The associations between subcomponents of physical activity and regional fat deposits in obese children and adolescents

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Resumé

På verdensplan estimeres det, at op mod 27,0% af drenge og 23,1% af piger mellem 5-19 år er overvægtige eller fede (Micha et al., 2020). Tidligere studier har vist, at større mængder af både visceralt fedt (VAT) og leverfedt medfører sundhedsrisici (Ding et al., 2018; Geurtsen et al., 2020), mens mængden af subkutant fedt ikke nødvendigvis er relateret til sundhedsrisici (Cali & Caprio, 2009; Neeland et al., 2013). Lokaliseringen af fedt er derfor en afgørende faktor for, hvor stor sundhedsrisiko overvægt medfører, og fedtdeponeringen er derfor vigtig at undersøge frem for total mængde kropsfedt (Neeland et al., 2018; Piché et al., 2018; Després et al., 2015).
Tidligere studier har påvist, at intensiteten af fysisk aktivitet er væsentlig i forhold til mængden af abdominalt fedt (Dencker et al., 2006; Dencker et al., 2008). Disse studier indikerer, at fysisk aktivitet med høj intensitet har en stærkere sammenhæng med mindre fedtophobning i det abdominale område sammenlignet med fysisk aktivitet ved lavere intensitet (Dencker et al., 2006; Dencker et al., 2008). Dog er litteraturen om sammenhængen mellem karakteristika ved fysisk aktivitet og regionale fedtdepoter (VAT, SAT og leverfedt) hos børn begrænset.
Formålet med dette studie var derfor at undersøge sammenhængen mellem specifikke karakteristika ved fysisk aktivitet og regionale fedtdepoter hos børn med fedme i alderen 10-15 år. Ydermere blev den metodiske tilgang og inter-rater reliabilitet undersøgt for fire forskellige tilgange til bestemmelse af leverfedt.

Studiet var opbygget som et tværsnitsstudie, og 20 forsøgspersoner (6 piger og 14 drenge) deltog. Alle forsøgspersoner var kategoriseret som fede, og gennemsnitsalderen var 12.1±1.6 år. Karakteristika ved fysisk aktivitet blev målt ved hjælp af accelerometri (avanceret skridttæller), som blev båret af forsøgspersonerne i syv dage. De undersøgte karakteristika ved fysisk aktivitet i dette studie var fysisk aktivitet med let intensitet (LPA), fysisk aktivitet med moderat til høj intensitet (MVPA), stillesiddende tid, total mængde fysisk aktivitet og kondital. Kondital blev beregnet på baggrund af en test til udmattelse udført på et cykelergometer.
De regionale fedtdepoter undersøgt i dette studie inkluderede VAT, SAT og leverfedt, som blev målt ved hjælp af magnetisk resonans imaging (MRI). Sammenhængen mellem LPA og MVPA i forhold til de regionale fedtdepoter blev undersøgt ved hjælp af multipel regression. Simpel lineær regression

blev anvendt til at undersøge sammenhængen mellem henholdsvis stillesiddende tid, total mængde af fysisk aktivitet samt kondital i forhold til de regionale fedtdepoter.
Til kvantificering af leverfedt blev fire forskellige metodetilgange anvendt. De fire metoder inkluderede: 2 interesseområder (2 ROIs) (venstre og højre leverlap), 4 ROIs (anterior, posterior, medialt og lateralt segment), 9 ROIs (ni Couinaud segmenter) og hele-lever ROI (hele tværsnitsarealet). To personer (ratere) udførte alle disse analyser for hver enkelt forsøgsperson, og inter-rater reliabilitet blev bestemt ved hjælp af Bland-Altman Plot og intraclass correlation coefficient (ICC). Ydermere blev det testet, om der var signifikante forskelle i resultaterne af de fire metoder ved hjælp af en two-way repeated measures ANOVA.

Resultaterne viste, at der var en signifikant omvendt sammenhæng mellem LPA og MVPA i forhold til SAT, det var dog kun den estimerede koefficient for LPA, der var signifikant. Der var ikke nogen signifikant sammenhæng for LPA og MVPA i forhold til de andre fedtdepoter. Den simple lineære regression viste en signifikant sammenhæng mellem kondital og VAT samt SAT, således at højere kondital var relateret til mindre mængde VAT og SAT. Der var ikke en signifikant sammenhæng mellem stillesiddende tid samt total mængde fysisk aktivitet og de regionale fedtdepoter.
Resultaterne for de fire metodetilgange til bestemmelse af leverfedt viste, at der ikke var signifikant forskel mellem de fire metodetilgange. Derudover forekom fremragende inter-rater reliabilitet for alle metoder udtrykt ved ICC. Bland-Altman plottet viste en smule bedre inter-rater reliabilitet ved at benytte 4 ROIs, 9 ROIs eller hele-lever ROI fremfor 2 ROIs.

Overordnet set indikerede resultaterne i dette studie, at mere tid i LPA var relateret til lavere mængde SAT, og et højere kondital var associeret med lavere mængde VAT og SAT. Det er dog nødvendigt med videre forskning, hvor flere forsøgspersoner inkluderes for at klarlægge sammenhængen mellem karakteristika ved fysisk aktivitet og regionale fedtdepoter. Derudover viste resultaterne, at metoderne med 4 ROIs, 9 ROIs og hele-lever ROI kan benyttes til kvantificering af leverfedt ud fra MRI.

Abstract

Aim: The aim of this study was 1) to examine the association between characteristics of physical activity and regional fat deposits including visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT) and liver fat in obese children and adolescents and 2) to examine the outcome and inter-rater agreement of four different methodological approaches for quantification of liver fat.

Methods: The participants of the study included 20 obese children and adolescents (girls n=6, boys n=14) aged 10-15 years (mean age: 12.1±1.6 years). The cross-sectional study examined the association of the independent variables light intensity physical activity (LPA), moderate to vigorous intensity physical activity (MVPA), sedentary time, total physical activity and cardiorespiratory fitness (CRF) with the dependent variables VAT, SAT and liver fat. Physical activity was measured with an accelerometer for seven days, and regional fat deposits were measured with magnetic resonance imaging (MRI). CRF was measured with a watt-max test on a bicycle ergometer. The four methods of determining liver fat included 2 regions of interest (ROIs) (right and left liver lobe), 4 ROIs (anterior, posterior, medial and lateral segment), 9 ROIs (nine Couinaud segments) and whole-liver ROI (whole cross-sectional area). Two raters performed all of these methodological approaches to determine inter-rater agreement assessed with Bland-Altman Plot and intraclass correlation coefficients (ICC).

Results: Multiple regression analysis showed a significant inverse association for LPA and MVPA with SAT (p=0.05), but only the coefficient of LPA was significant (p=0.02). LPA and MVPA were not significantly associated with liver fat and VAT. Simple linear regression showed a significant inverse association between CRF and VAT (p=0.04) as well as between CRF and SAT (p=0.0002). Sedentary time and total physical activity were not associated with the regional fat deposits.
For the four methods of liver fat quantification, ICC showed excellent agreement between the two raters (ICC>0.999). Bland-Altman Plot showed small advantages for 4 ROIs, 9 ROIs and whole-liver ROI compared to 2 ROIs. There were no significant differences in the results of liver fat quantification across methods.

Conclusion: The results indicated that more time spent engaging in LPA was associated with a lower amount of SAT, and higher CRF was associated with a lower amount of VAT and SAT. However, the association between elements of physical activity and regional fat deposits should be further examined in a larger sample. This study suggests using 4 ROIs, 9 ROIs or whole-liver ROI for liver fat quantification using MRI.

Introduction

According to worldwide estimates, overweight and obesity affects 27.0% of boys and 23.1% of girls aged 5-19 years (Micha et al., 2020). Overweight and obesity may lead to metabolic and cardiovascular diseases as well as negative psychological consequences. For instance, higher body mass index (BMI) among children has shown to be associated with higher risk of type 2 diabetes (Arslainan, 2000), dyslipidemia (Saha et al., 2011), higher levels of depression and lower self-esteem (Gibson et al., 2008).

Body mass index is a cheap and simple predictor of cardiovascular disease (Ortega et al., 2016). However, BMI does not provide information about the location of fat deposition. This information is provided by regional fat deposits and is relevant due to the interindividual differences in regional fat deposits at similar BMI (Neeland et al., 2018). Considering these interindividual differences is important since the regional fat deposits pose different levels of health risks (Neeland et al., 2018; Piché et al., 2018; Després et al., 2015). Studies in children and adolescents have shown that accumulation of visceral adipose tissue (VAT) is associated with a higher risk of metabolic syndrome (Druet et al., 2008; Cali & Caprio, 2009; Ding et al., 2018) and insulin resistance (Campos et al., 2019). Furthermore, liver fat has shown to be associated with insulin resistance (Saki & Karamizadeh, 2014; Geurtsen et al., 2020), higher blood pressure and total cholesterol (Geurtsen et al., 2020). In comparison, subcutaneous adipose tissue (SAT) shows weaker or no association with the health risks (Cali & Caprio, 2009; Neeland et al., 2013; Liu et al., 2010), which indicates that the consequences of SAT are less critical than those related to VAT and liver fat. These conflicting health consequences of different regional fat deposits underline the relevance of examining regional fat deposits rather than total body fat as a measure of obesity, since regional fat deposits provide a more accurate assessment of health risks (Neeland et al., 2018; Piché et al., 2018; Després et al., 2015).

Non-invasive and precise measurements of regional fat deposits can be provided by magnetic resonance imaging (MRI), which is well-suited for children due to no radiation exposure (Pietrobelli & Tató, 2005). However, the approach for measurement of these MRI-derived regional fat deposits lacks standardization. For the liver, inconsistencies exist on the locations, numbers and sizes of regions of interest (ROIs) (Campo et al., 2017). A study by Campo et al. (2017) examined different combinations of locations, numbers and sizes of ROIs including nine different analysis paradigms in adults. Three raters independently conducted the analyses, and the results were compared assessing inter- and intra-rater agreement. Generally, the results showed that the larger area of the liver covered in the analysis, the better reproducibility and repeatability. However, the study did not include whole-liver ROIs meaning that they did not manually mark the whole cross-sectional area of the liver. Therefore, it is relevant to compare the methods covering large parts of the liver used in the study by Campo et al. (2017) with whole-liver ROIs in children and adolescents. This methodological problem needs to be addressed to ensure a reliable method for measuring liver fat in children.

Cardiorespiratory fitness (CRF) provides a general measure of aerobic effect and is related to physical activity (Jackson et al., 2009; Dencker et al., 2008; Gutin et al., 2005). Studies have shown that CRF is inversely associated with VAT (Winsley et al., 2006; Lee & Arslanian, 2007) and SAT (Lee & Arslanian, 2007) in children. Objective measurements of physical activity provide more detailed information of the characteristics of physical activity. Detailed information of physical activity can be provided by accelerometers (Reilly et al., 2008; Corder et al., 2008) that permit analysis of sub-components of physical activity including intensity, duration and frequency (Reilly et al., 2008; Corder et al., 2008). Examining physical activity more thoroughly is relevant to investigate if specific characteristics of physical activity behavior are related to regional fat deposits and the associated health risks.

The association between physical activity measured with accelerometers and regional fat deposits was examined in a study by Saelens et al. (2007). The cross-sectional study showed an inverse relationship between total amount of physical activity and VAT, but no significant relationship between physical activity and SAT. Therefore, the results suggest that less active children accumulate more body fat as VAT while the amount of physical activity does not influence SAT. However, only the total amount of physical activity was examined, and subcomponents of physical activity such as intensity were not included (Saelens et al., 2007). The intensity has shown to be a covariate in studies examining the association between intensities of physical activity and abdominal adipose tissue (AAT) in children (Dencker et al., 2006; Dencker et al., 2008). These cross-sectional studies have shown a stronger inverse association between vigorous physical activity (VPA) and AAT in comparison with physical activity of lower intensities, indicating that higher intensities of physical activity may be associated with smaller regional fat deposits (Dencker et al., 2006; Dencker et al., 2008). However, to our knowledge the association between the characteristics of physical activity and these more specific regional fat deposits (VAT and SAT) determined with MRI has not previously been examined.

Equivocal findings are present on the association between total amount of physical activity and liver fat in children and adolescents. A study suggested that the total amount of physical activity was negatively associated with the risk of fatty liver (Anderson et al., 2016), while another study found no relation between physical activity level and liver fat (Maskarinec et al., 2021). However, the latter used self-reported physical activity which may be affected by reporter bias and tends to overestimate the level of physical activity (Dyrstad et al., 2014). Therefore, objective measurement of physical activity with accelerometers is required to further explore the association between the characteristics of physical activity and liver fat.

The influence of physical activity on regional fat deposits is relevant to examine for the purpose of clarifying which characteristics of physical activity behavior might be closely related to health risks in children and adolescents. Therefore, the primary aim of this cross-sectional study was to examine the association between characteristics of physical activity (measured with accelerometers) and MRI-derived regional fat deposits including VAT, SAT and liver fat in children and adolescents aged 10-15 years. The secondary aim of the study was to determine possible differences in the results and inter-rater agreement of four different methodological approaches for determining liver fat in children and adolescents.

Methods

This cross-sectional study used baseline data from a randomized controlled study that aims to examine metabolic consequences associated with obesity in children and adolescents and explain how lifestyle changes, including high intensity interval training influences the pathological mechanisms. The experimental study is part of a Ph.D project in partnership with the Department of Pediatrics at Aalborg University Hospital. The study was supposed to include 60 participants, but due to the Danish lock down as a consequence of the Corona pandemic, the collection of data got delayed and data from only 20 participants were included. Authors of this study were responsible for helping with magnetic resonance imaging (MRI) acquisition and were responsible for analysis of the fat deposits in these children and adolescents.

Data were collected on MRI-derived regional fat deposits, seven-day accelerometer-derived physical activity (Axivity AX3) and CRF using watt-max test conducted on a bicycle ergometer.

Participants

This study included baseline data from boys (n=14) and girls (n=6) aged 10-15 years (mean: 12.1±1.6 years). All participants were obese defined as sex and age specific BMI (isoBMI) > 30 and were excluded if they had any disabilities that prevented participating in the training intervention of the randomized controlled study. Participants were recruited from Videnscenter for Børn og Unge med Overvægt (VIBUO) at Aalborg University Hospital. Measurements of height, weight and blood pressure were collected by the primary investigator, Charlotte Nørkjær Eggertsen. The study protocol was approved by The North Denmark Region Committee on Health Research Ethics (reference no. N-2020035). The participants and guardian were informed about the protocol and written informed consent was obtained from the guardian.

Quantification of physical activity

To quantify the daily physical activity all participants were asked to wear Axivity AX3 (Axivity, Newcastle, UK). Axivity AX3 is a thigh-worn triaxial accelerometer and was set to a sampling frequency of 50 Hz, range ± 8 g. Participants were asked to wear the accelerometer 24 hours a day for seven consecutive days following the baseline visit. The accelerometer was placed on the front of the right thigh. With inspiration from Overgaard et al. (2018) the accelerometer was attached with tape (Hydrokolloider okklusiv 10x10cm) at the skin so it could not move around and Duoderm Extra thin was placed between the skin and accelerometer to avoid inconvenience. The accelerometer was attached by the primary investigator and participants got extra tape and a guide to fitting the accelerometer in case they had to take it off due to discomfort. A day was considered valid if the participants wore the accelerometer for at least eight hours.

Activity counts were summed over 10 sec epochs. Total physical activity assessed as counts per minute (cpm), sedentary time and time spent in the intensities light physical activity (LPA), moderate physical activity (MPA) and VPA were quantified. It was observed that the participants spent limited time in VPA, and due to this MPA and VPA were summed (MVPA). Sedentary time was defined as ≤100 cpm (<30% VO_{2max}), LPA was defined as 101-4970 cpm (30-40% VO_{2max}) and MVPA was defined as ≥4971 cpm (>40% VO_{2max}) (Toftager & Brønd, 2019). Physical activity of children and adolescents is characterized by sporadic movement patterns, and this type of physical activity poses a large part of the total amount of physical activity in children and adolescents (Toftager & Brønd, 2019). Accelerometers tend to estimate these movement patterns inaccurately (Staudenmayer et al., 2012). The explanation of this possible error is that acceleration is a mechanical measurement that

does not account for the energy expenditure of short periods of inactivity that split up periods of high intensity physical activity (Toftager & Brønd, 2019). In this study a relatively new method of processing accelerometer data was used. This method enables inclusion of the sporadic activity in the calculation of time spent in each intensity. The method uses an algorithm that incorporates the excess post-exercise oxygen consumption in the determination of time spent in each intensity (Toftager & Brønd, 2019). The processing of the accelerometer data for this study was conducted by Associate Professor at University of Southern Denmark, Jan Christian Brønd.
Axivity data for one participant were excluded due to missing data.

Estimation of cardiorespiratory fitness

Cardiorespiratory fitness was assessed with a watt-max test on a bicycle ergometer (Monark 894E). The watt-max test has been validated to estimate VO_{2max} in children (Hansen et al.,1989). With inspiration from Riddoch et al. (2005) development of protocol for watt-max test and data collection was conducted by the primary investigator. The height of the saddle was adjusted such that the participants were sitting comfortably and slightly bending the knee. Participants went through a short familiarization, to get used to biking on an ergometer and obtain the right cadence. The start load for the test was 30 watt with an increment of 30 watt every third minute. During the test the participants maintained a cadence of 60 rpm, and the cadence was monitored on a screen at the ergometer. The participants received verbal cheers during the test and the test ended when the participants could not maintain the cadence. During the test, heart rate was measured with a heart rate electrode in an elastic chest band (iQniter/Suunto, Finland).
Watt-max was determined with the equation from Hansen et al. (1989).

$$W_{max} = W_h + W_d * t\ (sec) * 180^{-1}$$

Where W_h is the workload in the last completed period, W_d is the increase in workload every third minute, and t is the time in seconds in the unfinished period.
Afterwards the formula from Hansen et al. (1989) was used to determine Vo_{2max}.

$$VO_{2max}(ml * min^{-1}) = 12 * W_{max}(W) + 5 * body\ mass\ (kg)$$

Data were presented as CRF (ml O_2*min^{-1}*kg^{-1}) by dividing the VO_{2max} with body mass.

Magnetic resonance imaging

To quantify liver fat, SAT and VAT magnetic resonance imaging was performed in a 3 Tesla MRI scanner (GE Premier Rx 19) at Aalborg University Hospital. The participants were lying in supine position with a 30 channel Aircoil placed over the abdomen and a 60 channel in-bed Aircoil posterior. Proton-density fat fraction images were obtained using a 3D IDEAL IQ sequence with following imaging parameters: 5-mm slice thickness 0 gap, repetition time (TR) 5.8 msec, echo times (TE) min full, number of echoes 6, number of excitations 2, NEX 0.5 and a 160 X 160 matrix. Participants were asked to hold their breath for 25 seconds during the sessions to minimize motion artifacts.

Quantification of liver fat

Images were analyzed using the standard software PACS (EazyViz v. 7.6.7-270, Karos Health A/ S, Valby, Denmark). With inspiration from Campo et al. (2017), four different methods of analysis were applied to quantify liver fat. The methods differed in numbers of ROIs and the location of ROIs. The methods are illustrated on Figure 1.

For all four methods, ROIs were covering the largest possible area of the liver avoiding inclusion of veins and disruptive image artifacts. For 2 ROIs, 4 ROIs and whole-liver ROI the slice with the biggest visible cross-sectional area of the liver was used. The first method included two circular ROIs, with one per right and left liver lobe (2 ROIs). The second method included four circular ROIs, with one per anterior, posterior, medial and lateral segment (4 ROIs). For the third method the region of interest was encircled manually with a mouse-controlled pointer and the whole cross-sectional area of the liver was marked as ROI (whole-liver ROI).

The fourth method included nine circular ROIs distributed on two slices to cover the nine Couinaud segments of the liver (9 ROIs) (Campo et al., 2017).

All analyses of liver fat were made independently by the two authors, to allow the assessment of inter-rater agreement.

For each of the two raters the estimated amount of fat in the liver was calculated for each participant by averaging the fat content in the ROIs for each of the four methods.

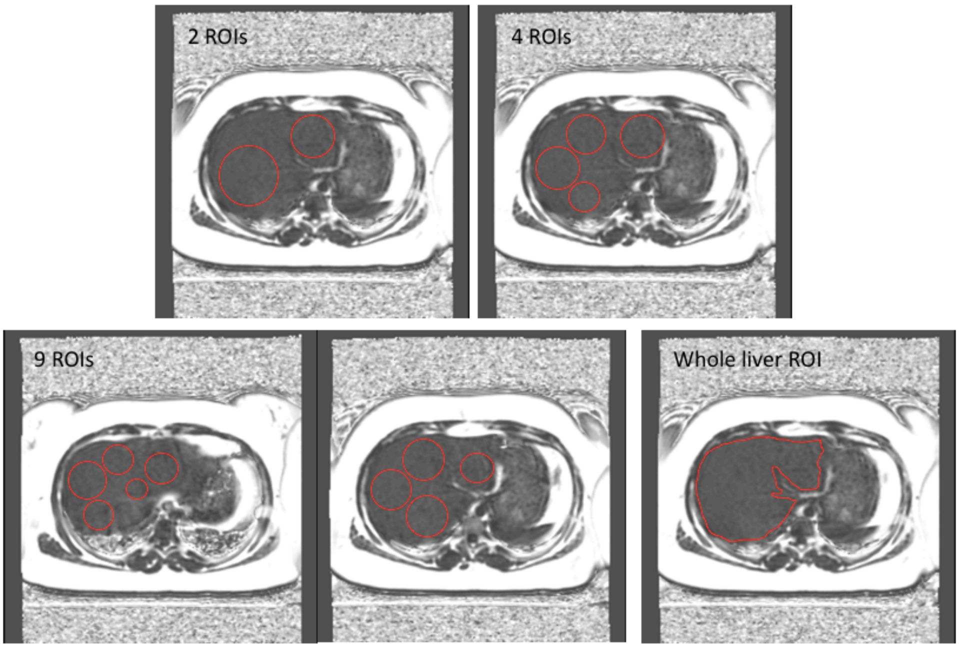

Figure 1. The four methods of quantifying liver fat.

Quantification of visceral adipose tissue and subcutaneous adipose tissue

The amount of VAT and SAT was determined in an image analysis software program produced in-house (MRIBodyComposition, Esben Bolvig Mark). For the analysis the fat fraction images were used at the level of L2-L3. When images of the participants at the level L2-L3 included kidneys the slice at the highest level without kidneys was used.

SAT was defined as adipose tissue in the abdominal area just beneath the skin, and VAT as the adipose tissue within the muscle wall surrounding the abdominal cavity (Jung & Song, 2018). In the software, pixels containing fat were defined by having an intensity >75 and marked with yellow if they were placed in the visceral area and blue if placed in subcutaneous area (Figure 2). The software made an estimate of the fat deposits based on the intensities and location, and the slices were afterwards manually checked and pixels outside the region of interest were excluded with a mouse-controlled pointer. The software quantified the amount of fat as cm^2 by summing the cross-sectional areas of the pixels containing fat in the slice of interest. Data from one participant was excluded due to an unusable image.

All analyses were made independently by the two authors, to allow measurements of inter-rater agreement.

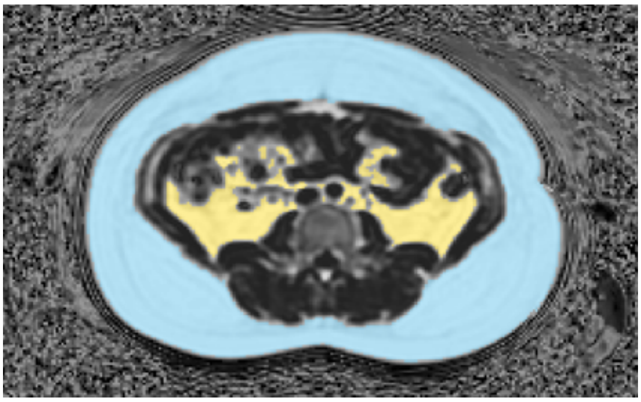

Figure 2. Screenshot of the cross-sectional area of the abdomen at L2-L3 in the software program, MRIbodycompostion. Yellow indicates VAT and blue indicates SAT.

Statistical analysis

Descriptive statistics was reported as means and standard deviations (SD). The criterion for statistical significance was $\alpha \leq 0.05$. All the statistical analyses were performed in SPSS statistics version 25 (IBM, Armonk, NY, USA). For all measurements of fat, the two-way random effect, absolute agreement intraclass correlation coefficient (ICC) and the corresponding 95% confidence interval (CI) was calculated. The ICC was chosen, based on the article by Koo and Li (2016). Outliers were defined as studentized residuals greater than ±3. Normality was assessed by checking QQ-plots and residual plots.

Quantification of liver fat

A Bland-Altman plot (Bland & Altman, 1986) was made including mean liver fat for each participant on the x-axis and the differences between rater 1 and rater 2 on the y-axis to analyze the agreement between the two raters. Data for all four methods are presented in a single Bland-Altman plot.

To test the interaction or main effect for rater and method used to quantify liver fat, a two-way repeated measures ANOVA was run with two within subject factors (method and rater). Since the two-way repeated measures ANOVA is fairly robust to violations of the assumption of normality the analysis was carried out regardless of violations of this assumption. Mauchly's test of sphericity was used to check the assumption of sphericity, and if the assumption of sphericity was violated, Greenhouse-Geisser correction was used.

Whole-liver ROI was used as the measure of liver fat for further data processing.

Regression analysis

To examine the association between characteristics of physical activity and each of the fat deposits, separate multiple linear regression models were made for each fat deposit (VAT, SAT and liver fat) with minutes spent in LPA and MVPA as independent variables. Furthermore, the following linear regression models were made for each regional fat deposit: three linear regressions for sedentary time (dependent variable: fat deposit, independent variable: sedentary), three linear regressions for total physical activity (dependent variable: fat deposit, independent variable: total physical activity) and three linear regressions for CRF (dependent variable: fat deposit, independent variable: CRF). To summarize the direction and strength of the associations across all the independent and dependent variables separate Pearson's correlation coefficients were calculated. For the significant multiple linear regressions models a post hoc power calculation was made to establish the achieved power and required sample size to achieve a power of 0.80. Power calculation was made in G*power (version 3.1.6.9).

Results

The descriptive characteristics of participants are shown in Table 1. The sample included six girls and 14 boys. For the axivity data five participants had six valid days and the rest of the participants had seven valid days.

Table 1. Descriptive characteristics of participants.

	Mean	**SD**
Age (years)	12.1	±1.6
Height (cm)	162.5	±10.6
Weight (kg)	76.7	±16.6
BMI	28.9	±4.7
BMI-SDS	2.6	±0.6
Systolic blood pressure (mmHg)	112.1	±7.0
Diastolic blood pressure (mmHg)	67.0	±4.7

Inter-rater reliability for liver fat

Mean percentages of liver fat are reported in Table 2. The Bland-Altman analysis (Figure 3) of the inter-rater agreement showed that number and placement of ROIs resulted in a maximum bias of

-0.2, indicating no systematic tendencies (Table 3). For the four methods combined data showed a bias of -0.1. Generally, the data of all four methods was fairly close spread around a difference between raters of zero. When comparing data for the four different methods, the 2 ROIs method showed a tendency for more variance in the difference between rater 1 and rater 2 compared to the other three methods.

Looking at limits of agreement, 2 ROIs had broader limits of agreement (-1.5;1.0) compared to the other three methods (Table 3) and the whole-liver ROI method had the narrowest limits of agreement (-0.5;0.5).

Table 2. Means and standard deviations of liver fat determined by the raters.

	2 ROIs (%)	4 ROIs (%)	9 ROIs (%)	Whole-live ROI (%)
Rater 1	5.9±8.8	6.2±8.7	6.2±8.7	6.2±9.0
Rater 2	6.2±9.2	6.3±8.8	6.2±8.9	6.2±8.7
Mean	6.0±9.0	6.3±8.8	6.2±8.8	6.2±8.8

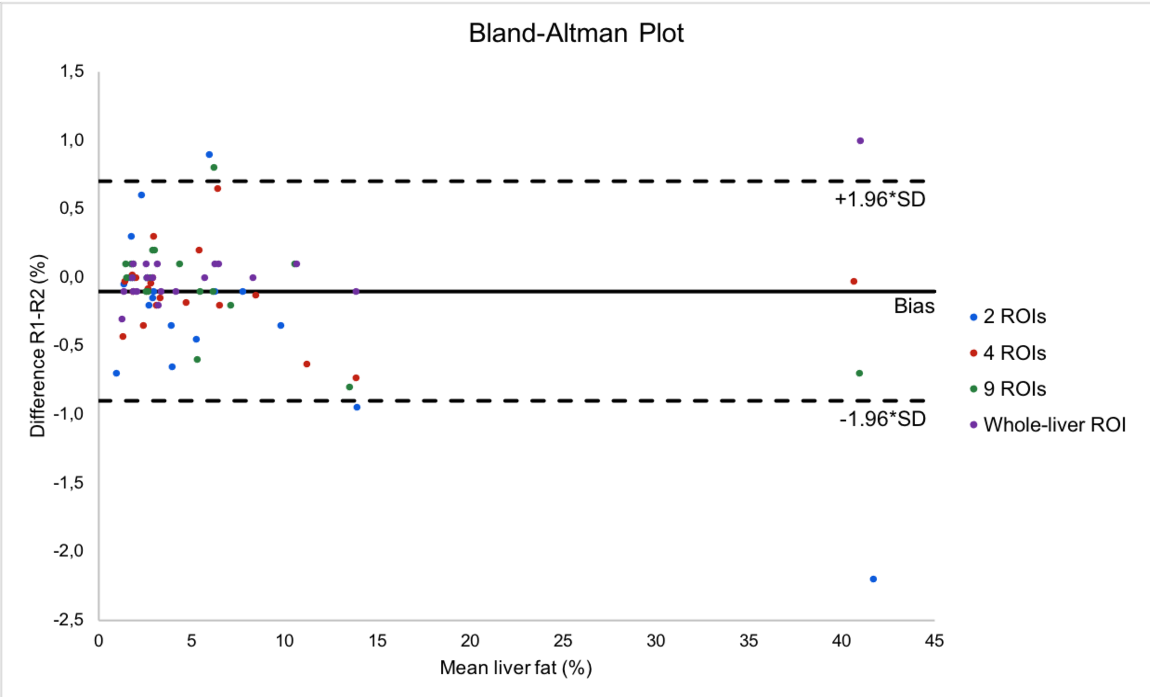

Figure 3. Bland-Altman plot of methods to quantify liver fat. Mean difference (solid line), upper and lower limits of agreement (dashed lines) are calculated on all data. R1: Rater 1, R2: Rater 2.

Table 3. Mean liver fat, bias, upper and lower limits of agreement (LOA) for each method.

	2 ROIs (%)	4 ROIs (%)	9 ROIs (%)	Whole-liver ROI (%)
Bias	-0.2	-0.1	0.0	0.0
Lower LOA	-1.5	-0.7	-0.7	-0.5
Upper LOA	1.0	0.5	0.6	0.5

The results of ICC confirmed high inter-rater reliability and showed excellent agreement for all four methods based on classifications of Koo & Li (2016) (Table 4).

Table 4. ICC and 95% confidence interval (CI) for each method.

	2 ROIs	**4 ROIs**	**9 ROIs**	**Whole-liver ROI**
ICC	0.999	1.000	1.000	1.000
95 % CI – Lower bound	0.997	0.999	0.999	0.999
95 % CI – Upper bound	0.999	1.000	1.000	1.000

Differences between methods and raters for liver fat

To determine any interaction or main effect for methods and raters on liver fat a two-way repeated measures ANOVA was conducted. Data are reported in Table 2. One outlier was identified, however since the researchers were confident this outlier was a genuinely unusual value and not due to error, this outlier was still included in the analysis. Mauchly's test of sphericity showed that the assumption of sphericity was violated, therefore Greenhouse-Geisser correction was used. There was no statistically significant interaction between methods and raters ($F(1.685, 32.010) = 1.501$, $p=0.238$). There were no significant main effect for rater ($F(1,19) = 3.047$, $p=0.097$) or method ($F(2.214,42.057) = 1.916$, $p=0.156$).

Associations between subcomponents of physical activity and regional fat deposits

ICC between the raters for VAT was 0.999 with 95% CI of 0.997 to 1.000. ICC between the raters for SAT was 1.000 with 95% CI 1.000 to 1.000. Mean values, standard deviations and range for characteristics of physical activity behavior, total physical activity, CRF and regional fat deposits are reported in Table 5.

Table 5. Means, standard deviations and range for physical activity behavior and regional fat deposits.

	Mean	**SD**	**Range**
VAT (cm^2) n=19	44.4	±26.3	3.9 - 100.2
SAT (cm^2) n=19	279.0	±81.4	150.9 - 448.2
Liver fat (%) n=20	6.2	±8.8	1.3 - 41.0
Total physical activity (cpm) n=19	581	±188	171 - 870
LPA (min/day) n=19	309.0	±80.7	100.9 - 428.2
MPA (min/day) n=19	9.7	±5.2	0.4 - 20.8
VPA (min/day) n=19	0.7	±0.5	0.0 - 1.8
MVPA (min/day) n=19	10.3	±5.5	0.4 - 21.4
Sedentary (min/day) n=19	505.3	±77.5	373.3 - 680.1
CRF (ml O_2*min^{-1}*kg^{-1})	27.4	±5.6	20 - 40

A multiple regression was conducted to examine the association between LPA and MVPA as independent variables and SAT as dependent variable. Significant multiple linear regression was established so that time spent in LPA and MVPA was significantly associated with the amount of SAT ($F(2,15) = 3.622$, $p=0.05$, $R^2=0.326$). Only the coefficient of LPA was significant ($p=0.02$). Regression equation was as follows:

$$SAT = 504.742 - 0.825 * LPA\ (min) + 3.545 * MVPA\ (min)$$

95% CI for coefficient of LPA was -1.485 to -0.165, and 95% CI for MVPA was -4.857 to 11.948.

Simple linear regression between CRF and regional fat deposits showed a significant association for VAT ($F(1,17) = 5.268$, $p=0.04$, $R^2=0.237$) and SAT ($F(1,17) = 21.877$, $p=0.0002$, $R^2=0.563$) (Figure 4). For liver fat, the association was not significant ($p=0.07$), however a tendency towards an inverse association was indicated.
Regression equations were as follows:

$$VAT = 109.891 - 2.357 * CRF\ (ml\ O_2 * min^{-1} * kg^{-1})$$
$$SAT = 591.890 - 11.260 * CRF\ (ml\ O_2 * min^{-1} * kg^{-1})$$

95% CI for the coefficient of VAT was -4.524 to -0.190 and for SAT -16.339 to -6.181.

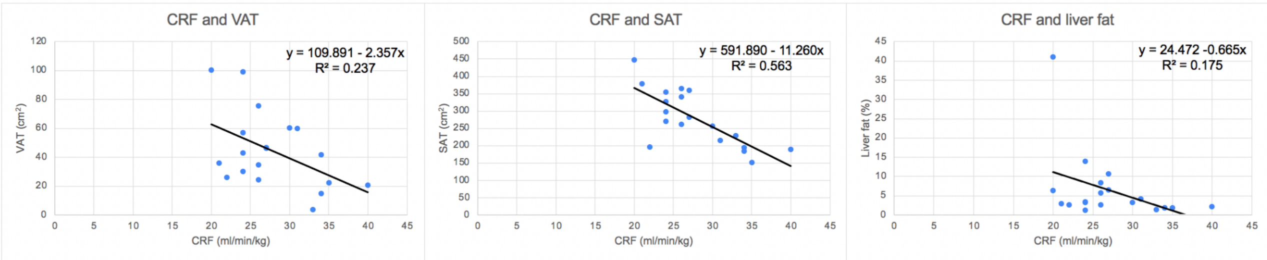

Figure 4. The regression for CRF and VAT, CRF and SAT as well as CRF and liver fat.

The remaining regression analyses described in methods (section: Regression analysis) were not significant. For an overview of the association between the variables the correlation coefficients are reported in Table 6.

Table 6. Correlation coefficients of all examined associations.

	LPA (min)	**MVPA** (min)	**Sedentary time** (min)	**CRF** (ml O_2*min^{-1}*kg^{-1})	**Total physical activity** (cpm)
Liver fat (%)	-0.032	0.353	-0.025	-0.419	0.180
VAT (cm^2)	-0.216	0.153	0.020	-0.486*	-0.066
SAT (cm^2)	-0.538*	-0.082	0.355	-0.750*	-0.293

*significant p-value

Data for subcomponents of physical activity and each regional fat deposit are presented in Figure 5.

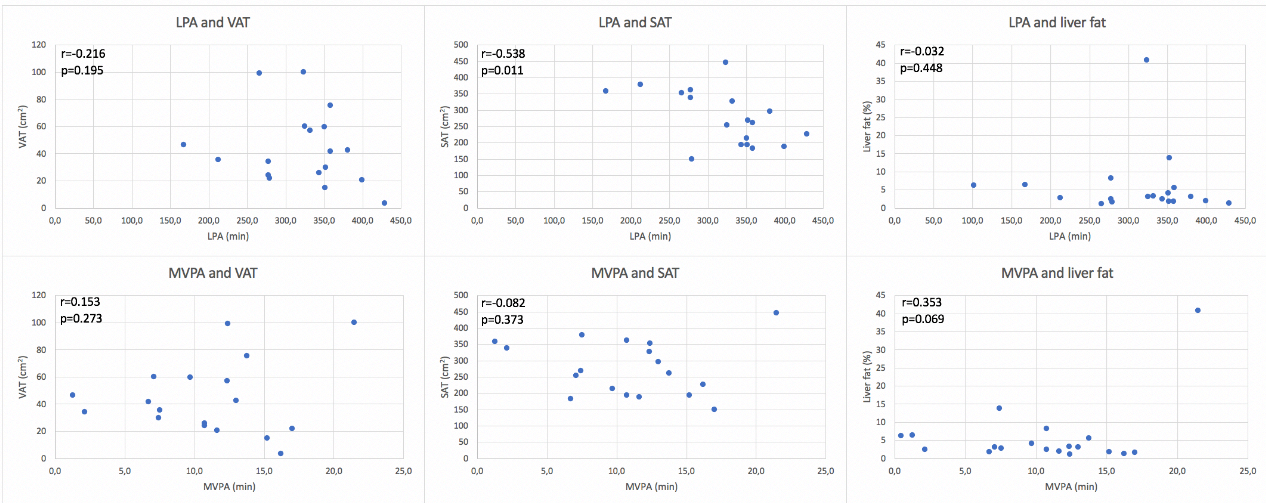

Figure 5. The associations between LPA as well as MVPA and VAT, SAT and liver fat, respectively. LPA and VAT: r=-0.216, LPA and SAT: r=-0.538*, LPA and liver fat: r=-0.032, MVPA and VAT: r=0.153, MVPA and SAT: r=-0.082, MVPA and liver fat: r=0.353.

*significant p-value

Discussion

This cross-sectional study showed a significant inverse multiple linear regression for LPA and MVPA associated with SAT in obese children and adolescents. However, the results showed that only the estimated coefficient for LPA was significant. The multiple regression for the association of LPA and MVPA with VAT and liver fat was not significant. The simple regression analyses showed significant inverse associations between CRF and VAT as well as between CRF and SAT. There was a trend for an inverse association between CRF and liver fat, yet not significant. Sedentary time and total physical activity were not significantly associated with any of the regional fat deposits. Additionally, the results of the two-way repeated measures ANOVA showed no significant difference between the four methods or the raters on the four methods of quantifying liver fat. Furthermore, ICC for inter-rater agreement was excellent for all four methods, however the Bland-Altman plot revealed small advantages of 4 ROIs, 9 ROIs and whole-liver ROI compared to 2 ROIs.

Subcomponents of physical activity

The multiple regression for LPA and MVPA associated with SAT was significant, however MVPA had a very broad 95% CI ranging from -4.857 to 11.948 which complicates making any conclusions based on this data. The results showed that LPA had 95% CI ranging from -1.485 to -0.165. This indicates that each minute spent in LPA was associated with 1.485 to 0.165 cm^2 less SAT. The finding that more time spent engaging in LPA was associated with lower SAT is in contrast with the intensity suggested most advantageous to obtain low amounts of AAT (Dencker et al., 2006; Dencker et al., 2008). The study by Dencker et al. (2006) examined the association between MPA and AAT as well as VPA and AAT in children. The results showed that VPA was inversely associated with AAT, while no significant association was found between MPA and AAT. In continuation of these results, Dencker et al. (2008) found a significant inverse association between VPA and AAT, while no significant association existed between MVPA and AAT. These results indicate that higher intensities of physical activity may be associated with lower AAT. However, it was evident that the study by Dencker et al. (2006) reported more physical activity (MPA range: 71-239 min/day, VPA range: 4-116 min/day) measured with an accelerometer for four days. The range of time spent doing physical activity in the current study was lower (MPA range: 0.4-20.8 min/day, VPA range: 0.0-1.8 min/day). The remarkably less time spent in MPA and VPA can be an explanation of the fact that no association between MVPA and any of the fat deposits was detected in this study since low variance can reduce the possibility of a significant regression. The reason for the differences in activity levels may be that

Dencker (2006) included a heterogeneous group of children including a variety of different BMI (mean boys: 17.4±2.8, mean girls: 17.4±2.9), while this study only included participants with an ISO-BMI > 30. Dencker et al. (2006) reported a significantly lower amount of mean physical activity and time spent engaging in VPA for children in the upper quartile of percent body fat compared to the lower quartile of percent body fat. This may explain the remarkably lower amount of MPA and VPA in the current study.

This cross-sectional study found no significant association for LPA and MVPA associated with VAT. The non-significant finding is in contrast with other studies that found a significant inverse association between MVPA and VAT. For instance, a study by Murabito et al. (2015) reported a significant inverse relationship between MVPA and VAT in 1249 adults. Furthermore, a study by Janz et al. (2017) examined the association between LPA and MVPA in relation to estimations of VAT conducted with dual-energy X-ray absorptiometry in 463 children and adolescents. The results showed that MVPA was inversely associated with VAT in boys, while no significant association between MVPA and VAT was found in girls. The non-significant finding in girls may be because the girls had lower absolute level and limited range of MVPA and VAT compared to the boys (Janz et al., 2017). No significant associations between LPA and VAT were found for boys or girls.

The finding of no significant association between physical activity and liver fat in the current study is similar to the findings of the study by Maskarinec et al. (2021). The study reported no significant association between self-reported physical activity and liver fat. However, a study by Dyrstad et al. (2014) compared self-reported and accelerometer-measured physical activity in adults. The results showed that the general agreement between self-reported and accelerometer-measured physical activity was poor, which may question the reliability of using self-reported physical activity compared to the objective method of using accelerometers (Dyrstad et al., 2014). A study by Saelens et al. (2007) examined the association between accelerometer-measured physical activity and self-reported physical activity in children. Self-reported physical activity estimated more than twice the amount of MPA and VPA than the accelerometer-measured physical activity. Using the objective accelerometer-measured physical activity, a study by Andersen et al. (2016) examined how total amount of physical activity and MVPA at age 12-14 years prospectively was associated with liver fat at mean age 17.8 years. The results showed that a larger amount of total physical activity and MVPA was associated with a lower risk of accumulating liver fat. These contrasting findings underline the need for more research to clarify the association between physical activity and liver fat.

Even though accelerometer-derived physical activity was not associated with VAT, the results showed that CRF was inversely associated with VAT and SAT. These results are similar to the results in the study by Lee and Arslanian (2007) that found CRF to be inversely associated with VAT and SAT in children. CRF is a measure of aerobic effect and related to physical activity (Jackson et al., 2009), therefore it seems likely that some elements of physical activity may be associated with both VAT and SAT. For obese children and adolescents, engaging in physical activity to improve CRF may be beneficial since higher CRF shows association with lower VAT and SAT.

The seven days of measured physical activity in this study only covers a small part of the accumulated physical activity that may influence regional fat deposits. It could be speculated if the limited accelerometer data is enough to reflect the physical activity level of the participants. A study by Trost et al. (2000) found that adolescents show more day-to-day variation in MVPA compared to children. They reported that wearing the accelerometer for 4-5 days in children was enough to obtain an ICC of 0.8 for the day-to-day variability in MVPA, while 8-9 days were necessary in adolescents. Therefore, since this study included both children and adolescents, the seven days may not have been sufficient to obtain an ICC of 0.8. On the other hand, Trost et al. (2000) reported that seven days provide a reliable estimate for both children and adolescents (ICC>0.7), so in that perspective the seven days should be acceptable to reflect the habitual physical activity level.

Methods of quantifying liver fat

This study found no significant differences in liver fat across the four methods used. Excellent inter-rater agreement for all methods were established which suggests suitability of all four methods to quantify liver fat. Excellent ICC was evident because the variation due to rater was very small compared to the variation due to other things such as variation across participants. The Bland-Altman plot showed narrower limits of agreement and less variation around a difference of zero between raters for the methods of 4 ROIs, 9 ROIs or whole-liver ROI compared to 2 ROIs. This suggest that including a larger portion of the liver is preferable for determining liver fat in obese children and adolescents. The above-mentioned results suggest that when using MRI the method of 4 ROIs, 9 ROIs and whole-liver ROIs can be used for future studies and clinical work, for example to diagnose fatty liver.

A study of Campo et al. (2017) reported higher inter- and intra-rater agreement for methods covering larger areas of the liver. The current study extends these results by showing that the method of using whole-liver ROIs provides similar results to the other methods. The participants in the study by

Campo et al. (2017) were adults, therefore this study adds that the inter-rater agreement in children and adolescents is high as well. However, it should be emphasized that this study did not compare the methods to the gold standard of liver biopsy to diagnose and grade nonalcoholic fatty liver disease (NAFLD) (Vu et al., 2016) or to MRS that is considered the noninvasive reference standard for quantification of liver fat (Longo et al., 1995; Vu et al., 2016). Therefore, the reliability analysis relies on inter-rater agreement of the same method which is less precise than comparison with the gold standard. However, a study by Vu et al. (2016) compared the proton-density fat fraction MRI with ROIs as measure points, as in the current study, with magnetic resonance spectroscopy (MRS) on determining liver fat. The study showed excellent ICC (0.916) between the liver fat quantification determined with MRI and MRS techniques which validates MRI as a suitable method for noninvasive quantification of liver fat (Vu et al., 2016).

Participants

Generally, the participants spent limited time in MVPA (mean: 10.3 min/day) and the highest registered amount of time spent in MVPA was 21.4 min/day. According to national guidelines this age group should be engaging in at least 60 min of MVPA per day (Pedersen & Andersen, 2018). Considering these guidelines, none of the participants are close to reaching the national guidelines.

This study found a prevalence of 35% for fatty liver (>5% liver fat) among the 20 children and adolescents included in the analysis. Similarly, a study by Schwimmer et al. (2006) found a prevalence of 38% among obese children and adolescents in the United States. In comparison, fatty liver was present in 5% of the normal weight children and adolescents (Schwimmer et al., 2006).

The amount of VAT (44.4±26.3 cm^2) and SAT (279.0±81.4 cm^2) in this study is similar to levels of VAT (47.7±15.05 cm^2) and SAT (298.3±48.22 cm^2) reported in participants of similar age (mean: 13.9±0.94 years) and BMI (mean: 29.1±2.32 kg/m^2) in the study by Jung & Song (2018). Therefore, these comparisons indicate that the regional fat deposits of the children and adolescents in this study are similar to other cohorts of obese children and adolescents at similar age.

Health perspective of regional fat deposits

As previously mentioned, the deposition of body fat described as regional fat deposits influences the level of health risks (Neeland et al., 2018; Piché et al., 2018; Després, 2015). Therefore, it is relevant to establish guidelines for favorable intensities, durations and frequencies of physical activity intended to reduce specific regional fat deposits and associated health risks in children and adolescents. The current study was able to find a significant association for LPA and MVPA associated with SAT. From a health perspective, lower amounts of SAT may not decrease health risks, since SAT has been shown to be weakly or not associated with health risks (Cali & Caprio, 2009; Neeland et al., 2013; Liu et al., 2010).

Guidelines to achieve a low amount of VAT and liver fat are highly relevant, since these fat deposits are associated with health risks such as insulin resistance (Campos et al., 2019; Saki & Karamizadeh, 2014; Geurtsen et al., 2020) and metabolic syndrome (Druet et al., 2008; Cali & Caprio, 2009; Ding et al., 2018). The results of the current study were unable to add new evidence to the guidelines of how physical activity can be related to the amount of VAT and liver fat. However, an intervention study by Jung and Song (2018) examined the effect of a 16 week taekwondo training program on VAT and SAT in adolescents. The results showed that training significantly reduced VAT and SAT. Therefore, the study indicates that physical activity influences both VAT and SAT even though this cross-sectional study only found a significant association for the estimated coefficient for LPA associated with SAT.

Furthermore, two studies examined the effect of a three months training intervention on liver fat in adolescents (Lee et al., 2012; Lee et al., 2013). Both studies examined the effect of a resistance training intervention and an aerobic training intervention compared to a control group. Lee et al. (2012) reported a significant decrease of liver fat after the resistance training intervention but not after aerobic resistance training intervention. Conversely, Lee et al. (2013) reported a significant decrease of liver fat after aerobic training intervention but not after a resistance training intervention. These conflicting results along with the results for liver fat in the current study support the need for further research to establish the association between the subcomponents of physical activity and liver fat in children and adolescents for the purpose of reducing the amount of liver fat.

Limitations

The current study included 20 participants. This small sample size could be a limitation since the study may not be sufficiently powered to identify associations between subcomponents of physical activity and regional fat deposits. Insufficient sample size was supported by post hoc power calculation that showed a power of 0.26 for the significant multiple regression. To obtain a power of 0.80 the sample size had to be at least 69 participants. Therefore, one possible explanation for the different results between other studies and the current study may be the smaller sample size in the current study which decreased the ability of identifying possible associations. The participants of this study had a similar absolute level and low range of time spent engaging in MVPA, which may have affected the ability of the study to find significant results.

Furthermore, the cross-sectional nature of this study prevents inferring causalities. To infer causality between subcomponents of physical activity and regional fat deposits, randomized controlled trials are necessary.

Practical applications and conclusion

This cross-sectional study found a significant inverse association for LPA and MVPA associated with SAT in obese children and adolescents. However, the results showed that only the estimated coefficient for LPA was significant. Therefore, more time spent doing LPA was associated with a lower amount of SAT. The broad 95% CI of MVPA complicated making conclusions about MVPA based on the current data. LPA and MVPA were not significantly associated with VAT and liver fat. Furthermore, the results showed a significant inverse association between CRF and VAT as well as between CRF and SAT. There was a trend for an inverse association between CRF and liver fat, however the variables were not significantly related. Therefore, a higher CRF may be related to lower health risks due to smaller regional fat deposits. The study found no significant associations between sedentary time as well as total physical activity and the regional fat deposits. However, small sample size and highly homogeneous physical activity level for the participants may have affected these finding. Therefore, more research is needed examining a larger population to establish the relationship between these subcomponents of physical activity behavior and regional fat deposits. Research on this problem may lead to establishment of guidelines of physical activity intended to reduce health risks that are associated with regional fat deposits in children and adolescents.

Furthermore, this study found similar outcomes of four different methods of quantifying liver fat and excellent inter-rater agreement of all methods. However, the results indicate small advantages in inter-rater agreement of methods 4 ROIs, 9 ROIs and whole-liver ROI in comparison with 2 ROIs. This finding suggests that the methods of using 4 ROIs, 9 ROIs and whole-liver ROIs may be used by clinicians and for future studies to quantify liver fat when using MRI.

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