KANDIDATSPECIALE

ECTOPIC FAT DEPOSITION IN CHILDREN AND ADOLESCENTS: EFFECTS OF WEIGHT STATUS AND LIFESTYLE INTERVENTION IN COMBINATION WITH HIGH-INTENSITY INTERVAL TRAINING

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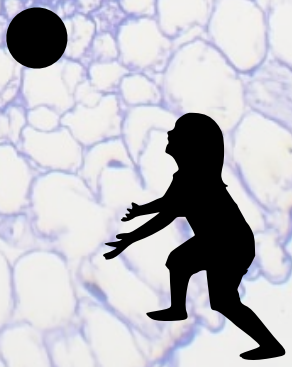

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Synopsis:

Baggrund: Fedme og overvægt blandt børn og unge er et stigende problem, hvor deponering af ekstopisk fedt er associeret med en række metaboliske komplikationer.

Formål: At sammenligne deponeringen af ektopisk fedt samt fysisk aktivitetsniveau mellem normal- og overvægtige børn og unge (studie 1), samt at undersøge effekten på ektopisk fedt og fysisk aktivitetsniveau ved at tilføje HIIT til livsstilsinterventionen TCOCT (studie 2).

Metode: Deponering af ektopisk fedt i leveren, pancreas, psoas-musklen samt VAT og SAT blev målt med MRI-PDFF. Fysisk aktivitetsniveau (MVPA) blev vurderet med accelerometre over perioder på 7 dage. I studie 1 fik 30 normalvægtige og 57 overvægtige børn og unge (9-16 år) sammenlignet BMI z-score, ektopisk fedt, aktivitetsniveau samt VO_2peak (watt-max test) ved baseline. I studie 2 gennemgik 20 børn og unge TCOCT, mens 21 børn og unge gennemgik TCOCT i kombination med HIIT (3 gange ugentligt, 4x4 min, 90-95 % VO_2peak) over 12 uger. De samme parametre, som i studie 1, blev målt ved henholdsvis baseline og i eller efter uge 12.

Resultater: I studie 1 havde børn og unge med overvægt signifikant forhøjet ektopisk fedt i leveren, pancreas, psoas-musklen, VAT, SAT samt en lavere VO2peak. Børn og unge med overvægt havde signifikant mindre fysisk aktivitet i MVPA. I studie 2 blev BMI z-score samt ektopisk fedt i leveren, pancreas og VAT formindsket samt VO_2peak forhøjet over 12 uger men uden en forøget effekt af HIIT. HIIT havde ingen signifikant effekt på MVPA.

Konklusion: Ektopisk fedt er forhøjet i børn og unge med overvægt sammenlignet med normalvægtige. Livsstilsinterventionen TCOCT reducerer ektopisk fedt men uden en additiv effekt ved tilføjelse af HIIT. Højere aktivitetsniveau, målt på MVPA, ser ud til at mindske ektopisk fedt.

Forord til kandidatspecialet

Dette kandidatspeciale er udarbejdet på 10. semester af kandidatuddannelsen i Idræt på Aalborg Universitet som et eksternt samarbejde med Børne- og Ungeafdelingen, Aalborg Universitetshospital. Specialet består indledningsvist af en videnskabelig artikel, der er det bærende element i specialet. Herefter følger en række arbejdsblade (placeret til sidst i dette dokument), der uddyber de teoretiske, metodiske og perspektiverende dele præsenteret i den videnskabelige artikel. Det anbefales indledningsvist at læse den videnskabelige artikel, der følger herunder, hvorefter arbejdsbladene læses som supplement. Kandidatspecialets data er indhentet som en del af et Ph.d.-projekt hos Børne- og Ungeafdelingen på Aalborg Universitetshospital i samarbejde med Aalborg Universitet. Forfatteren bag dette kandidatspeciale har ikke bidraget til dataindsamlingen (da data blev indsamlet mellem 2020-2023) men har været ansvarlig for data management, statistiske analyser og repræsentation af den anvendte data samt efterfølgende fortolkning af resultaterne. Til specialet hører et bilag med data anvendt i de statistiske analyser (se bilag 1).

Der skal lyde en stor tak til Børne- og Ungeafdelingen, Aalborg Universitetshospital og Aalborg Universitet for samarbejdet samt tak til vejleder for god vejledning og sparring under specialeforløbet.

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Ectopic fat deposition in children and adolescents: Effects of weight status and lifestyle intervention in combination with high-intensity interval training

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Abstract

Background and aim: Children and adolescents with obesity is a health problem. Accumulation of ectopic fat within the liver, pancreas, and muscles is associated with metabolic complications such as pre-diabetes, non-alcoholic fatty liver disease (NAFLD), and non-alcoholic fatty pancreas disease (NAFPD). Therefore, knowledge about the deposition of ectopic fat and strategies to reduce ectopic fat are of high relevance. The study aimed to 1) compare ectopic fat depositions between children and adolescents with and without obesity, 2) investigate the effect of a multidisciplinary family-based intervention in combination with high-intensity interval training (HIIT) on ectopic fat deposition, and 3) explore associations between physical activity behavior and ectopic fat depositions.

Methods: The study was divided into two parts. Study 1 compared ectopic fat depositions in 30 normal weight children and adolescents with 57 children and adolescents with obesity (9-16 years) at baseline. In study 2, 53 obese children and adolescents followed either The Children's Obesity Clinic´s Treatment (TCOCT) (TCOCT, n=26) or TCOCT in combination with HIIT (TCOCT+HIIT, n=27) for 12 weeks. The HIIT protocol consisted of 4x4 minute intervals of ball games, running-based, or strength-based exercises aimed at reaching 90-95% of HRmax three times a week. The primary outcomes were ectopic fat fractions measured by magnetic resonance imaging (MRI) of the liver, pancreas, psoas muscle, visceral adipose tissue (VAT), and subcutaneous adipose tissue (SAT). Secondary outcomes were BMI z-score and VO_2peak. Physical activity level was measured with accelerometers for two occasions: 7 days prior to the intervention (baseline) and for 7 days in week 12.

Results: Children and adolescents with obesity had increased BMI z-score and ectopic fat fractions within the liver, pancreas, psoas, and increased VAT and SAT compared to normal weights ($p<0.001$). Furthermore, the normal weight children and adolescents had increased moderate to

vigorous (MVPA) activity levels ($p<0.001$), and inverse relationships between MVPA and ectopic fat depositions were observed ($r=-0.406$ to 0.510; $p<0.001$). At the end of 12 weeks, both TCOCT and TCOCT+HIIT had decreased BMI z-score, hepatic fat fraction, pancreatic fat fraction, VAT, SAT, and VO_2peak ($p<0.05$). However, the addition of HIIT to TCOCT did not augment the effect on any of the parameters and did not change physical activity levels.

Conclusion: Ectopic fat depositions within the liver, pancreas, psoas, VAT and SAT are increased in children and adolescents with obesity. TCOCT decreases ectopic fat depositions but without an additional effect of adding HIIT to TCOCT. More time spent in MVPA seems to decrease deposition of ectopic fat.

Introduction

Overweight and obesity are health problems, and it is estimated that about 20 % of Danish children are overweight (isoBMI > 25) and 5 % obese (isoBMI > 30) (Pearson et al., 2010). Obesity can lead to excessive fat accumulation early in an individual's life and cause lipotoxicity; defined as an enhanced influx of excessive free fatty acids to non-adipose cells in or outside organs (Rada et al., 2020; Trauner et al., 2010). Theoretically, accumulation of fat in non-adipose cells can affect the function of the relevant organs (Rada et al., 2020; e Silva et al., 2020). Being overweight, obese, and physical inactive are associated with a higher risk of lipotoxicity and excessive fat accumulation (Rada et al., 2020).

In children and adolescents accumulation of ectopic fat inside vital organs like the liver, pancreas, and muscles is associated with metabolic disease like pre-diabetes (Hagman et al., 2014). Visceral adipose tissue (VAT), excess fat lining the internal organs, is associated with deposition of ectopic fat and development of non-alcoholic fatty liver disease (NAFLD) (Johansen et al., 2022; Chabanova et al., 2017;) and non-alcoholic fatty pancreas disease (NAFPD) (Simo˜es e Silva et al., 2020). VAT and ectopic fat are considered to be more metabolically active and associated with a higher risk of metabolic diseases compared to subcutaneous adipose tissue (SAT) (Neeland et al., 2018; Suliga, 2009). Therefore, it is particularly relevant to distinguish between SAT, VAT, and ectopic fat. Studies have shown significant differences between children and adolescents with and without obesity (aged 8-18 years) in VAT, SAT, hepatic fat fraction, and ectopic muscle fat fractions (Fonvig et al., 2015). Fonvig et al., 2015 reported that boys with overweight or obesity have a significantly higher amount of VAT and hepatic fat fraction compared to girls with overweight or obesity (Fonvig et al., 2015). There is limited information about the deposition of pancreatic fat in children and adolescents, but comparisons of ectopic fat deposition in the pancreas between normal weight children and children with obesity are needed.

Daily physical activity level could be a factor influencing ectopic fat deposition. A study shows an association between daily sedentary time obtained and NAFLD biomarkers, indicating that less sedentary time is associated with a lower risk of NAFLD in children with obesity (Valerie et al., 2022). In addition, children with lower sedentary time and more time with moderate to vigorous activity (MVPA) had significantly less hepatic fat (Valerie et al., 2022). Consequently, it is relevant

to explore associations between physical activity behavior and ectopic fat deposition in children and adolescents.

Treatment of obesity includes lifestyle interventions and/or implementation of exercise programs (Brown et al., 2019). Firstly, The Children's Obesity Clinic´s Treatment (TCOCT), which is used for the treatment of childhood obesity in obesity clinics in Denmark, is a multidisciplinary family-based lifestyle intervention introducing a lifestyle modification approach that encompasses different aspects of daily living, including nutritional guidance, daily activity recommendations, sleep optimization, screen time management, and addressing social issues (Holm et al., 2011). TCOCT has shown a great effect on body composition based on reductions in BMI z-scores (Hvidt et al., 2014; Mollerup et al., 2017; Holm et al., 2011). Additionally, by following TCOCT for 12 months VAT, hepatic fat and ectopic muscle fat decreased significantly (Fonvig et al., 2015). Secondly, it has been reported that different types of regular exercise, including High-Intensity Interval Training (HIIT), are advantageous in reducing fat mass for young individuals with obesity (Wang et al., 2024). Medrano et al., 2019 and Labayen et al., 2019 added 90 minutes of high-intensive exercise at >75 % of HRmax 3 times a week to a family-based lifestyle and psycho-educational intervention in 22 weeks designed for children with overweight. Compared to controls who only completed the lifestyle intervention the responder rates for hepatic fat reduction increased and an additional decrease in hepatic fat fraction was observed in participants who added exercise to the lifestyle intervention (Medrano et al., 2019; Labayen et al., 2019). Furthermore, Tas et al., 2023 found a decrease in hepatic fat after 4 weeks of HIIT, but only in children suffering from NAFLD (hepatic fat fraction > 5 %) (Tas et al., 2023). Taken together, these results indicate that adding exercise training to lifestyle interventions may amplify the reductions in ectopic fat depositions in children and adolescents with obesity.

It is expected that adding HIIT to TCOCT enhances daily activity levels regarding MVPA. MVPA can increase total energy expenditure and induce energy deficits, which can contribute to an increased reduction of ectopic fat depositions (Boutcher, 2011). On the other hand, Lee et al., 2012 observed that adolescent boys with obesity decreased their ectopic liver fat and VAT after 3 months of aerobic exercise, but without a significant reduction in BMI (Lee et al., 2012), suggesting that changes in ectopic fat can occur independent of changes in body composition. However, a compensatory response of following a scheduled exercise intervention is reported, indicating that prescribed exercise can lower daily non-exercise physical activity patterns (Mansfeldt et al., 2023).

This evidence suggests that HIIT can reduce MVPA in daily living, highlighting the relevance of investigating whether the addition of HIIT contributes to an increase in daily time spent with MVPA intensity.

Based on data from the project *HIIT med Kiloene,* the purpose of this study is three-fold: 1) to compare ectopic fat depositions between children and adolescents with and without obesity, and 2) to investigate the effect of 12 weeks of HIIT combined with TCOCT on fat deposition in children and adolescents with obesity, and 3) to explore associations between physical activity behavior and ectopic fat depositions.

Methods

The data for this project was collected for a cross-sectional study and a randomized controlled trial (RCT), that was initiated as a part of a PhD project at *The Department of Children and Adolescents* at Aalborg University Hospital in collaboration with Aalborg University. The author of this article was responsible for data management and the statistical analysis of the results presented in this article and did not participate in data collection and analysis of MRI scans.

Study design

This study is divided into two parts. Study 1 is a cross-sectional study that aims to compare fat depositions between children and adolescents with and without obesity, and Study 2 is an RCT that investigates the effect of adding 12 weeks of HIIT to TCOCT on ectopic fat depositions. In Study 1 baseline measurements of fat depositions and activity level were compared between a normal weight control group and a group of children and adolescents with obesity (the same participants who participated in Study 2).

In Study 2 the children and adolescents with obesity were randomized to either an intervention group (TCOCT+HIIT) or a control group (TCOCT). TCOCT underwent an intervention of a family-based educational lifestyle intervention called *The Children's Obesity Clinic's Treatment* (TCOCT) (Holm et al., 2011). TCOCT+HIIT underwent a supervised exercise program consisting of high-intensity interval training (HIIT) in combination with TCOCT. The participants had their fat content quantified, anthropometrics collected, and daily activity levels measured at baseline and after 12 weeks (see figure 1). Height was assessed to the closest 0.1 cm utilizing a wall-mounted

stadiometer, while weight was determined to the closest 0.1 kg by a calibrated scale (Out-patient clinic, Aalborg University Hospital: seca 799, Hamburg, Germany, and municipal obesity clinic: Tanita DC 360S, Soeborg, Denmark) with participants wearing light indoor clothes, excluding shoes. Body mass index (BMI) was calculated (kg/m^2) and converted into BMI z-scores (WHO, 2006).

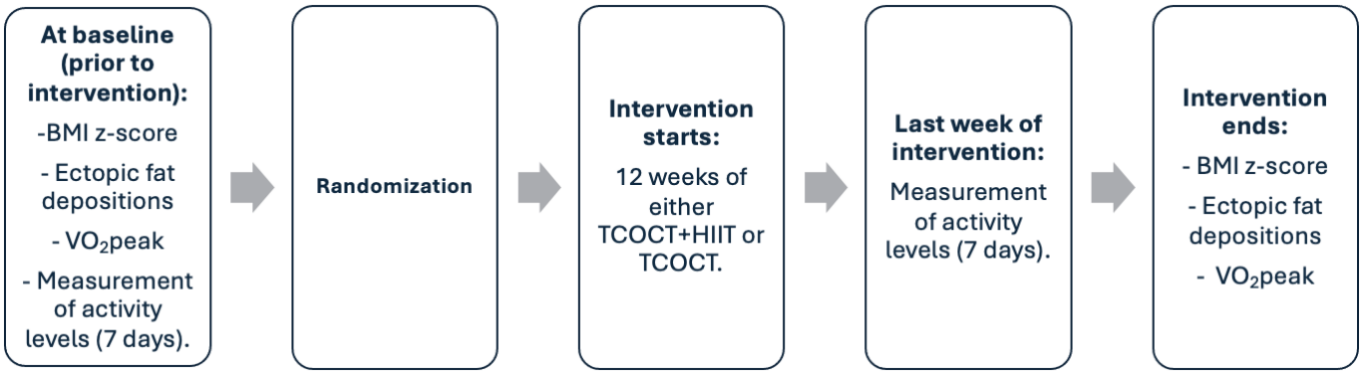

Figure 1: Timeline of procedures and measurements in study 2.

Participants

For study 1, 30 age-matched children with normal weight (isoBMI < 30) were recruited. Study 1 (n=57) and study 2 (n=53) involved children and adolescents with obesity (isoBMI > 30) (WHO, 2006) (9-16 years). Because of dropouts and insufficient data collection- and analysis, the number of participants varies between the different test parameters. All the children and adolescents for both studies were recruited from *Videncenter for Børn og unge med overvægt* (VIBUO) at Aalborg University Hospital. The protocol adhered to the principles defined in the Helsinki Declaration, and approval was obtained from the Local Ethics Committee of North Jutland Region, *Videnskabsetisk Komité*. The parents of the children signed an informed consent.

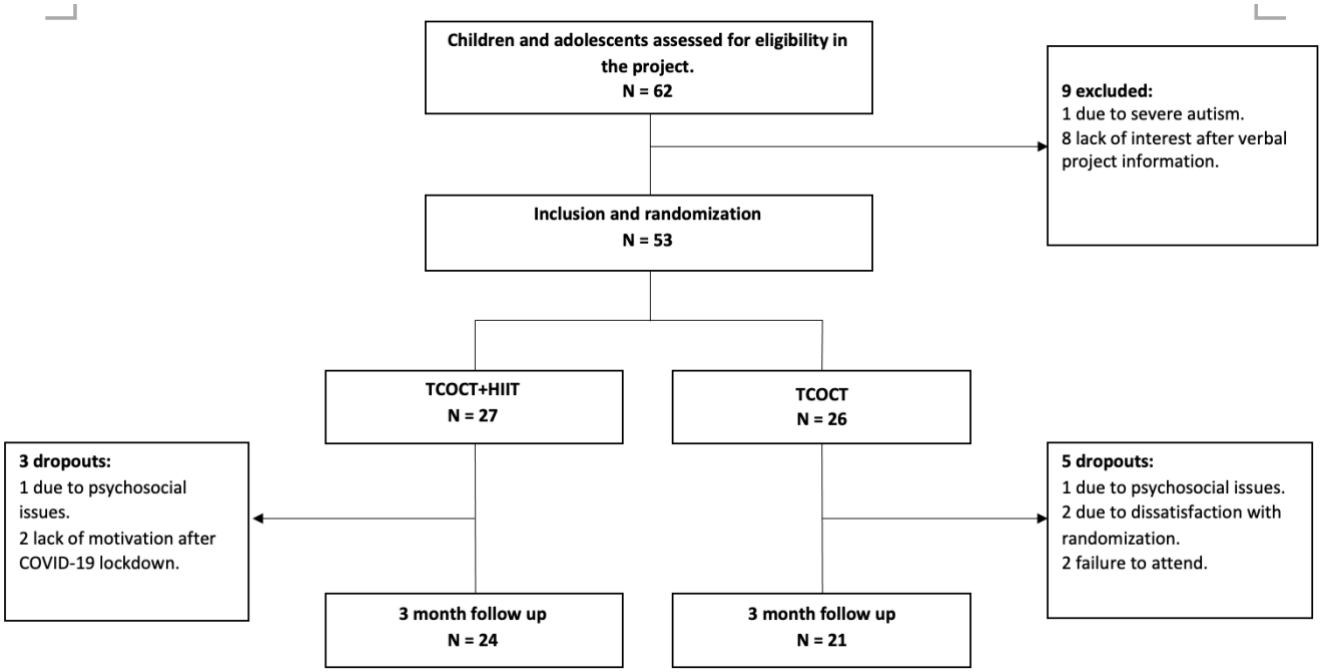

Figure 2: CONSORT flow chart for study 2.

TCOCT

Both TCOCT+HIIT and TCOCT in study 2 underwent the TCOCT intervention focusing on an educational approach to a healthy lifestyle (Holm et al., 2011). All consultations were conducted on an individual basis with each family, starting with a comprehensive questionnaire-based interview designed to identify lifestyle modifications necessary to optimize the child's daily routine for achieving weight loss. These modifications to lifestyle were integrated into an individualized treatment plan for each family consisting of health-related information about nutrition, sleep, exercise, daily activities, and mental health. All consultations were conducted with nurses and dietitians as needed, both in terms of content and frequency (Holm et al., 2011).

HIIT

TCOCT+HIIT in study 2 went through a 12-week supervised HIIT intervention of 40-minute sessions performed three times a week in local sports halls. Each session included 6-10 participants and 2 supervisors. The HIIT sessions consisted of a warmup followed by 4x4 minute intervals aimed at reaching 90-95% of HRmax. The breaks between the intervals were 1-3 minutes performed as active recovery. The exercises performed by the participants involved different activities, such as ball games, running-based exercises, or strength-based exercises inspired by CrossFit. The activities were supervised by students in Sport Science and Physiotherapy in a playful

and non-competitive way. The specific activities varied to maintain motivation. The intensity and duration of intervals were adjusted based on the perceived difficulty of the training by the participants. The participants wore heart rate monitors (iQNiter ApS, Aalborg, Denmark) around the chest. HRmax was predicted by age (208 – (0.7 * age)) (Tanaka et al., 2001).

Daily activity level

To investigate the level of daily activity in both study 1 and study 2, all participants wore an accelerometer (Axivity, AX3, Newcastle, UK) on the front thigh for 7 days prior to the intervention (baseline) and again in the last week of the intervention (at week 12 in study 2). The measurements were obtained 24 hours a day (5 weekdays and 2 weekend days). The activity level was grouped into defined categories with specific cut-points measured in counts per minute (cpm): sedentary (≤100 cpm), light (101-4970 cpm), moderate (≥4.971 cpm), and vigorous (≥8.452 cpm). MVPA was defined as a summation of moderate and vigorous. The counts were obtained simultaneously using 10 seconds periods from acceleration data. Cut-points were determined from a calibration study comparing energy expenditure measured by indirect calorimetry and acceleration data (Brønd et al., 2019). The outcomes were processed in MATLAB (The MathWorks Inc., Natick, MA, USA) and are presented in minutes per day (24 h) as a mean of 7 days. Because of insufficient data collection- and analysis, 48 of the children and adolescents with obesity completed the measurement of activity levels at baseline and 35 children and adolescents had activity levels measured at baseline + week 12.

VO_2peak

VO_2peak was estimated with an ergometer (Monark 894E) watt max test at baseline and at the end of the intervention. The saddle was adjusted so the participants had slightly bent knees. The participants went through a short familiarization with the ergometer. The watt max test started at a workload of 30 watts and increased by 30 watts every third minute aiming to maintain a constant cadence of 60 repetitions per minute. The test was stopped when the participants could not maintain the cadence. VO_2peak was estimated with the equation from Hansen et al., 1989:

$$VO_2\,max = 12 * W_{max} + 5 * body\ mass$$

Where,

$$W_{max} = W_h + W_d + t * 180^{-1}$$

W_h is the workload in the last completed period, W_d is the increased workload (30 watts), and t is the time (seconds) completed in the last unfinished period (Hansen et al., 1989).

Quantification of fat

The participants underwent magnetic resonance imaging (MRI) of the abdomen to access hepatic fat, pancreatic fat, intramuscular fat in the psoas, VAT, and SAT at baseline and after 12 weeks. A 3 Tesla MRI scanner (Signa Premier, General Electrics, Milwaukee, WI, USA) with a 30-channel Aircoil (AIR™) and a 60-channel in-bed Aircoil was used with participants lying in supine position. The scanning was performed with a breath-hold to avoid artifacts from movements. The images were obtained using a multi-point Dixon sequence (IDEAL IQ) with a slice thickness of 5 mm, a repetition time of 5.7 ms., a field of view of 44 cm, a flip angle of 3°, and an acquisition matrix of 160×160. Proton density fat fraction (PDFF) maps were generated by this technique (Steinkohl, 2021). The measurements were performed by a professional health staff blinded to the identity of the data.

The PDFF maps were analyzed using the software PACS Vitrea Read (v.8.3.53-55, Canon Medical Informatics Inc., Minnetonka, Minnesota, USA). In this software, hepatic fat was estimated using 4 regions of interests (ROIs) calculating a mean PDFF value. Pancreatic fat was estimated by the mean of 3 ROIs. Ectopic fat in psoas was estimated from the bilateral psoas muscle by drawing ROIs in a single slice. Ectopic fat content in psoas was analyzed by manually encircling the muscle, where the entire cross-sectional area of the muscle was marked. VAT and SAT were quantified on PDFF maps by a semi-automatic software (VikingSlice, Aalborg University Hospital, Aalborg, Denmark) written in MATLAB (The MathWorks Inc., Natick, MA, USA) (Kipp, 2019). For each participant, a single slice at the L3 level was chosen and segmented by extracting the cross-sectional areas of VAT and SAT, respectively. VAT was defined as the adipose tissue located within the abdominal cavity, excluding fat within organs and back muscles. SAT was defined as the adipose tissue outside the abdominal muscles. Manual corrections of PDFF were made to exclude tissues that did not correspond to either VAT or SAT (Nemeth, 2019).

Statistical analysis

All statistical tests were performed in SPSS-version 28 (IBM Corp. Released 2021. IBM SPSS Statistics for IOS, Version 28.0. Armonk, NY: IBM Corp). Significance was determined with a $p \leq$

0.05. Firstly, normal distribution was assessed by Shapiro-Wilk tests. Demographic data and baseline data between TCOCT+HIIT and TCOCT are presented in Table 3. The groups are compared by a nonparametric unpaired t-test (Mann-Whitney U-test) to assess variability after randomization (Moher et al., 2012).

For study 1 a Two-Way ANOVA (group, sex, group × sex interaction) was processed to compare differences in hepatic fat, pancreatic fat, intramuscular fat in psoas, VAT, SAT, BMI z-score, and daily activity levels at baseline between participants with and without obesity and to investigate possible sex differences. Two Spearman's Rank Order Correlation tests (non-parametric data) were performed to investigate correlations between activity level and fat depositions in data from children and adolescents with normal weight and obesity and a Spearman's Rank Order Correlation test on data only from children and adolescents with obesity.

For Study 2 the data from TCOCT+HIIT and TCOCT at baseline and after 12 weeks were processed by a Two-Way ANOVA Repeated Measures (group, time, group × time interaction) to test for an interaction effect between the 'between factor' (groups) and the 'within factor' (time) regarding the measured variables (hepatic fat, pancreatic fat, ectopic fat in the psoas muscle, VAT, SAT, BMI z-score, VO_2peak, and daily activity levels). Additionally, a Two-Way ANOVA Repeated Measures evaluated hepatic fat from participants suffering from NAFLD, defined as a hepatic fat fraction of > 5%, to investigate if NAFLD influences the effect of the interventions regarding fat fractions (Tas et al., 2023). The association between changes in fat depositions and changes in daily activity level, BMI z-score, and VO_2peak was investigated by Spearman's Rank Order Correlations. All these values were converted into delta-values (Δ) (baseline - 12 weeks) to investigate associations.

Results

Study 1: Comparisons of normal weight and obese children and adolescents

For study 1, 57 children and adolescents with obesity (12.8 ± 1.9 years; BMI z-score 2.7 ± 0.6) and 30 normal weights (12.8 ± 2.2 years; BMI z-score -0.1 ± 0.8) were recruited at baseline. When comparing children and adolescents with and without obesity an effect of group was observed, indicating a significantly higher BMI z-score, hepatic fat fraction, pancreatic fat fraction, psoas fat

fraction, VAT, and SAT in children and adolescents with obesity compared to normal weights ($p<0.001$). No differences between sexes were found (Figure 3 and Table 1).

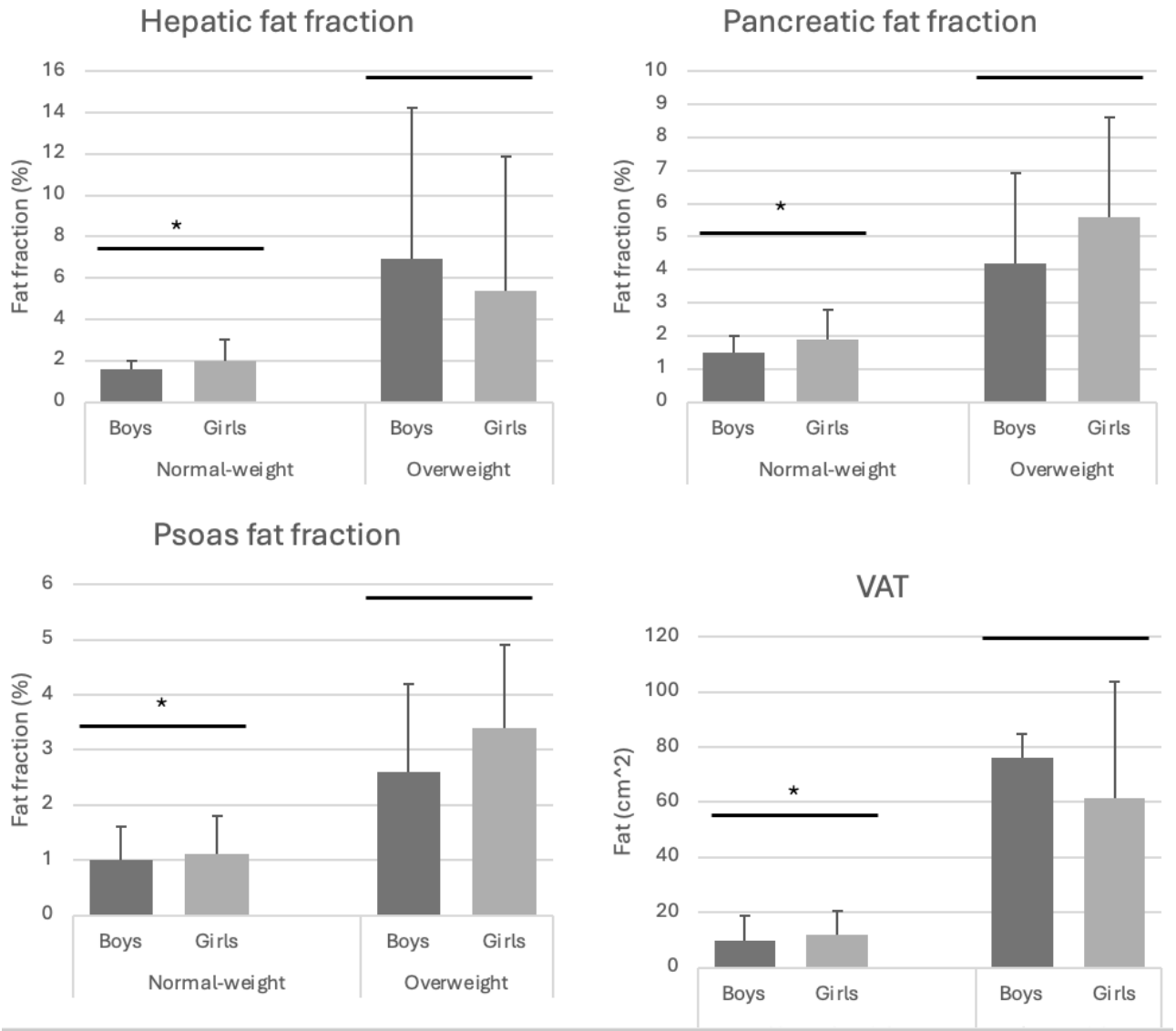

Figure 3: Fat fractions from baseline between children and adolescents with normal weight (boys: n=18, girls: n=12) and with obesity (boys: n=33, girls: n=15). Presented as mean ± SD. *Main effect of group (Two-Way ANOVA), $p<0.05$.

The main effect of group on MVPA shows that the normal weight children and adolescents spent more daily time in MVPA compared to children and adolescents with obesity (Table 1).

Table 1: Comparisons of SAT, BMI z-score, and activity levels between boys and girls with and without obesity. Presented as mean ± SD. P-values from Two-Way ANOVA. *p<0.05

	Normal weight		Obese		group x sex	Group	sex
	Boys (n=17)	Girls (n=12)	Boys (n=32)	Girls (n=15)			
SAT (cm^2)	313.6 ± 94.1	302.9 ± 102.5	38.1 ± 28.2	65.6 ± 43.9	0.331	0.001*	0.103
BMI z-score	-0.1 ± 0.7	-0.1 ± 0.8	2.7 ± 0.6	2.7 ± 0.7	0.927	0.001*	0.749
Sedentary	476.7 ± 101.9	430.8 ± 61.8	421.2 ± 79.7	457.4 ± 86.2	0.061	0.504	0.823
Light	230.4 ± 58.9	243.1 ± 48.1	211.3 ± 66.4	233 ± 61.7	0.765	0.335	0.256
Moderate	53.3 ± 21.2	39.3 ± 18.0	32.7 ± 18.0	31.1 ± 12.2	0.159	0.001*	0.078
Vigorous	24.6 ± 13.1	23.5 ± 15.8	8.2 ± 8.8	11.6 ± 8.6	0.416	0.001*	0.664
MVPA	77.9 ±33.1	62.8±30.3	40.9 ±22.0	42.7 ±18.8	0.184	0.001*	0.297

In Table 2a, a significant inverse correlation between time spend on MVPA and fat depositions occurred when including data from both children and adolescents with and without obesity.

Table 2a: Correlations between activity level and fat depositions in children and adolescents with and without obesity at baseline. n=75. r-values and p-values from a Spearman's rank order correlation test. *p<0.05

	Hepatic fat (%)		Pancreatic fat (%)		Psoas (%)		VAT (cm^2)		SAT (cm^2)	
	r	p	r	p	r	p	r	p	r	p
BMI z-score	0.721	0.001*	0.656	0.001*	0.693	0.001	0.881	0.001*	0.904	0.001*
Sedentary	-0.207	0.322	0.047	0.824	0.299	0.177	0.172	0.412	-0.052	0.807
Light	0.189	0.365	-0.006	0.978	-0.046	0.838	-0.211	0.312	-0.120	0.568
Moderate	-0.154	0.453	0.023	0.912	0.349	0.103	-0.017	0.933	0.147	0.473
Vigorous	-0.222	0.286	-0.330	0.107	0.057	0.801	-0.373	0.066	-0.255	0.218
MVPA	-0.406	0.001*	-0.433	0.001*	-0.411	0.001*	-0.485	0.001*	-0.510	0.001*

Conversely, when including data only from children and adolescents with obesity, there were significant inverse correlations between vigorous intensity and hepatic fat and VAT as well as between time spent in moderate intensity and pancreatic fat and VAT. No significant correlations between MVPA and fat fractions were observed (Table 2b). Furthermore, positive correlations between BMI z-score and most ectopic fat depositions, VAT, and SAT were observed in both correlation analyses (Table 2a and 2b).

Table 2b: Correlations between activity level and fat depositions in children and adolescents with obesity at baseline. n=46. r-values and p-values from a Spearman's rank order correlation test. *p<0.05

	Hepatic fat (%)		Pancreatic fat (%)		Psoas (%)		VAT (cm^2)		SAT (cm^2)	
	r	p	r	p	r	p	r	p	r	p
BMI z-score	0.573	0.001*	0.215	0.137	0.361	0.012*	0.690	0.001*	0.703	0.001*
Sedentary	0.254	0.089	0.082	0.598	-0.187	0.229	0.214	0.159	0.295	0.049*
Light	-0.354	0.016*	-0.195	0.205	0.019	0.903	-0.286	0.057	-0.380	0.010*
Moderate	-0.125	0.408	-0.390	0.009*	-0.032	0.837	-0.297	0.047*	-0.302	0.044
Vigorous	-0.380	0.009*	-0.273	0.073	-0.007	0.964	-0.388	0.008*	-0.476	0.001
MVPA	-0.119	0.430	-0.242	0.114	-0.224	0.144	-0.205	0.176	-0.256	0.090

Study 2: Effect of TCOCT+HIIT on fat depositions and activity level

Table 3 shows baseline values from TCOCT+HIIT and TCOCT in study 2. There were no significant differences in baseline values of age, BMI z-score, VO_2peak, and ectopic fat depositions between groups in study 2.

Table 3: Baseline values for study 2. Presented as mean ± SD. * p<0,05 of independent t-tests and Mann Whitney tests for differences between TCOCT+HIIT and TCOCT at baseline.

	TCOCT+HIIT	**TCOCT**	**P-value**
n	27	26	
Age	12.7 ± 1.9	12.8 ± 1.9	0.944
BMI	29.6 ± 5.0	29.7 ± 5.00	0.972
BMI z-score	2.7 ± 0.7	2.7 ± 0.5	0.956
Hepatic fat (%)	6.9 ± 2.1	6.6 ± 1.3	0.534
Pancreatic fat (%)	5.1 ± 0.7	3.8 ± 0.7	0.190
Psoas (%)	3.4 ± 0.4	2.2 ± 0.2	0.071
VAT (cm^2)	69.3 ± 8.2	70.3 ± 11.1	0.969
SAT (cm^2)	304.4 ± 24.2	298.8 ± 22.8	0.466
VO_2peak	23.7 ± 1.4	25.2 ± 1.0	0.135

The average attendance rate in HIIT sessions of the participants in TCOCT+HIIT (n=21) was 74 % (mean 0.74 ± 0.20; range 0.03-0.92). Seven-teen (81%) of the participants reached >70 % in attendance rate. When comparing TCOCT+HIIT and TCOCT main effects of time occurred indicating that both TCOCT+HIIT and TCOCT reduced BMI z-score (p=0.001), hepatic fat fraction (p=0.045), pancreatic fat fraction (p=0.007), VAT (p=0.045), and increased VO_2peak (p=0.001). But no time x group interactions were observed (see Figure 4 and Table 4).

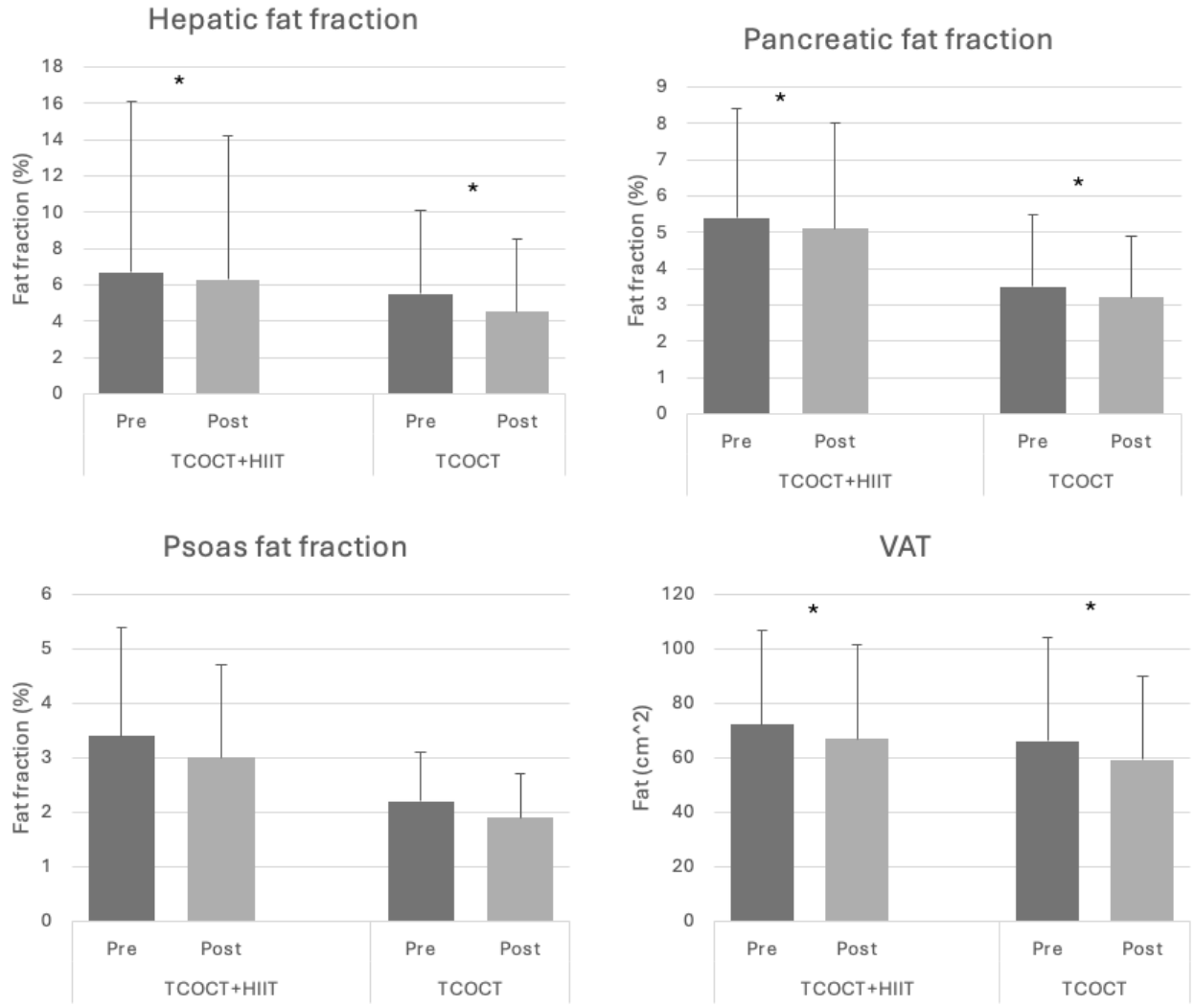

Figure 4: Fat fractions from baseline (pre) to 12 weeks (post) between TCOCT+HIIT (n=21) and TCOCT (n=20). *main effect of "time" (Two-Way ANOVA Repeated measures), $p<0.05$.

Table 4: Effect of TCOCT+HIIT and TCOCT on BMI z-score, SAT, and VO_2peak. Presented as mean ± SD. P-values from a Two-Way ANOVA Repeated measures. *$p<0.05$.

	TCOCT+HIIT (n=21)		TCOCT (n=20)		time x group	group	time
	Pre	Post	Pre	Post			
BMI z-score	2.67 ± 0.7	2.55 ± 0.7	2.66 ± 0.5	2.57 ± 0.6	0.678	0.969	0.001*
SAT (cm^2)	319.5 ± 106.9	313.1 ± 115.5	278.7 ± 79.8	270.3 ± 91.3	0.904	0.172	0.364
VO_2peak (ml/min/kg)	23.8 ± 5.9	25.6 ± 7.0	25.3 ± 4.2	26.4 ± 4.3	0.406	0.511	0.001*

When isolating participants with NAFLD (hepatic fat fraction of > 5%) both TCOCT+HIIT and TCOCT significantly reduced BMI z-score (p=0.045), hepatic fat (p=0.026), and increased VO_2peak (p=0.046) (Table 5).

Table 5: Effect of TCOCT+HIIT and TCOCT on fat depositions, BMI z-score and VO_2peak in children and adolescents *with NAFLD* (hepatic fat > 5%). Presented as mean ± SD. P-values from a Two-Way ANOVA Repeated measures. *p<0.05.

	TCOCT+HIIT (n=10)		TCOCT (n=7)		time x group	group	time
	Pre	Post	Pre	Post			
BMI z-score	3.16 ± 0.58	3.09 ± 0.55	2.90 ± 0.39	2.81 ± 0.47	0.745	0.294	0.045*
Hepatic fat (%)	11.1 ± 12.4	9.84 ± 10.5	11.1 ± 2.9	8.5 ± 4.5	0.386	0.887	0.026*
Pancreatic fat (%)	6.1 ± 3.3	5.8 ± 3.2	4.2 ± 1.5	3.5 ± 1.3	0.369	0.130	0.064
Psoas fat (%)	4.0 ± 2.5	3.8 ± 2.0	2.4 ± 1.0	1.9 ± 1.1	0.710	0.057	0.390
VAT (cm^2)	95.1 ± 24.6	90.7 ± 25.8	98.9 ± 41.7	87.1 ± 29.4	0.578	0.994	0.240
SAT (cm^2)	374.0 ± 90.8	384.0 ± 81.1	297.1 ± 70.0	284.9 ± 87.5	0.479	0.036*	0.942
VO_2peak (ml/min/kg)	20.2 ± 3.7	21.5 ± 5.7	22.4 ± 4.2	24.5 ± 5.0	0.640	0.270	0.046*

No differences between TCOCT+HIIT and TCOCT of daily MVPA or other physical activity levels were detected (Figure 5).

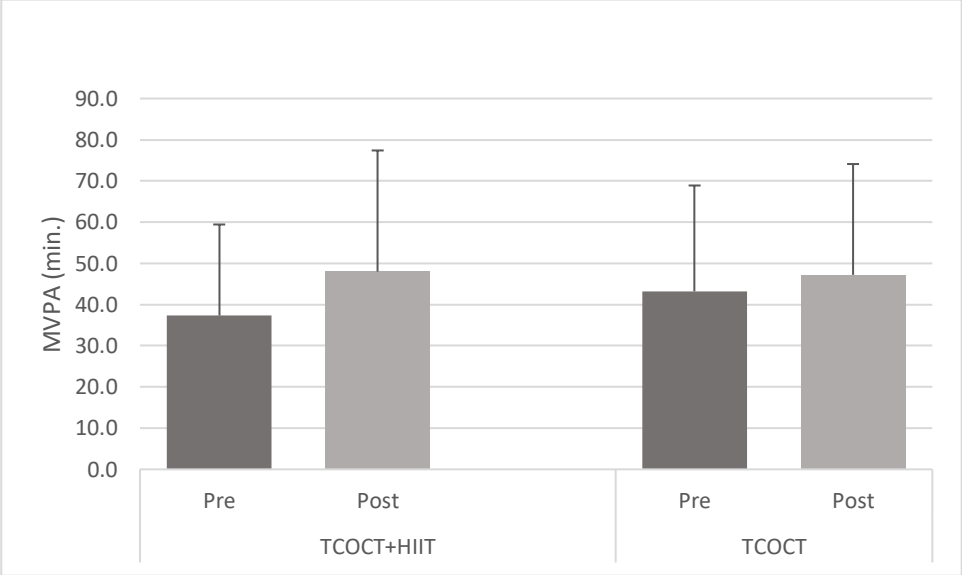

Figure 5: Minutes of MVPA from baseline (pre) to 12 weeks (post) between TCOCT+HIIT (n=18) and TCOCT (n=15). Presented as mean + SD. No significant differences were detected from a Two-Way ANOVA Repeated measure (p<0.05).

When analyzing changes from before to after the intervention no significant correlations between fat depositions and activity level and VO_2peak were observed, but positive associations between changes in BMI z-score and changes in the different fat depositions occurred (Table 6).

Table 6: Correlations between changes (Δ) in activity level and changes in BMI z-score, fat depositions, and VO_2peak in children and adolescents with obesity. r-values and p-values from a Spearman's rank order correlation test. *p<0.05. n=30

	Δ Hepatic fat (%)		Δ Pancreatic fat (%)		Δ Psoas (%)		Δ VAT (cm^2)		Δ SAT (cm^2)	
	r	p	r	p	r	p	r	p	r	p
Δ BMI z-score	0.311	0.065	-0.045	0.795	0.042	0.808	0.476	0.003*	0.525	0.001*
Δ Sedentary	0.067	0.732	0.098	0.612	-0.055	0.775	0.037	0.847	0.159	0.395
Δ Light	-0.019	0.921	-0.261	0.171	0.043	0.825	-0.044	0.825	-0.362	0.054
Δ Moderate	0.240	0.202	-0.293	0.116	0.150	0.428	-0.050	0.793	-0.161	0.395
Δ Vigorous	-0.192	0.319	-0.182	0.345	0.153	0.427	-0.212	0.270	-0.212	0.270
Δ MVPA	0.171	0.367	-0.230	0.222	0.246	0.190	-0.038	0.840	-0.143	0.449
Δ VO_2peak	0.171	0.327	0.046	0.794	0.308	0.092	-0.025	0.889	-0.089	0.613

Discussion

Summary

This study showed that children and adolescents with obesity had increased ectopic fat depositions compared to children and adolescents without obesity at baseline. Furthermore, the normal weight children and adolescents were more physically active and associations between MVPA and fat depositions were found, suggesting that time spent in MVPA may be a target for reducing ectopic fat depositions. 12 weeks of TCOCT resulted in significant reductions in BMI z-score and ectopic fat deposition in hepatic fat, pancreatic fat, and VAT. A novel aspect was to investigate if the addition of a HIIT intervention to TCOCT, would augment the reductions in ectopic fat deposition. No significant differences between TCOCT+HIIT and TCOCT in ectopic fat depositions nor in physical activity level were observed, indicating that 12 weeks of HIIT performed three times a week did not have an additive effect on the reduction of ectopic fat deposition and enhancement of daily physical activity level. No associations between changes in physical activity level and changes in ectopic fat depositions were found, indicating that other factors than activity level may be responsible for the decrease in ectopic fat depositions observed in study 2.

Study 1

Comparisons of ectopic fat depositions between children and adolescents with and without obesity

Our study showed that children and adolescents with obesity had increased hepatic fat fraction, pancreatic fat fraction, ectopic fat fraction in psoas, VAT, and SAT compared to the normal weight control group. A few studies have investigated potential differences in ectopic fat depositions between children and adolescents with and without obesity. Chabanova et al., 2017 observed that children and adolescents with obesity (8-18 years, BMI SDS between 1.28 and 2.33) had higher hepatic fat fractions than normal weight children and adolescents (boys: $0.9 \pm 0.16\%$ vs. $0.5 \pm 0.04\%$; girls: $1.1 \pm 0.24\%$ vs. $0.5 \pm 0.03\%$) measured with 1H-MRS (Chabanova et al., 2017). Fonvig et al., 2015 found higher prevalence rates of hepatic and muscular steatosis (>5 % hepatic or muscle fat fraction) in children with obesity compared to normal weight controls. Muscular steatosis was assessed in the psoas muscle like the present study and found significantly higher muscle fat fractions in children and adolescents with obesity compared to normal weights (Fonvig et al., 2015).

The current study is the first study to investigate differences in pancreatic fat fractions between children and adolescents with and without obesity. The elevated pancreatic fat content in obese children is in accordance with Gjela et al., 2024, who found a higher pancreatic fat fraction in adults with obesity (8.0% (6.1; 13.3%); mean BMI of 36.0) compared to normal weight adults (2.6% (1.7; 3.9%); mean BMI of 23.9) based on PDFF from MRI-scans. The pancreatic fat fractions in adults seem to be higher than the values observed in children and adolescents in the current study. This could be explained by the recruitment of adults with greater BMI values compared to our participants (Gjela et al., 2024). Other studies support this finding of greater pancreatic fat fractions in adult populations with obesity compared to normal weight adults (Pieńkowska et al., 2019; Sijens et al., 2010; Rossi et al., 2012). An explanation of the increased ectopic fat accumulation in children with obesity could be due to lipotoxicity, wherein an elevated circulation of free fatty acids causes an influx of free fatty acids into non-adipose cells within specific organs (Rada et al., 2020). Hepatic and pancreatic fat accumulation is associated with a higher risk of developing pre-diabetes (Hagman et al., 2014). Ectopic fat accumulation within the pancreas is linked to beta-cell dysfunction or apoptosis affecting the regulation of insulin levels (Simo˜es e Silva et al., 2020). Furthermore, a dysfunction of hepatic cells and muscle cells in the psoas muscle and other skeletal muscles can cause metabolic complications like insulin resistance affecting glucose metabolism

(Taylor et al., 2019; Krssak et al., 1999). Therefore, it is highly relevant to investigate the development of ectopic fat deposition in vital organs like the liver, pancreas, and skeletal muscles.

The present study found strong positive associations between ectopic fat depositions and BMI z-score, which could indicate that the BMI z-score is a valid measure to estimate the risk of developing increased ectopic fat. This agrees with Fonvig et al., 2015, who observed a significant positive correlation between liver fat content and BMI SDS in 287 children and adolescents with overweight or obesity (Fonvig et al., 2015).

Association between daily physical activity behavior and ectopic fat depositions

Daily activity data shows that the normal weight controls performed a significantly higher amount of time within MVPA intensity compared to children and adolescents with obesity. Furthermore, significant associations between MVPA and hepatic fat, pancreatic fat, ectopic fat in the psoas muscle, and VAT based on data from both normal weight controls and children and adolescents with obesity were observed. This behaviour (increased MVPA) could contribute to the decreased ectopic fat depositions in the normal weight controls probably in combination with other lifestyle factors. More time spent with higher activity intensity can increase total energy expenditure and induce energy deficits, which can contribute to an increased reduction of fat depositions (Boutcher, 2011). To this, a study shows associations between overall sedentary time and alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which are biomarkers for NAFLD. This indicates that less sedentary time is associated with a lower risk of NAFLD and hepatic fat accumulation (Valerie et al., 2022). Furthermore, children with lower sedentary time and more time with MVPA had significantly less hepatic fat content (Valerie et al., 2022). The present study 1 finds a significant inverse association between MVPA and ectopic fat, which could indicate that MVPA is decisive for minimizing ectopic fat depositions. A prospective study by Anderson et al., 2016 supports this finding as greater time spent in MVPA (>3600 cpm) at ages 12 to 14 years was associated with a lower risk of accumulating liver fat at a mean age of 17.8 years (Anderson et al., 2016). There is no existing evidence for the relationship between daily activity levels and pancreatic fat in children and adolescents, why it is new evidence that the present study confirms a significant inverse association between MVPA and pancreatic fat content.

Sex differences in children and adolescents with and without obesity

There were no sex differences in ectopic fat depositions, which is similar to Chabanova et al. 2017, who did not find differences in hepatic fat fractions between sexes (Chabanova et al., 2017). On the other hand, Fonvig et al., 2015 reported higher hepatic fat fractions and VAT in boys compared to girls (Fonvig et al., 2015). Nissen et al., 2016 observed higher hepatic fat fractions in boys, but without any sex differences in ectopic fat content in the psoas muscle (Nissen et al., 2016). However, sex-specific variations in different hormone levels are known. Estrogen promotes proliferation of adipocytes which contributes to increase fat depositions, while androgens stimulate lipolysis which theoretically can decrease fat accumulation (Gavin et al., 2020; Blouin et al., 2008). At this point, we have no information on hormone levels in our study. Future studies are warranted to investigate the influence of hormone levels and ectopic fat deposition in this cohort. On the other hand, puberty and maturation status could reduce these hormonal sex differences meaning that less mature children and adolescents produce less sex hormones and growth hormones compared to more mature individuals (Roemmich et al., 1998).

Study 2

TCOCT+HIIT and BMI z-score

The present study showed a 12-week reduction in BMI z-score in both TCOCT+HIIT and TCOCT without differences between groups. TCOCT has shown a great effect on body composition based on BMI z-score with an absolute decrease of 0.21 in 12 months (Hvidt et al., 2014). Furthermore, Mollerup et al., 2017 found absolute decreases of 0.38 in boys and 0.18 in girls after 1.5 years of TCOCT, and Holm et al., 2011 found an absolute reduction of 0.32 and 0.23 in boys and girls, based on BMI SDS in children with obesity and overweight (Mollerup et al., 2017; Holm et al., 2011). It must therefore be confirmed that TCOCT as a treatment is effective in changing body composition. Nevertheless, TCOCT+HIIT did not achieve an additional effect on BMI z-score by adding HIIT to TCOCT. Implementation of specific exercise interventions alongside lifestyle interventions has earlier been investigated by the EFIGRO-studies. By comparing a group of children with overweight and obesity following a multidisciplinary lifestyle intervention with an intervention group following the same lifestyle intervention plus addition of 22 weeks of exercise (>75 % of HRmax), both groups reduced their BMI. However, no statistical comparisons between the two groups were presented, why it cannot be confirmed if the addition of high intense exercise had an additive effect on BMI (Labayen et al., 2019).

TCOCT+HIIT and ectopic fat depositions

Our study observed a reduction in hepatic fat, pancreatic fat, and VAT in both TCOCT+HIIT and TCOCT, but without differences between groups. This must indicate that TCOCT alone can decrease ectopic fat content within specific organs. This agrees with the results from Fonvig et al., 2015, who found a reduction in hepatic fat, VAT, and ectopic fat in the psoas muscle after 12 months of TCOCT. TCOCT helps children and families to introduce lifestyle modifications regarding physical activity, nutrition, sleep habits, and mental health (Holm et al., 2012).

It may seem surprising that the implementation of HIIT alongside TCOCT did not have an additive effect on the reduction of ectopic fat depositions. The EFIGRO studies found that following 22 weeks of intensive supervised exercise (>75 % of HRmax) in combination with a multidisciplinary lifestyle intervention contributed to reducing hepatic fat fractions. No changes in hepatic fat were observed in a control group only following the lifestyle intervention (Labayen et al., 2019). By investigating responders for hepatic fat accumulation, the responder rates for hepatic fat reduction increased by adding intensive supervised exercise to the same multidisciplinary lifestyle intervention (Medrano et al., 2019). The duration of the exercise intervention in the EFIGRO studies was longer (22 weeks, 3 times a week) and consisted of a larger volume pr. training session but with a lower intensity (90 minutes). In the present study, it is possible that 12 weeks of HIIT provided insufficient stimulus for an additional decrease in ectopic fat deposition. Our study did not find differences in ectopic fat between TCOCT+HIIT and TCOCT in the subgroup analysis by investigating children with a baseline hepatic fat fraction of >5 % (NAFLD). In addition, only hepatic fat was significantly decreased in both TCOCT+HIIT and TCOCT (Table 5). It cannot be denied that the small sample size and large baseline variability (groups were not matched for levels of NAFLD at baseline) in this subgroup analysis led to a type 2 error. Tas et al., 2023 implemented HIIT for 4 weeks and found a reduction in hepatic fat accumulation in children and adolescents with obesity, but only in children with NAFLD. The protocol consisted of 10x1 minute aerobic work at 80-90 % of HRmax (Tas et al., 2023). This could indicate that the degree of hepatic fat content could influence the response rates to exercise interventions. Regarding pancreatic fat deposition, the literature lacks in assessing the effect of HIIT and other exercise interventions and lifestyle interventions on children and adolescents. However, evidence suggests that moderate-intensity continuous exercise decreases pancreatic fat and improves beta cell function in a group of sedentary adults (40-55 years) (Heiskanen et al., 2018). It is a new finding that TCOCT induced reductions in

pancreatic fat content in children and adolescents with obesity. The pancreas plays a crucial role in glucose metabolism, and the accumulation of pancreatic fat may lead to beta cell dysfunction affecting glucose metabolism and increasing the risk of diabetes (Simo˜es e Silva et al., 2020). The results support that TCOCT could be a treatment for reducing pancreatic fat and metabolic risks.

The present study found significant positive associations between changes in BMI z-score and changes in VAT and SAT, respectively. But no associations with changes in ectopic fat parameters. Similarly, Lee et al., 2012 observed that adolescent boys (12-18 years) with obesity decreased their ectopic liver fat and VAT after 3 months of aerobic exercise but without a significant reduction in BMI (Lee et al., 2012). Taken together these results suggest that changes in ectopic fat can occur independent of changes in BMI z-score.

HIIT and ectopic fat depositions

There was no augmented effect of adding HIIT to TCOCT in the present study. The lack of additional effect was despite a relatively high attendance rate (74 ± 20 %) for HIIT sessions. This attendance rate for HIIT sessions is similar to the EFIGRO study (72.0 ± 16.1%) over 22 weeks (Labayen et al., 2019). Focusing on isolated HIIT interventions, the literature suggests that 12 weeks of HIIT performed alone contributes to reducing VAT in children and adolescents with obesity (Cao et al., 2022). The protocol consisted of two sets of eight bouts of 15 seconds run at maximal aerobic speed three times a week. However, the study did not assess ectopic fat (Cao et al., 2022). In adult populations Winn et al. found a decrease in hepatic fat after 4 weeks of HIIT by 4 min intervals at 80% of VO_2peak (Winn et al., 2018). The knowledge of the effect of HIIT on ectopic fat deposition in specific organs in children and adolescents is sparse and must be expanded.

However, several mechanisms induced by HIIT and time spent in MVPA could contribute to a reduction in ectopic fat depositions. Generally, prolonged aerobic training with moderate intensity leads to a relatively high acute energy contribution from fat oxidation via lipolysis and beta-oxidation, whereas this contribution is lower in HIIT. However, the overall energy expenditure relative to working time is greater in HIIT due to its higher intensity (Boutcher, 2011). Additionally, post-exercise excess consumption (EPOC) is a mechanism observed to be higher after high-intensity training sessions. EPOC demonstrates increased oxygen consumption in the hours following exercise, which may stimulate increased lipolysis and beta-oxidation (Boutcher, 2011). The liver is important in meeting the ATP demands of working muscles by releasing glucose into

the bloodstream from liver glycogen and through gluconeogenesis. Gluconeogenesis synthesizes glucose from glycerol, pyruvate, and lactate and the process requires ATP, which is derived from oxidation of fatty acids within the liver. Because of this, it is supposed that physical activity increases the turnover and decreases the storage of hepatic fatty acids (Thyfault et al., 2020).

As mentioned, ectopic fat depositions could be decreased without changes in BMI z-score and body composition (Lee et al., 2012). It may be caused by exercise-induced mechanisms independent of an energy deficit. Several hormones stimulate the oxidation of fat and are exercise intensity-dependent, with higher work intensities leading to increased hormone release. HIIT has been shown to increase the release of such hormones like catecholamines, growth hormone, cortisol, and glucagon which stimulate lipolysis (Arner, 2005; Trapp et al., 2007; Pritzlaff et al., 2000). The hormone adiponectin is released from adipose tissue and is related to an upregulation of lipolysis of hepatic lipids by binding to hepatic adiponectin receptors (Cho et al., 2016). A meta-analysis found that serum adiponectin concentrations were altered by exercise in children and adolescents with obesity compared to non-exercising controls. Greater exercise intensities resulted in greater serum adiponectin concentrations (Zhang et al., 2023). Furthermore, cytokines and exerkines that are released from muscle tissue and other tissues as a response to exercise could mediate reductions of ectopic fat by enhancing insulin sensitivity, beta-oxidation, and lipid metabolism (Thyfault et al., 2020; Wang et al., 2023). For instance, the hormones fibroblast growth factor 21 (FGF21) and Interleukin 6 (IL-6) is elevated by exercise and is correlated with hepatic fat content and VAT (Wang et al., 2023; Wedell-Neergaard et al., 2019). It is possible that HIIT did not stimulate these pathways and mechanisms to induce additional effects. Most likely because of the lack of differences in MVPA between TCOCT+HIIT and TCOCT.

Physical activity level

Daily activity levels were investigated prior to and in the last week of TCOCT+HIIT and TCOCT interventions. No significant differences in activity levels were found between TCOCT+HIIT and TCOCT. This means that both groups executed the same time span in each intensity category throughout daily living and time spent on exercising. The intention was to investigate if the addition of HIIT to TCOCT would enhance MVPA. A minor numerical increase in MVPA from baseline to week 12 was observed in both TCOCT+HIIT and TCOCT, but not significant. The lack of significant differences in MVPA between TCOCT+HIIT and TCOCT could be an explanation for

the absence of interactions on ectopic fat depositions. Mansfeldt et al., 2023 demonstrated a compensatory response to a scheduled exercise intervention, suggesting that prescribed exercise can reduce subsequent physical activity time influenced by physical and/or psychological factors (Mansfeldt et al., 2023). It is possible that the addition of HIIT to TCOCT led to a compensatory response that reduced the possible TCOCT-induced MVPA outside the scheduled HIIT sessions. This could explain why a significant increase in MVPA compared to TCOCT was not observed. Our study did not find significant correlations between changes in activity levels and changes in fat depositions over 12 weeks. This could be explained by other lifestyle factors, possibly introduced to daily life by the TCOCT intervention, that must be responsible for the decrease in fat depositions in both groups. All elements of TCOCT may contribute to reduce fat mass and it cannot be concluded which elements, that are the main factors of inducing the observed reductions in ectopic fat content.

VO_2peak

The present study did not find an association between changes in VO_2peak and changes in any of the fat deposition parameters. Medrano et al., 2020 found that higher cardiorespiratory fitness was associated with a lower percentage of hepatic fat in children with overweight (Medrano et al., 2020). VO_2peak is a measure of cardiorespiratory effect and Jones et al., 2019 found that VO_2peak is associated with MVPA in children (Jones et al., 2019). Because of this, it could be expected that VO_2peak was associated with ectopic fat depositions. Otherwise, a significant increase in VO_2peak after 12 weeks was observed in both TCOCT+HIIT and TCOCT, but without differences between groups. In the same way, by following a lifestyle and psycho-education intervention, the EFIGRO studies observed a significant increase in VO_2peak (pre: 35.9 ± 6.3; post: 38.7 ± 6.8 (mL/kg/min)) over 22 weeks (Labayen et al., 2019). Thus, it seems like lifestyle interventions alone can increase VO_2peak. Furthermore, HIIT interventions have shown a greater effect on VO_2peak compared to continuous exercise with moderate intensity (Wisløff et al., 2009). However, the present study did not observe an additive effect of HIIT on VO_2peak, likely due to comparable MVPA values. Another factor contributing to increases in VO_2peak (values defined as mL/kg/min) is the observed weight loss, meaning that a potential association between VO_2peak and ectopic fat depositions may be influenced by the fact that reductions in weight could increase VO_2peak.

Strengths and limitations

A strength of this study is the inclusion of activity data to determine possible differences in daily activity levels between groups. Furthermore, the use of an RCT design in study 2 allows us to

determine possible HIIT-induced changes for the different parameters. A limitation is the lack of assessment of other lifestyle factors like nutrition, sleep, and mental health, which could contribute to changes in fat depositions. In the same way, there is a risk that the participants changed their behavior unnaturally by wearing the activity tracker as well as the awareness of participating in an intervention.

Conclusion

In conclusion, this study found that hepatic fat, pancreatic fat, ectopic fat in psoas, VAT, and SAT were elevated in children and adolescents with obesity compared to normal weight controls (study 1). These differences in ectopic fat can be influenced by time spent in MVPA**,** where an inverse association between MVPA and ectopic fat depositions in children and adolescents with and without obesity was observed. Moreover, no augmented effect on ectopic fat depositions, BMI z-scores, and VO_2peak was observed by adding 12 weeks of HIIT to TCOCT (study 2). This can be explained by the fact that activity levels (MVPA) were not increased by adding HIIT to TCOCT. This is the first study to find elevated ectopic fat content in the pancreas in children and adolescents with obesity, but it seems like TCOCT as treatment can reduce ectopic fat content independent of the addition of HIIT sessions. Furthermore, it seems plausible that increasing MVPA is a decisive factor in reducing ectopic fat. More investigations are needed to determine the relationship between exercise interventions and ectopic fat depositions in children and adolescents.

References

Blouin, K., Boivin, A., & Tchernof, A. (2008). Androgens and body fat distribution. Journal of Steroid Biochemistry and Molecular Biology, 108(3), 272-280. doi:10.1016/j.jsbmb.2007.09.001

Boutcher, S. H. (2011). High-intensity intermittent exercise and fat loss. Journal of Obesity, 2011, 1-10. doi:10.1155/2011/868305

Brown, T., Moore, T. H., Hooper, L., Gao, Y., Zayegh, A., Ijaz, S., et al. (2019). Interventions for preventing obesity in children. Cochrane Database of Systematic Reviews, 2019(7), CD001871. doi:10.1002/14651858.CD001871.pub4

Brønd, J. C., Aadland, E., Andersen, L. B., Resaland, G. K., Andersen, S. A., & Arvidsson, D. (2019). The ActiGraph counts processing and the assessment of vigorous activity. Clinical Physiology and Functional Imaging, 39(4), 276-283. doi:10.1111/cpf.12571

Cao, M., Tang, Y., & Zou, Y. (2022). Integrating high-intensity interval training into a school setting improve body composition, cardiorespiratory fitness and physical activity in children with obesity: A randomized controlled trial. Journal of Clinical Medicine, 11(18), 5436. doi:10.3390/jcm11185436

Chabanova, E., Fonvig, C. E., Bøjsøe, C., Holm, J., & Thomsen, H. S. (2017). 1H MRS assessment of hepatic fat content: Comparison between normal- and excess-weight children and adolescents. Academic Radiology, 24(8), 982-987. doi:10.1016/j.acra.2017.02.010

Cho, J., Koh, Y., Han, J., Kim, D., Kim, T., & Kang, H. (2016). Adiponectin mediates the additive effects of combining daily exercise with caloric restriction for treatment of non-alcoholic fatty liver. *International Journal of Obesity, 40*(11), 1760-1767. doi:10.1038/ijo.2016.104

e Silva, L. d. L. S., Fernandes, M. S. d. S., Lima, E. A. d., Stefano, J. T., Oliveira, C. P., & Jukemura, J. (2021). Fatty pancreas: Disease or finding? Clinics (São Paulo, Brazil), 76, e2439. doi:10.6061/clinics/2021/e2439

Fonvig, C. E., Chabanova, E., Andersson, E. A., Ohrt, J. D., Pedersen, O., Hansen, T., et al. (2015a). 1H-MRS measured ectopic fat in liver and muscle in danish lean and obese children and adolescents. PloS One, 10(8), e0135018. doi:10.1371/journal.pone.0135018

Fonvig, C. E., Chabanova, E., Ohrt, J. D., Nielsen, L. A., Pedersen, O., Hansen, T., et al. (2015b). Multidisciplinary care of obese children and adolescents for one year reduces ectopic fat content in liver and skeletal muscle. BMC Pediatrics, 15(196), 196. doi:10.1186/s12887-015-0513-6

Gavin, K. M., & Bessesen, D. H. (2020). Sex differences in adipose tissue function. Endocrinology and Metabolism Clinics of North America, 49(2), 215-228. doi:10.1016/j.ecl.2020.02.008

HAGMAN, E., REINEHR, T., KOWALSKI, J., EKBOM, A., MARCUS, C., & HOLL, R. W. (2014). Impaired fasting glucose prevalence in two nationwide cohorts of obese children and adolescents. International Journal of Obesity, 38(1), 40-45. doi:10.1038/ijo.2013.124

Heiskanen, M. A., Motiani, K. K., Mari, A., Saunavaara, V., Eskelinen, J., Virtanen, K. A., et al. (2018). Exercise training decreases pancreatic fat content and improves beta cell function regardless of baseline

glucose tolerance: A randomised controlled trial. Diabetologia, 61(8), 1817-1828. doi:10.1007/s00125-018-4627-x

Holm, J., Gamborg, M., Bille, D. S., Grønbæk, H. N., Ward, L. C., & Faerk, J. (2011). Chronic care treatment of obese children and adolescents. International Journal of Pediatric Obesity, 6(3-4), 188-196. doi:10.3109/17477166.2011.575157

Hvidt, K. N., Olsen, M. H., Ibsen, H., & Holm, J. (2014). Effect of changes in BMI and waist circumference on ambulatory blood pressure in obese children and adolescents. Journal of Hypertension, 32(7), 1470-1477. doi:10.1097/HJH.0000000000000188

Johansen, M. J., Vonsild Lund, M. A., Ängquist, L., Fonvig, C. E., Holm, L. A., Chabanova, E., et al. (2022). Possible prediction of obesity-related liver disease in children and adolescents using indices of body composition. Pediatric Obesity, 17(10), e12947-n/a. doi:10.1111/ijpo.12947

Jones, M. A., Skidmore, P. M., Stoner, L., Harrex, H., Saeedi, P., Black, K., et al. (2020). Associations of accelerometer-measured sedentary time, sedentary bouts, and physical activity with adiposity and fitness in children. Journal of Sports Sciences, 38(1), 114-120. doi:10.1080/02640414.2019.1685842

Julian, V., Bergsten, P., Ennequin, G., Forslund, A., Ahlstrom, H., Ciba, I., et al. (2022). Association between alanine aminotransferase as surrogate of fatty liver disease and physical activity and sedentary time in adolescents with obesity. European Journal of Pediatrics, 181(8), 3119-3129. doi:10.1007/s00431-022-04539-z

Keating, S. E., Hackett, D. A., Parker, H. M., O'Connor, H. T., Gerofi, J. A., Sainsbury, A., et al. (2015). Effect of aerobic exercise training dose on liver fat and visceral adiposity. Journal of Hepatology, 63(1), 174-182. doi:10.1016/j.jhep.2015.02.022

Kipp, J. P., Olesen, S. S., Mark, E. B., Frederiksen, L. C., Drewes, A. M., & Frøkjær, J. B. (2019). Normal pancreatic volume in adults is influenced by visceral fat, vertebral body width and age. Abdominal Imaging, 44(3), 958-966. doi:10.1007/s00261-018-1793-8

KRSSAK, M., PETERSEN, K. F., DRESNER, A., DIPIETRO, L., VOGEL, S. M., ROTHMAN, D. L., et al. (1999). Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans : A 1H NMR spectroscopy study. Diabetologia, 42(1), 113-116. Retrieved from MEDLINE database. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10027589

Labayen, I., Medrano, M., Arenaza, L., Maíz, E., Osés, M., Martínez-Vizcaíno, V., et al. (2020). Effects of exercise in addition to a family-based lifestyle intervention program on hepatic fat in children with overweight. Diabetes Care, 43(2), 306-313. doi:10.2337/dc19-0351

LEE, S., BACHA, F., HANNON, T., KUK, J. L., BOESCH, C., & ARSLANIAN, S. (2012). Effects of aerobic versus resistance exercise without caloric restriction on abdominal fat, intrahepatic lipid, and insulin sensitivity in obese adolescent boys: A randomized, controlled trial. *Diabetes (New York, N.Y.), 61*(11), 2787-2795. doi:10.2337/db12-0214

Mansfeldt, J. M., & Magkos, F. (2023). Compensatory responses to exercise training as barriers to weight loss: Changes in energy intake and non-exercise physical activity. Current Nutrition Reports, 12(2), 327-337. doi:10.1007/s13668-023-00467-y

Medrano, M., Arenaza, L., Migueles, J. H., Rodríguez-Vigil, B., Ruiz, J. R., & Labayen, I. (2020a). Associations of physical activity and fitness with hepatic steatosis, liver enzymes, and insulin resistance in children with overweight/obesity. Pediatric Diabetes, 21(4), 565-574. doi:10.1111/pedi.13011

Medrano, M., Arenaza, L., Ramírez-Vélez, R., Ortega, F. B., Ruiz, J. R., & Labayen, I. (2020b). Prevalence of responders for hepatic fat, adiposity and liver enzyme levels in response to a lifestyle intervention in children with overweight/obesity: EFIGRO randomized controlled trial. Pediatric Diabetes, 21(2), 215-223. doi:10.1111/pedi.12949

Moher, D., Hopewell, S., Schulz, K. F., Montori, V., Gøtzsche, P. C., Devereaux, P. J., et al. (2012). CONSORT 2010 explanation and elaboration: Updated guidelines for reporting parallel group randomised trials. International Journal of Surgery (London, England), 10(1), 28-55. doi:10.1016/j.ijsu.2011.10.001

Mollerup, P. M., Gamborg, M., Trier, C., Bøjsøe, C., Nielsen, T. R. H., Baker, J. L., et al. (2017). A hospital-based child and adolescent overweight and obesity treatment protocol transferred into a community healthcare setting. PloS One, 12(3), e0173033. doi:10.1371/journal.pone.0173033

Neeland, I., Poirier, P., & Després, J. (2018). Cardiovascular and metabolic heterogeneity of obesity: Clinical challenges and implications for management. Circulation (New York, N.Y.), 137(13), 1391-1406. doi:10.1161/CIRCULATIONAHA.117.029617

Nemeth, A., Segrestin, B., Leporq, B., Seyssel, K., Faraz, K., Sauvinet, V., et al. (2019). 3D chemical Shift-Encoded MRI for volume and composition quantification of abdominal adipose tissue during an overfeeding protocol in healthy volunteers. Journal of Magnetic Resonance Imaging, 49(6), 1587-1599. doi:10.1002/jmri.26532

Nissen, A., Fonvig, C. E., Chabanova, E., Bøjsøe, C., Trier, C., Pedersen, O., et al. (2016). 1H-MRS measured ectopic fat in liver and muscle is associated with the metabolic syndrome in danish girls but not in boys with overweight and obesity. Obesity Science and Practice, 2(4), 376-384. doi:10.1002/osp4.61

Pearson, S., Hansen, B., Sørensen, T. I., & Baker, J. L. (2010). Overweight and obesity trends in copenhagen schoolchildren from 2002 to 2007. Acta Paediatrica, 99(11), 1675-1678. doi:10.1111/j.1651-2227.2010.01897.x

Physical activity is prospectively associated with adolescent nonalcoholic fatty liver disease.(2017). Journal of Pediatric Gastroenterology and Nutrition, 64(5), 844. doi:10.1097/MPG.0000000000001569

Pieńkowska, J., Brzeska, B., Kaszubowski, M., Kozak, O., Jankowska, A., & Szurowska, E. (2019). MRI assessment of ectopic fat accumulation in pancreas, liver and skeletal muscle in patients with obesity, overweight and normal BMI in correlation with the presence of central obesity and metabolic syndrome. Diabetes, Metabolic Syndrome and Obesity, 12, 623-636. doi:10.2147/DMSO.S194690

Rada, P., González-Rodríguez, Á, García-Monzón, C., & Valverde, Á M. (2020). Understanding lipotoxicity in NAFLD pathogenesis: Is CD36 a key driver? Cell Death & Disease, 11(9), 802-802. doi:10.1038/s41419-020-03003-w

ROEMMICH, J. N., CLARK, P. A., VU MAI, BERR, S. S., WELTMAN, A., VELDHUIS, J. D., et al. (1998). Alterations in growth and body composition during puberty: III. influence of maturation,

gender, body composition, fat distribution, aerobic fitness, and energy expenditure on nocturnal growth hormone release. The Journal of Clinical Endocrinology and Metabolism, 83(5), 1440-1447. doi:10.1210/jc.83.5.1440

Rossi, A. P., Fantin, F., Zamboni, G. A., Mazzali, G., Rinaldi, C. A., Giglio, M., et al. (2011). Predictors of ectopic fat accumulation in liver and pancreas in obese men and women. Obesity, 19(9), 1747-1754. doi:10.1038/oby.2011.114

Sijens, P. E., Edens, M. A., Bakker, S. J. L., & Stolk, R. P. (2010). MRI-determined fat content of human liver,pancreas and kidney. World Journal of Gastroenterology : WJG, 16(16), 1993-1998. doi:10.3748/wjg.v16.i16.1993

Steinkohl, E., Olesen, S. S., Hansen, T. M., Drewes, A. M., & Frøkjær, J. B. (2021). T1 relaxation times and MR elastography-derived stiffness: New potential imaging biomarkers for the assessment of chronic pancreatitis. Abdominal Imaging, 46(12), 5598-5608. doi:10.1007/s00261-021-03276-5

Suliga, E. (2009). Visceral adipose tissue in children and adolescents: A review. Nutrition Research Reviews, 22(2), 137-147. doi:10.1017/S0954422409990096

Tanaka, H., Monahan, K. D., & Seals, D. R. (2001). Age-predicted maximal heart rate revisited. Journal of the American College of Cardiology, 37(1), 153-156. doi:10.1016/S0735-1097(00)01054-8

Tas, E., Landes, R. D., Diaz, E. C., Bai, S., Ou, X., Buchmann, R., et al. (2023). Effects of short-term supervised exercise training on liver fat in adolescents with obesity: A randomized controlled trial. Obesity (Silver Spring, Md.), 31(11), 2740-2749. doi:10.1002/oby.23887

Taylor, R., Al-Mrabeh, A., & Sattar, N. (2019). Understanding the mechanisms of reversal of type 2 diabetes. The Lancet. Diabetes & Endocrinology, 7(9), 726-736. doi:10.1016/S2213-8587(19)30076-2

Thyfault, J. P., & Rector, R. S. (2020). Exercise combats hepatic steatosis: Potential mechanisms and clinical implications. Diabetes, 69(4), 517-524. doi:10.2337/dbi18-0043

Trauner, M., Arrese, M., & Wagner, M. (2010). Fatty liver and lipotoxicity. Biochimica Et Biophysica Acta, 1801(3), 299-310. doi:10.1016/j.bbalip.2009.10.007

Wang, Z., Sun, T., Yu, J., Li, S., Gong, L., & Zhang, Y. (2023). FGF21: A sharp weapon in the process of exercise to improve NAFLD. *Frontiers in Bioscience (Landmark. Print), 28*(12), 351. doi:10.31083/j.fbl2812351

Wang, Y., Wang, S., Meng, X., & Zhou, H. (2024). Effect of high-intensity interval training and moderate-intensity continuous training on cardiovascular risk factors in adolescents: Systematic review and meta-analysis of randomized controlled trials. Physiology & Behavior, 275, 114459. doi:10.1016/j.physbeh.2024.114459

Wedell-Neergaard, A., Lang Lehrskov, L., Christensen, R. H., Legaard, G. E., Dorph, E., Larsen, M. K., et al. (2019). Exercise-induced changes in visceral adipose tissue mass are regulated by IL-6 signaling: A randomized controlled trial. *Cell Metabolism, 29*(4), 844-855.e3. doi:10.1016/j.cmet.2018.12.007

WHO child growth standards. (2006).

Winn, N. C., Liu, Y., Rector, R. S., Parks, E. J., Ibdah, J. A., & Kanaley, J. A. (2018). Energy-matched moderate and high intensity exercise training improves nonalcoholic fatty liver disease risk independent of changes in body mass or abdominal adiposity — A randomized trial. Metabolism, 78, 128-140. doi:10.1016/j.metabol.2017.08.012

Wisløff, U., Ellingsen, Ø, & Kemi, O. J. (2009). High-intensity interval training to maximize cardiac benefits of exercise training? Exercise and Sport Sciences Reviews, 37(3), 139-146. doi:10.1097/jes.0b013e3181aa65fc

Zhang, Y., Wu, Y., Fei, X., Li, Y., Li, Y., & Yan, X. (2023). Effects of aerobic exercise on serum adiponectin concentrations in children and adolescents with obesity: A systematic review and meta-analysis. *Life (Basel, Switzerland), 13*(8), 1772. doi:10.3390/life13081772

ARBEJDSBLADE

Kandidatspeciale

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1.0 Forord til arbejdsblade

De følgende arbejdsblade uddyber projektets teoretiske, metodiske og perspektiverende grundlag. Det anbefales, at artiklen læses først, hvorefter arbejdsbladene supplerer artiklens indhold. Arbejdsbladene indeholder indledningsvist en præsentation af forskningsprojektet *HIIT med Kiloene*, der danner grundlaget for dette kandidatspeciale. Derefter afdækkes projektets teoretiske baggrund med afsæt i forskningslitteraturen, herunder fedtmetabolismen samt mekanismerne bag deponering af ektopisk fedt samt effekterne af træningsinterventioner. Endelig suppleres projektets metoder og datagrundlag, herunder MRI-scanninger og analyse af disse, samt der følger en uddybning af de anvendte statistiske analyser. Slutteligt præsenteres en række perspektiverende tanker med fokus på fremtidige undersøgelser.

2.0 HIIT med kiloene

Dette kandidatspeciale er udarbejdet på baggrund af forskningsprojektet *HIIT med Kiloene*, der er udført som en del af Charlotte Eggertsens Ph.d.-forløb på Aalborg Universitet og Børne- og Ungeafdelingen, Aalborg Universitets Hospital. Formålet med forskningsprojektet er at undersøge effekten af en livsstilsintervention i kombination med *højintens intervaltræning (HIIT)* på fedtdeponeringer i overvægtige børn og unge.

HIIT med Kiloene forløb over en treårig periode og bestod af to studier. Dette speciale omhandler det andet studie, der indledningsvist involverede 60 overvægtige børn og unge (isoBMI > 30) samt 30 normalvægtige børn og unge som kontrolgruppe. I studiet blev de overvægtige børn og unge randomiseret i to grupper. Den ene gruppe fungerede som kontrolgruppe, der kun gennemgik en livsstilsintervention (TCOCT), mens den anden gruppe gennemgik en kombination af TCOCT og HIIT i 12 uger. Desuden blev der indsamlet data på den normalvægtige kontrolgruppe, der giver mulighed for at foretage en sammenligning af fedtdeponeringer i normal- og overvægtige børn.

2.1 The Children's Obesity Clinic's Treatment (TCOCT)

TCOCT er en livsstilintervention, der også er kendt som *Holbæk-metoden.* Metoden er anvendt i flere vægttabsklinikker i Danmark med stor succes. Overvægt anses som en kompleks og kronisk lidelse grundet de medicinske (eks. udvikling af det metaboliske syndrom) og psykosociale (Health-related quality of life) komplikationer overvægt kan medføre, og som kan besværliggøre forsøg på vægttab (Weiss et al., 2004; Schwimmer et al., 2003). Derfor bygger interventionen på omfattende kronisk behandling med elementer som vejledning i kost, søvnvaner, fysisk aktivitet samt social og mental sundhed og velvære. TCOCT fungerer som en familiebehandling, der fokuserer på børnene og udvikler individuelle behandlingsplaner, der tilgodeser det enkelte barns samt dets families situation og behov. Det er således intentionen, at børnene og forældrene aktivt inkorporerer disse planer i hverdagen og gør det til deres eget valg at foretage en livsstilsændring. Behandlingsplanen bygger på en række konkrete individuelle livsstilsmæssige ændringer, typisk 15-25 ændringer, som familien forsøger at overholde. Tabel 1 viser eksempler på typiske ændringer, der kan implementeres i en behandlingsplan (Holm et al., 2011).

Tabel 1: Liste med eksempler på konkrete livsstilsændringer til en behandlingsplan i et TCOCT-forløb. Udarbejdet på baggrund af Holm et al., 2011.

Morgenmad	Middagsmad	Eftermiddag	Aftensmad	Aktivitet	Generelt
Spis morgenmad	Reducer spisning foran TV/PC	Reducer smør og erstat i stedet med margarine	Reducer sodavand, juice og iste	1 times daglig aktivitet	1-2 stykker frugt dagligt
Reducer fed yoghurt	Reducer indtag af chokolade via forskellige fødevarer	Øg mængden af mørkt brød	Portions-anret i køkkenet	Cykle/gå til skole og sport	Kage max én gang om ugen
			Sikre normal størrelse portioner: 20% kød, 40 % kartofler/ris, 40 % grøntsager	Dans til musik derhjemme	Reducer alkohol
				Start til organiseret idræt i ex en forening (x antal gange om ugen)	Fast food maks. én gang om måneden
				Sænk skærmtid til maks. 3 timer dagligt	

TCOCT udføres i praksis ved, at familierne tilbydes én-til-én konsultationer med en sygeplejerske og diætist. Forløbet starter med et spørgeskema-baseret interview til at identificere de livsstilsændringer, der anses som nødvendige. Herefter planlægges løbende konsultationer til at vejlede og følge op på planen. Frekvensen på konsultationerne varierer efter behov hvor familier, der oplever større vanskeligheder med at fuldføre planen, tilbydes konsultationerne med en højere frekvens og omvendt (Holm et al., 2011).

Forskning på effekterne af TCOCT har vist positive resultater på vægttab og andre fysiologiske sundhedsparametre. Tabel 2 opsummerer de vigtigste fund fra forskningslitteraturen.

Tabel 2: Sammenfatning af positive fysiologiske effekter af TCOCT på overvægtige børn og unge (6-18 år). Effekterne er statistisk signifikante $p<0.05$. ↓ = værdi for testparameter mindskes, → = værdi for testparameter uændret. Parenteser angiver ændringens størrelse (gennemsnit).

Reference	Forsøgsdesign	Interventionsperiode	Effekter
Holm et al., 2011	Longitudinal observational study, n= 617	12 mdr.	BMI SDS ↓ (-0.23 i piger og -0.32 i drenge)
Mollerup et al., 2017	Prospective study, n=1001	18 mdr.	BMI SDS ↓ (-0.18 i piger og -0.38 i drenge)
Hvidt et al., 2014	Longitudinal observational study, n=61	12 mdr.	BMI z-score ↓ (-0.21)
Kloppenborg et al., 2017	Longitudinal observational study, n= 569	13 mdr.	BMI z-score ↓ (-0.31) Insulin koncentration ↓ Insulin sensitivitet (HOMA2-IS) → Betacelle funktion (HOMA2-B) →
Mollerup et al., 2017	Longitudinal observational study, n= 663	12 mdr.	Blodtryk ↓ (på personer med hypertension ved baseline)
Most et al., 2015	Longitudinal observational study, n=313	24 mdr.	BMI SDS ↓ (-0.19 i piger og -0.30 i drenge)
Fonvig et al., 2015	Longitudinal observational study, n=40	12 mdr.	BMI SDS ↓ (0.24) Leverfedt ↓ (0.0 procentpoint) Muskelfedt ↓ (2.6 procentpoint) VAT ↓ (10 cm3) Prævalens af hepatic steatosis ↓ (8 procentpoint) Prævalens af muscular steatosis ↓ (30 procentpoint) HDL / LDL ↓
Nielsen et al., 2018	Longitudinal observational study, n=876	Median på 1.8 år (range 0.4–7.4 år)	BMI SDS ↓ hos 68% af deltagerne. LDL / HDL ↓ (hos deltagere hvor BMI SDS ↓)

2.2 HIIT-protokol

High-Intensity Interval Training (HIIT) blev i projektet implementeret for interventionsgruppen i kombination med TCOCT. HIIT blev udført tre gange om ugen med aktiviteter, der fordrede en legende og ikke-konkurrerende tilgang. Tabel 3 viser den overordnede træningsplan gennemført

over interventionens 12 uger. Der blev således arbejdet med tre forskellige træningstemaer, herunder boldspil, crossfit og løberelaterede øvelser. I de valgfrie moduler kunne der frit vælges mellem disse tre træningstemaer. Et træningspas, uanset valg af træningstema, bestod af opvarmning efterfulgt af 4x4 minutters intervaller med intensionen om at ramme 90-95 % af HRmax.

Tabel 3: Skematisk plan for HIIT-interventionen. Fra 'HIIT med Kiloene'-projektet.

Uge	Træning 1	Træning 2	Træning 3
1	Introduktion /cykeltest	Introduktion /cykeltest	Valgfri aktivitetsform / evt. cykeltest
2	Boldspil	Crossfit	Løbemodul
3	Valgfri aktivitetsform	Valgfri aktivitetsform	Valgfri aktivitetsform
4	Valgfri aktivitetsform	Valgfri aktivitetsform	Valgfri aktivitetsform
5	Valgfri aktivitetsform	Valgfri aktivitetsform	Valgfri aktivitetsform
6	Crossfit	Løbemodul	Boldspil
7	Valgfri aktivitetsform	Valgfri aktivitetsform	Valgfri aktivitetsform
8	Valgfri aktivitetsform	Valgfri aktivitetsform	Valgfri aktivitetsform
9	Valgfri aktivitetsform	Valgfri aktivitetsform	Valgfri aktivitetsform
10	Valgfri aktivitetsform	Valgfri aktivitetsform	Valgfri aktivitetsform
11	Løbemodul	Boldspil Påsætning af Axivity	Crossfit
12	Valgfri aktivitetsform	Valgfri aktivitetsform / Cykeltest Aftagning af Axivity	Valgfri aktivitetsform / Cykeltest Afslutning

HIIT er forbundet med flere sundhedsmæssige effekter i børn og unge, herunder normalisering af BMI, fedtmasse, systolisk og diastolisk blodtryk, forbedring af VO_2max samt mindskning af mængden af triglycerider, LDL- og HDL-kolesterol og normalisering af blodglukose og insulinkoncentrationen (Wang et al., 2024). Der er desuden foretaget forskning af HIIT og effekterne på ektopiske fedtdeponeringer, hvilket opridses i afsnit 5, omend forskningslitteraturen er divergerende og mindre omfattende på dette parametre.

3.0 Fedtmetabolismen og deponering af fedt

For at forstå baggrunden for deponering af fedt i kroppens væv, samt effekterne af HIIT og TCOCT på fedtdeponering, følger her en kort gennemgang af *fedtmetabolismen*. Fedt er kroppens primære energikilde, hvorfor optagelse, deponering og nedbrydning af fedt er normale og vigtige processer. Størstedelen af fedtet deponeres som *subkutant fedt (SAT)* i adipocytter placeret under huden. Men fedt kan også deponeres omkring kroppens organer som *visceralt fedt (VAT)* og inde i organerne som *ektopisk fedt*. Eksempelvis kan fedt deponeres i *leveren* og *pancreas* som ektopisk fedt, imens der kan deponeres mindre mængder af ektopisk fedt i muskelvævet. Fedtet deponeres primært som triglycerol (TG) i de forskellige væv. Deponeringen af triglycerol foregår ved, at frie fedtsyrer fra blodet transporteres til adipocytter i fedtvævet eller til non-adipøse celler i organerne og deponeres ved at tre frie fedtsyrer bindes til et glycerolmolekyle. Dette kaldes en esterificering, og processen er katalyseret af enzymet triglycerid-syntase (Frayn et al., 2003).

Øgning af fedtmassen kan forårsages af flere faktorer relateret til livsstil, genetik, hormonelle faktorer, alder og sygdom. Metabolisk energioverskud eller positiv energibalance opstår, når den totale mængde energi, der indtages gennem kosten, overstiger den samlede mængde energi kroppen forbruger i form af hvilemetabolismen, fysisk aktivitet og hormonelle faktorer. I denne situation vil den overskydende energi oftest deponeres som triglycerol i fedtvævet (Frayn et al., 2003).

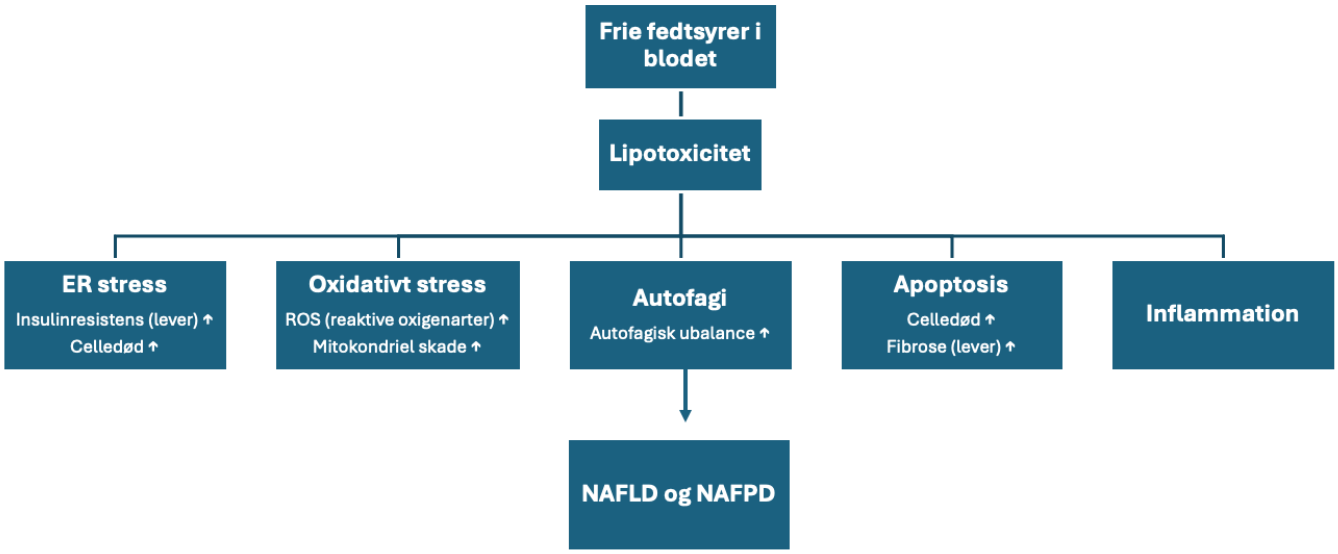
3.1 Beta-oxidation

Fra fedtvævet kan TG igen nedbrydes til glycerol og frie fedtsyrer i en proces kaldet *lipolyse* vha. en række enzymer med fællesbetegnelsen ´lipaser´. Herefter transporteres fedtsyrerne til kroppens celler, via blodbanen, til dannelse af adenosine triphosphate (ATP) ved beta-oxidation (Arner, 2005). Beta-oxidation øges ved situationer med fysisk aktivitet og/eller metabolisk energiunderskud for at dække cellernes energibehov. I en muskelcelle transporteres de frie fedtsyrer fra blodbanen ind over muskelcellemembranen ved at fedtsyren palmintin bindes til proteinet albumin. Optaget til muskelcellen kan ske via tre forskellige transportproteiner (FAT, FATP og FABP). I muskelcellen reagerer palmintin med CoenzymA og danner palmitoyl-CoA (aktiveret fedtsyre). Den aktiverede fedtsyre bindes til carnitin gennem enzymet CPT-1 og transporteres ind i mitokondriet via translokase i membranen. Herefter spalter CPT-2 carnitin og palmitoyl-CoA fra hinanden igen (Shaw et al., 2010).

Efterfølgende beta-oxideres palmitoyl-CoA ved at syrergruppen og 2 kulstofatomer fraspaltes. Denne fraspaltning gentages, hvorved acetyl-CoA dannes og overføres til Kreb´s cyklus og afgiver co-faktorer til elektrontransportkæden. Fedtsyren reduceres på denne måde, indtil der er to kulstofatomer tilbage. Denne proces katalyseres af enzymet Beta-Hydroxy-Acyl-CoA-Dehydrogenase (HAD) (Shaw et al., 2010).

3.2 Lipotoxicitet

I sunde og raske individer er der normalvis ligevægt mellem mængden af frie fedtsyre i blodet samt oxidationen i cellernes mitokondrier og/eller transporten og deponeringen i kroppens adipocytter. I situationer med forhøjede niveauer af frie fedtsyrer i blodet, kan adipocytterne være begrænset i optaget af frie fedtsyrer. For at kompensere optages de frie fedtsyrer derfor i non-adipøse celler placeret i kroppens organer og muskelvæv. I cellerne omdannes de frie fedtsyrer til triglycerider og ophobes gradvist. Kapaciteten for optag af fedtsyrer er i disse celler begrænset, hvor en overskridning af kapaciteten kan føre til cellulær dysfunktion eller celledød. Dette fænomen benævnes *lipotoxicitet* og kan have negative fysiologiske konsekvenser for vævet og individets sundhed. Risikoen for lipotoxicitet øges ved et stort fedtindtag fra kosten, overvægt, genetik og fysisk inaktivitet (Rada et al., 2020). Lipotoxicitet kan forstyrre reguleringen af de oxidative processer i mitokondrierne, der kan resultere i oxidativt stress, celleskader og aktivering af inflammatoriske og apoptotiske signalveje. Desuden kan de naturlige signaleringsveje i endoplasmatisk reticulum (ER) i cellemembranen forstyrres, hvilket blandt andet er associereret med udvikling af insulinresistens. Endeligt kan en konsekvens af øget ektopisk fedtdeponering være en ubalance i de autofagiske processer i lysosomerne, der naturligt nedbryder og regulerer uønskede eller beskadigede dele af cellerne. Denne ubalance øger sandsynligheden for celleskader (Rada et al., 2020), jf. figur 1.



Figur 1: Overblik over lipotoxicitet og de potentielle biokemiske følgevirkninger med betydning for udvikling af *non-alcoholic fatty liver disease (NAFLD)* og *non-alcoholic fatty pancreas disease (NAFPD)*. En øget koncentration af frie fedtsyrer i blodet inducerer lipotoxicitet, der kan medføre stress i endoplasmatisk reticulum, oxidativt stress i mitokondrierne, autofagi, apoptose og inflammatoriske tilstande. ↑ = opreguleres/øges. Figuren er udarbejdet med inspiration fra Rada et al., 2020.

3.3 Subkutan, visceral og ektopisk fedtdeponering

Generelt indikerer litteraturen, at forekomsten af VAT og ektopisk fedt medfører en række risikofaktorer afhængigt af, i hvilke væv ophobningen finder sted, jf. nedenstående. Ophobningen af VAT anses om en markør for ektopisk fedtdeponering, jf. lipotoxicitet. Således viser et studie, at VAT er positivt associeret med ektopisk leverfedt (Fonvig et al., 2015). SAT er ikke relateret til øgede sundhedsmæssige risikofaktorer (Neeland et al., 2018; Deprés et al., 2015).

3.4 Fedtdeponering i leveren og pancreas

Ophobning af frie fedtsyrer i leverceller øger risikoen for udvikling af en række leverlidelser som *ikke-alkoholisk leversygdom (NAFLD)*, steatose (fedtlever), steatohpatitis (inflammation i leveren) og leverfibrose. Dette kan kædes sammen med lipotoxicitet og forekomsten af oxidativt stress i levercellerne, stress i endoplasmatisk reticulum, apoptosis og inflammatoriske tilstande, jf. figur 1 (Rada et al., 2020; Trauner et al., 2010). Hertil er en fedtfraktion i leveren på > 5 % definitionen på NAFLD (Tas et al., 2023). *Alaninaminotransferase (ALT)* og *aspartataminotransferase (AST)* er to enzymer, som ofte anvendes som biomarkører for diverse leversygdomme og skader, hvor koncentrationen af disse i blodbanen er set at være associeret med deponering af fedt i leveren

(Johansen & Gade et al., 2020; Valerie et al., 2022). Enzymerne har en naturlig funktion i aminosyreromsætningen, særligt i levercellerne men også i mindre grad i andre væv. Ved leversygdomme, som NAFLD, hvor levercellerne beskadiges, trænger ALT og AST ud af levercellerne og videre ud i blodbanen og kan herefter påvises via en blodprøve som biomarkør for leverskade (Weiss et al., 2014). De nævnte lidelser og mekanismer kan resultere i udvikling af insulinresistens i levercellerne, som kan være medvirkende til udvikling af type 2 diabetes (Dongiovanni et al., 2018; Taylor et al., 2019).

I pancreas har ophobning af frie fedtsyrer i beta-cellerne en skadelig effekt på evnen til at frigive insulin til blodbanen. Dette sker ved, at beta-cellernes funktionsevne påvirkes eller dør. Dette kan på sigt føre til *non-alcoholic fatty pancreas disease (NAFPD)* med udvikling af diabetes som en mulig konsekvens (e Silva et al., 2020). Overvægt og inaktivitet er en særlig risikofaktor for udvikling af NAFPD (Sepe et al., 2011).

3.5 Leverens og pancreas rolle i udvikling af diabetes som konsekvens af fedtdeponering

Type 2 diabetes kan altså i denne sammenhæng forårsages af henholdsvis insulinresistens i leveren og dysfunktion af beta-cellerne i pancreas. Insulins funktion er at stimulere optag af glukose til levercellerne, hvorfor udvikling af insulinresistens i leveren påvirker reguleringen af glukosemetabolismen. Dette fører til øgede koncentrationer af blodglukose, da leverens optag af blodglukose mindskes samtidig med, at leverens naturlige frigivelse af glukose til blodbanen i mindre grad inhiberes af insulin sammenlignet med normale forhold. Pancreas kompenserer ved at øge koncentrationen af insulin i blodbanen. Samtidig stimulerer insulin omdannelsen af kulhydrat til fedt i leveren, hvor overskydende triglycerider i leveren omdannes til frie fedtsyrer og ophobes i andre væv, eksempelvis beta-cellerne i pancreas. Da dette hæmmer beta-cellernes akutte evne til at producere og frigive insulin, vil konsekvenserne være perioder med forhøjede koncentrationer af blodglukose. Over tid øges frigivelsen af insulin og bidrager til yderligere dannelse af fedt i leveren og deponering af overskydende fedt i andre væv. Denne cyklus fortsætter indtil beta-cellerne ikke kan producere insulin i en mængde, der kompenserer insulinresistensen (Taylor et al., 2019).

Studier indikerer, at cyklussen kan vendes ved at mindske deponeringen af fedt i leveren og i pancreas (Taylor et al., 2019; Lim et al., 2011). Herefter normaliseres levercellernes respons på

insulin og beta-cellernes funktionsevne normaliseres ved redifferentiation. Det viste sig, at 8 ugers regulering af kosten hos overvægtige individer med type 2 diabetes førte til vægttab, mindskning af triglycerider i leveren samt pancreas og bidrog med markante forbedringer af insulinsensitiviteten i leveren (Lim et al., 2011). Derfor anses vægttab og mindskelse af ektopisk fedt som afgørende for normalisering af glukosemetabolismen blandt overvægtige (Taylor et al., 2019; Lim et al., 2011). Hertil er frigivelse af forskellige træningsafhængige cytokiner (benævnt *exerkines*), ex fibroblast growth factor 21 (FGF21) og Interleukin 6 (IL-6), set at modvirke flere af de nævnte mekanismer, som er oplistet i Figur 1 (Wang et al., 2023).

3.6 Fedtdeponering i muskulaturen

En mindre mængde af fedt, sammenlignet med SAT og VAT, kan deponeres i skeletmuskulaturen som triglycerol (ektopisk fedt). Type I muskelfibre indeholder to-tre gange så meget intramuskulært triglycerol sammenlignet med type II muskelfibre. Dette kan forbindes med type I fibrenes øgede oxidative kapacitet, blandt andet til beta-oxidering af frie fedtsyrer (van Loon, 2004).

Deponering af ektopisk fedt i muskulaturen kan føre til muskulær steatose, hvilket kan påvirke muskelcellernes evne til at reagere på insulin ved forstyrrelser i insulinens signaleringsveje til muskelcellen. Dette påvirker optagelsen og omsætningen af glukose lokalt i muskelvævet og er derfor en stærk risikofaktor for udvikling af type 2 diabetes (Shaw et al., 2010). Desuden viser undersøgelser, at fedtdeponering i muskelvævet er positivt korreleret med glykosyleret hæmoglobin (HbA1c). HbA1c er et hæmoglobin-molekyle, hvor et sukkerstof spontant binder sig til. Dette ses eksempelvis ved diabetes. HbA1c øger mængden af reaktive frie radikaler i blodcellerne, hvilket ændrer blodcellernes membraner og funktionsevne. Derfor er fedt i muskulaturen forbundet med øget risiko for kardiovaskulære komplikationer (Fonvig et al., 2015; Saleh, 2015).

4.0 Træningsinducerede mekanismer med betydning for fedtdeponering

Træning kan altså have potentialet til at mindske mængden af ektopisk fedt og VAT i overvægtige børn og unge. Flere mekanismer, induceret af træning, kan være medvirkende til dette. Generelt vil længerevarende aerob træning med moderat intensitet medføre et relativt højt akut energibidrag fra forbrænding af fedt via lipolysen og beta-oxidering, hvorimod denne er lavere ved HIIT. Til gengæld er det samlede energiforbrug, relativt til arbejdstid, større ved HIIT grundet den højere intensitet (Boutcher, 2011). Hertil er efterforbrænding en mekanisme, som ses at være højere efter træning med højere intensiteter. Efterforbrændingen er påvirket af `excess post-excercise consumption` (EPOC), der viser en forhøjet iltoptagelse i timerne efter træning. Dette kan stimulere til øget lipolyse og beta-oxidation (Boutcher, 2011).

4.1 Hormonelt repsons

En række hormoner virker stimulerende på fedtforbrændingen. HIIT har vist sig at øge frigivelsen af katekolaminer, der stimulerer lipolysen (Arner, 2005; Trapp et al., 2007). Katekolaminer er en fællesbetegnelse for en række hormoner og neurotransmittere, bla. adrenalin, nor-adrenalin og dopamin, der ved frigivelse øger nedbrydningen af deponeret fedt og glykogen samt opregulerer blodgennemstrømningen i muskulaturen (Trapp et al., 2007). Der er desuden observeret en øgning af koncentrationen af væksthormon (GH) i blodet efter træning. Denne koncentrationsøgning har en relation til intensitet, hvor højere arbejdsintensiteter øger frigivelsen af GH (Pritzlaff et al., 2000). GH har en katabolsk effekt, hvilket øger efterforbrændingen ved en accelereret fedtoxidation samt forbrændingen af VAT og ektopisk fedt (Pritzlaff et al., 2000; Boutcher, 2011). Derudover bliver hormonerne cortisol og glukagon ligeledes frigivet ved træning og har en opregulerende effekt på lipolysen. I denne proces omdannes triglyceriderne fra fedtvævet til frie fedtsyrer og transporteres via blodbanen til muskelcellerne og beta-oxidation i mitokondrierne. På samme måde beta-oxideres frie fedtsyrer fra kosten og mindsker akkumuleringen af disse i adipocytter og modvirker lipotoxicitet (Kramer, 2023).

Det viser sig, at både HIIT og aerob træning med moderat intensitet over længere arbejdsperioder kan frigive disse lipolytiske hormoner, der bidrager til at øge beta-oxidationen (Brun et al., 2022). Dog er HIIT forbundet med et stort bidrag fra glykolysen til gendannelse af ATP under

muskelarbejdet. Glykolysen er en anaerob proces, der forløber under arbejde med høj intensitet. Ved aktivering af glykolysen frigives enzymet Acetyl-CoA carboxylase, der katalyserer dannelsen af malonyl-CoA. Dette stof inhiberer enzymet CPT-1, således translokasen af frie fedtsyrer gennem mitokondriets membran hæmmes (se afsnit 3.1). Dermed bidrager glykolysen til at reducere beta-oxidationen af frie fedtsyrer, hvor glukose i stedet bliver den primære energikilde (Brun et al., 2022). Dette vil på kort sigt mindske forbrændingen af fedtvæv og opregulerer forbrændingen af allerede tilgængelig glukose.

Det er påvist, at HIIT påvirker appetitreguleringen målt ved angivelse af en såkaldt `sult-score` samt ved rapportering af det samlede energiindtag efter forskellige typer træning med forskellige intensiteter. Det blev påvist, at træning med høj intensitet sænker følelsen af sult og påvirker adfærden, således der indtages mindre energi i tiden efter træning. Appetitreguleringen menes at være påvirket af en øget frigivelse af interleukin-6 og laktat, der igangsætter en række signalkaskader, der nedregulerer neuroner i CNS og fordøjelsessystemet med betydning for appetitregulering (Panissa et al., 2019). Det er muligt, at denne mekanisme nedsætter det samlede energiindtag over tid. Frigivelsen af cytokinet interleukin-6 fra muskelcellerne, efter muskelarbejde med høj intensitet, menes desuden at stimulere til øget lipolyse og deraf forbrænding af VAT og ektopisk fedt (Wedell-Neergaard et al., 2019).

4.2 Fysisk aktivitet til mindskelse og forebyggelse af leverfedt

Ved fysisk aktivitet øges musklernes akutte behov for ATP, hvor leveren danner og frigiver glukose til blodbanen fra glykogendepoterne og via *glukoneogenese (GNG)* for at opretholde blodsukkerkoncentrationen. GNG er en proces, hvor glukose dannes ud fra glycerol, laktat, pyruvat og aminosyrer. Processen kræver energi i form af ATP, hvilken kommer fra *oxidation af leverfedtsyrer (FAO)*. GNG øges gradvist og opreguleres ved længerevarende fysisk arbejde (Wasserman et al., 1991), men der observeres desuden en akut øget frigivelse af glukose fra leveren via GNG ved start af et fysisk arbejde (Trefts et al., 2015). På denne måde bidrager fysisk aktivitet til nedbrydelse af leverfedtsyrer via FAO (Thyfault et al., 2020). Derudover kan regelmæssig fysisk aktivitet på sigt øge den aerobe kapacitet, hvor kendte adaptationer til dette er en øget kapacitet for mitokondriel respiration, der har en positiv effekt på evnen til at omsætte og forbrænde fedtsyrer (jf. afsnit 3.1). Disse effekter er også observeret i levercellerne (Thyfault et al., 2020).

Overordnet er der altså flere mulige fysiologiske og biokemiske faktorer, som potentielt stimuleres af HIIT og har betydning for deponeringen af fedt og forekomsten af lipotoxicitet. Det bør dog påpeges, at ikke alle de involverede mekanismer er undersøgt på børn, hvorfor det ikke med sikkerhed kan vides, hvorvidt de samme mekanismer er gældende i denne målgruppe.

5.0 Træningsstudier og fedtdeponering

Følgende afsnit præsenterer en litteraturgennemgang af forskellige typer af træningsinterventioner og effekten på deponering af ektopisk fedt i lever, pancreas, muskler samt VAT og SAT. Målgruppen er børn og unge, men studier på voksne anvendes som supplement, da forskningen er begrænset på børn angående træningsinterventioner og ektopisk fedt. Alle studier er foretaget på overvægtige individer.

5.1 HIIT samt længerevarende kontinuerlig aerob træning

Studier indikerer, at HIIT-interventioner kan have positiv effekt på deponeringen af ektopisk fedt og VAT hos børn. Andelen af VAT faldt efter 12 uger med 3 ugentlige HIIT-sessioner bestående af 2x8x15 sekunders løb med 90 ~ 100% af maximal aerobic hastighed. Der blev samtidig ikke fundet ændringer i VAT for en gruppe, der udførte 30 minutters kontinuerligt løb på 60 ~ 70% af maximal aerob hastighed. Interventionen blev foretaget på overvægtige drenge (11.2 ± 0.7 år) (Cao et al., 2022).

Over en periode på 4 uger med HIIT 3 gange ugentligt hos overvægtige unge (13-18 år), blev der ikke fundet en effekt af HIIT på koncentrationen af triglycerider i leveren (intrahepatic triglyceride content (IHTG) (Tas et al., 2023). HIIT-protokollen bestod af 10x1 minut på cross-trainer, ergometercykel eller løbebånd med 80-90 % af HRmax. Dog faldt IHTG ved kun at inddrage forsøgsdeltagere med NAFLD, defineret ved en leverfraktion på > 5%, efter 4 ugers HIIT (Tas et al., 2023). Dette kan tyde på, at der kan være en sammenhæng mellem mængden af deponeret ektopisk fedt og træningsinterventioners respons på ektopisk fedt. Davis et al., 2011 undersøgte effekten af 16 ugers cirkeltræning udført to gange om ugen på overvægtige unge (15.8 ± 1.1 år). Cirkeltræningen bestod af en kombination af styrketræningsøvelser og aerob træning på tid. Den samlede træningstid steg progressivt over ugerne fra 60 til 90 minutter og intensiteten blev fastholdt på 70-85 % af HRmax. Sammenlignet med en kontrolgruppe blev SAT, VAT samt markører for insulinresistens signifikant forbedret (Davis et al., 2011)

Modsat findes studier, der indikerer, at effekten af HIIT på deponering af fedt er begrænset. Dias et al., 2017 fandt ingen effekt af HIIT (4x4 minutter, 85-95 % HRmax) og/eller kontinuerlig aerob træning (44 minutter, 60-70 % HRmax) på VAT og SAT efter 3 ugentlige træningssessioner over

12 uger. Studiet blev udført på 7-16-årige overvægtige børn og unge, hvor studiedesignet placerer sig tæt op ad indeværende undersøgelses. Både HIIT-gruppen og gruppen med kontinuerlig aerob træning modtog sideløbende kostvejledning (Dias et al., 2017). Ved anvendelse af interventioner bestående af længerevarende aerob træning med moderat intensitet, fandt Van der Heijden et al., 2010, modsat Cao et al., 2022, en positiv effekt på fedtdeponering over 12 uger. Træningsinterventionen blev udført på unge overvægtige drenge og piger (15.6 +/- 0.4 år), og bestod af 4 x 30 minutter om ugen ved 70% af VO_2-peak. Der blev fundet en effekt på leverfedt og VAT men ikke på intramuskulært fedt. I en kontrolgruppe af normalvægtige alders- og kønsmatchede unge blev der ikke fundet effekter af træningen (Van der Heijden et al., 2010).

Ovennævnte studier er foretaget på børn og unge. Litteraturen er generelt mere omfattende ved undersøgelse af den voksne population. Ved at sammenligne HIIT med træning ved moderat intensitet i to studier i henholdsvis 4 uger (4 minutters intervaller ved 80% af VO_2peak vs. 60 minutter ved 55 % af VO_2peak) og 6 uger (10x1 minut ved 100% af VO_2max vs. 30 minutter ved 65% af VO_2max) blev der ikke fundet ændringer i VAT og SAT (Winn et al., 2018; Gerosa-Neto et al., 2019). Dog observerede Winn et al., 2018 en reduktion af IHTG efter 4 uger ved begge typer træning (Winn et al., 2018). Begge studier matchede træningsgruppernes samlede energiforbrug ved udførsel af træningssessionerne. Modsat fandt Johnson et al., 2009 et signifikant fald i VAT, IHTG og frie fedtsyrer i blodet efter 4 ugers aerob cykling efter de generelle sundhedsanbefalinger (30-45 min, 50-70 % af VO_2peak 3 gange ugentligt). Der blev ikke fundet en effekt på SAT og intramuskulært fedt i dette studie (Johnson et al., 2009).

To nyere metaanalyser konkluderer, at HIIT og aerob træning med høj intensitet er mest effektive for at reducere VAT og SAT (Houttu et al., 2022; Xiaoke et al., 2023). Modsat slår en metaanalyse byggende på RCT-studier fast, at der ikke kan findes betydende forskelle i træningseffekterne mellem HIIT og kontinuerlig aerob træning, når effektmålet er VAT (Kramer, 2023). Litteraturen angiver altså blandede effekter af træning på fedtdeponeringer, og generelt er litteraturen begrænset, hvad angår trænings effekt på ektopisk fedt blandt børn og unge. Det skal bemærkes, at der er forskel på interventionernes varighed, træningsmodaliteter samt metoder til kvantificering af fedtdeponeringer, hvilket der bør tages højde for i en sammenligning af de forskellige forskningsresultater.

6.0 Uddybning af metode

I det følgende uddybes en række af de metoder, der er anvendt i projektet, og afsnittet vil således supplere artiklens metodeafsnit. Afsnittet berører BMI z-score, uddyber og sammenligner forskellige metoder og analysemetoder til at kvantificere fedtdeponeringer, samt uddyber indsamlingen af aktivitetsdata og udførelsen af de statistiske tests.

6.1 BMI z-score

Til vurdering af vægtstatus blev der i projektet anvendt *BMI z-score*, da denne vurderer BMI i forhold til den øvrige population af børn og unge. Som referenceramme anvendtes WHO Growth Chart fra 2006 (WHO, 2006). BMI z-score blev beregnet ud fra følgende formel:

$$z\_score = \frac{(\mathrm{X} - \mathrm{m})}{\mathrm{SD}}$$

, hvor X er den observerede værdi (BMI) mens m og SD er henholdsvis gennemsnittet og standardafvigelsen fra referencepopulationen (Martinez-Millana et al., 2018). Ved en normalfordelt referencepopulation bliver z-scoren et mål for afstanden fra den givne BMI-værdi til gennemsnittet for populationen. Således er en z-score på +1 én standardafvigelse over gennemsnittet, mens en z-score på -1 er én standardafvigelse under gennemsnittet (Martinez-Millana et al., 2018). Et individ med en positiv BMI z-score repræsenterer derfor et BMI, der er højere end gennemsnittet. Fordelen ved anvendelsen af BMI z-score til børn og unge er, at modellen, via dens reference til den øvrige population, tager højde for alder, køn og vækst (WHO, 2006).

6.2 Metoder til kvantificering af fedtdeponeringer

Til at kvantificere deponeringen af fedt i de forskellige væv, er data i dette projekt indsamlet ved Dixon *magnetic resonance imaging (MRI)*. Den originale Dixon MRI fungerer ved at bruge to tværsnitsbilleder af et væv med en modificeret spin-echo impulssekvens, en med vand- og fedtsignaler i fase og en impulssekvens med signalerne ude af fase med 180°. På grund af sekvensforskellene mellem fedt og vand bliver det muligt at kvantificere mængden af fedt i kroppens forskellige væv (Ma, 2008).

Nuclear magnetic resonance spectroscopy (benævnt MRS eller 1H-MRS) er også en ikke-invasiv metode. I denne måling dannes et magnetfelt omkring det undersøgte område, mens fotoner tilføjes. Dette gør det muligt at se strukturen af molekylerne pba. frekvensen på de returnerede fotoner. I stedet for at skabe et billede, som Dixon MRI, skaber det et spektrum af forskellige molekyler i det undersøgte område (Gujar et al., 2005).

Det er også muligt at bestemme mængden af fedt i de forskellige væv ved biopsier, som til gengæld er en invasiv metode. Anvendelsen af biopsier anses for at være golden standard inden for kvantificering af fedt i væv (Bohte et al., 2011). En meta-analyse af Bohte et al., 2011 har forsøgt at undersøge forskellige metoder til påvisning af leverfedt, herunder MRI, MRS og leverbiopsi. De fandt, at MRI og MRS er nøjagtige metoder til at give en medicinsk vurdering af leversygdommen hepatisk steatose, som diagnosticeres ud fra mængden af fedt i leveren. Ovenstående resultater indikerer derfor, at MRI er en acceptabel metode til at måle mængden af leverfedt (Bohte et al., 2011).

6.3 MRI: Imaging analysis

Til analyse af scanningsbillederne fra MRI blev *proton density fat fractions (PDFF)* estimeret ved at anvende gennemsnitsværdier af en række *regions of interests (ROIs)*. ROIs blev indtegnet vha. af softwaren PACS Vitrea Read (v.8.3.53-55, Canon Medical Informatics Inc., Minnetonka, Minnesota, USA). Antallet af identificerede områder af det undersøgte væv, som indgik i gennemsnittet, varierede mellem de forskellige undersøgte væv. Leverfedt blev estimeret ud fra fire ROIs i henholdsvis det anterior, posterior, mediale og laterale segment af leveren med en gennemsnitlig størrelse på 8.2 ± 1.8 cm^2 pr. ROI (se figur 2). Fedt i pancreas blev estimeret ud fra tre ROIs: én i toppen, én i midten og én i bunden af pancreas med en gennemsnitlig størrelse på 0.7 ± 0.3 cm^2 (se figur 3). Ektopisk fedt i *psoas-musklen* blev estimeret ved manuelt at indtegne musklen (freedraw). VAT og SAT blev estimeret ved at analysere den fremkomne PDFF via en semi-automatisk software udviklet i MatLab (The MathWorks Inc., Natick, MA, USA) (Kipp, 2019). VAT bestod af fedtvævet i bughulen (fraregnet fedt i organerne og rygmusklerne), mens SAT bestod af fedtvævet placeret på ydersiden af de abdominale muskler (se figur 4).

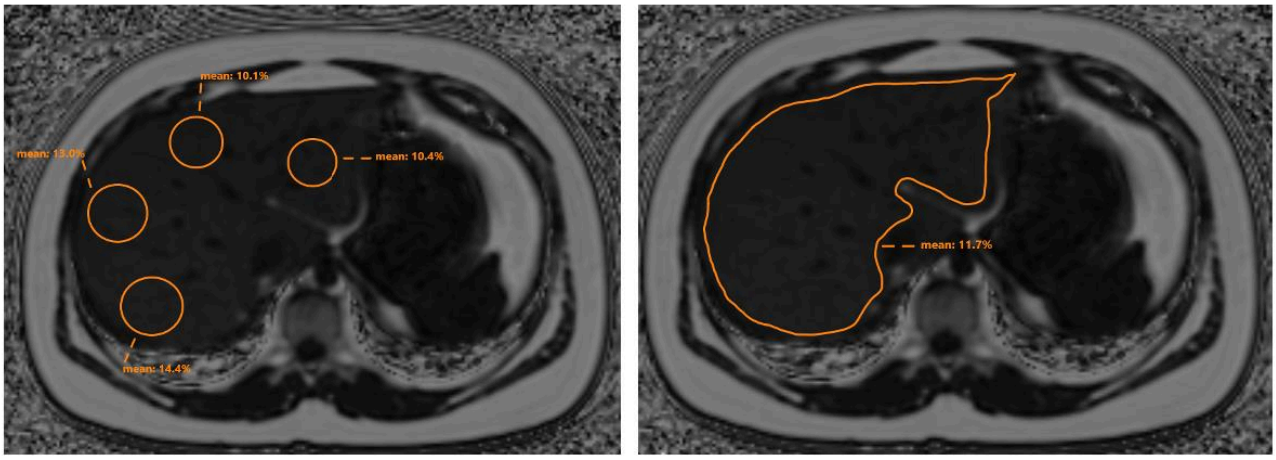
Som et alternativ til kvantificering af fedtdeponeringerne ved indtegnelse af ROIs og beregning af mean, kan de undersøgte væv analyseres ved manuelt at indtegne hele vævet (*freedraw*). De to

metoder er illustreret på figur 2 for leveren og figur 3 for pancreas. De mest præcise estimeringer af fedtdeponeringer i leveren indikeres dog at opnås ved at indtegne så stort et areal af leveren som muligt, eksempelvis ved at indtegne så store ROIs som muligt eller øge antallet af ROIs, dog med sigte på at undgå at indtegne større blodkar (Campo et al., 2017).

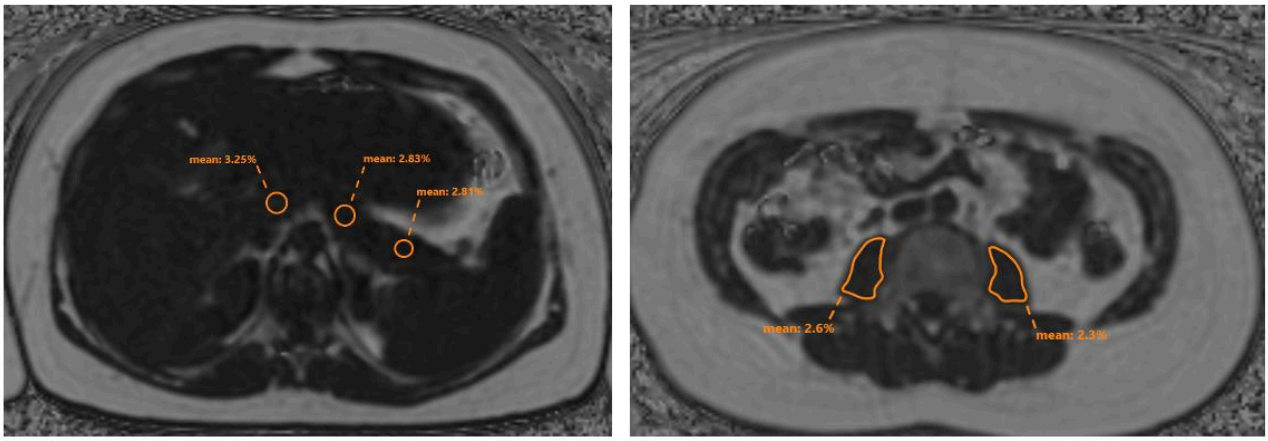
MRI-scanningerne fra leveren og pancreas blev i dette projekt indledningsvist analyseret med begge metoder (mean og freedraw), hvor kun *mean* blev anvendt i de endelige statistiske analyser. Ved statistisk at sammenligne de to metoders resultater på fedtdeponeringer på dette projekts datagrundlag ved hjælp af en *Spearman's Rank Order Correlations*, fandtes en god overensstemmelse mellem de to metoder (se tabel 4). Derfor må begge metoder anses som anvendelige. Desuden blev metoderne sammenlignet vha. en *intraclass-correlation-coefficient (ICC)* analyse. ICC-analysen blev udført som *two-way random effect, absolute agreement* for at sammenligne forskellige målemetoders resultater på de samme subjekter (Terry & Mae, 2016). Metoden viste en fremragende enighed mellem metoderne (jf. tabel 4) vurderet ud fra skalaen af Terry & Mae, 2016, hvor en ICC på 0,00-0,49 indikerer en svag pålidelighed, 0,50-0,74 en moderat pålidelighed, 0,75-0,89 en god pålidelighed og 0,90-1 indikerer en fremragende pålidelighed (Terry & Mae, 2016).

Tabel 4: Sammenligning af de to metoder (mean og freedraw) ved Spearman's Rank Order Correlations og ICC. Præsenteret som mean ± SD. *p<0.05

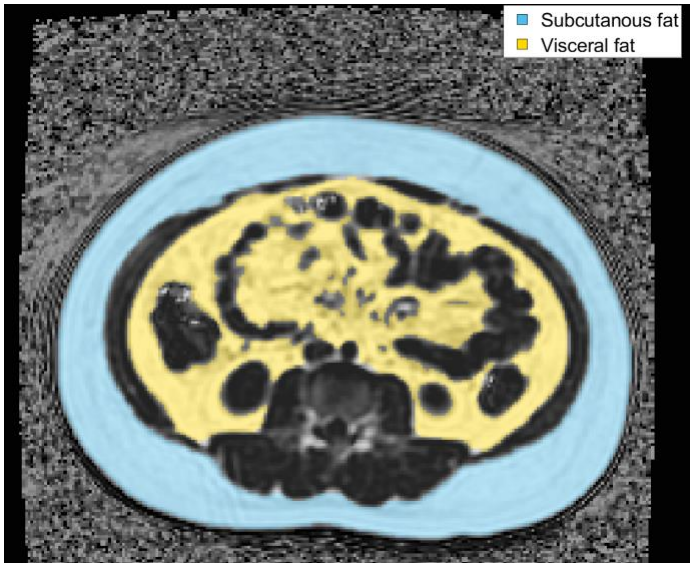
	Methods		**Spearman's Rank Order Correlation**		**Intraclass Correlation Coefficient**
	Mean	**Freedraw**	**r**	**p**	**ICC**
Hepatic fat fraction (%)	6.1 ± 0.9	6.1 ± 0.9	r=0.995	p=0.001*	1.000
Pancreatic fat fraction (%)	4.8 ± 0.4	4.6 ± 0.4	r=0.965	p=0.001*	0.992



Figur 2: PDFF fra MRI-scanninger af leveren. Til venstre ses metoden *mean* med indtegnelse af fire ROI's. Til højre ses metoden *freedraw*.



Figur 3: PDFF fra MRI-scanninger af pancreas. Til venstre ses metoden *mean* med indtegnelse af tre ROI's. Til højre ses metoden *freedraw*.



Figur 4: Illustration af VAT (gul markering) og SAT (blå markering) på baggrund af MRI.

6.4 Måling af aktivitetsniveau - Axivity

Til kvantificering af deltagernes aktivitetsniveau blev et tri-aksialt accelerometer (*Axivity*, *AX3*, Newcastle, UK) anvendt med placering på låret. Det vurderes, at placeringen på låret giver en mere nøjagtig måling og genkendelse af forskellige typer aktiviteter sammenlignet med en placering på håndleddet (Toftager & Brønd, 2019). Måling af acceleration til kvantificering af aktivitetsniveau er relaterbart til energiforbrug, hvor intensitet, varighed og frekvens registreres.

Måleenheden for accelerometrets data er *counts per minute* (cpm) og disse målinger blev konstant summeret over 10 sekunders perioder. Axivity målte med en frekvens på 50 HZ. Aktivitetsniveauet blev klassificeret ud fra forskellige forudbestemte intensitetsintervaller på baggrund af cpm. Intensitetsintervallernes størrelse (i cpm) er estimeret via et kalibreringsstudie (ikke udgivet, men beskrevet i Brønd et al., 2019) på børn, hvor deltagerne udførte forskellige aktiviteter med samtidig måling af accelerationer samt energiforbrug ved kalorimetri. På denne måde bliver de målte accelerationer relateret til et estimeret energiforbrug (Toftager & Brønd, 2019). Denne metode muliggør at medregne energiforbrug ved kortere perioder med inaktivitet, som ofte forekommer under aktiviteter (ex boldspil og crossfit). Almindelig accelerometri medregner ikke disse inaktive pauser som bidragende til det samlede aktivitetsniveau, men energiforbruget vil i disse situationer være forhøjet grundet EPOC (Toftager & Brønd, 2019). Således blev cut-points med det refererende energiforbrug (% af VO_2max) defineret som sedentary (≤100 cpm; 30 % VO_2max), light (101-4970 cpm; 30-40 % VO_2max), moderate (≥4.971 cpm; >40 % VO_2max) og vigorous (≥8.452 cpm) (Toftager & Brønd, 2019). Processeringen og klargøring af data fra Axivity blev til dette projekt udført af Syddansk Universitet i softwaren MatLab (The MathWorks Inc., Natick, MA, USA).

I undersøgelsen blev aktivitetsniveauet beregnet som et gennemsnit over 7 sammenhængende dage. Ud af en interventionsperiode på 12 uger, udgør målingerne på 7 dage en forholdsvis lille del af det samlede akkumulerede fysiske aktivitetsniveau. Derfor er det nærliggende at diskutere, hvorvidt 7 dage giver et validt estimat af det samlede fysiske aktivitetsniveau, når der må forventes dag-til-dag variationer eller periodevise variationer i et individs aktivitetsmønstre. En undersøgelse (Trost et al., 2000) slog fast, at børn (yngste skoleklasser i USA) udviste færre dag-til-dag variationer i MVPA sammenlignet med unge (ældste skoleklasser i USA) målt med accelerometre. Hertil var målinger i 4-5 dage tilstrækkeligt hos børn og 8-9 dage hos unge for at opnå en reliabilitets-koefficient på 0.8, der i undersøgelsen anslås som tilstrækkelig for opnå et validt estimat af aktivitetsniveauet over tid.

Ved at inddrage både børn og unge i undersøgelsen (alle klassetrin), var 7 dage tilstrækkeligt, hvorfor indeværende undersøgelses 7 dages målinger med Axivity må anses som acceptabelt (Trost et al., 2000). Inddragelsen af både hverdage og weekenddage er tiltænkt at afdække en almindelige 7 dages uge bedst muligt. Trost et al., 2000 fandt netop forskelle i aktivitetsniveauet mellem weekenddage og hverdage hos børn og unge, hvorfor dette parametre er vigtigt at tage højde for (Trost et al., 2000).

6.5 Uddybning af statistiske tests

Følgende afsnit uddyber de statistiske analyser udført i projektet. Alle analyser blev udført i SPSS (IBM Corp. Released 2021. IBM SPSS Statistics for IOS, Version 28.0. Armonk, NY: IBM Corp). Alt data blev indledningsvist testet for, om det afviger signifikant fra en normalfordeling ved hjælp af en *Shapiro-Wilk test* (statistics.laerd.com (a)). Herefter gennemgik de forskellige data parametriske og non-parametriske tests på baggrund af normalfordelingstestens udfald, hvilke beskrives herunder.

6.5.1 Statisk test for variabilitet

For at undersøge en mulig variabilitet mellem grupperne ved baseline, gennemgik baselinedata *uparrede t-tests*, da de enkelte Shapiro-Wilk tests viste, at data var normalfordelt. Mens ikke-normalfordelt data gennemgik en *Mann- Whitney test* som non-parametrisk t-test (statistics.laerd.com (b; c)). Dette blev gjort for at teste, om der var en variabilitet mellem grupperne i studie 2 ved baseline på de forskellige måleparametre, da dette kan influere resultaterne (Moher et al., 2012).

6.5.2 Two-Way ANOVA

I studie 1 blev en *Two-Way ANOVA* anvendt til at sammenligne grupperne samt eventuelle forskelle mellem køn. Testen sammenholder to uafhængige variabler (gruppe og køn) samt én afhængig variabel (fedtdeponering eller aktivitetsniveau) (statistics.laerd.com (d)). I denne test beregnes *main effects* for gruppe, hvilken indikerer om der er signifikante forskelle mellem normal- og overvægtige, samt main effects for køn, hvilken indikerer forskelle mellem køn uafhængigt af gruppe. Desuden testes for en interaktionseffekt mellem gruppe og køn, der angiver, om der er signifikante forskelle mellem grupperne, når køn er med som en faktor.

6.5.3 Two-Way ANOVA Repeated measures

I studie 2 blev der anvendt en *Two-Way ANOVA Repeated measures* (også kaldt *Mixed ANOVA*) med to uafhængige variabler (gruppe og tid) samt én afhængig variabel (fedtdeponering eller aktivitetsniveau). Således fungerer gruppe som *between factor* samt tid som *with-in factor*. En Two-Way ANOVA Repeated measures angiver, om der findes en signifikant interaktionseffekt mellem den angivne *between factor* og *within factor* på den afhængige variabel (statistics.laerd.com (e)).

6.5.4 Korrelationsanalyser

Der blev i projektet udarbejdet korrelationsanalyser for at undersøge sammenhængen mellem fedtdeponeringer og aktivitetsniveau (studie 1) samt ændringer i fedtdeponeringer og ændringer i aktivitetsniveau (studie 2). Til dette blev *Spearman's Rank Order Correlation tests* anvendt, da data ikke at var normalfordelt (statistics.laerd.com (f)). Til medicinsk og fysiologisk data, har Hopkins et al., 2009 udarbejdet en modificeret skala til vurdering af korrelationskoefficienter (r). Den angiver, at når $r < 0.1$ er der tale om en meget lille sammenhæng; 0.1–0.3 lille-; 0.3–0.5 moderat-; 0.5–0.7 stor-; 0.7–0.9 meget stor-; 0.9 næsten perfekt-; og endelig 1.0 perfekt sammenhæng (Hopkins et al., 2009).

7.0 Resultater (komplet samling)

Herunder følger en komplet samling af projektets resultater fra de statiske analyser. Alt data er præsenteret i tabeller. I det vedhæftede bilag (Bilag 1) findes det data, de statistiske analyser er foretaget på baggrund af.

7.1 Study 1

Table 5: Comparisons of BMI z-score and fat deposition between normal weight and obese boys and girls. Presented as mean ± SD. P-values from a Two Way ANOVA. *p<0.05

	Obese		Normal weight		group x sex	group	Sex
	Boys (n=33)	Girls (n=15)	Boys (n=18)	Girls (n=12)			
BMI z-score	2.7 ± 0.6	2.7 ± 0.7	-0.1 ± 0.7	-0.1 ± 0.8	0.927	0.001*	0.749
Hepatic fat (%)	6.9 ± 7.3	5.4 ± 6.5	1.6 ± 0.4	2.0 ± 1.0	0.475	0.002*	0.766
Pancreatic fat (%)	4.6 ± 2.7	5.6 ± 3.0	1.5 ± 0.5	1.9 ± 0.9	0.591	0.001*	0.236
Psoas fat (%)	2.6 ± 1.6	3.4 ± 1.5	1.0 ± 0.6	1.1 ± 0.7	0.313	0.001*	0.420
VAT (cm^2)	76.0 ± 8.9	61.4 ± 42.5	9.7 ± 8.9	11.7 ± 8.6	0.265	0.001*	0.107
SAT (cm^2)	313.6 ± 94.1	302.9 ± 102.5	38.1 ± 28.2	65.6 ± 43.9	0.331	0.001*	0.103

Table 6: Comparisons of activity level between normal weight and obese boys and girls. Presented as mean ± SD. P-values from a Two Way ANOVA. *p<0.05

	Obese		Normal weight		group x sex	group	sex
	Boys (n=32)	Girls (n=15)	Boys (n=17)	Girls (n=12)			
Sedentary	421.2 ± 79.7	457.4 ± 86.2	476.7 ± 101.9	430.8 ± 61.8	0.061	0.504	0.823
Light	211.3 ± 66.4	233 ± 61.7	230.4 ± 58.9	243.1 ± 48.1	0.765	0.335	0.256
Moderate	32.7 ± 18.0	31.1 ± 12.2	53.3 ± 21.2	39.3 ± 18.0	0.159	0.001*	0.078
Vigorous	8.2 ± 8.8	11.6 ± 8.6	24.6 ± 13.1	23.5 ± 15.8	0.416	0.001*	0.664
Sitting	516.0 ± 110.0	459.8 ± 69.3	445.4 ± 62.9	457.2 ± 76.8	0.123	0.098	0.313
MVPA	40.9 ±22.0	42.7 ±18.8	77.9 ±33.1	62.8±30.3	0.184	0.001*	0.297

Table 7: Correlations between activity level and fat deposition in normal weight and obese children and adolescents. r-values and p-values from a Spearman's rank order correlation test. N=75. *p<0.05

	Hepatic fat (%)		Pancreatic fat (%)		Psoas (%)		VAT (cm^2)		SAT (cm^2)	
	r	P	r	P	r	P	r	P	r	P
BMI z-score	0.721	0.001*	0.656	0.001*	0.693	0.001	0.881	0.001*	0.904	0.001*
Sedentary	-0.207	0.322	0.047	0.824	0.299	0.177	0.172	0.412	-0.052	0.807
Light	0.189	0.365	-0.006	0.978	-0.046	0.838	-0.211	0.312	-0.120	0.568
Moderate	-0.154	0.453	0.023	0.912	0.349	0.103	-0.017	0.933	0.147	0.473
Vigurous	-0.222	0.286	-0.330	0.107	0.057	0.801	-0.373	0.066	-0.255	0.218
MVPA	-0.406	0.001*	-0.433	0.001*	-0.411	0.001*	-0.485	0.001*	-0.510	0.001*
Sitting	-0.251	0.226	-0.177	0.397	0.171	0.446	0.192	0.359	-0.001	0.997

Table 8: Correlations between activity level and fat deposition in obese children and adultescents. r-values and p-values from a Spearman's rank order correlation test. N=46. *p<0.05

	Hepatic fat (%)		Pancreatic fat (%)		Psoas (%)		VAT (cm^2)		SAT (cm^2)	
	r	P	r	P	r	P	r	P	r	P
BMI z-score	0.573	0.001*	0.215	0.137	0.361	0.012*	0.690	0.001*	0.703	0.001*
Sedentary	0.254	0.089	0.082	0.598	-0.187	0.229	0.214	0.159	0.295	0.049*
Light	-0.354	0.016*	-0.195	0.205	0.019	0.903	-0.286	0.057	-0.380	0.010*
Moderate	-0.125	0.408	-0.390	0.009*	-0.032	0.837	-0.297	0.047*	-0.302	0.044
Vigurous	-0.380	0.009*	-0.273	0.073	-0.007	0.964	-0.388	0.008*	-0.476	0.001
MVPA	-0.119	0.430	-0.242	0.114	-0.224	0.144	-0.205	0.176	-0.256	0.090
Sitting	0.370	0.011*	0.105	0.496	-0.139	0.375	0.357	0.016*	0.300	0.045

7.2 Study 2

Table 9: Effect of TCOCT+HIIT and TCOCT on fat deposition, BMI z-score and VO_2peak. Presented as mean ± SD. P-values from a Two-Way ANOVA Repeated measures. *p<0.05.

	TCOCT+HIIT (n=21)		TCOCT (n=20)		time x group	group	time
	Pre	Post	Pre	Post			
BMI z-score	2.67 ± 0.7	2.55 ± 0.7	2.66 ± 0.5	2.57 ± 0.6	0.678	0.969	0.001*
Hepatic fat (%)	6.7 ± 9.4	6.3 ± 7.9	5.5 ± 4.6	4.5 ± 3.9	0.465	0.483	0.045*
Pancreatic fat (%)	5.4 ± 3.0	5.1 ± 2.9	3.5 ± 2.0	3.2 ± 1.7	0.865	0.019*	0.007*
Psoas fat (%)	3.4 ± 2.0	3.0 ± 1.72	2.2 ± 0.9	1.9 ± 0.8	0.629	0.014*	0.063
VAT (cm^2)	72.3 ± 34.6	66.9 ± 34.8	66.0 ± 38.2	59.2 ± 30.7	0.819	0.504	0.045*
SAT (cm^2)	319.5 ± 106.9	313.1 ± 115.5	278.7 ± 79.8	270.3 ± 91.3	0.904	0.172	0.364
VO_2peak (ml/min/kg)	23.8 ± 5.9	25.6 ± 7.0	25.3 ± 4.2	26.4 ± 4.3	0.406	0.511	0.001*

Table 10: Effect of TCOCT+HIIT and TCOCT on fat deposition, BMI z-score and VO_2peak **in children and adolescents with NAFLD at baseline** (hepatic fat > 5%). Presented as mean ± SD. P-values from a Two-Way ANOVA Repeated measures. *p<0.05.

	TCOCT+HIIT (n=10)		TCOCT (n=7)		time x group	group	time
	Pre	Post	Pre	Post			
Hepatic fat (%)	11.1 ± 12.4	9.84 ± 10.5	11.1 ± 2.9	8.5 ± 4.5	0.386	0.887	0.026*
BMI z-score	3.16 ± 0.58	3.09 ± 0.55	2.90 ± 0.39	2.81 ± 0.47	0.745	0.294	0.045*
Pancreatic fat (%)	6.1 ± 3.3	5.8 ± 3.2	4.2 ± 1.5	3.5 ± 1.3	0.369	0.130	0.064
Psoas fat (%)	4.0 ± 2.5	3.8 ± 2.0	2.4 ± 1.0	1.9 ± 1.1	0.710	0.057	0.390
VAT (cm^2)	95.1 ± 24.6	90.7 ± 25.8	98.9 ± 41.7	87.1 ± 29.4	0.578	0.994	0.240
SAT (cm^2)	374.0 ± 90.8	384.0 ± 81.1	297.1 ± 70.0	284.9 ± 87.5	0.479	0.036*	0.942
VO_2peak (ml/min/kg)	20.2 ± 3.7	21.5 ± 5.7	22.4 ± 4.2	24.5 ± 5.0	0.640	0.270	0.046*

Daily activity level

Table 11: Effect of TCOCT+HIIT and TCOCT on activity levels. Presented as mean ± SD. P-values from a Two-Way ANOVA Repeated measures. *p<0.05

	TCOCT + HIIT (n= 18)		TCOCT (n= 15)		time x group	group	time
	Pre	Post	Pre	Post			
Sedentary	436.3 ± 88.1	475.5 ± 71.4	477.8 ± 58.7	451.6 ± 90.3	0.108	0.777	0.66
Light	216.9 ± 70.7	238.3 ± 58.6	232.8 ± 68.9	207.4 ± 53.9	0.036*	0.92	0.487
Moderate	29.2 ± 14.0	35.9 ± 18.3	33.6 ± 17.4	35.7 ± 15.9	0.555	0.292	0.59
Vigorous	8.1 ± 8.1	12.1 ± 11.1	9.6 ± 8.3	11.5 ± 11.0	0.513	0.328	0.768
Sitting	466.6 ± 119.6	506.6 ± 63.0	531.4 ± 88.8	496.0 ± 137.0	0.106	0.939	0.242
MVPA	37.3 ± 22.1	48.0 ± 29.4	43.2 ± 25.7	47.2 ± 26.9	0.444	0.245	0.61

Table 12: Correlations between changes (Δ) in activity level and changes in BMI z-score, fat deposition and VO_2peak in normal weight and obese children and adolescents. r-values and p-values from a Spearman's rank order correlation test. *p<0.05. n=30.

	Δ Hepatic fat (%)		Δ Pancreatic fat (%)		Δ Psoas (%)		Δ VAT (cm^2)		Δ SAT (cm^2)	
	r	P	r	P	r	P	r	P	r	P
Δ BMI z-score	0.311	0.065	-0.045	0.795	0.042	0.808	0.476	0.003*	0.525	0.001*
Δ Sedentary	0.067	0.732	0.098	0.612	-0.055	0.775	0.037	0.847	0.159	0.395
Δ Light	-0.019	0.921	-0.261	0.171	0.043	0.825	-0.044	0.825	-0.362	0.054
Δ Moderate	0.240	0.202	-0.293	0.116	0.150	0.428	-0.050	0.793	-0.161	0.395
Δ Vigurous	-0.192	0.319	-0.182	0.345	0.153	0.427	-0.212	0.270	-0.212	0.270
Δ Sitting	0.177	0.358	-0.067	0.729	-0.093	0.632	0.076	0.696	0.296	0.120
Δ MVPA	0.171	0.367	-0.230	0.222	0.246	0.190	-0.038	0.840	-0.143	0.449
Δ VO_2peak	0.171	0.327	0.046	0.794	0.308	0.092	-0.025	0.889	-0.089	0.613

Deltagelsesrate

Følgende supplerende analyse, er udført som en undersøgelse af deltagelsesraten i HIIT-sessionerne, og dets betydning for udviklingen af ektopisk fedt, BMI z-score og VO_2peak hos TCOCT+HIIT. Deltagelsesraten (%) er udregnet som andelen af gennemførte HIIT-sessioner planlagt over de 12 uger (37-38 HIIT-sessioner). Der blev udført en Two-Way ANOVA Repeated measures med gruppe som 'between factor' samt tid som 'with-in factor'. Grupperne blev opdelt efter en cut-off værdi på 70 % for deltagelsesraten. Den gennemsnitlige deltagelsesrate i HIIT-sessionerne for TCOCT+HIIT var 74 ± 20 %. Ifølge Tabel 13 blev der ikke fundet en interaktionseffekt mellem gruppe (deltagelsesrate) og tid (præ-test til post-test). Det er muligt, at en lille statistisk power i denne analyse influerer resultatet.

Table 13: Effect of attendance rate (TCOCT+HIIT) on fat deposition, BMI z-score and VO_2peak. Presented as mean ± SD. P-values from a Two-Way ANOVA Repeated measures. *p<0.05.

	Attendance rate >70% (n=17)		Attendance rate <70% (n=4)		time x group	group	time
	Pre	Post	Pre	Post			
BMI z-score	2.7 ± 0.75	2.6 ± 0.8	2.6 ± 0.5	2.4 ± 0.7	0.351	0.795	0.015*
Hepatic fat (%)	7.1 ± 10.4	6.4 ± 8.7	5.2 ± 2.3	5.6 ± 3.4	0.474	0.784	0.807
Pancreatic fat (%)	5.2 ± 3.2	4.9 ± 3.0	6.3 ± 2.4	5.7 ± 2.6	0.574	0.573	0.098
Psoas fat (%)	3.6 ± 2.1	3.2 ± 1.8	2.3 ± 0.7	1.6 ± 0.7	0.746	0.200	0.264
VAT (cm^2)	74 ± 38	69 ± 37	62 ± 8	55 ± 23	0.895	0.484	0.227
SAT (cm^2)	324 ± 113	319 ± 118	300 ± 89	287 ± 117	0.808	0.649	0.611
VO_2peak (ml/min/kg)	24.8 ± 6.1	27.1 ± 6.6	20.0 ± 3.9	20.1 ± 6.0	0.102	0.066	0.083

8.0 Perspektiver og fremtidige undersøgelser

Studie 1 viste, at overvægt er forbundet med øget ektopisk fedtdeponering i leveren, pancreas og psoas-musklen i børn og unge. Dette er forbundet med øget risiko for komplikationer (NAFLD, NAFPD, insulinresistens, nedsat beta-celle funktion mv.). Derfor er det vigtigt med interventioner, der kan medføre en reduktion af ektopiske fedtdeponeringer. TCOCT har længe vist sig velegnet til vægttab (Holm et al., 2011), og studie 2 tyder på, at TCOCT har effekt på ektopisk fedt. Disse resultater bakker altså op om, at TCOCT er en effektiv metode til at fremme sundheden blandt børn og unge med overvægt.

Tilføjelsen af HIIT til TCOCT viste sig ikke at øge effekten og øgede samtidigt heller ikke børnenes aktivitetsniveau målt på MVPA. Fremtidige undersøgelser af effekten af HIIT på ektopisk fedt kan med fordel fokusere på HIIT interventioner over længere perioder end 12 uger for at undersøge, om tidsperspektivet influerer på responset. Hertil kan det være relevant at tilføje en interventionsgruppe, der gennemfører en HIIT-intervention uden TCOCT, for at undersøge den isolerede effekt af HIIT på ektopisk fedt hos overvægtige børn og unge, da forskningen er begrænset på dette område, jf. afsnit 5. Dette kan desuden give flere perspektiver via en direkte sammenligning af effekten fra henholdsvis HIIT og TCOCT, hvor andre livsstilsfaktorer, introduceret i TCOCT, formentlig i mindre grad influerer resultaterne i HIIT-gruppen. Der kan også spekuleres i, om valget af 4x4 minutter er den mest effektive. Andre protokoller har fokuseret på kortere arbejdsperioder, eksempelvis 15 sekunders løbeintervaller (Cao et al., 2022) og 10x1 minut på cross-trainer, ergometercykel eller løbebånd med 80-90 % af HRmax (Tas et al., 2023). Fremtidige undersøgelser kan undersøge effekten af forskellige arbejdsintervaller og intensiteter.

Endelig kan pubertetsstatus påtænkes at have betydning for deponering af ektopisk fedt, da pubertets- og vækststatus har betydning for produktionen af kønshormoner og væksthormoner (Roemmich et al., 1998). Fremtidige undersøgelser af TCOCT og HIIT kan inddrage pubertetsstatus som en faktor i undersøgelsen af ektopisk fedt blandt børn og unge, da forskningslitteraturen er begrænset, hvad angår denne faktor.

9.0 Litteratur til arbejdsblade

Arner, P. (2005). Human fat cell lipolysis: Biochemistry, regulation and clinical role. *Best Practice & Research Clinical Endocrinology & Metabolism, 19*(4), 471-482. doi:10.1016/j.beem.2005.07.004

Bohte, A. E., van Werven, J. R., Bipat, S., & Stoker, J. (2011). The diagnostic accuracy of US, CT, MRI and 1H-MRS for the evaluation of hepatic steatosis compared with liver biopsy: A meta-analysis. *European Radiology, 21*(1), 87-97. doi:10.1007/s00330-010-1905-5

Boutcher, S. H. (2011). High-intensity intermittent exercise and fat loss. *Journal of Obesity, 2011*, 1-10. doi:10.1155/2011/868305

Brønd, J. C., Aadland, E., Andersen, L. B., Resaland, G. K., Andersen, S. A., & Arvidsson, D. (2019). The ActiGraph counts processing and the assessment of vigorous activity. *Clinical Physiology and Functional Imaging, 39*(4), 276-283. doi:10.1111/cpf.12571

Brun, J., Myzia, J., Varlet-Marie, E., Raynaud de Mauverger, E., & Mercier, J. (2022). Beyond the calorie paradigm: Taking into account in practice the balance of fat and carbohydrate oxidation during exercise? *Nutrients, 14*(8), 1605. doi:10.3390/nu14081605

Campo, C. A., Hernando, D., Schubert, T., Bookwalter, C. A., Pay, A. J. V., & Reeder, S. B. (2017). Standardized approach for ROI-based measurements of proton density fat fraction and R2 in the liver. *American Journal of Roentgenology, 209*(3), 592-603. doi:10.2214/ajr.17.17812

Cao, M., Tang, Y., & Zou, Y. (2022). Integrating high-intensity interval training into a school setting improve body composition, cardiorespiratory fitness and physical activity in children with obesity: A randomized controlled trial. *Journal of Clinical Medicine, 11*(18), 5436. doi:10.3390/jcm11185436

Chen, X., He, H., Xie, K., Zhang, L., & Cao, C. (2024). Effects of various exercise types on visceral adipose tissue in individuals with overweight and obesity: A systematic review and network meta-analysis of 84 randomized controlled trials. *Obesity Reviews, 25*(3), e13666-n/a. doi:10.1111/obr.13666

DAVIS, J. N., GYLLENHAMMER, L. E., VANNI, A. A., MEIJA, M., TUNG, A., SCHROEDER, E. T., et al. (2011). Startup circuit training program reduces metabolic risk in latino adolescents. *Medicine & Science in Sports & Exercise, 43*(11), 2195-2203. doi:10.1249/mss.0b013e31821f5d4e

Després, Jean-Pierre, PhD, FAHA, FIAS. (2015). Obesity and cardiovascular disease: Weight loss is not the only target. *Canadian Journal of Cardiology, 31*(2), 216-222. doi:10.1016/j.cjca.2014.12.009

Dias, K. A., Ingul, C. B., Tjønna, A. E., Keating, S. E., Gomersall, S. R., Follestad, T., et al. (2018). Effect of high-intensity interval training on fitness, fat mass and cardiometabolic biomarkers in children with obesity: A randomised controlled trial. *Sports Medicine (Auckland), 48*(3), 733-746. doi:10.1007/s40279-017-0777-0

Dongiovanni, P., Stender, S., Pietrelli, A., Mancina, R. M., Cespiati, A., Petta, S., et al. (2018). Causal relationship of hepatic fat with liver damage and insulin resistance in nonalcoholic fatty liver. *Journal of Internal Medicine, 283*(4), 356-370. doi:10.1111/joim.12719

e Silva, L. d. L. S., Fernandes, M. S. d. S., Lima, E. A. d., Stefano, J. T., Oliveira, C. P., & Jukemura, J. (2021). Fatty pancreas: Disease or finding? *Clinics (São Paulo, Brazil), 76*, e2439. doi:10.6061/clinics/2021/e2439

Fonvig, C. E., Chabanova, E., Ohrt, J. D., Nielsen, L. A., Pedersen, O., Hansen, T., et al. (2015). Multidisciplinary care of obese children and adolescents for one year reduces ectopic fat content in liver and skeletal muscle. *BMC Pediatrics, 15*(196), 196. doi:10.1186/s12887-015-0513-6

Frayn, K. N., Karpe, F., Fielding, B. A., Macdonald, I. A., & Coppack, S. W. (2003). Integrative physiology of human adipose tissue. *International Journal of Obesity, 27*(8), 875-888. doi:10.1038/sj.ijo.0802326

Gerosa-Neto, J., Panissa, V. L. G., Monteiro, P. A., Inoue, D. S., Ribeiro, J. P. J., Figueiredo, C., et al. (2019). High- or moderate-intensity training promotes change in cardiorespiratory fitness, but not visceral fat, in obese men: A randomised trial of equal energy expenditure exercise. *Respiratory Physiology & Neurobiology, 266*, 150-155. doi:10.1016/j.resp.2019.05.009

Gujar, S. K. M., Maheshwari, S. M., Björkman-Burtscher, I. M., & Sundgren, P. C. M. (2005). Magnetic resonance spectroscopy. *Journal of Neuro-Ophthalmology,* doi:10.1097/01.wno.0000177307.21081.81

Heijden, G. v. d., Wang, Z. J., Chu, Z. D., Sauer, P. J. J., Haymond, M. W., Rodriguez, L. M., et al. (2010). 12-week aerobic exercise program reduces hepatic fat accumulation and insulin resistance in obese, hispanic adolescents. *Obesity, 18*(2), 384-390. doi:10.1038/oby.2009.274

Holm, J., Gamborg, M., Bille, D. S., Grønbæk, H. N., Ward, L. C., & Faerk, J. (2011). Chronic care treatment of obese children and adolescents. *International Journal of Pediatric Obesity, 6*(3-4), 188-196. doi:10.3109/17477166.2011.575157

HOPKINS, W. G., MARSHALL, S. W., BATTERHAM, A. M., & HANIN, J. (2009). Progressive statistics for studies in sports medicine and exercise science. *Medicine & Science in Sports & Exercise, 41*(1), 3-12. doi:10.1249/mss.0b013e31818cb278

Houttu, V., Bouts, J., Vali, Y., Daams, J., Grefhorst, A., Nieuwdorp, M., et al. (2022). Does aerobic exercise reduce NASH and liver fibrosis in patients with non-alcoholic fatty liver disease? A systematic literature review and meta-analysis. *Frontiers in Endocrinology (Lausanne), 13*, 1032164. doi:10.3389/fendo.2022.1032164

Hvidt, K. N., Olsen, M. H., Ibsen, H., & Holm, J. (2014). Effect of changes in BMI and waist circumference on ambulatory blood pressure in obese children and adolescents. *Journal of Hypertension, 32*(7), 1470-1477. doi:10.1097/HJH.0000000000000188

Johansen, M. J., Gade, J., Stender, S., Frithioff-Bøjsøe, C., Lund, M. A. V., Chabanova, E., et al. (2020). The effect of overweight and obesity on liver biochemical markers in children and adolescents. *The Journal of Clinical Endocrinology and Metabolism, 105*(2), 430-442. doi:10.1210/clinem/dgz010

Johnson, N. A., Sachinwalla, T., Walton, D. W., Smith, K., Armstrong, A., Thompson, M. W., et al. (2009). Aerobic exercise training reduces hepatic and visceral lipids in obese individuals without weight loss. *Hepatology, 50*(4), 1105-1112. doi:10.1002/hep.23129

Julian, V., Bergsten, P., Ennequin, G., Forslund, A., Ahlstrom, H., Ciba, I., et al. (2022). Association between alanine aminotransferase as surrogate of fatty liver disease and physical activity and sedentary

time in adolescents with obesity. *European Journal of Pediatrics, 181*(8), 3119-3129. doi:10.1007/s00431-022-04539-z

Keating, S. E., Hackett, D. A., Parker, H. M., O'Connor, H. T., Gerofi, J. A., Sainsbury, A., et al. (2015). Effect of aerobic exercise training dose on liver fat and visceral adiposity. *Journal of Hepatology, 63*(1), 174-182. doi:10.1016/j.jhep.2015.02.022

Kipp, J. P., Olesen, S. S., Mark, E. B., Frederiksen, L. C., Drewes, A. M., & Frøkjær, J. B. (2019). Normal pancreatic volume in adults is influenced by visceral fat, vertebral body width and age. *Abdominal Imaging, 44*(3), 958-966. doi:10.1007/s00261-018-1793-8

Kloppenborg, J. T., Gamborg, M., Fonvig, C. E., Nielsen, T. R. H., Pedersen, O., Johannesen, J., et al. (2018). The effect of impaired glucose metabolism on weight loss in multidisciplinary childhood obesity treatment. *Pediatric Diabetes, 19*(3), 366-374. doi:10.1111/pedi.12605

Koo, T. K., & Li, M. Y. (2016). A guideline of selecting and reporting intraclass correlation coefficients for reliability research. *Journal of Chiropractic Medicine, 15*(2), 155-163. doi:10.1016/j.jcm.2016.02.012

Kramer, A. M., Martins, J. B., de Oliveira, P. C., Lehnen, A. M., & Waclawovsky, G. (2023). High-intensity interval training is not superior to continuous aerobic training in reducing body fat: A systematic review and meta-analysis of randomized clinical trials. *Journal of Exercise Science and Fitness, 21*(4), 385-394. doi:10.1016/j.jesf.2023.09.002

Lim, E. L., Hollingsworth, K. G., Aribisala, B. S., Chen, M. J., Mathers, J. C., & Taylor, R. (2011). Reversal of type 2 diabetes: Normalisation of beta cell function in association with decreased pancreas and liver triacylglycerol. *Diabetologia, 54*(10), 2506-2514. doi:10.1007/s00125-011-2204-7

MA J. (2008). Dixon techniques for water and fat imaging. *J Magn Reson Imaging, 28*, 543-558.

Martinez-Millana, A., Hulst, J. M., Boon, M., Witters, P., Fernandez-Llatas, C., Asseiceira, I., et al. (2018). Optimisation of children z-score calculation based on new statistical techniques. *PloS One, 13*(12), e0208362. doi:10.1371/journal.pone.0208362

Moher, D., Hopewell, S., Schulz, K. F., Montori, V., Gøtzsche, P. C., Devereaux, P. J., et al. (2012). CONSORT 2010 explanation and elaboration: Updated guidelines for reporting parallel group randomised trials. *International Journal of Surgery (London, England), 10*(1), 28-55. doi:10.1016/j.ijsu.2011.10.001

Mollerup, P. M., Lausten-Thomsen, U., Fonvig, C. E., Baker, J. L., & Holm, J. (2017). Reductions in blood pressure during a community-based overweight and obesity treatment in children and adolescents with prehypertension and hypertension. *Journal of Human Hypertension, 31*(10), 640-646. doi:10.1038/jhh.2017.36

Mollerup, P. M., Gamborg, M., Trier, C., Bøjsøe, C., Nielsen, T. R. H., Baker, J. L., et al. (2017). A hospital-based child and adolescent overweight and obesity treatment protocol transferred into a community healthcare setting. *PloS One, 12*(3), e0173033. doi:10.1371/journal.pone.0173033

Most, S. W., Højgaard, B., Teilmann, G., Andersen, J., Valentiner, M., Gamborg, M., et al. (2015). Adoption of the children's obesity clinic's treatment (TCOCT) protocol into another danish pediatric obesity treatment clinic. *BMC Pediatrics, 15*(1), 13. doi:10.1186/s12887-015-0332-9

Neeland, I., Poirier, P., & Després, J. (2018). Cardiovascular and metabolic heterogeneity of obesity: Clinical challenges and implications for management. *Circulation (New York, N.Y.), 137*(13), 1391-1406. doi:10.1161/CIRCULATIONAHA.117.029617

Nielsen, T. R. H., Fonvig, C. E., Dahl, M., Mollerup, P. M., Lausten-Thomsen, U., Pedersen, O., et al. (2018). Childhood obesity treatment; effects on BMI SDS, body composition, and fasting plasma lipid concentrations. *PloS One, 13*(2), e0190576. doi:10.1371/journal.pone.0190576

Panissa, V. L. G., Julio, U. F., St-Pierre, D. H., Tavares da Silva Gomes, A., Caldeira, R. S., Lira, F. S., et al. (2019). Timing of high-intensity intermittent exercise affects ad libitum energy intake in overweight inactive men. *Appetite, 143*, 104443. doi:10.1016/j.appet.2019.104443

Pritzlaff, C. J., Wideman, L., Blumer, J., Jensen, M., Abbott, R. D., Gaesser, G. A., et al. (2000). Catecholamine release, growth hormone secretion, and energy expenditure during exercise vs. recovery in men. *Journal of Applied Physiology, 89*(3), 937-946. doi:10.1152/jappl.2000.89.3.937

Rada, P., González-Rodríguez, Á, García-Monzón, C., & Valverde, Á M. (2020). Understanding lipotoxicity in NAFLD pathogenesis: Is CD36 a key driver? *Cell Death & Disease, 11*(9), 802-802. doi:10.1038/s41419-020-03003-w

ROEMMICH, J. N., CLARK, P. A., VU MAI, BERR, S. S., WELTMAN, A., VELDHUIS, J. D., et al. (1998). Alterations in growth and body composition during puberty: III. influence of maturation, gender, body composition, fat distribution, aerobic fitness, and energy expenditure on nocturnal growth hormone release. *The Journal of Clinical Endocrinology and Metabolism, 83*(5), 1440-1447. doi:10.1210/jc.83.5.1440

Saleh, J. (2015). Glycated hemoglobin and its spinoffs: Cardiovascular disease markers or risk factors? *World Journal of Cardiology, 7*(8), 449-453. doi:10.4330/wjc.v7.i8.449

Schwimmer, J. B., Burwinkle, T. M., & Varni, J. W. (2003). Health-related quality of life of severely obese children and adolescents. *Jama, 289*(14), 1813-1819. doi:10.1001/jama.289.14.1813

Sepe, P. S., MD, Ohri, A., Sanaka, S., MD, Berzin, T. M., MD, Sekhon, S., MD, Bennett, G., MD, et al. (2011). A prospective evaluation of fatty pancreas by using EUS. *Gastrointestinal Endoscopy, 73*(5), 987-993. doi:10.1016/j.gie.2011.01.015

Shaw, C. S., Clark, J., & Wagenmakers, A. J. M. (2010). Effect of exercise and nutrition on intramuscular fat metabolism and insulin sensitivity. *Annual Review of Nutrition, 30*(1), 13-34. doi:10.1146/annurev.nutr.012809.104817

Statistic laerd, a. Testing for Normality using SPSS Statistics. Retrieved May 5, 2024. Retrieved from: https://statistics.laerd.com/spss-tutorials/testing-for-normality-using-spss-statistics.php

Statistic laerd, b. Independent t-test using SPSS Statistics using SPSS Statistics. Retrieved May 5, 2024. Retrieved from: https://statistics.laerd.com/spss- tutorials/independent-t-test-using-spss-statistics.php

Statistic laerd, c. Mann-Whitney U Test using SPSS Statistics. Retrieved May 5, 2024. Retrieved from: https://statistics.laerd.com/spss-tutorials/mann-whitney-u-test-using-spss-statistics.php

Statistic laerd, d. Two-way ANOVA in SPSS Statistics. Retrieved May 5, 2024, from: https://statistics.laerd.com/spss-tutorials/two-way-anova-using-spss-statistics.php

Statistic laerd, e. Mixed ANOVA using SPSS Statistics. Retrieved May 5, 2024. Retrieved from: https://statistics.laerd.com/spss-tutorials/mixed-anova-using-spss-statistics.php

Statistic laerd, f. Spearmans-Rank-Order-Correlation using SPSS Statistics. Retrieved May 5, 2024. Retrieved from: https://statistics.laerd.com/spss-tutorials/spearmans-rank-order- correlation-using-spss-statistics.php

Tas, E., Landes, R. D., Diaz, E. C., Bai, S., Ou, X., Buchmann, R., et al. (2023). Effects of short-term supervised exercise training on liver fat in adolescents with obesity: A randomized controlled trial. *Obesity (Silver Spring, Md.), 31*(11), 2740-2749. doi:10.1002/oby.23887

Taylor, R., Al-Mrabeh, A., & Sattar, N. (2019). Understanding the mechanisms of reversal of type 2 diabetes. *The Lancet. Diabetes & Endocrinology, 7*(9), 726-736. doi:10.1016/S2213-8587(19)30076-2

Thyfault, J. P., & Rector, R. S. (2020). Exercise combats hepatic steatosis: Potential mechanisms and clinical implications. *Diabetes, 69*(4), 517-524. doi:10.2337/dbi18-0043

Toftager, M., & Brønd, J. C. (2019). *Fysisk aktivitet og stillesiddende adfærd blandt 11-15-årige:National monitorering med objektive målinger* Sundhedsstyrelsen.

Trapp, E. G., Chisholm, D. J., & Boutcher, S. H. (2007). Metabolic response of trained and untrained women during high-intensity intermittent cycle exercise. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology, 293*(6), R2370-R2375. doi:10.1152/ajpregu.00780.2006

Trauner, M., Arrese, M., & Wagner, M. (2010). Fatty liver and lipotoxicity. *Biochimica Et Biophysica Acta, 1801*(3), 299-310. doi:10.1016/j.bbalip.2009.10.007

Trefts, E., Williams, A. S., & Wasserman, D. H. (2015). Exercise and the regulation of hepatic metabolism. *Progress in Molecular Biology and Translational Science, 135*, 203-225. doi:10.1016/bs.pmbts.2015.07.010

TROST, S. G., PATE, R. R., FREEDSON, P. S., SALLIS, J. F., & TAYLOR, W. C. (2000). Using objective physical activity measures with youth: How many days of monitoring are needed? *Medicine and Science in Sports and Exercise, 32*(2), 426-431. doi:10.1097/00005768-200002000-00025

van Loon, L. J. C. (2004). Use of intramuscular triacylglycerol as a substrate source during exercise in humans. *Journal of Applied Physiology, 97*(4), 1170-1187. doi:10.1152/japplphysiol.00368.2004

Wang, Y., Wang, S., Meng, X., & Zhou, H. (2024). Effect of high-intensity interval training and moderate-intensity continuous training on cardiovascular risk factors in adolescents: Systematic review and meta-analysis of randomized controlled trials. *Physiology & Behavior, 275*, 114459. doi:10.1016/j.physbeh.2024.114459

Wasserman, D. H., & Cherrington, A. D. (1991). Hepatic fuel metabolism during muscular work: Role and regulation. *American Journal of Physiology-Endocrinology and Metabolism, 260*(6), 811. doi:10.1152/ajpendo.1991.260.6.e811

Wedell-Neergaard, A., Lang Lehrskov, L., Christensen, R. H., Legaard, G. E., Dorph, E., Larsen, M. K., et al. (2019). Exercise-induced changes in visceral adipose tissue mass are regulated by IL-6 signaling: A randomized controlled trial. *Cell Metabolism, 29*(4), 844-855.e3. doi:10.1016/j.cmet.2018.12.007

Weiss, J., Rau, M., & Geier, A. (2014). Non-alcoholic fatty liver disease. *Dtsch Arztebl Int.,* doi:10.3238/arztebl.2014.0447

Weiss, R., Dziura, J., Burgert, T. S., Tamborlane, W. V., Taksali, S. E., Yeckel, C. W., et al. (2004). Obesity and the metabolic syndrome in children and adolescents. *New England Journal of Medicine, 350*(23), 2362-2374. doi:10.1056/NEJMoa031049

WHO child growth standards. (2006).

Winn, N. C., Liu, Y., Rector, R. S., Parks, E. J., Ibdah, J. A., & Kanaley, J. A. (2018). Energy-matched moderate and high intensity exercise training improves nonalcoholic fatty liver disease risk independent of changes in body mass or abdominal adiposity — A randomized trial. *Metabolism, 78*, 128-140. doi:10.1016/j.metabol.2017.08.012