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The effect of diet or exercise on ectopic adiposity in children and adolescents with obesity: a systematic review and meta-analysis.

Introduction

Overweight and obesity remain one of the most prevalent chronic health conditions in children and adolescents.[1, 2] The worldwide prevalence of overweight and obesity is increasing rapidly, with the fastest rise in low and middle-income countries.[1]

The increasing prevalence of childhood obesity is associated with the raise of metabolic and cardiovascular comorbidities including hypertension, dyslipidemia and type 2 diabetes mellitus.[1, 3] Since disease progression into adulthood is plausible, this current situation constitutes a challenge for future demands on health services.[3-7]

However, children and adolescents with a "metabolically healthy obesity" (MHO) phenotype exist. These individuals are currently not diagnosed with any common metabolic complication such as dyslipidemia, insulin resistance or arterial hypertension.[8-10] Comparable to adults, there are numerous reasons why some children and adolescents with obesity do not develop any metabolic complications.[11] One of the possible contributing factors is a difference in fat distribution. Individuals with MHO have a better ability to absorb free fatty acids in adipocytes and store less ectopic fat than individuals with unhealthy metabolic obesity.[12] Ectopic adiposity is defined as excess of fat in places not classically associated with adipose tissue storageand and may contribute to inflammation and insulin resistance.[13-16] Furthermore, ectopic fat deposition is associated with an increased risk of cardiovascular disease and insulin resistance.[17-19]

Consequently, in addition to body weight and whole-body fat mass, a stronger focus on ectopic adiposity is necessary in the follow-up of children and adolescents with overweight or obesity. In adults, ectopic adiposity has been described in the abdomen, skeletal muscles, liver, heart and kidneys and such ectopic fat accumulation may lead to metabolic and cardiovascular diseases.[20, 21]

Fat deposits in the liver of children and adolescents can lead to paediatric Non-Alcoholic Fatty Liver Disease (NAFLD) and Non-Alcoholic Steatohepatitis (NASH) [22-24] Since a liver biopsy is still the gold standard for the diagnosis of NAFLD, the prevalence of NAFLD amongst children is relatively unknown due to its invasive character. Estimations, however, suggest that worldwide, 38% to 90% of all children with obesity develop NAFLD.[22, 25-27] Consequently, early diagnosis and treatment of paediatric NAFLD should be mandatory to prevent the development of NASH.[23, 24] It is equally important to obtain knowledge of the effect size of a liver steatosis treatment.

Diet or exercise have a significant effect on the decrease of ectopic adiposity in adults with overweight and obesity and simultaneously improve the cardio metabolic profile.[28-30] Although research in this area is still scarce concerning children or adolescents with overweight or obesity, previous investigation supports a decrease in visceral adiposity in children and adolescents.[31] Moreover, guidelines highlight the importance of weight loss and lifestyle modification in children and adolescents with overweight or NAFLD.[32-35]

The aim of this systematic review and meta-analysis is to summarize the evidence for the use of a non-invasive weight loss intervention (diet and/or exercise) in children and adolescents with overweight or obesity and its effect on ectopic adiposity located in and around skeletal muscles, liver, heart, pancreas and kidneys.

Methods

This systematic review and meta-analysis is designed according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analysis) statement.[36] The protocol of this review has been registered in PROSPERO under the number CRD42014015381.

Search Strategies

The PubMed, PEDro and Cochrane databases were used to run an electronic search specified to each anatomical fat deposition area.

Key words were based on the PICO acronym and were combined with BOOLEAN operators “OR” and “AND”. The search strategy is shown in Table 1. When applicable, limits were set on “clinical trials” and “children”.

Study Selection and Quality Assessment

The three databases were systematically searched using a priori defined in- and exclusion criteria. To obtain consistent results, only clinical trials in which the outcome measurement was ectopic fat were included. Studies in which echography evalulated hepatic adiposity were excluded because no quantifiable data were reported. Since histological abnormalities in liver biopsies are not always paired with elevated liver enzymes in children with NAFLD [37, 38], studies in which liver enzymes were used as an indication of liver adiposity were excluded. Papers describing children or adolescents (mean age<19y) with obesity-related complications such as impaired glucose tolerance, NAFLD or impaired liver function were included. Overweight and obesity were identified in agreement with established international paediatric cut-off criteria and curves.[39, 40] This meta-analysis focuses on lifestyle interventions aiming to reduce body weight including the achievement of a negative energy balance by implementing a hypocaloric diet, exercise, the combination of diet and exercise or healthy lifestyle advice. Studies or study arms in which medication or nutritional supplements were a part of the treatment, were excluded. The Cochrane risk of bias tool was used by two independent investigators to assess the study quality.[41]

Screening and Data Extraction

Titles, abstracts and full-text articles were screened by two independent investigators. Studies fulfilling the criteria mentioned above were included. Figure 1 illustrates the flow diagram of the systematic reviewing process. A standardized data extraction form was used to compile Tables 2 and 3. Whenever methods or data were not clearly reported, the corresponding authors were contacted.

Statistical Analysis

The extracted data was entered into the CMA-2 software (Comprehensive Meta-Analysis 2nd

version, Biostat, Englewood, USA). A random-effects model was used to pool the individual study results and to examine the overall weighted effect size of a lifestyle intervention on ectopic adiposity. Effect sizes (changes in ectopic adiposity) were calculated as standardized mean differences. It is likely that the analysis, based on small study groups, results in an overestimation of the effect size. Therefore, a correction was made with a factor g, expressed as Hedges' g.[42] A negative or positive value for Hedges' g indicates a decrease or increase in ectopic adiposity, respectively. The value of the effect size is defined as 0.2=small, 0.5=moderate and 0.8=large.[41]

The 95% confidence intervals [95%CI] were calculated for the individual studies and the overall weighted estimate. Using a correlation coefficient of 0.7 between pre- and post-intervention values and a random-effects model, a balanced and conservative approach is maintained which allows true variations in the effect size and heterogeneity across included studies.[43] The Cochran's Q statistic and its corresponding p-value were calculated for heterogeneity testing, and the I^2 statistic was assessed to express the degree of heterogeneity across studies. To facilitate the clinician's interpretation of the overall effect of lifestyle intervention on hepatic adiposity, the value of Hedges' g was re-expressed to Intra Hepatic Lipids (IHL) and described as proton density fat fraction (%). Baseline % IHL standard deviations of the intervention and control groups from the Lee et al. study[44] were pooled and multiplied by the pooled standardized mean difference. Two additional subgroup analyses were performed based on commonly accepted confounding variables such as study design (Randomized controlled trials versus non-randomized controlled trials) and the ethnicity of subjects. Finally, an additional sensitivity analysis was done in which one study was excluded.

P-values less than 0.05 were considered significant (2-tailed).

Results

Study Selection

The initial search resulted in 18 hits in the search strategy of muscular adiposity (Intra MyoCellular Lipids-IMCL) (search strategy a), 99 hits in the search strategy of hepatic adiposity (search strategy b)

and nine hits in the search strategy of pancreatic adiposity (search strategy c). The search strategy of ectopic adiposity of the heart and kidneys (search strategy d and e) yielded three hits each.

After removing duplicates and eliminating papers based on the eligibility criteria, 14 studies remained available for full-text analysis. Due to insufficient data reporting, one article was excluded.[45] After completion of the full-text screening, nine articles on the effect of lifestyle interventions on hepatic fat (320 patients) and three articles on IMCL (55 patients) remained for the meta-analysis. No articles were found on lifestyle interventions and the deposition of ectopic fat in/around the heart, kidneys or pancreas.

Risk of bias

Four clinical trials and six randomized controlled clinical trials were included in this meta-analysis. The results of the risk of bias assessment are shown in Table 4. Since the aspect of blinding was often inadequately explained and the results were repeatedly not transparently presented, a risk of bias was plausible. Only two papers report the adherence to the exercise program or dietary regime.[46, 47]

Population characteristics

According to classification criteria of overweight and obesity in children and adolescents [39, 40], all articles addressed a lifestyle intervention in children or adolescents with obesity. Teenagers (Tanner stage between 4 and 5) without cardiometabolic comorbidities were examined in most studies. In three studies, (part of the) subjects were diagnosed with NAFLD or NASH.[47-49] Most studies described the exact number of ethnic groups to the total population.

Intervention Characteristics

In the included studies, supervised physical activity or the advice to increase physical activity was a part of the lifestyle intervention. Study duration ranged between 3 and 12 months, and the weekly used exercise volume ranged between 90 and 180 minutes.

Anthropometric Data

A statistically significant reduction in BMI or BMI z-score was described in almost all studies. In only two studies conducted by Lee et al., the aerobic training [44, 50] and a strength training [44] did not result in statistically significant BMI decreases. BMI changes were not reported in one study.[51] Changes in whole-body fat mass and fat-free mass were reported in the majority of studies. Only in one study, whole-body fat mass did not change.[52] These anthropometric parameters were not reported in three studies.[46, 47, 51]

Adiposity of the liver

Hepatic adiposity was evaluated in nine studies including 320 subjects (table 2). A forest plot of this analysis is shown in figure 2. A lifestyle intervention led to a decrease in hepatic adiposity (-0.54 Hedges' g [95% CI: -0.69 to -0.38] with p<0.0001). By re-expressing the observed overall weighted effect size based on the population variability of Lee et al's research.[44] , it was confirmed that diet and/or exercise interventions resulted in an absolute IHL reduction of 2% in children and adolescents with obesity. No between-study heterogeneity was observed (Cochran's Q = 10.19, df(Q) = 12, p=0.6; I^2 = 0%).

a) Subgroup analysis study design

A first subgroup analysis based on study design (non-randomized versus randomized clinical trials) showed a higher, non-significant overall weighted effect size (p=0.71) (-0.55 Hedges' g (CI) versus -0.48 Hedges' g)

b) Subgroup analysis modality of the intervention

In a second subgroup analysis, groups were compared by intervention modality. Exercise training seemed to lead to the greatest reductions in hepatic adiposity (-0.64 [95% CI: -1.00 to -0.27]) compared to the combination of diet and exercise (-0.54 [95% CI: -0.74 to -0.34]) or diet-only (-0.47 [95% CI: -1.00 to 0.05]). Though, the differences in effect size between groups were not significant

(p=0.86). There was no heterogeneity between the exercise-only studies or other study groups (with Cochran's Q = 1.79, df (Q) = 4, p=0.38; I^2 =5.48%) and heterogeneity in the studies applying diet and exercise was negligible (Cochran's Q = 4.23, df (Q) = 4, p=0.76; I^2 = 0%).

Heterogeneity was moderate (albeit not statistically significant) in diet-only studies (Cochran's Q = 3.65, df (Q) = 2, p=0.16; I^2 = 45.3%). Hasson et al's study [51] was the only study in which dietary advice was not described, changes in BMI or total whole-body fat mass were not reported and strength training was applied. Hereby it was uncertain that subjects obtained a negative energy balance. Moreover, since no decrease in hepatic adiposity was observed, it was considered to be an outlier. In a sensitivity analysis, Hasson et al. were therefore excluded. This analysis suggested that a hypocaloric diet has a greater effect on reducing hepatic adiposity (-0.76 [95% CI: -1.27 to -0.25]) compared to exercise-only (-0.64 [95% CI: -1.01 to -0.27]) or to the combination of diet and exercise (-0.55 [95% CI: -0.81 to -0.30]). However, the differences between intervention groups were not statistically significant (p = 0.77) (Figure 3).

Intramyocellular lipids (IMCL)

The effect of an intervention on IMCL was measured in three studies including 55 subjects (Table 3). The overall weighted mean effect size of diet or exercise on IMCL, expressed as Hedges' g was -0.03 [95% CI: -0.52 to 0.47].

Further analysis showed moderate, non significant heterogeneity across studies (Cochran's Q = 4.99, df(Q) = 3, p=0.17; I^2 = 39.9%).

Discussion

Although the link between overweight or obesity and metabolic diseases in childhood obesity could be provoked by body fat distribution and ectopic adiposity[15], research on ectopic adiposity patterns in children and adolescents is scarce.

This meta-analysis concerns only data of hepatic adiposity (nine studies, including 392 subjects) and intramyocellular lipids (three studies, including 76 subjects. The impact of lifestyle intervention on other anatomic sites of ectopic adiposity in children and adolescents with overweight or obesity remains to be studied.

Results of this meta-analysis demonstrate for the first time that a lifestyle intervention (diet and/or exercise) of at least 3 months may yield towards a 2% decrease in intra hepatic lipid content in children and adolescents with obesity. The effect of lifestyle interventions on changes in hepatic fat seems to be smaller compared to adults with overweight and obesity (5-10% IHL reduction).[29] The intra hepatic lipid content is expressed as proton density fat fraction (IHL = (lipid/ (lipid+water)*100). Nevertheless, this is an absolute value of lipid quantification in the liver and an absolute 2% decrease has been observed. In reference to Lee et al's study [44] which was used for the re-expression of Hedges' g, baseline values range between 2.0±1.3% and 3.0±5.4%. Hereby, an absolute reduction by 2% means a relative reduction of more than 50% of existing liver fat. Hepatic adiposity reduction involving lifestyle interventions may be as high as 77% in children and adolescents with obesity (Table 2).[44] Therefore, a lifestyle intervention does lead to substantial and clinically relevant reductions in IHL in children and adolescents with obesity.

Moreover, it is observed that baseline hepatic adiposity content is much lower in children and adolescents with obesity compared to adults with obesity. Furthermore, Lange et al.'s previous research confirms that the mean IHL of children with obesity is more than one order of magnitude smaller than the IHL content in adults with obesity (1.0±0.5% vs 17.0±8.7%).[53] This can clinically be explained by the fact that severe or fibrotic NASH need substantial time to develop. Therefore the prevalence is higher in adults with obesity than in children or adolescents with obesity.[54]
Since NAFLD can evolve towards NASH, it is important to observe the NAFLD progression during treatment of young patients by validated imaging techniques.[23, 24] In clinical settings, liver enzymes are used as a non-invasive screening tool for NAFLD in children.[55] Nevertheless, cohort studies in

children and adults show normal liver enzymes values in nearly 80% of patients with established fatty liver disease. Moreover, cut-off values in children and adolescents with NAFLD based on blood liver enzymes are discussable.[24, 37, 38, 55-59] Therefore, we preferred to analyze data based on direct measurements of hepatic adiposity. Studies in which liver enzymes were only used as markers of hepatic adiposity, were excluded. Despite the fact that ultrasound techniques have an important clinical value, it was not possible to use ultrasound results in this meta-analysis, because no quantifiable data were reported. Echography results are operator dependent and limit therefore sensitivity and specificity in mild NAFLD.[60-63] The most common technique to assess liver adiposity is Magnetic Resonance Spectroscopy (^{1}H-MRS) which is considered to be a valid and accurate assessment method with good reproducibility. However, it is time-consuming and requires complex data analysis.[64-67] No clinical trials were found in which the effect of a conservative treatment (diet or exercise) on hepatic fat content was assessed by liver biopsy.

According to the different intervention stages described by Barlow et al., weight loss is a key factor in the treatment of pubertal children with obesity.[68, 69] Nevertheless, this meta-analysis shows that a BMI reduction does not relate to a decrease in hepatic adiposity. Shorter (up to 12 weeks) exercise-only studies did not result in significant BMI reductions while significant reductions of IHL were observed.[44, 50, 70] It can be explained by the fact that physical activity sensitizes muscles to insulin and modifies hepatic lipids.[71-73] Furhtermore, it should be noted that a reduction in whole-body fat mass is achieved in these studies.

The variations in program duration, exercise modalities, exercise volume and degree of caloric intake made it difficult to conduct direct comparisons between studies and to identify the most effective intervention to reduce hepatic adiposity in children and adolescents with obesity. In order to overcome this limitation, a subgroup analysis was performed and outliers were detected. Since it was possible that no negative energy balance was obtained in Hasson et al.'s[51], this research was considered to be an outlier. However, a sensitivity analysis without this study did not change our

results. Although there seems to be a difference in effect size between different study designs, this difference was not statistically significant.

The limited number of studies (with each small sample sizes) in the subgroup analyses evoked large confidence intervals partially explaining why the between-groups difference in effect size was not statistically significant.

It is remarkable that the exercise volume (90-180 minutes/week) applied in the exercise study groups did not often comply with the recommended guideline of one hour per day of exercise in children and adolescents with obesity.[68, 74, 75] It could be that a more rigorous exercise regimen would yield better results. It may be argued that the impact of lifestyle interventions on ectopic fat is underestimated in children and adolescents with obesity.

Although there were significant improvements in insulin resistance or sensitivity in all intervention groups, neither endurance nor resistance exercise training yielded significant changes in IMCL. This finding supports the results found by Larson-Meyer et al.[76], who stated that IMCL content is metabolically inert and should not be considered as a determinant of insulin resistance in skeletal muscles. In this regard, it can be assumed that skeletal muscle oxidative capacity plays a role in the association between insulin resistance and excess IMCL in people with overweight or obesity.[77, 78]

In only three studies, (part of the) subjects were diagnosed with NAFLD or NASH.[47-49] Since NAFLD is defined as IHL content higher than 5.6% measured by ^{1}H-MRS [79], only Lee et al.'s research addresses with children without liver disease.[44, 50]

One of the most challenging aspects for healthcare professionals in paediatric weight management programs is the difficulty in obtaining sustained long-term results. Rates of attrition are reported between 27% and 75%.[80, 81] Unfortunately, no long-term studies or studies with follow-up measurements were found.

One of the strengths of this study is the extensive systematic review of the literature providing a meta-analysis revealing the effects of lifestyle interventions on all well documented anatomic sites of ectopic adiposity in children and adolescents with obesity. In addition, the results of lifestyle interventions on hepatic adiposity were made clinically interpretable by re-expressing Hedges'g as absolute values of IHL. In general, clinical and statistical heterogeneity among the included studies was low.

There are, however, also some limitations to this study. The quality of this systematic review and meta-analysis is limited by the methodological quality of the included studies. In the majority of included studies, a risk of bias is plausible because due to inadequate reporting of applied methodology and patient adherence. In most studies, the prevalence of insulin resistance, type 2 diabetes or liver diseases was not reported.

Finally, the included studies described rather small study populations.

To facilitate future systematic reviews and meta-analyses, researchers should be encouraged to report their methods and observed outcomes transparently (as well in changes as in means with standard deviations). Given the fact that long-term effectiveness of a lifestyle intervention is dependent on the sustainability of behaviour change, it is important that adherence to the prescribed intervention protocol is adequately assessed and reported. A comparison with habitual diet and exercise behaviour can result in a correct interpretation of the intervention effect.

Conclusion

This meta-analysis shows that diet and/or exercise is effective in reducing hepatic adiposity in children and adolescents with obesity, even without a BMI reduction. This reaffirms existing clinical guidelines in which complete lifestyle modification is promoted in the management of paediatric obesity. Although there were significant ameliorations in insulin sensitivity in all intervention groups, no significant changes in IMCL were found.

References

1 Lobstein T, Jackson-Leach R, Moodie ML, Hall KD, Gortmaker SL, Swinburn BA*, et al.* Child and adolescent obesity: part of a bigger picture. *Lancet*. 2015; 385: 2510-20.

2 van Stralen MM, te Velde SJ, van Nassau F, Brug J, Grammatikaki E, Maes L*, et al.* Weight status of European preschool children and associations with family demographics and energy balance-related behaviours: a pooled analysis of six European studies. *Obesity reviews : an official journal of the International Association for the Study of Obesity*. 2012; 13 Suppl 1: 29-41.

3 Lobstein T, Jackson-Leach R. Estimated burden of paediatric obesity and co-morbidities in Europe. Part 2. Numbers of children with indicators of obesity-related disease. *Int J Pediatr Obes*. 2006; 1: 33-41.

4 Kiess W, Bottner A, Raile K, Kapellen T, Muller G, Galler A*, et al.* Type 2 diabetes mellitus in children and adolescents: a review from a European perspective. *Horm Res*. 2003; 59 Suppl 1: 77-84.

5 Malecka-Tendera E, Erhardt E, Molnar D. Type 2 diabetes mellitus in European children and adolescents. *Acta Paediatr*. 2005; 94: 543-6.

6 Simmonds M, Llewellyn A, Owen CG, Woolacott N. Predicting adult obesity from childhood obesity: a systematic review and meta-analysis. *Obesity reviews : an official journal of the International Association for the Study of Obesity*. 2016; 17: 95-107.

7 Singh AS, Mulder C, Twisk JW, van Mechelen W, Chinapaw MJ. Tracking of childhood overweight into adulthood: a systematic review of the literature. *Obesity reviews : an official journal of the International Association for the Study of Obesity*. 2008; 9: 474-88.

8 Bluher S, Molz E, Wiegand S, Otto KP, Sergeyev E, Tuschy S*, et al.* Body mass index, waist circumference, and waist-to-height ratio as predictors of cardiometabolic risk in childhood obesity depending on pubertal development. *The Journal of clinical endocrinology and metabolism*. 2013; 98: 3384-93.

9 Roberson LL, Aneni EC, Maziak W, Agatston A, Feldman T, Rouseff M*, et al.* Beyond BMI: The "Metabolically healthy obese" phenotype & its association with clinical/subclinical cardiovascular disease and all-cause mortality -- a systematic review. *BMC public health*. 2014; 14: 14.

10 van Vliet-Ostaptchouk JV, Nuotio ML, Slagter SN, Doiron D, Fischer K, Foco L*, et al.* The prevalence of metabolic syndrome and metabolically healthy obesity in Europe: a collaborative analysis of ten large cohort studies. *BMC endocrine disorders*. 2014; 14: 9.

11 Kramer CK, Zinman B, Retnakaran R. Are metabolically healthy overweight and obesity benign conditions?: A systematic review and meta-analysis. *Annals of internal medicine*. 2013; 159: 758-69.

12 Bluher S, Schwarz P. Metabolically healthy obesity from childhood to adulthood - Does weight status alone matter? *Metabolism: clinical and experimental*. 2014; 63: 1084-92.

13 Van Name M, Santoro N. Type 2 diabetes mellitus in pediatrics: a new challenge. *World J Pediatr*. 2013; 9: 293-9.

14 Alderete TL, Toledo-Corral CM, Goran MI. Metabolic basis of ethnic differences in diabetes risk in overweight and obese youth. *Curr Diab Rep*. 2014; 14: 455.

15 Samara A, Ventura EE, Alfadda AA, Goran MI. Use of MRI and CT for fat imaging in children and youth: what have we learned about obesity, fat distribution and metabolic disease risk? *Obesity reviews : an official journal of the International Association for the Study of Obesity*. 2012; 13: 723-32.

16 Britton KA, Fox CS. Ectopic fat depots and cardiovascular disease. *Circulation*. 2011; 124: e837-41.

17 Larson-Meyer DE, Newcomer BR, Ravussin E, Volaufova J, Bennett B, Chalew S*, et al.* Intrahepatic and intramyocellular lipids are determinants of insulin resistance in prepubertal children. *Diabetologia*. 2011; 54: 869-75.

18 Lionetti L, Mollica MP, Lombardi A, Cavaliere G, Gifuni G, Barletta A. From chronic overnutrition to insulin resistance: the role of fat-storing capacity and inflammation. *Nutrition, metabolism, and cardiovascular diseases : NMCD*. 2009; 19: 146-52.
19 Schwimmer JB, Pardee PE, Lavine JE, Blumkin AK, Cook S. Cardiovascular risk factors and the metabolic syndrome in pediatric nonalcoholic fatty liver disease. *Circulation*. 2008; 118: 277-83.
20 Despres JP, Lemieux I, Bergeron J, Pibarot P, Mathieu P, Larose E*, et al.* Abdominal obesity and the metabolic syndrome: contribution to global cardiometabolic risk. *Arteriosclerosis, thrombosis, and vascular biology*. 2008; 28: 1039-49.
21 Mathieu P, Boulanger MC, Despres JP. Ectopic visceral fat: a clinical and molecular perspective on the cardiometabolic risk. *Reviews in endocrine & metabolic disorders*. 2014; 15: 289-98.
22 Alisi A, Manco M, Vania A, Nobili V. Pediatric nonalcoholic fatty liver disease in 2009. *The Journal of pediatrics*. 2009; 155: 469-74.
23 Feldstein AE, Charatcharoenwitthaya P, Treeprasertsuk S, Benson JT, Enders FB, Angulo P. The natural history of non-alcoholic fatty liver disease in children: a follow-up study for up to 20 years. *Gut*. 2009; 58: 1538-44.
24 Molleston JP, White F, Teckman J, Fitzgerald JF. Obese children with steatohepatitis can develop cirrhosis in childhood. *The American journal of gastroenterology*. 2002; 97: 2460-2.
25 Barshop NJ, Sirlin CB, Schwimmer JB, Lavine JE. Review article: epidemiology, pathogenesis and potential treatments of paediatric non-alcoholic fatty liver disease. *Aliment Pharmacol Ther*. 2008; 28: 13-24.
26 Fan JG. Epidemiology of alcoholic and nonalcoholic fatty liver disease in China. *J Gastroenterol Hepatol*. 2013; 28 Suppl 1: 11-7.
27 Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. *Pediatrics*. 2006; 118: 1388-93.
28 Wu FZ, Wu CC, Kuo PL, Wu MT. Differential impacts of cardiac and abdominal ectopic fat deposits on cardiometabolic risk stratification. *BMC cardiovascular disorders*. 2016; 16: 20.
29 Hens W, Taeyman J, Cornelis J, Gielen J, Van Gaal L, Vissers D. The Effect of Lifestyle Interventions on Excess Ectopic Fat Deposition Measured by Non-Invasive Techniques in Overweight and Obese Adults: A Systematic Review and Meta-Analysis. *J Phys Act Health*. 2015.
30 Snel M, Jonker JT, Schoones J, Lamb H, de Roos A, Pijl H*, et al.* Ectopic fat and insulin resistance: pathophysiology and effect of diet and lifestyle interventions. *Int J Endocrinol*. 2012; 2012: 983814.
31 Vissers DH, Wendy; Hansen, Dominique; Taeymans, Jan. The Effect of Diet or Exercise on Visceral Adipose Tissue in Overweight Youth. *Medicine & Science in Sports & Exercise, Post Acceptance: January 30, 2016*. 2016.
32 Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K*, et al.* The diagnosis and management of non-alcoholic fatty liver disease: Practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *The American journal of gastroenterology*. 2012; 107: 811-26.
33 Hansen D, Hens W, Peeters S, Wittebrood C, Van Ussel S, Verleyen D*, et al.* Physical Therapy as Treatment for Childhood Obesity in Primary Health Care: Clinical Recommendation From AXXON (Belgian Physical Therapy Association). *Physical therapy*. 2016; 96: 850-64.
34 AlKhater SA. Paediatric non-alcoholic fatty liver disease: an overview. *Obesity reviews : an official journal of the International Association for the Study of Obesity*. 2015; 16: 393-405.
35 August GP, Caprio S, Fennoy I, Freemark M, Kaufman FR, Lustig RH*, et al.* Prevention and treatment of pediatric obesity: an endocrine society clinical practice guideline based on expert opinion. *The Journal of clinical endocrinology and metabolism*. 2008; 93: 4576-99.
36 Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP*, et al.* The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *Annals of internal medicine*. 2009; 151: W65-94.

37 Molleston JP, Schwimmer JB, Yates KP, Murray KF, Cummings OW, Lavine JE*, et al.* Histological abnormalities in children with nonalcoholic fatty liver disease and normal or mildly elevated alanine aminotransferase levels. *The Journal of pediatrics*. 2014; 164: 707-13 e3.
38 Schwimmer JB, Dunn W, Norman GJ, Pardee PE, Middleton MS, Kerkar N*, et al.* SAFETY study: alanine aminotransferase cutoff values are set too high for reliable detection of pediatric chronic liver disease. *Gastroenterology*. 2010; 138: 1357-64, 64 e1-2.
39 Barlow SE, Dietz WH. Obesity evaluation and treatment: Expert Committee recommendations. The Maternal and Child Health Bureau, Health Resources and Services Administration and the Department of Health and Human Services. *Pediatrics*. 1998; 102: E29.
40 Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ*. 2000; 320: 1240-3.
41 Higgins JPT GS. The Cochrane Collaboration: Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0.
42 Michael Borenstein LVH, Julian P. T. Higgins, Hannah R. Rothstein. References, in Introduction to Meta-Analysis. Chichester, UK: John Wiley & Sons, Ltd 2009.
43 Borenstein M, Hedges LV, Higgins JP, Rothstein HR. A basic introduction to fixed-effect and random-effects models for meta-analysis. *Research synthesis methods*. 2010; 1: 97-111.
44 Lee S, Deldin AR, White D, Kim Y, Libman I, Rivera-Vega M*, et al.* Aerobic exercise but not resistance exercise reduces intrahepatic lipid content and visceral fat and improves insulin sensitivity in obese adolescent girls: a randomized controlled trial. *American Journal of Physiology-Endocrinology and Metabolism*. 2013; 305: E1222-E29.
45 Davis JN, Gyllenhammer LE, Vanni AA, Meija M, Tung A, Schroeder ET*, et al.* Startup circuit training program reduces metabolic risk in Latino adolescents. *Med Sci Sports Exerc*. 2011; 43: 2195-203.
46 Pozzato C, Verduci E, Scaglioni S, Radaelli G, Salvioni M, Rovere A*, et al.* Liver fat change in obese children after a 1-year nutrition-behavior intervention. *Journal of pediatric gastroenterology and nutrition*. 2010; 51: 331-5.
47 Ramon-Krauel M, Salsberg SL, Ebbeling CB, Voss SD, Mulkern RV, Apura MM*, et al.* A low-glycemic-load versus low-fat diet in the treatment of fatty liver in obese children. *Child Obes*. 2013; 9: 252-60.
48 Pacifico L, Arca M, Anania C, Cantisani V, Di Martino M, Chiesa C. Arterial function and structure after a 1-year lifestyle intervention in children with nonalcoholic fatty liver disease. *Nutrition, metabolism, and cardiovascular diseases : NMCD*. 2013; 23: 1010-6.
49 Vitola BE, Deivanayagam S, Stein RI, Mohammed BS, Magkos F, Kirk EP*, et al.* Weight loss reduces liver fat and improves hepatic and skeletal muscle insulin sensitivity in obese adolescents. *Obesity*. 2009; 17: 1744-8.
50 Lee S, Bacha F, Hannon T, Kuk JL, Boesch C, Arslanian S. 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*. 2012; 61: 2787-95.
51 Hasson RE, Adam TC, Davis JN, Kelly LA, Ventura EE, Byrd-Williams CE*, et al.* Randomized controlled trial to improve adiposity, inflammation, and insulin resistance in obese African-American and Latino youth. *Obesity*. 2012; 20: 811-8.
52 McCormack SE, McCarthy MA, Harrington SG, Farilla L, Hrovat MI, Systrom DM*, et al.* Effects of exercise and lifestyle modification on fitness, insulin resistance, skeletal muscle oxidative phosphorylation and intramyocellular lipid content in obese children and adolescents. *Pediatric obesity*. 2014; 9: 281-91.
53 Lange T, Buechert M, Baumstark MW, Deibert P, Gerner S, Ryden H*, et al.* Value of MRI and MRS fat measurements to complement conventional screening methods for childhood obesity. *Journal of magnetic resonance imaging : JMRI*. 2015; 42: 1214-22.
54 Xanthakos SA, Jenkins TM, Kleiner DE, Boyce TW, Mourya R, Karns R*, et al.* High Prevalence of Nonalcoholic Fatty Liver Disease in Adolescents Undergoing Bariatric Surgery. *Gastroenterology*. 2015; 149: 623-34 e8.

428 55 Rodriguez G, Gallego S, Breidenassel C, Moreno LA, Gottrand F. Is liver transaminases
429 assessment an appropriate tool for the screening of non-alcoholic fatty liver disease in at risk obese
430 children and adolescents? *Nutr Hosp*. 2010; 25: 712-7.
431 56 Fishbein MH, Miner M, Mogren C, Chalekson J. The spectrum of fatty liver in obese children
432 and the relationship of serum aminotransferases to severity of steatosis. *Journal of pediatric*
433 *gastroenterology and nutrition*. 2003; 36: 54-61.
434 57 Guha IN, Parkes J, Roderick PR, Harris S, Rosenberg WM. Non-invasive markers associated
435 with liver fibrosis in non-alcoholic fatty liver disease. *Gut*. 2006; 55: 1650-60.
436 58 Colantonio DA, Kyriakopoulou L, Chan MK, Daly CH, Brinc D, Venner AA*, et al.* Closing the
437 gaps in pediatric laboratory reference intervals: a CALIPER database of 40 biochemical markers in a
438 healthy and multiethnic population of children. *Clinical chemistry*. 2012; 58: 854-68.
439 59 Park JH, Kim SH, Park S, Park MJ. Alanine aminotransferase and metabolic syndrome in
440 adolescents: the Korean National Health and Nutrition Examination Survey Study. *Pediatric obesity*.
441 2014; 9: 411-8.
442 60 Shannon A, Alkhouri N, Carter-Kent C, Monti L, Devito R, Lopez R*, et al.* Ultrasonographic
443 quantitative estimation of hepatic steatosis in children With NAFLD. *Journal of pediatric*
444 *gastroenterology and nutrition*. 2011; 53: 190-5.
445 61 Strauss S, Gavish E, Gottlieb P, Katsnelson L. Interobserver and intraobserver variability in the
446 sonographic assessment of fatty liver. *AJR American journal of roentgenology*. 2007; 189: W320-3.
447 62 Saadeh S, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M*, et al.* The utility of
448 radiological imaging in nonalcoholic fatty liver disease. *Gastroenterology*. 2002; 123: 745-50.
449 63 Awai HI, Newton KP, Sirlin CB, Behling C, Schwimmer JB. Evidence and recommendations for
450 imaging liver fat in children, based on systematic review. *Clin Gastroenterol Hepatol*. 2014; 12: 765-
451 73.
452 64 Vajro P, Lenta S, Socha P, Dhawan A, McKiernan P, Baumann U*, et al.* Diagnosis of
453 nonalcoholic fatty liver disease in children and adolescents: position paper of the ESPGHAN
454 Hepatology Committee. *Journal of pediatric gastroenterology and nutrition*. 2012; 54: 700-13.
455 65 Longo R, Ricci C, Masutti F, Vidimari R, Croce LS, Bercich L*, et al.* Fatty infiltration of the liver.
456 Quantification by 1H localized magnetic resonance spectroscopy and comparison with computed
457 tomography. *Investigative radiology*. 1993; 28: 297-302.
458 66 Ricci C, Longo R, Gioulis E, Bosco M, Pollesello P, Masutti F*, et al.* Noninvasive in vivo
459 quantitative assessment of fat content in human liver. *Journal of hepatology*. 1997; 27: 108-13.
460 67 Thomsen C, Becker U, Winkler K, Christoffersen P, Jensen M, Henriksen O. Quantification of
461 liver fat using magnetic resonance spectroscopy. *Magnetic resonance imaging*. 1994; 12: 487-95.
462 68 Barlow SE, Expert C. Expert committee recommendations regarding the prevention,
463 assessment, and treatment of child and adolescent overweight and obesity: summary report.
464 *Pediatrics*. 2007; 120 Suppl 4: S164-92.
465 69 Torres DM, Harrison SA. Diagnosis and therapy of nonalcoholic steatohepatitis.
466 *Gastroenterology*. 2008; 134: 1682-98.
467 70 van der Heijden GJ, Wang ZJ, Chu ZD, Sauer PJ, Haymond MW, Rodriguez LM*, et al.* A 12-
468 week aerobic exercise program reduces hepatic fat accumulation and insulin resistance in obese,
469 Hispanic adolescents. *Obesity*. 2010; 18: 384-90.
470 71 Garcia-Hermoso A, Saavedra JM, Escalante Y, Sanchez-Lopez M, Martinez-Vizcaino V.
471 Endocrinology and Adolescence: aerobic exercise reduces insulin resistance markers in obese youth:
472 a meta-analysis of randomized controlled trials. *European journal of endocrinology*. 2014; 171: R163-
473 71.
474 72 Mikines KJ, Sonne B, Farrell PA, Tronier B, Galbo H. Effect of physical exercise on sensitivity
475 and responsiveness to insulin in humans. *The American journal of physiology*. 1988; 254: E248-59.
476 73 Dela F, Mikines KJ, von Linstow M, Secher NH, Galbo H. Effect of training on insulin-mediated
477 glucose uptake in human muscle. *The American journal of physiology*. 1992; 263: E1134-43.
478 74 Baker JL, Farpour-Lambert NJ, Nowicka P, Pietrobelli A, Weiss R, Childhood Obesity Task
479 Force of the European Association for the Study of O. Evaluation of the overweight/obese child--

practical tips for the primary health care provider: recommendations from the Childhood Obesity Task Force of the European Association for the Study of Obesity. *Obes Facts*. 2010; 3: 131-7.
75 Richardson L, Paulis WD, van Middelkoop M, Koes BW. An overview of national clinical guidelines for the management of childhood obesity in primary care. *Prev Med*. 2013; 57: 448-55.
76 Larson-Meyer DE HL, Redman LM. Effect of calorie restriction with or without exercise on insulin sensitivity, beta-cell function, fat cell size, and ectopic lipid in overweight subjects. *Diabetes Care* 2006; 29: 1337-44.
77 Goodpaster BH, He J, Watkins S, Kelley DE. Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. *The Journal of clinical endocrinology and metabolism*. 2001; 86: 5755-61.
78 Malenfant P, Joanisse DR, Theriault R, Goodpaster BH, Kelley DE, Simoneau JA. Fat content in individual muscle fibers of lean and obese subjects. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity*. 2001; 25: 1316-21.
79 Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S*, et al.* Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *American journal of physiology Endocrinology and metabolism*. 2005; 288: E462-8.
80 Skelton JA, Beech BM. Attrition in paediatric weight management: a review of the literature and new directions. *Obesity reviews : an official journal of the International Association for the Study of Obesity*. 2011; 12: e273-81.
81 Skelton JA, Irby MB, Beech BM, Rhodes SD. Attrition and family participation in obesity treatment programs: clinicians' perceptions. *Acad Pediatr*. 2012; 12: 420-8.