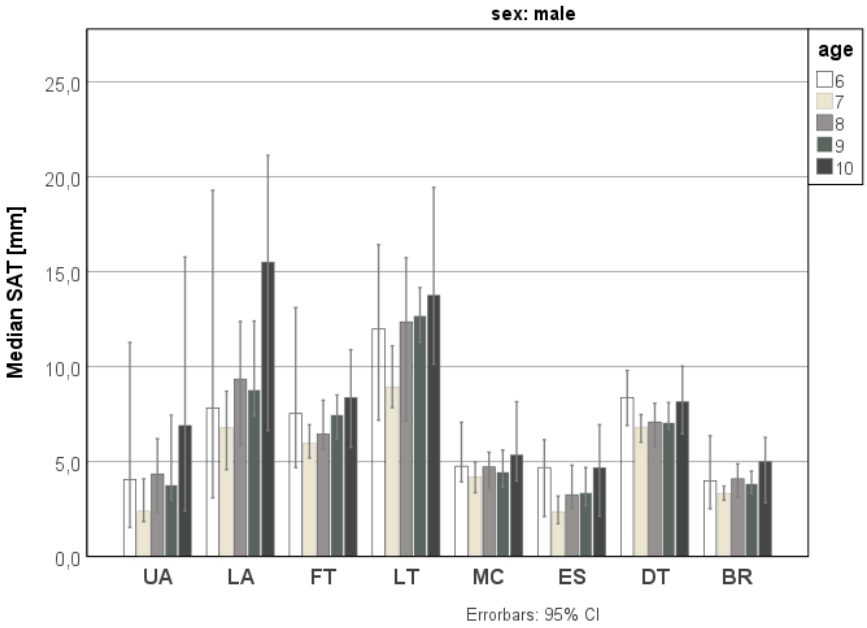

Figure 11: Median SAT for all eight sites in 6-10 year old boys

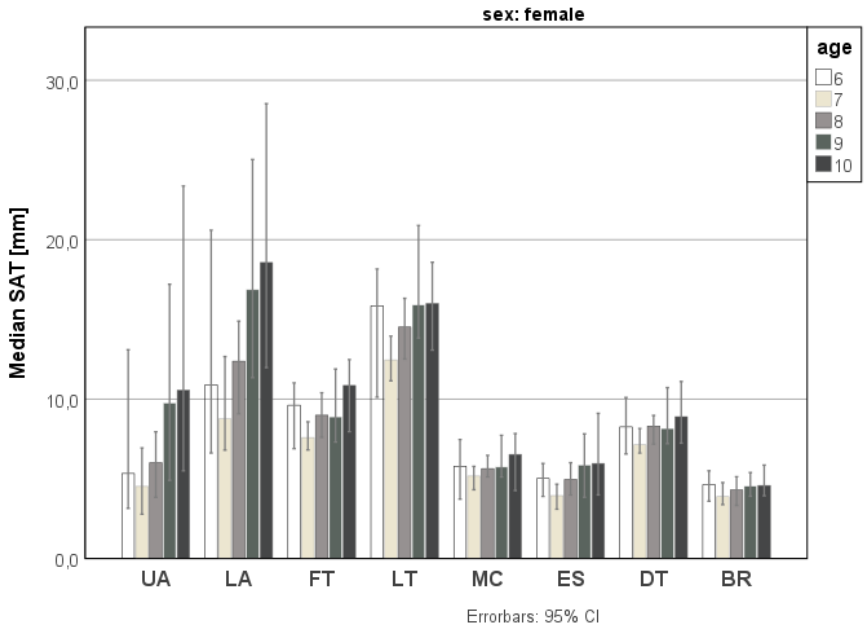

Figure 12: Median SAT for all eight sites in 6-10 year old girls

5. Discussion

The present study tried to provide reference values for the amount of SAT in children aged six to ten years. In chapter 4, the different amounts of SAT at the 8 standardized sites are presented. The tables also contain the minimum, maximum, standard deviation and the mean at the eight standardized sites, for each age-group and split by sex.

5.1 Children excluded for data analysis

The anthropometric data of the children showed that they differ greatly depending on their origin. The Afro-Caribbean children in particular had longer legs on average than the others ($p < 0.001$). The comparison of the body height revealed also significant differences, with higher values for the Afro-Caribbean children. These 34 children were therefore excluded from the investigation.

Four children in the sample were of Asian origin and two were Hispanic. As these are very few compared to the overall sample, they were also excluded from the reference values.

The sample also included 15 11-year-olds. This number is quite low for the purpose of deriving reference values. The reference values should also relate to primary school children. As 11-year-olds are normally no longer in elementary school, this group was also excluded from the results.

5.2 Reference values

To create reference values, the results were broken down by age group and sex. The tables show the SAT values in mm for the 8 sites, but also for the sum of all 8 measurement points. The respective values of the age group were then divided into percentiles. The tables show a total of 11 percentiles (1 to 99) plus minimum and maximum. Depending on the measured SAT values, the children were assigned to the respective percentiles. The 25th, 50th and 75th percentiles are highlighted in the middle of each table.

With the help of these percentiles, reference values for children can now be visualized. In addition, they can be used to display not only the entire SAT, but also the SAT at the eight sites. It also makes it possible to compare other results obtained using the same method.

5.3 SAT at different ages

In recent years, overweight and obesity in children has steadily increased worldwide.
WHO data has shown that in 37 countries in Europe, 25% of children aged 6-9 are overweight. In Austria, it was also exactly 25% of the children examined (78). These data corresponds very well with the data we collected in the present study. Table 3 shows that 22.28% of the children who took part in the SAT measurement were overweight. Since in particular children with overweight tended to refuse measurement of SAT, the number of children with overweight and obesity was higher with 23.48% for the total sample.

In another study in 2021, subcutaneous fat was measured using ultrasound in children between the ages of 7 and 10 in South Africa. The SAT was measured and evaluated under the same conditions as in the present study.
A total of 73 children took part in the measurements. Of these, 50 were female and 23 male. The sum of SAT at the 8 points including fibrous structures (D_I) was D_I: 54.02 mm with a standard deviation of ± 21.71 mm for the girls. Our results show a D_I of 76.78 mm ± 39.34 mm for girls aged 6-11. This results in a difference of 22.76 mm in relation to the entire SAT at the 8 points.
For the boys, the D_I in the South African sample was 34.42 mm with a standard deviation of 15.84 mm. Our sample showed a D_I of 65.14 mm and a standard deviation of 41.97 mm. The difference is therefore 30.72 mm for all measuring points.

These findings does not correspond to what would be expected as the South African children were slightly older on average and thus more SAT would have been expected. The mean age for girls was 9.64 ± 0.94 years compared to 8.18 ± 1.32 years and the boys were 9.78 ± 0.90 years compared to 8.44 ± 1.31 years (2).

The same study also examined 85 adolescents aged 13-17 years. The 66 girls studied had a mean D_I = 140.86 ± 59.4 mm and the 19 boys of D_I = 79.48 ± 75.6 mm. This also shows that the proportion of SAT increases significantly with age. Furthermore, the difference between girls and boys in this age group is much more pronounced. In our sample, the difference in D_I was only 11.64 mm in the total sample (?). In the older age group, however, the difference was D_I = 61.38 mm. This is probably due to the fact that adolescents are already in puberty and from this point onwards hormones, especially in girls, cause a change in body composition. In addition, the study participants were African children. However, only

Caucasians were included in our study. This could also influence the average SAT values examined and their differences (2,79,80).

In a different study in Germany, the SAT of 274 children aged 3-5 years was measured according to the same standards as in our study. The results showed a significant difference between girls and boys in this age group with values of 6.3 ± 2.0 mm for girls and 5.3 ± 2.0 mm for boys. These values are significantly lower than those we collected. Given that both samples have the same cultural background, and based on the results of the SAT measurements on South African adolescents, it can be suggested that subcutaneous fat tissue increases as development progresses (74).

5.4 Sex differences

This study, but also all others mentioned, showed a significant difference between the two sexes. Girls showed a higher proportion of subcutaneous adipose tissue than boys. As reported by Kenso et al. (2019, 2020), this difference can already be observed in 3-year-old children and continues throughout childhood and adolescence. These data suggest that females generally have more SAT than males. Other methods, such as DXA and MRI, were able to obtain similar results. Although the sample's age of 12.3 ± 3.4 year was slightly higher than here, the SAT values for girls were still considerably higher than those for boys (81).

One reason for this finding could be the female hormones, especially estrogens. Studies have shown that estrogens determine body fat distribution. With an increase in estrogen during puberty, the SAT in the gluteofemoral area also increases. Furthermore, due to the influence of estrogens, fat accumulation in SAT was higher than on the abdominal VAT, which again shows that estrogens have much influence on body fat distribution (80).
The fact that girls have an earlier onset of puberty than boys may also be a factor in the significantly higher SAT scores of girls in our results (79).
In addition to hormones, an individual's genes also play a major role. Studies of twins, families and adoptions have shown that between 40 and 70% of obesity are influenced by genes (82).

In another study, the SAT of 6 to 18-year-olds was examined using MRI. It was shown that SAT in boys increased continuously until the age of 18. In girls, however, a dynamic increase was observed starting from the maturity-onset (between 7 and 11 years), which then stabilized around the age of 14. This also underscores the significant influence hormones have on fat distribution, especially in girls (83).

Not just the sum of SAT for boys and girls showed significant differences. Our data also expose that the SAT growth from one year to another differs greatly between girls and boys. The increase is not continuous but varies considerably between the age groups.
For girls, the greatest increase is between the age of 8 to 9 years. An average growth of 17 mm was observed here. One year later this was only 1.5 mm, one year earlier the change was 10.3 mm.
In boys, on the other hand, the greatest growth occured between the ages of 7 and 8 years, with an average change of 17.6 mm. In the following year, this value was just 1.5 mm. One year later it was again 10.6 mm. This shows that physical development in childhood is very individual, but also that there is a clear difference between the two sexes.

Table 5 and Table 6 show the SAT values at the 8 sites for boys and girls. The upper abdomen ($p = 0.015$), lower abdomen ($p = 0.004$), front thigh ($p = 0.003$), lateral thigh ($p = 0.010$), medial calf ($p = 0.009$) and erector spinae ($p < 0.001$) show significant sex differences. No sex difference was found at the distal triceps ($p = 0.179$) and brachioradialis ($p = 0.216$). The mean SAT thickness at the distal triceps was 8.76 mm for the girls and 8.25 mm for the boys. For the brachioradialis it was 4.60 mm for the girls and 4.36 mm for the boys. Both measurement points are located on the upper arm. In contrast to the other 6 measuring points, there is physiologically less fat here. The other measuring points are all located more centrally on the abdomen or in the gluteofemoral region. These are the typical locations where most of the body fat is stored. This could explain why only these 6 measurement points differed significantly (80,84).

5.4.1 Sex differences in under 6-year-olds

Our results show no significant sex differences between boys and girls at the age of 6. The first sex-specific differences can only be observed from the age of 7. From this timepoint onwards, girls have higher total SAT scores than boys for all age groups. It could therefore be assumed that there are no sex differences before the age of 6. However, the above-

mentioned study by Kelso et al. showed that a significant sex difference can already be observed in children aged 3-5 years. One explanation for this finding might be the fact that our sample was smaller than the one in the Kelso's study. In our group of 6-year-olds, there was a total of 35 children, 20 of whom were female and 15 male. In contrast, the study by Kelso et al. included a total of 274 children. Thus, the effect might have just not shown up in this small sample.

5.5 Anthropometric indices compared to SAT

In addition to the SAT scores, the children's anthropometric data were collected. There was a significant difference in the height of the 6-year-olds. In the 8-year-olds, on the other hand, significant differences could be seen in the WHtR and hip circumference.

All other anthropometric data, such as BMI or MI, showed no significant differences in children between 6-11 years. These results would suggest that there are no differences in body composition at this age

The SAT resulst, however, showed a different picture. In most age groups, the total SAT differed significantly between the sexes. Girls had higher SAT values than boys across all age groups.

In the group of 9-year-olds, the girls had an average SAT value of D_i mean = 89 mm for all 8 positions. The boys, on the other hand, only 66.7 mm. On average, the girls therefore had 22.3 mm more SAT than the boys. However, the anthropometric measurements showed no significant differences. With this method, differences in body composition can be identified before they can be measured using one of the other methods mentioned before.

Especially in comparison to the BMI, the most widely used method when it comes to body composition and the quantification of obesity, clear advantages can be seen with this method. The BMI suggests that there is virtually no difference between the two sexes. Due to the high sensitivity of the method, however, clear differences can be made visible. This also shows that BMI has severe limitations and may not be the best way to measure body fat.

5.6 Ultrasound measurements compared to other techniques

The BMI is the most widely used parameter for determining overweight and obesity. Due to its ease of use, it has established itself as the gold standard for categorizing weight status.

Much more important, however, is the composition of the body and the distribution of fat. Only with this information specific statements about overweight and obesity can be made.

However, this is not possible with the BMI, as it does not directly measure fat tissue or fat distribution in the body, but only shows the ratio of body weight to height.
In our study, we were able to show that a much more accurate representation of the human body is possible using ultrasound. It provides a direct, location-specific measurement of subcutaneous fat. In addition, the different types of tissue can be distinguished from each another. Already in childhood, a high body fat percentage is a significant risk factor for metabolic syndrome and early-onset of type2 diabetes. These risk factors cannot be displayed by the BMI alone, which is why ultrasound measurements have much more predictive power for these diseases. As children are still growing, their height and weight are subject to constant fluctuations. This in turn makes it very difficult to compare the BMI of children of the same age. With ultrasound measurement of SAT, it is easier to compare the values with each other, as they are measured independently of height or weight (85).

The major technical methods such as MRI and CT provide the most detailed images of the inner side of the body. They can be used to visualize all compartments of the body. For certain questions, these methods might be better suited than ultrasound, but for measurement of fat, ultrasound examinations are sufficient. Various studies have shown that these methods are highly accurate and reliable. The intra- and inter-observer mean difference of the MRI measurement, for example, was only 1% for the SAT. As these values are comparable with ultrasound measurement, MRI offers no advantage here.
The biggest limitation of both methods is probably their very high cost. As the devices are also tied to a specific location, they are not suitable for use in the field and therefore cannot be used for large examinations or case numbers.
In addition, CT examinations involve radiation exposure. This alone makes it unsuitable for studies in children (57,65,83).

5.7 Limitations

Measuring subcutaneous adipose tissue using B-mode ultrasound has proven to be a very good method. However, in addition to the high accuracy, the excellent intra- and interrater reliability and the highly trained personnel executing the measures, there are also some limitations.

First, the anthropometric data of the participants must be determined. Various measurements were carried out for this purpose, all of which are prone to error. In addition, the 8 sites on the body have to be marked, and here too, the measurement points can differ slightly from one another. The preparations for the measurements therefore require trained personnel and a certain amount of time to carry them out. These could represent a major obstacle to the widespread use of the method, for example at the doctor's or in other areas of daily life.
For elite sports, on the other hand, the method could be very advantageous, as it is often the little things that determine success. Especially in sports where body weight and fat distribution are important, the accuracy of the method can be an advantage. Due to its high reliability, changes in body composition can also be tracked (76,86).

Table 7 shows the correlation between the BMI and the respective body part measured. The highest correlation is given for the upper and lower abdomen. For the upper abdomen the value is r = 0.902 and for the lower abdomen r = 0.896. For use in the field and in everyday life, it would therefore be conceivable to use only these two values. This would allow the measurements to be carried out much more quickly and would therefore be more practicable. Instead of measuring the error-prone BMI, these two values can be used to make better statements about body composition and obesity.

It should also be noted that the backgrounds of the children varied significantly between schools. At one of the schools, there were hardly any children with migration background. At the other two schools, the proportion was well over half, at one school, it was even close to 100%.
Chapter 5.1 outlines the criteria for excluding certain children from the data analysis. The reference values collected therefore only refer to Caucasian children.

5.8 Conclusions

As stated above, this thesis is the first to establish reference values for the amount of subcutaneous adipose tissue in children measured by B-mode ultrasound. The fat values were recorded at 8 different, standardized locations for the age groups from 6 to 10 years. In addition, information was provided on the total amount of body fat in children. It was also shown that the amount of fat increases steadily over the years.

The distribution of fat was equally striking. Girls showed more fat than boys in all age groups examined. Furthermore, the methodology of assessing subcutaneous adipose tissue using B-mode ultrasound is clearly illustrated and described.

The aim of this study, as well as the present thesis, was also to find a new, simple method for measuring body fat in children.
The thesis demonstrated that while BMI is the most widely used method, it also has significant limitations. For determining body composition and making statements about different tissues, it is entirely unsuitable. The subcutaneous fat measurement using B-mode ultrasound has proven to be a method that, despite its simple handling, demonstrates very high precision, validity and reliability. Due to its high intra- and interrater reliability, it is also excellently suited for comparisons between different samples and measurements.

However, standardized measurement protocols and trained personnel are necessary to achieve such high values.
In addition, specific software is needed to carry out automatic thickness measurements of the SAT layers. To obtain comparable results, this software hast to be always the same.
Due to these limitations, the ultrasound method will probably not be able to replace the BMI as the standard method in the near future. However, with the technical developments such as small hand-held ultrasound devices, it could initially be used, for example, by doctors who already have experience in ultrasound measurement. There are also very good opportunities to establish the method in competitive sports.
With further research, ultrasound fat measurement could be considered as a new standard method for examining and definition of adiposity.

5.9 Perspectives

Overweight and obesity in children are on the rise. It is therefore even more important to find new strategies for dealing with this problem. It is not only important to find good methods to measure obesity, but also to evolve interventions to counteract the development. In a first step, the public awareness for this problem should be raised. At the same time, children should be educated at school about the health problems of being overweight. Regardless of the root cause, every child should be screened for lifestyle-related risk factors, and any complications linked to obesity should be identified as early as possible.

The SAT measurements via B-Mode ultrasound could represent a non-invasive option for early diagnosis. Another major potential could be its use in professional sports. As a widely applicable and very inexpensive method, there is great potential and should therefore definitely be considered in future research.

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