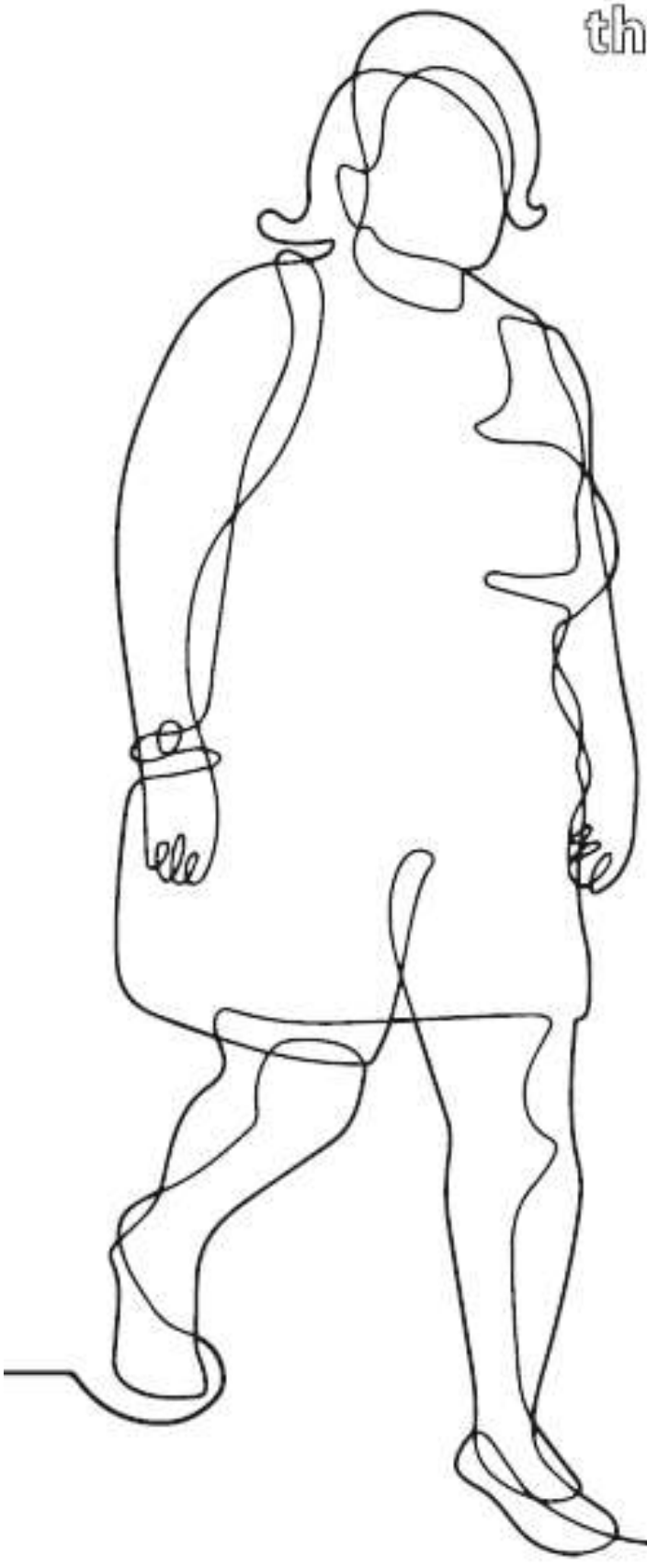
Exercise, ectopic fat and the metabolic profile in women with **overweight** and **obesity**



proefschrift voorgelegd voor het behalen van de graad van Doctor in de Medische Wetenschappen aan de Universiteit Antwerpen te verdedigen door

Wendy Hens

Voor mijn lief, William,

en onze kinderen Laïs, Lucie en Flo…

Zonder jullie steun was deze studie niet mogelijk geweest.

EXERCISE, ECTOPIC FAT AND THE METABOLIC PROFILE IN WOMEN WITH OVERWEIGHT AND OBESITY

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OEFENTHERAPIE, ECTOPISCHE VETOPSTAPELING EN HET METABOOL PROFIEL VAN VROUWEN MET OVERGEWICHT EN OBESITAS

Thesis
submitted in fulfilment of the requirements of the degree of Doctor in Medical Sciences at the University of Antwerp

Proefschrift
voorgelegd tot het behalen van de graad van Doctor in de Medische Wetenschappen aan de Universiteit Antwerpen

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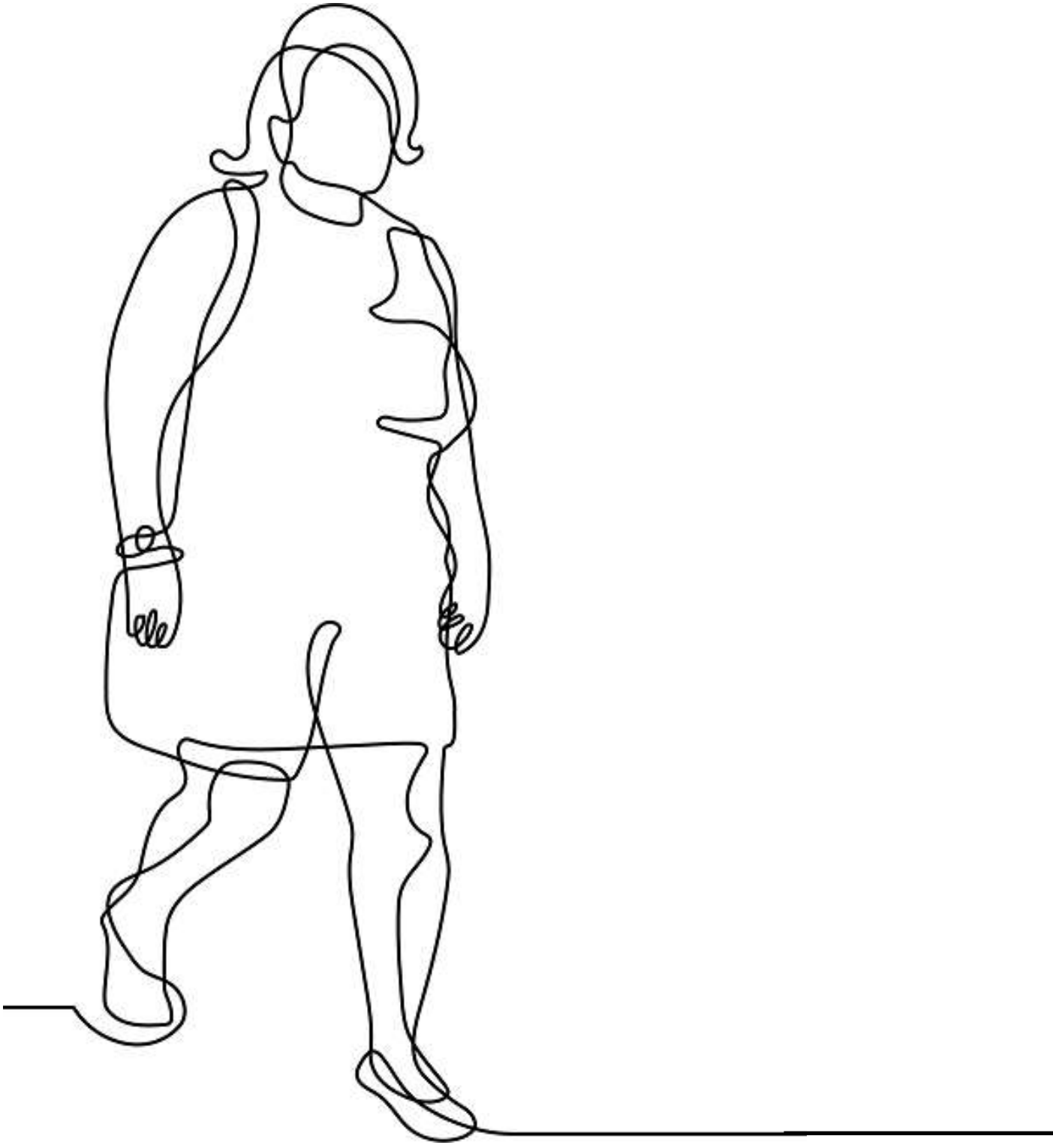
"If we could give every individual the right amount of nourishment and exercise, not too little and not too much, we would have found the safest way to health"

Hippocrates (460-370 BC)

CHAPTER 1 Introduction and outline of the thesis



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1.1 Overweight and obesity: prevalence and etiology

Last WHO report stated that approximately 39% of adults worldwide are overweight (BMI ≥ 25.0 kg.m^{-2}). At the same time, the prevalence of obesity (BMI ≥ 30.0 kg.m^{-2}) has tripled since 1975 to the alarming rate of 13%. This results in the fact that in most countries worldwide, overweight and obesity kills more people than underweight. [1, 2]
Prevalence rates of obesity vary considerably between different regions and countries, from <5% in Africa and parts of Asia to >20% in Europe and >30% in the U.S.A. and some countries in the Middle East. [1] The low prevalence rates in Africa and parts of Asia should be interpreted carefully. Overall, nationally representative data are scarce in the developing world. It is seen that when data are available, the prevalence has increased over the past two decades particularly amongst urban and high Social Economic Status (SES) groups. The association between obesity and SES varies by gender, age, and country. In general, SES groups with greater access to energy-dense diets (low-SES in industrialized countries and high-SES in developing countries) are at increased risk of being obese than their counterparts. [3, 4]

Overweight and obesity in children and adolescents is diagnosed based on normative BMI percentiles and growth charts. [5] Use of percentile and growth charts allow a uniform metric across all ages, relative to age- and sex-matched peers. [6] In this way, children of all countries, ethnicity, … can be compared against a single standard thus assessment becomes more objective and easy to compare. However, the disadvantage of using charts is that they are likely to over diagnose underweight in a large number of apparently normal children in developing countries and under diagnose overweight in the Middle East. [7]
Recent studies shows that childhood overweight and obesity prevalence is high (23% in high-income countries) but may have plateaued, especially in high-income countries. [2, 8-12] The stability in obesity prevalence might mask meaningful increases in the prevalence of severe obesity. Studies have shown a marked increase in severe obesity in preschool-aged children (2-5 years). [10, 13, 14]

In Belgium, the Health Interview Survey estimates that prevalence of adult overweight is 48% and prevalence of obesity amongst adults is 14%. [15]

Of all Belgian children and adolescents, 20% have overweight and 7% suffer from obesity. [15]

It is widely accepted that a chronic positive energy balance leads to overweight and obesity. Behavioral influences e.g. high energy density diet, low physical activity and the adoption of a sedentary lifestyle as well as eating disorders are considered as important risk factors for increments in body weight and overall adiposity. Changes in diet or physical activity can be induced by particular life change events, professional situations, psychological stress, poor sleep, etc. [16-22]
Also, endocrine disruptors (known as obesogens; in plastics, pesticides, detergents, …) are potentially involved in weight gain by altering lipid homeostasis and promoting adipogenesis and lipid accumulation. [23, 24]
Besides this, changes in the composition of the intestinal microbiota and different obesity genes/genetic variants were found contributing to obesity. [25-28] Continuously, new information about genes involved in explaining long-term weight development is published. In the concept of gene-environment interaction, is estimated that 50% of the variation in obesity phenotype can be explained by genetic factors. [28] However, this estimate can give the false impression that half of obesity cases have a genetic origin. It is more correct to say that the response or the adaptation to the environment, a behavior, or a change in behavior is conditional on the genotype. [29]
Moreover, an obesogenic environmental that stimulates to eat more and move less goes way further than individual responsibility.[30-35] In the obesogenic environment-concept, different themes are included: urban planning, public transport, education, food advertisement and marketing, etc. [35] This paves the road for the strengthening of research in the areas of behavior change and the implementation of "health-in-all" policy approaches.

Concluding, complex interactions between biological, behavioral and environmental factors are involved in the regulation of energy balance and fat stores. The rapid increase in the prevalence of obesity over the past thirty years might mainly be the result of the combination of behavioral and environmental influences. [28, 36, 37]

1.2 Comorbidities of overweight and obesity

Overweight and obese people are at risk of a number of medical conditions leading to further morbidity and premature mortality. A meta-analysis identified 18 co-morbidities attributable to an increased BMI: type 2 diabetes, different types of cardiovascular diseases, osteoarthritis, gallbladder disease, asthma, chronic back pain and different types of cancer (table 1). [38] A higher disease severity e.g. more comorbidities or more serious expression of obesity-related comorbidities or functional impairment is associated with higher mortality. [39, 40]

In late twentieth century, the importance of the clustering of cardiovascular risk factors was defined. It was stated that the Metabolic Syndrome (MetS), a constellation of interrelated risk factors, culminate adverse outcomes when occurring together. This combination of underlying risk factors predispose the risk of the development of type 2 diabetes by fivefold and the development of cardiovascular diseases by twofold in the next 5-10 years leading to an approximately 1.6 fold increase in mortality. [41-43]
Currently, there are several definitions used as set out by the International Diabetes Federation, the World Health Organization, the National Cholesterol Education Program (NCEP-ATP III), the American Heart Association and others. [42, 44-46] Because of the inconsistency in cut-off points between these organizations, the true prevalence of MetS is hard to determine. However, it is generally accepted that the prevalence of MetS is increasing with increasing body mass index (BMI) and age. [47] Worldwide prevalence of MetS is estimated from <10% to 84% depending on the region, urban or rural environment, population characteristics (sex, age, race and ethnicity) and the used definition. [43, 48] The prevalence of the MetS is investigated in more than 12 000 American adults of different ethnicities. Overall, 4.6%, 22.4% and 59.6% of normal weight, overweight and obese men meet the metabolic syndrome diagnostic criteria defined by the NCEP-ATP III. Results are similar in women with prevalence rates of 6.2%, 28.1% and 50.0% respectively. [49]

Co-morbidity	Measure	Overweight		Obesity	
		Male	Female	Male	Female
Type II Diabetes*	**BMI**	2.40 (2.12–2.72)	3.92 (3.10–4.97)	6.74 (5.55–8.19)	12.41 (9.03–17.06)
	WC	2.27 (1.67–3.10)†	3.40 (2.42–4.78)	5.13 (3.81–6.90)†	11.10 (8.23–14.96)
Cancer					
Breast, Postmenopausal	**BMI**	-	1.08 (1.03–1.14)	-	1.13 (1.05–1.22)
Colorectal	**BMI**	1.51 (1.37–1.67)	1.45 (1.30–1.62)	1.95 (1.59–2.39)	1.66 (1.52–1.81)
Endometrial	**BMI**	-	1.53 (1.45–1.61)	-	3.22 (2.91–3.56)
Esophageal	**BMI**	1.13 (1.02–1.26)	1.15 (0.97–1.36)	1.21 (0.97–1.52)	1.20 (0.95–1.53)
Kidney	**BMI**	1.40 (1.31–1.49)	1.82 (1.68–1.98)	1.82 (1.61–2.05)	2.64 (2.39–2.90)
Ovarian	**BMI**	-	1.18 (1.12–1.23)	-	1.28 (1.20–1.36)
Pancreatic	**BMI**	1.28 (0.94–1.75)	1.24 (0.98–1.56)	2.29 (1.65–3.19)	1.60 (1.17–2.20)
Prostate	**BMI**	1.14 (1.00–1.31)	-	1.05 (0.85–1.30)	-
Cardiovascular Diseases					
Hypertension*	**BMI**	1.28 (1.10–1.50)	1.65 (1.24–2.19)	1.84 (1.51–2.24)	2.42 (1.59–3.67)
	WC	NA	1.38 (1.27–1.51)	NA	1.90 (1.77–2.03)
Coronary Artery Disease*	**BMI**	1.29 (1.18–1.41)†	1.80 (1.64–1.98)	1.72 (1.51–1.96)†	3.10 (2.81–3.43)
	WC	1.41 (1.16–1.72)†	1.82 (1.41–2.36)	1.81 (1.45–2.25)†	2.69 (2.05–3.53)
Congestive Heart Failure*	**BMI**	1.31 (0.96–1.79)	1.27 (0.68–2.37)†	1.79 (1.24–2.59)	1.78 (1.07–2.95)†
Pulmonary Embolism	**BMI**	1.91 (1.39–2.64)	1.91 (1.39–2.64)	3.51 (2.61–4.73)	3.51 (2.61–4.73)
Stroke*	**BMI**	1.23 (1.13–1.34)†	1.15 (1.00–1.32)†	1.51 (1.33–1.72)†	1.49 (1.27–1.74)†
Other					
Asthma	**BMI**	1.20 (1.08–1.33)†	1.25 (1.05–1.49)†	1.43 (1.14–1.79)†	1.78 (1.36–2.32)†
Gallbladder Disease*	**BMI**	1.09 (0.87–1.37)‡	1.44 (1.05–1.98)‡	1.43 (1.04–1.96)‡	2.32 (1.17–4.57)‡
	WC	1.61 (1.40–1.85)†	NA	2.38 (2.06–2.75)†	NA
Osteoarthritis	**BMI**	2.76 (2.05–3.70)	1.80 (1.75–1.85)†	4.20 (2.76–6.41)	1.96 (1.88–2.04)†
Chronic Back Pain	**BMI**	1.59 (1.34–1.89)†	1.59 (1.34–1.89)†	2.81 (2.27–3.48)†	2.81 (2.27–3.48)†

With: BMI: body mass index; WC: waist circumference † If indicated, the relative risks calculated from the ratios of proportions (RR-Ps) were used; otherwise, the incidence rate ratios (IRRs) were used; ‡ Both RR-Ps and IRRs were used *WC measures were considered to be the better risk predictor than BMI measures Cancer: cases, not mortality and indicated by physician diagnosis of cancer; Coronary Artery Disease: indicated by Myocardial Infarction or Angina; Osteoarthritis: indicated by joint replacement; Chronic Back Pain: indicated by early retirement due to back pain; NA: Not available; "-" Not applicable

Table 1: Relative co-morbidity risks related to overweight or obesity; adapted from Guh. et al. [38]

Also in childhood obesity, comorbidities are common and long-term health complications often result. Research shows adverse effects of childhood obesity on cardiovascular structure and function. Growing evidence supports the fact that obesity in childhood and adolescence leads to an increased lifetime risk for type 2 diabetes, hypertension, dyslipidemia, and cardiovascular diseases even when obesity is "grown out". [50-52]
There are different definitions to describe the MetS in children. Despite differences in the prevalence across various studies, factors such as sex, age/pubertal status, ethnicity, and obesity status tends to determine prevalence. In 2014, prevalence rate was defined in more than 3000 American adolescents using the Joliffe and Janssen metabolic syndrome thresholds. [53] This report revealed that MetS prevalence was less than 1% in normal-weight boys, 6.8 % in overweight boys, and 34.5 % in obese boys. In girls, the MetS prevalence rates were 1.7 % in those of normal weight, 9.2 % in the overweight, and 24.6 % in the obese girls. [54]

Besides physical problems, overweight and obesity leads to psychological consequences. The dominant cultural narrative around obesity, fuels assumptions about personal irresponsibility upon individuals living with obesity. [55] This leads to weight bias (= negative attitudes toward and beliefs about others because of their weight) which is manifested through stereotypes and prejudice towards people with obesity.[56] Weight bias can lead to obesity stigma and discrimination and its negative consequences are highly relevant issues within society. [57] The increased obesity risk in low-SES further affects chances and opportunities and ultimately leads to social, economic and health inequities. [58-60]

Although the relationship between several pathologies and increased BMI is well known, some obese people seem to have a healthy phenotype which is called "metabolically healthy obesity" (MHO). [61] Commonly, these people would be obese but not have insulin resistance, elevated blood lipids etc. Prevalence of this phenotype is between 7% and 70% in people with obesity, depending on the used definition. [62]
In this MHO population, the relative risk to develop cardiovascular pathologies is lower than the relative risk in metabolically unhealthy people with obesity. However, the relative risk is still higher than in healthy people without overweight or obesity. [63] This means that obese persons are at increased risk for adverse long-term outcomes even in the absence of metabolic abnormalities. [64, 65]

In this regard, healthy obesity can be described as an intermediate stage of disease progression with increased risk of type 2 diabetes and chronic kidney disease later in life. [63, 66-70] Research on the NHANES III database confirms that there is a strong positive association between overweight duration and the number of obesity-related comorbidities in men and women. [71] Concluding, getting sick from an increased BMI is just a matter of time.

1.3 Burden of overweight and obesity

The rising prevalence of overweight and obesity and its comorbidities represents an important public health issue. Since the causal relation between increased BMI and chronic diseases, overweight and obesity put a financial burden on health services. There is a gradient between increasing BMI, comorbidities and direct and indirect costs. [72]
Direct costs are the costs of the diagnosis and treatment, while the indirect costs are those resulting from productivity loss such as work absenteeism, presenteeism, early retirement, and the lost value of life due to premature mortality. [72-74]

Cost-Of-Illness (COI) studies help policy makers to understand the economic burden of a specific disease in order to formulate and prioritize health care policies. These studies are conducted from different perspectives that determine the types of costs. These perspectives measure costs to the society, health care system, participants and third-party payers. [75]
The total cost related to a disease is the sum of the direct, indirect and intangible costs. (figure 1) Intangible costs refer to costs related to pain or psychological suffering (e.g. stigmatization).
The McKinsey Global Institute estimated the worldwide economic cost of obesity and found that this cost was about 2.0 trillion dollars which is comparable with the cost from smoking or an armed conflict. [76] The report showed that the direct medical cost which is related to obesity responds to 20-27% of all health care expenditures. In this report, the total cost of obesity was underestimated because intangible costs were omitted.
A recent COI in Germany revealed that the annual direct costs of obesity is approximately 29,39 billion and the indirect costs to an additional 33,65 billion euros. Each year, a total of 102 000 subjects die prematurely because of obesity in Germany. From a lifetime perspective, every obese man is equal to an additional burden of 166 911 euros and each woman of 206 526 euros for the social security system in Germany. [73] Unemployment and absenteeism are the main cost drivers of the total indirect cost. Health care costs are the most important cost driver in the direct costs.

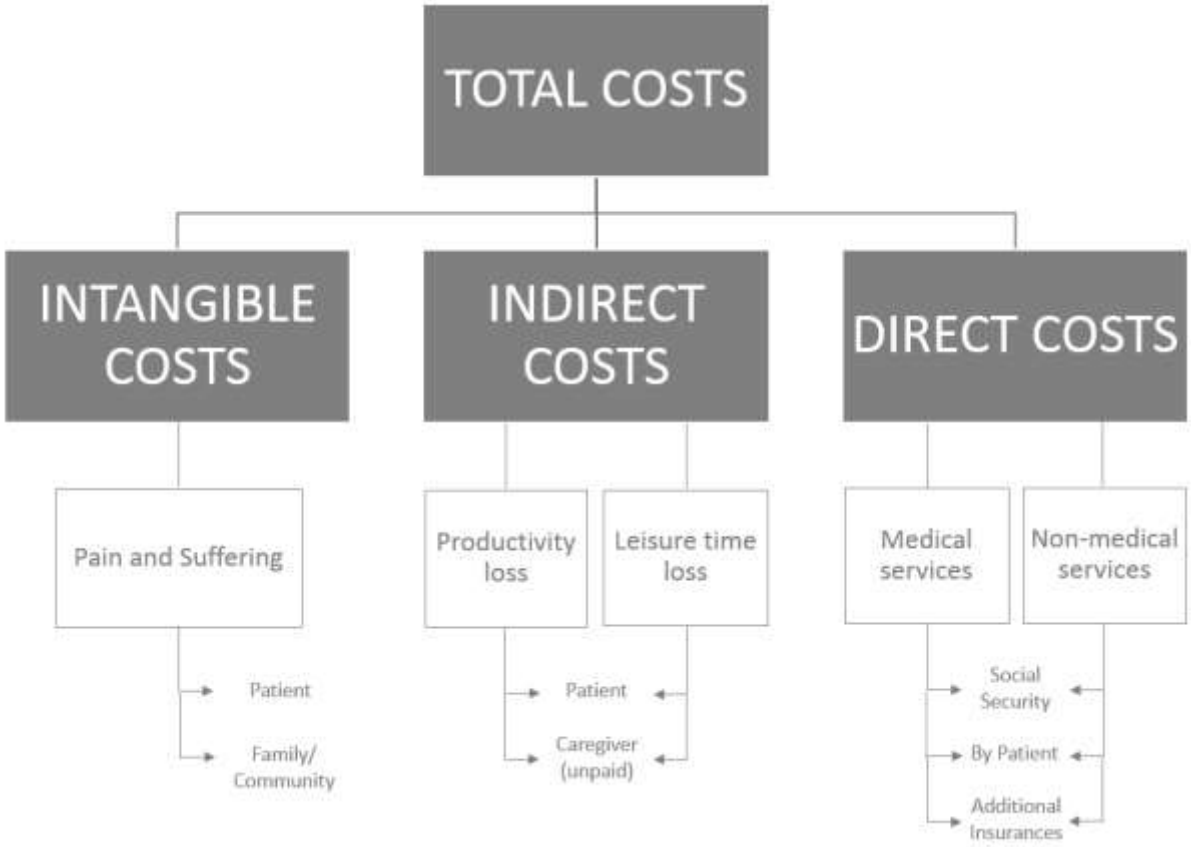

Figure 1: Overview of all costs, which can be included in a Cost-Of-Illness analysis

Besides the COI, health care decision-makers are requiring economic data along with clinical information, to make informed decisions on the allocation of the health care resources. Such economic evaluations aim to compare the costs and benefits associated with one therapeutic option over another. [77]

Conducting economic evaluation alongside clinical trials can be an efficient way of getting valid and reliable information with minimum assumptions made during data collection. [78] Different types of economic analyses, widely described in the literature are distinguished by the approach that they take to measuring outcomes. [79] Cost-benefit analysis assigns a monetary value to benefits. Cost-utility analysis (CUA) uses a single utility measure encompassing both duration of life and quality of life, usually quality-adjusted life-years (QALYs). Cost-minimization analysis applies if two interventions have equivalent effectiveness enabling them to be compared solely on cost. [79]

Cost-effectiveness analysis uses some unit of health as the outcome measure, for example life-years saved or cases detected and can be shown graphically on the cost-effectiveness plane (fig. 2). This compares the incremental costs (Y-axis) and incremental effects (X-axis)

of an intervention with those of a comparator. The position on the cost-effectiveness plane has implications for decision-making. An intervention located in the top-left quadrant is less effective and more costly than the comparator, which should consequently be the preferred option. An intervention that lies in the bottom-right quadrant is more effective and cheaper and will be preferred. An intervention in the bottom-left quadrant is cheaper and less effective. If an intervention lies in the top-right quadrant, it is more costly and more effective. In the last two cases, the question is whether or not the intervention is cost-effective, that is, are extra benefits gained worth the extra cost (top right) or are cost savings worth the benefits forgone (bottom left). [79]

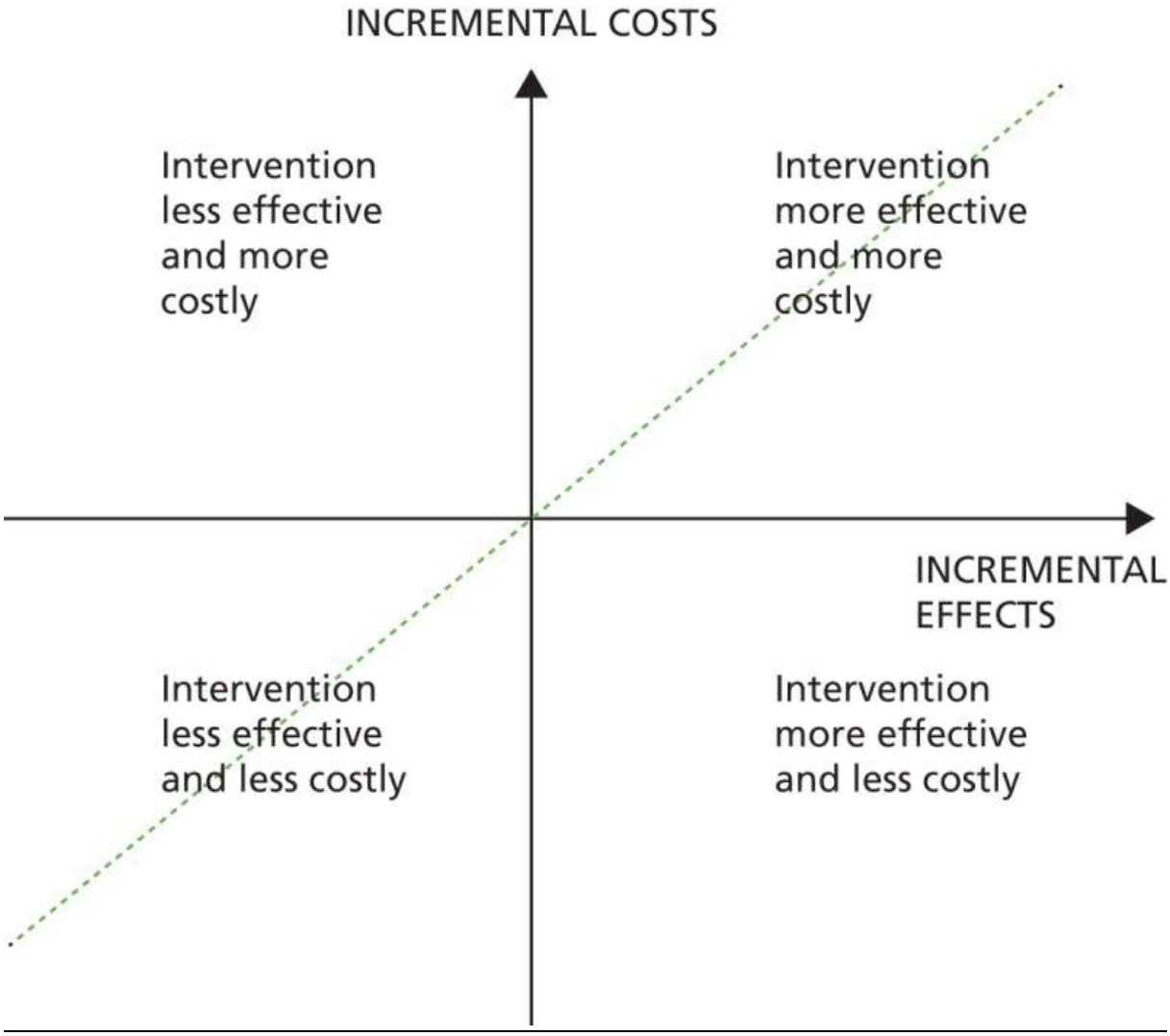

Figure 2: Cost-effectiveness acceptability plane, adapted from Drummond et al. [79]

1.4 Visceral adiposity

Although it is clear that an increasing BMI worsens the major cardiovascular risk factors it is important to look beyond body weight and BMI. [33] Differences in body composition rather than increased BMI might define the obesity phenotype. [80] BMI cannot make the distinction between an elevated body weight due to high levels of lean vs. fat body mass. [81, 82] Total body fatness or body fat distribution is associated with numerous lifestyle and behavioral factors, directly trough adaptations in dietary regimen or activity level or indirectly by factors which influence dietary regimen or physical activity e.g. smoking habits, life change events, poor sleep, etc.

Over the last several years, increasing attention has been paid to the relationship between adipose tissue and metabolic health. Adipose tissue is recognized as an endocrine and paracrine organ that plays an active role in energy homeostasis. [83] The release of a large number of bioactive mediators that influence insulin resistance, lipid levels, blood pressure, coagulation, fibrinolysis and inflammation leads to the development of endothelial dysfunction and atherosclerosis. [84]

In addition to total body fatness, the accumulation of visceral adipose tissue (VAT) independently increases cardiovascular risk and predicts all-cause mortality. [84, 85]

It is seen that individuals with a greater subcutaneous fat distribution are more insulin sensitive than subjects who have their fat distributed predominantly viscerally. "The portal vein hypothesis" starts from the differences in the characteristics and metabolic effects of subcutaneous adipose tissue (SAT) and VAT. VAT is composed of small adipocytes, in contrast to subcutaneous adipose tissue, which is comprised of larger cells. Small adipocytes are metabolically more active than large adipocytes and they secrete increased amounts of adipokines. As a result, visceral fat is more lipolytic than subcutaneous fat and is also less sensitive to the anti-lipolytic effect of insulin. [86] This leads to an increased delivery of free fatty acids (FFAs) to the liver leading to hepatic insulin resistance and induce liver steatosis.[87] Also, VAT is characterized by hypertrophy and hyperplasia of adipocytes leading to hypertriglyceridemia, inflammation and the development of atherosclerosis. [88-90]

The gold standard test to assess VAT is by imaging techniques e.g. Computed Tomography (CT), Magnetic Resonance Imaging (MRI), Dual-energy X-ray Absorptiometry (DXA). [91] An overview can be found in table 3.

VAT thresholds to indicate metabolic disturbances vary between 125 cm² and 140 cm² in men and between 70 cm² and 163 cm² in women. [88, 89, 92-94] Differences in identified thresholds are the result of differences in measurement technique and protocols for VAT, outcomes used to assess metabolic risk and analytic approaches used to determine the thresholds and the population which was studied.

In clinical practice, waist circumference (WC) is used to get an indication of VAT. [90, 95] WC is a good predictor of cardiovascular diseases and type 2 diabetes and reductions in WC are related to VAT reductions in a dose-response manner. [95-97] The National Institutes of Health Guidelines indicate that the relative health risk increases when moving from the normal-weight through obese BMI categories, [98, 99] and that within each BMI category men and women with high WC values are at a greater health risk than are those with normal WC values (table 2). [100] The most commonly used cutoffs among Caucasians for WC are 102 cm for men and 88 cm for women. [44, 101]

			Disease Risk* Relative to Normal Weight and Waist Circumference	
	BMI (kg/m²)	Obesity Class	Men 102 cm (40 in.) or less / Women 88 cm (35 in.) or less	Men >102 cm (40 in.) / Women >88 cm (35 in.)
Underweight	<18.5		–	–
Normal	18.5–24.9		–	–
Overweight	25.0–29.9		Increased	High
Obesity	30.0–34.9	I	High	Very high
	35.0–39.9	II	Very high	Very high
Extreme obesity	40.0†	III	Extremely high	Extremely high

Table 2: Classification of Overweight and Obesity by BMI, Waist Circumference and Associated Disease Risk, adapted from the NIH. [100]

Some studies have found that waist-to-height ratio is better than WC or BMI at predicting cardiovascular risk, diabetes risk and mortality. [102-104] Since the adjustment for age or sex is not necessary, this might be a useful index to identify high metabolic risk in overweight children. [105]

1.5 Ectopic fat deposition

According to the "ectopic fat" model (Fig. 3), the body's ability to cope with the surplus of calories might determine the individual's susceptibility to developing metabolic syndrome.

Healthy adipose tissue buffers the daily influx of dietary fatty acids. However, a long-term positive energy balance, leading to body weight gain, will increase adipocyte size. Since adipose tissue is an endocrine organ, adipocyte hypertrophy is accompanied by disturbances in lipid metabolism and alterations in adipokine secretion, which a shift toward a pro-inflammatory phenotype. The secretion of pro-inflammatory factors, is further boosted by the infiltration of several adaptive and innate immune cells into the adipose tissue in obesity. Together, the impairments in lipid metabolism and the secretory function of adipose tissue induce insulin resistance locally. Moreover, together with adipose tissue dysfunction, there will be lipid accumulation at undesirable sites (ectopic fat deposition). This contributes to systemic low-grade inflammation with detrimental effects at the whole-body e.g. obesity-related insulin resistance and chronic diseases.
In case that the extra energy can be stocked into insulin-sensitive subcutaneous adipose tissue, the individual is characterized to have a MHO phenotype. [106-109]

Ectopic fat depots that are particularly large or that occur in organs that have central functions in the metabolism (such as the liver) are considered to be primarily systemically acting fat depots. [110]
Ectopic fat deposition is associated with insulin resistance, type 2 diabetes, cardiovascular diseases and stroke in adults and type 2 diabetes and liver diseases in children. [84, 106, 111-113] But in the case of fatty liver, it is also described that this is a consequence of insulin resistance. [114]

Sometimes, abdominal obesity is seen as an inducer of ectopic fat accumulation and metabolic abnormalities associated with obesity. [115] In other opinions, VAT is stated to be a "spill-over" site for lipids when the subcutaneous sites are insufficient and can hereby be seen as a region of "ectopic fat". In this point of view, VAT can be used as a marker of ectopic fat. [115]

Various imaging techniques can be used to measure fat in different compartments of the human body, e.g. CT, MRI, Magnetic Resonance Spectroscopy (MRS), ultrasound. [107] An overview of all techniques with advantages and disadvantages can be found in table 3.

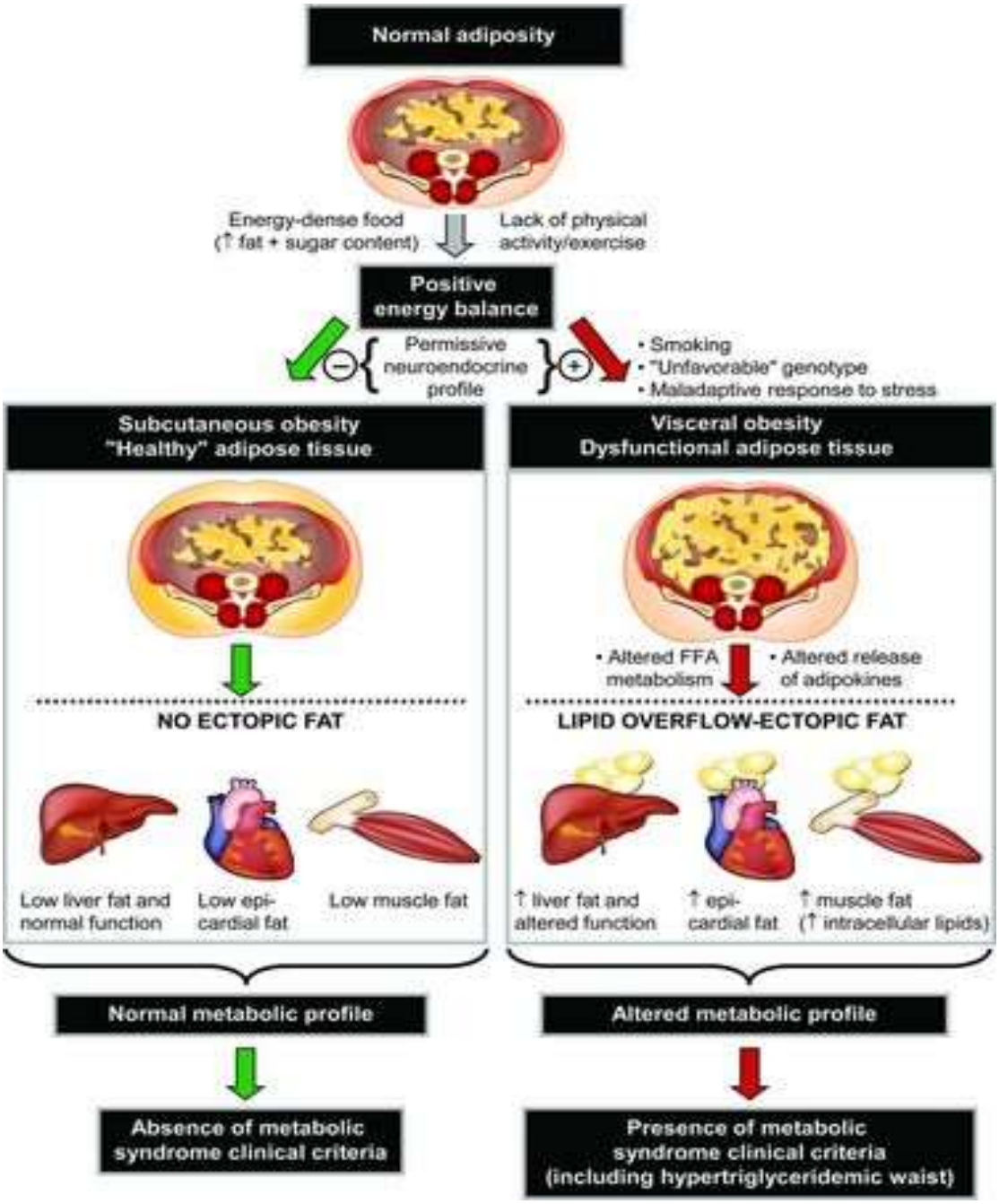

Figure 3: The Lipid overflow ectopic fat model, adapted from Després et al. [106]

	Method	Advantage	Disavantage
Intra-muscular fat	*Biopsy*	Biochemical analysis	Invasive
	CT	Accuracy Reproducibility	Radiation
	MRI	Accuracy	Expensive Facility Scan time
	DXA	Upper vs. Lower extremity	Lower spatial resolution Facility
	MRS	High accuracy Intra- vs extramyocellular	Expensive Facility Scan time Performed for research
Abdominal fat	*CT*	VAT vs SAT Volumetry	Radiation
	MRI	VAT vs SAT Volumetry	Expensive Facility Scan time Less tolerated
	DXA	Relatively low cost Volumetry	Lower spatial resolution Facility
Fat in the liver	*Biopsy*	Biochemical analysis	Invasive
	Ultrasound	Noninvasive Relatively cheap No radiation	Less quantitative Manual labor
	CT	Reproducibility Volumetry	Radiation Less quantitative
	MRI	Accuracy Volumetry	Expensive Facility Scan time
	MRS	High accuracy Most reliable	Expensive Facility Scan time Performed for research
Peri- and epicardial fat	*Ultrasound*	Easy to perform Safe No radiation Relatively cheap	Less reproducible Less quantitative
	CT	Pericardial vs epicardial Volumetry	Radiation
	MRI	Pericardial vs epicardial volumetry	Cost Facility Scan time

Table 3: Methods used to measure ectopic fat depots, adapted from Lim et al. [107]

with CT, computed tomography; MRI, magnetic resonance imaging; DXA, dual X-ray energy absorptiometry; MRS, magnetic resonance spectroscopy; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; circ., circumference; subcut., subcutaneous.

Metabolic alterations within muscle play a role in the reduced skeletal muscle fatty acid oxidation and increased accumulation of triglycerides within the skeletal muscle fibers, called Intra MyoCellular Lipids (IMCL). [116] This accumulation is apparent with obesity and other insulin-resistant states such as type 2 diabetes. [117] The flux of muscular fatty acids as a source of oxidative energy may play a pivotal role into the development of the abnormalities of muscle and whole-body energy metabolism. [118]

Cardiac fat depots are classified into epicardial fat located on the surface of the myocardium, pericardial fat located between the parietal and visceral pericardium, and paracardial fat located outside the pericardium. Besides these fat depots, intramyocardial fat within cardiomyocytes is described. [119] Cardiac fat depots have systemic but particularly local effects that may contribute to the development and progression of coronary atherosclerosis. [120, 121] The volume of epicardial fat is correlated with visceral adiposity, coronary artery disease, the metabolic syndrome, diabetes, fatty liver disease and cardiac changes. [122, 123] Pericardial fat is associated with poorer cardiovascular prognosis and left ventricular remodeling, independent of insulin resistance and inflammation. [124]

Fat accumulation in the liver, the so-called fatty liver, is a prevalent condition and has multiple effects on cardio metabolic risk factors. [125, 126] Obese individuals have a 3.5-fold increased risk of developing Non-Alcoholic Fatty Liver Disease (NAFLD), and there is an obvious dose-dependent relationship between BMI and NAFLD risk. [127] The lipid effects of fat depots in the liver include an increased free fatty acid release into the liver, hepatic insulin resistance and systemic inflammation. [128]

1.6 Management of overweight and obesity: current guidelines

Obesity fits the definition of a chronic, relapsing, progressive disease. Although it is widely accepted that obesity is more than just a "lifestyle choice", it is mostly not recognized as a chronic disease. [129] By recognizing obesity as a disease, effective prevention strategies could be realized and the environment could be modified in order to support and sustain changes in behavior. [130-132] By reducing the abundance of agents that cause obesity and promoting changes to perceptions, the obesity epidemic might be controlled. Such "health-in-all" policy changes in different areas would require collaboration between governments, obesity professionals, local health services, environmental authorities, the food industry, and others. [133-135] An example is the nudging concept in which population health can improve by designing a "choice architecture" that alters people's behavior in a predictable way without forbidding any options. [136]

Now, obesity management focuses primarily on education and personal responsibility of the patient. In obesity guidelines, body weight reduction and management is one of the main goals. This can be achieved by different strategies, going from non-invasive forms of lifestyle intervention (diet, exercise and psychosocial guidance) to more invasive forms of pharmacotherapy and weight loss surgery. [137] An overview of the stepwise management of overweight and obese adults is shown in fig.4.
Although more attention has to be paid to the improvement in body composition, quality of life and well-being, the goal setting in people with overweight and obesity is often primarily based on body weight management. [83] In a recent paper in which Look AHEAD data were published, the transition from metabolically unhealthy obesity to MHO would require a weight loss of 10%. This can be the first goal setting in obesity treatment because the achievement of MHO is protective against obesity-related chronic diseases and results in decreased direct and indirect costs related to obesity. [72, 138]

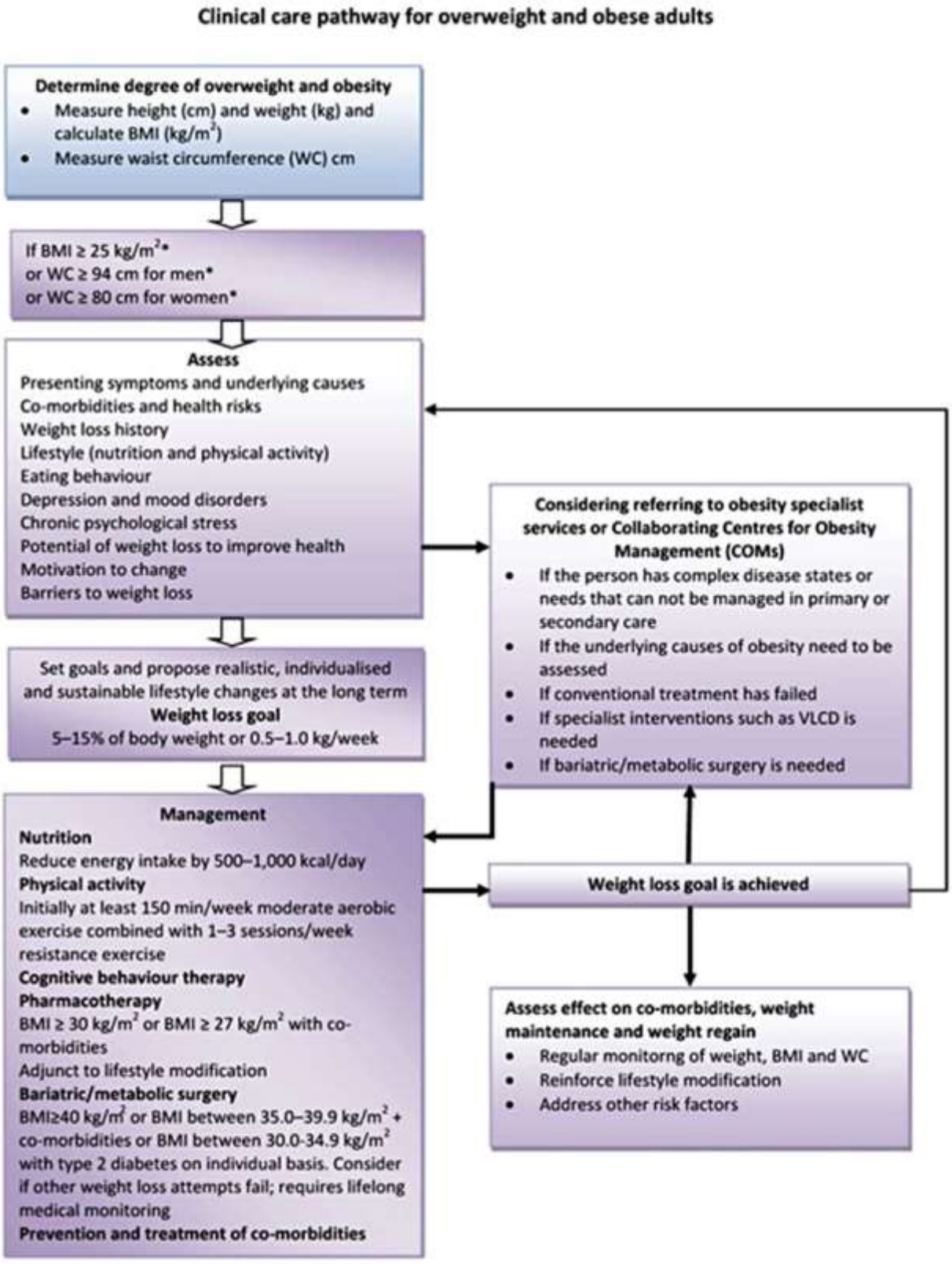

Figure 4: Algorithm for the assessment and stepwise management of overweight and obese adults, adapted from Yumuk et al. [139]

Note: BMI and WC cut-off points are different for some ethnic groups

It is difficult to obtain a body weight reduction of 5-10% by increased calorie expenditure alone. To reach this goal, a very high training volume is needed (moderate intensity training up to 250-420 min/week). [140] Since the amount of physical activity needed to prevent weight gain after weight loss is even higher, dietary modification is considered to be the cornerstone in the non-invasive body weight management. [141] Although guidelines highlight the combined use of diet, exercise and behavioral therapy in order to achieve long-term weight loss [142], planned exercise training is often not emphasized in clinical practice. Frequently, a more general public health approach of physical activity promotion and advise ("You should exercise more regularly") is used instead of individualized exercise prescription and training.[143]

This advise is mostly based on existing guidelines in which it is recommend that at least 150 min/week of moderate aerobic exercise (or 75 minutes of vigorous-intensity exercise training) should be combined with three weekly sessions of resistance exercise to increase muscle strength.[68, 69, 139, 144]

Some researchers argue that this gentle recommendation still sets the bar too high for sedentary people, and that guidelines should instead focus on making small incremental increases in physical activities in daily life. [118] Moreover, more attention can be given to reducing sitting time and increasing non-leisure time physical activity (active transport, physical activity during occupation or housework). [145]

In children and adolescents with overweight and obesity, guidelines focus on promoting healthy, safe pediatric lifestyle modification that include family involvement, with potential wide-reaching benefits. [5] Physiotherapists are encouraged to prescribe and support intensive, age-appropriate, culturally sensitive, family-centered exercise therapy based on clinical evaluations and tests. [146] It is stated that 60 minutes of daily moderate to vigorous physical activity together with a calorie-controlled diet leads to a reduction of the BMI or maintenance of weight loss. In the absence of caloric restriction, moderate exercise will not result in weight loss but may confer health benefits. Besides increasing physical activity, screen time should be reduced. [5, 147]

Following the EOSS system, the intensity of the individualized obesity management plan can be based on disease severity. [148]

1.7 Research objectives

The high prevalence of overweight and obesity and its comorbidities puts a financial burden on health services. There is a gradient between increasing BMI, comorbidities and direct and indirect healthcare costs. Although MHO seems to be an intermediate stage of disease progression, direct and indirect costs are lower than in unhealthy obesity.
It is hypothesized that ectopic fat deposition is the driver to the development of metabolically unhealthy obesity and can be influenced by lifestyle.
In this thesis, the cost of obesity is estimated. Furthermore, the effects of lifestyle on ectopic fat and metabolic risk are investigated, A rationale is given for the implementation of exercise in the treatment of people with overweight and obesity, taken all (obesity-related) costs into account.

The goals of this doctoral research:

- **Goal 1: Providing insights on the effect of lifestyle (diet and exercise) on metabolic parameters and ectopic fat deposition.**
  In **chapter 2**, a literature review and meta-analysis regarding the effects of exercise (alone or in combination with diet) on ectopic fat is presented in children, adolescents and adults with overweight and obesity.
  A randomized controlled trial was designed. The methods are presented in **chapter 3**.
  In **chapter 4**, the effectiveness and cost-effectiveness of a hypocaloric diet intervention whether or not in combination with unsupervised exercise training is described in premenopausal women with overweight and obesity. Primary outcome is the change in ectopic fat deposition.
- **Goal 2: Assessing the cost of obesity.**
  In **chapter 4**, a comprehensive Cost-of-Illness analysis links metabolic parameters, obesity related comorbidities and direct and indirect costs.
- **Goal 3: Assessing dynamics between different ectopic fat regions and metabolic profile.**
  In **chapter 4**, correlations between ectopic fat deposition and metabolic parameters are described.

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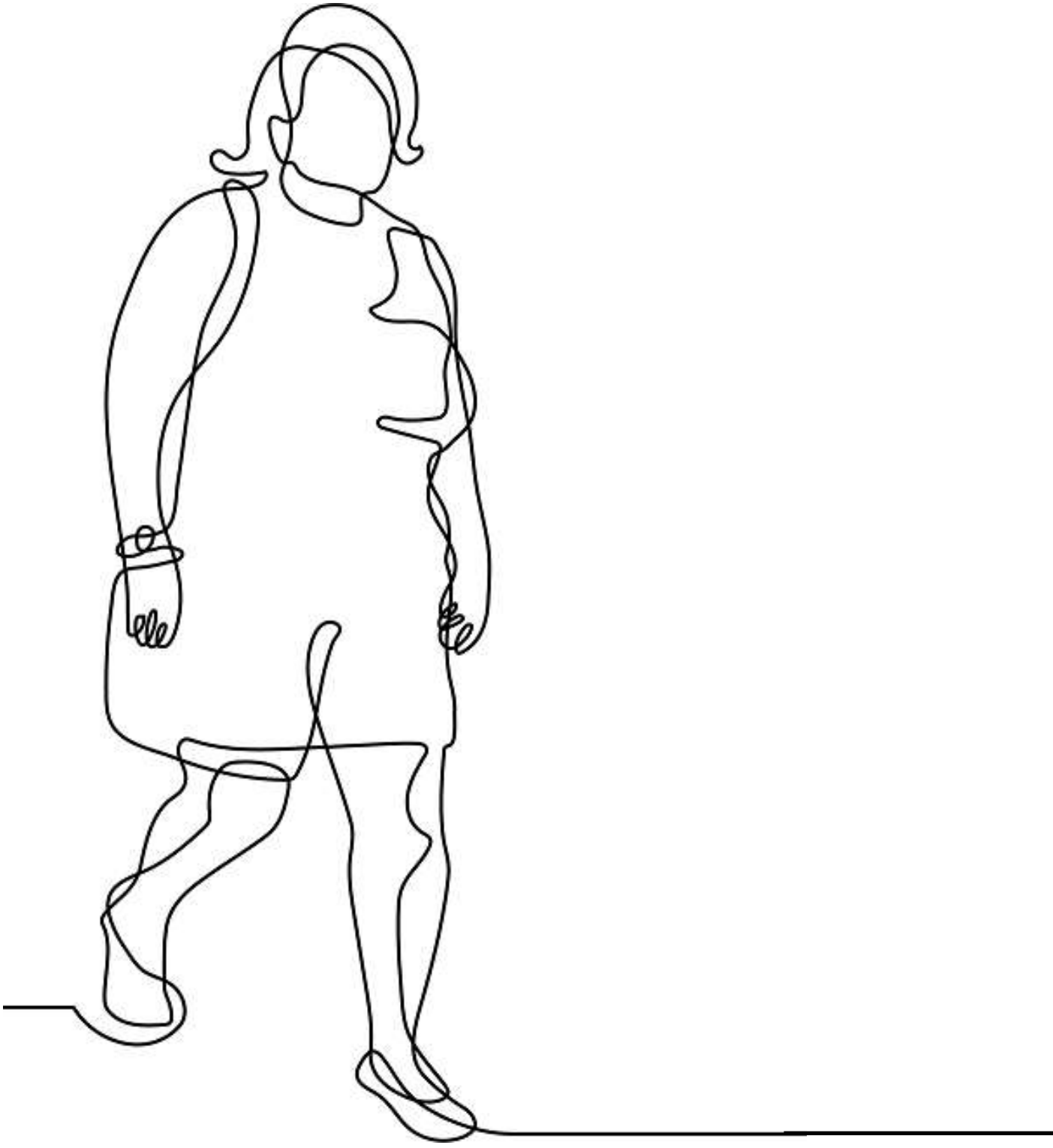
CHAPTER 2

Literature Study:
Exercise and Ectopic Fat



- Publication:

  Hens Wendy, Taeymans Jan, Cornelis Justien, Gielen Jan, Van Gaal Luc, Vissers Dirk

  "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"

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- Publication:

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2.1 Exercise and ectopic fat deposition in overweight and obese adults

Introduction

In Europe, obesity has reached epidemic proportions and is considered a severe medical disorder due to its associated risk of cardiovascular diseases and premature death. [1, 2] Guidelines for treatment of overweight and obesity outline the importance of the integration of an appropriate dietary regimen together with physical activity. [3] Weight loss interventions that succeed in decreasing body weight by 5 to 10%, can also have a significant effect on cardiovascular health.[4, 5] Nevertheless, it is recommended to 'look beyond weight loss' to evaluate the success of a weight loss program.[6-8] Despite the clear link between unhealthy Body Mass Index (BMI) and increased cardiovascular mortality [9], not every obese patient is characterized by worse cardiovascular risk factors. [10] BMI alone may not be suitable to predict health-oriented outcomes due to its inability to distinguish between metabolically healthy and unhealthy persons. [11, 10] Differences in regional fat distributions could possibly contribute to the heterogeneity in metabolic risk profiles across people with a comparable BMI. [12]

It is seen that subcutaneous adipose tissue (sometimes referred to as functional adipose tissue) has less harmful characteristics than internal or dysfunctional adipose tissue. [13] Ectopic fat deposition is defined as the excess of internal adipose tissue in locations not classically associated with adipose tissue storage. [14] This ectopic lipid overload in and around vital organs has destructive effects.[15, 16]

Recently, there is an increased focus on visceral adipose tissue (VAT). Visceral obesity is seen as a complex phenotype leading to ectopic fat accumulation and metabolic abnormalities. [17, 18, 13] A couple of mechanisms are involved in this process. Firstly, VAT seems to be more lipolytic and overexposes the liver (through the portal circulation) to non-esterified fatty acids. This results in an impairment in the liver metabolism and systemic hyperinsulinemia. [19, 20] Secondly, adipose tissue is a highly endocrine organ which contributes to the insulin resistant, pro-inflammatory, thrombotic and hypertensive state of visceral obesity. [21]

Moreover, excess visceral adiposity may be a marker of dysfunctional subcutaneous adipose tissue. The possibility to store excess lipids in subcutaneous adipose tissue seems to have a protective role. When lipids can not be stored in the already saturated subcutaneous adipose tissue (due to its inability to expand or its dysfunctional state), lipids will be stored in VAT

and other ectopic sites such as the liver,
the heart or the skeletal muscles. [21] This kind of fat infiltration can be considered as an important manifestation of the metabolic syndrome. [22-26]
Little is known about the effect of conventional non-invasive weight loss programs on ectopic fat deposition. A previous meta-analysis showed that exercise even without an hypocaloric diet, has the potential to reduce VAT in overweight and obese adults. [27] However, little is known about the effect of lifestyle weight loss programs on ectopic fat deposition in internal organs and muscles. Therefore this meta-analysis systematically searched literature for lifestyle interventions (including at least diet, exercise or the combination of both) that describe the effect on adiposity of the liver, the skeletal muscles (Intra Myocellular Lipids - IMCL), the heart, the pancreas, the kidneys or blood vessels in overweight or obese adults.

Methods

This systematic review and meta-analysis was written following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement. [28]

Search strategies

A separate electronic search for each ectopic fat location, based on the PICO acronym, was conducted in PubMed, PEDro and the Cochrane database between January 2014 and July 2014 and updated in December 2014. Often, VAT is seen as the accumulation of all intrathoracic adipose tissue and intra-abdominopelvic adipose tissue. [29] However, in numerous CT- studies the radiation dose is restricted by using single slice CT scans focusing primary on intra-abdominal fat. This implies that specific regions (e.g. fat around the heart, pancreas or kidneys) are not included. Since there is a substantial variation in the absolute amount of VAT depending on the location of the cross sectional slice taken. [30-32]
We wanted to be sure that all specific areas of ectopic fat deposition were searched. This was done by defining key words, depending on the specific region of ectopic fat (table 1). These keywords were combined and used in the PubMed database in the following combination: ("diabetes mellitus, type 2" OR "overweight" OR "insulin resistance" OR "metabolic Syndrome X") AND ("sports" OR "exercise therapy" OR "exercise" OR "lifestyle intervention" OR "anaerobic training" OR "diet" OR "aerobic training). Whenever possible,

Medical Subject Headings (MeSH) were used. Limits were set on "clinical trials". This search strategy was adapted to the Cochrane and Pedro databases.

Study selection

The three databases were systematically searched for clinical trials using a priori defined inclusion and exclusion criteria (table 2). To make sure that the majority of the participants in the different studies were clearly overweight, mean BMI at baseline had to be ≥ 26.5 kg/m2. Since an intervention duration of at least four weeks was considered to be a critical time span to obtain results on insulin sensitivity [33] and because insulin sensitivity correlates well with excessive adiposity [34, 20], only studies with a minimum duration of four weeks were eligible for inclusion. In order to obtain more homogeneous results, only studies using non-invasive imaging techniques for ectopic adiposity were included in this meta-analysis. Studies were included if valid and reliable imaging techniques were used, such as hydrogen based Magnetic Resonance Spectroscopy (H-MRS), Magnetic Resonance Imaging (MRI) and Computerized Tomography (CT). [35-38] Despite the fact that ultrasound can be a useful screening tool, it was excluded as a measurement tool because of reported difficulties to quantify fat and its debatable sensitivity.[36] An exception was made for epicardial fat thickness measured by echocardiography because this measurement technique shows a high agreement with epicardial fat volume and has a good reproducibility. [39-41] This systematic review and meta-analysis focused on interventions based on healthy lifestyle (diet, exercise or a combination of both) and therefore overfeeding studies were excluded. For the present study, exercise was defined as any coordinated or supervised exercise program aiming to reduce weight or body fat. Physical activity intervention studies based on "advise only" were not included because literature showed an uncertain effect. [42]

Quality assessment

The Effective Public Health Practice Project (EPHPP) quality assessment tool was used by two independent investigators to assess the study quality. [43] This tool was chosen because it has an excellent inter-rater agreement of the final rate and can be used to score randomized clinical trials as well as cohort intervention trials, in which parameters before and after an intervention were described. [44, 45] The decision was made not to exclude articles based on quality assessment or assuming skewness of results.

Manuscript screening and data-extraction

The achieved citations were screened by two independent investigators. Studies fulfilling the above mentioned criteria were included and reference lists were checked for relevant studies. Figure 1 presents the flow diagram of the systematic reviewing process. All data concerning the primary outcome parameters (changes in ectopic fat deposition) and related information necessary to pool study results were obtained. A standardized data-extraction form was used to construct tables 3 to 6. Whenever methods or primary outcome data were not reported clearly, the corresponding author was contacted. If reported by the authors, significance (S) or non-significance (NS) was noted in table 3 to 6 and exact p-values were retrieved.

Statistical analysis

The extracted data was entered into the CMA-2 software (Comprehensive Meta-Analysis 2nd version, Biostat, Englewood, USA). Expecting an important degree of between studies heterogeneity (due to different measurement techniques, specific patient characteristics and differences in treatment interventions), a random-effects model was chosen to pool the individual study results and to examine the overall effect size of a lifestyle intervention on ectopic fat depots. Effect sizes (changes in ectopic adiposity) were calculated as standardized mean differences and expressed as Hedges' g to correct for overestimating the true effect in small studies. The 95% confidence intervals [95%CI] were calculated for the individual studies and the overall estimate. Raw data from the research of Shea et al. [46] were used to calculate the correlation coefficient between pre- and post-intervention values. In the case of heart adiposity, this correlation was set on 0.9 while for liver and muscular adiposity this correlation was set on 0.7.

The search strategy for excess liver fat deposition yielded 27 articles, allowing subgroup analysis and meta-regression to assess the possible confounding effects of covariates such as the change in BMI or insulin resistance, the study design or the intervention modality on the overall estimate.

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. Publication bias was assessed when 10 or more studies were available per anatomical

site through visual analysis of the funnel plot and formal testing for funnel plot asymmetry ('trim and fill' and 'fail 'n safe' algorithms).

To facilitate the interpretation of the overall estimate of the liver studies for the clinician, its value was re-expressed to Intra Hepatic Lipids (IHL) referred to water in % (=unit as measured by the H-MRS technique). Baseline % IHL standard deviations of the intervention and control groups from the Hallsworth et al. study [47] were pooled and multiplied by the pooled standardized mean difference.

Thus, the pooled effect was re-expressed in the original units of the H-MRS instrument with the goal to interpret clinical relevance and impact of the intervention more easily. [48] P-values less than 0.05 were considered significant.

Results

Study selection

The initial search in the three databases identified publications resulted in articles about the adiposity of the liver (399 articles), the heart (11 articles), the pancreas (48 articles), the kidneys (47 articles), the IMCL (266 articles) and perivascular fat (165 articles).

After removing duplicates and eliminating papers based on the eligibility criteria, 37 studies remained for further analysis. In these citations, the effect of an intervention on liver, heart, muscular or pancreatic fat was described. No studies were found in which the effect of an intervention on renal or perivascular fat was investigated. Four more citations were excluded as they reported on the same study population. [49-52]

For the qualitative and quantitative analysis, the results of 33 trials were examined. In 27 articles, the effect of a lifestyle intervention on hepatic fat was discussed. [47, 46, 50, 53-76] In the case of muscle [77, 61, 64, 66, 68, 70, 72, 76], heart[40, 46, 54, 74, 78-81] and pancreas[63] adiposity, the number of included papers was eight, eight and one respectively. The effects on multiple ectopic fat locations (e.g. the combination of heart and liver fat) were reported in 11 studies.

Methodological quality and study characteristics

The majority of the included studies (21 studies) were classified to have a strong design (randomized controlled trials or controlled trials - table 7). Nine articles were generally

methodologically rated as "strong", 16 articles were rated as "moderate" and eight articles were rated as "weak". The most common shortcomings were inadequate or missing information addressing the eligible population group, blinding or controlling for confounders. Adiposity of the liver, the heart, skeletal muscles or the pancreas was assessed including 1146, 157, 336 and 87 participants respectively in the intervention groups.

Applying the BMI-classification criteria [82], most studies assessed people with class 1 obesity ($30.0 \leq BMI \leq 34.9$). Only in three trials [56, 59, 75] the effect of an intervention in people with class 3 obesity ($BMI \geq 40$) was investigated.
In 19 of the 33 included trials, the subjects did not suffer from overweight comorbidities (or possible prevalence percentage of the metabolic syndrome, (pre-)diabetes or an impaired liver function was not reported).

Intervention characteristics

In the majority of the papers, the participants or the study groups received either an exercise intervention or a hypocaloric diet. The combination of hypocaloric diet and exercise was only assessed in nine studies. Training volume could generally be interpreted as "moderate". The duration of physical activity was primarily between 60 and 80 min/week with a moderate intensity of 50-80% VO2peak.

Study outcomes: ectopic fat adiposity

Adiposity of the liver

An overview of all 27 studies concerning hepatic adiposity is presented in table 3. The overall effect of a lifestyle intervention on hepatic adiposity, expressed as Hedges' g, was -0.53 [95% CI: -0.65 to -0.40] ($p<0.001$). (figure 2)
By re-expressing the observed overall effect size based on the population variability of the research of Hallsworth et al. [47] , it could be concluded that an intervention with a duration of eight weeks had the ability to decrease IHL with 5%. Heterogeneity analysis showed high heterogeneity (Cochran's Q= 160, df(Q) =41, $p<0.001$; I2 = 74.4%). The 'fail'n safe' algorithm reported a low risk for publication bias because 2448 extra non-significant studies would be needed to lower the p-value to the alpha level.

To evaluate a possible confounding effect of body weight reduction on the effect size, a meta-regression of the individual studies effect sizes over BMI-change was conducted (figure 3). Analyzing the studies with a significant decrease in BMI or body weight, the corresponding change in hepatic adiposity expressed that the decrease in hepatic adiposity was larger in studies with a greater decrease in BMI or body weight. The regression coefficient was -0.11 [95% CI = -0.16 to -0.07] ($p<0.0001$).

Since insulin sensitivity was characterized to have a possible confounding effect on hepatic adiposity, a meta-regression of the individual studies effect sizes over the change in insulin sensitivity was conducted. Through meta-regression, no relation was found between the reduction in insulin resistance and decrease in hepatic adiposity (regression coefficient = 0.0057 with $p = 0.97$).

Further subgroup analysis presented that the decrease in hepatic adiposity was higher in the uncontrolled studies (Hedges' g = -0.55 [95% CI: -0.69 to -0.41], $p<0.001$) compared to the controlled studies (Hedges' g = -0.45 [95% CI: -0.73 to -0.17], $p=0.002$).

Finally, a subgroup analysis was made based on the categorization of the nature of the intervention (figure 4). Study groups were classified in "diet-only studies" (D), "exercise-only studies" (E) or the combination of "diet and exercise" (D+E). There was a high significant difference ($p<0.001$) in effect between the three intervention groups with the strongest effect in the diet-only groups (Hedges' g = -0.77 [95% CI: -0.97 to -0.57], $p<0.001$) and the weakest effect in the exercise-only groups (Hedges' g = -0.25 [95% CI: -0.49 to -0.01], $p=0.004$). Though, it should be emphasized that all intervention types showed a significant reduction on hepatic fat.

Adiposity of the heart

The majority of studies describing the effect of a lifestyle intervention on cardiac fat were uncontrolled trials (table 4). In seven studies, cardiac adiposity decreased after an intervention with an overall effect of -0.72 Hedges' g [95%CI = -1.10 to -0.35] ($p<0.001$). Heterogeneity analysis showed a very high between studies heterogeneity (Cochran's Q= 124.9, df(Q) =10, $p<0.001$; I2 = 92%). Due to the anatomically differences between the fat around the heart (epicardial and pericardial fat) and intramyocardial fatty infiltration (cardiac lipid content measured by H-MRS), a subgroup analysis was made in which these results were split up. The overall effect of an intervention on cardiac lipid content was not significant

(Hedges'g = -0.27 [95%CI = -0.97 to 0.38], p=0.391). The overall effect of an intervention on epicardial and pericardial fat was more pronounced with -1.26 Hedges'g [95%CI = -1.87 to -0.65] (p<0.001) and -0.565 Hedges'g [95%CI = -0.92 to -0.21] (p<0.001) resp.

Adiposity of the pancreas

Only one study described the effect of a lifestyle intervention (aerobic exercise) on adiposity in the pancreas (table 5). Although there was a trend towards a decrease in pancreatic fat in all intervention subgroups in this controlled study, the overall decrease was not significant (Hedges' g = -0.55 [95% CI: -1.21 to 0.10], p=0.098).

Adiposity of skeletal muscle (Intramyocellular lipids)

Table 6 depicts the characteristics of the studies in which muscular adiposity is measured after a lifestyle intervention. In this meta-analysis, slow twitch oxidative (type I, M. Gastrocnemius) and fast twitch glycolytic (type II, M. Tibialis Anterior, M. Vastus Lateralis) muscle fibers have been analyzed separately. Only two studies found significant changes in IMCL after an intervention[66, 76]. In the majority of the studies, a non-significant decrease was found. The overall effect of a lifestyle intervention on muscular adiposity in the M. Soleus (predominantly type I fibers) was Hedges' g = -0.28 [95% CI: -0.46 to -0.10] (p =0.002) (Figure 5). The overall effect was lower in the fast twitch muscle fibers (M. Tibialis Anterior: Hedges' g = -0.19 [95% CI: -0.36 to -0.01] (p = 0.041) and M. Vastus Lateralis: Hedges' g = 0.13 [95% CI: -0.79 to 1.06] (p = 0.78)).

Heterogeneity analysis showed a low but non-significant between studies heterogeneity (Cochran's Q= 20.5, df(Q) =14, p = 0.116; I2 = 31.6%).

Discussion

Diet and exercise are the cornerstones of a lifestyle treatment in people with overweight. By re-expressing the results, it can be concluded that interventions involving physical activity or hypocaloric diet could lead to a decrease in cardiac adiposity and a decrease in intra hepatic lipids of 5%.

It was remarkable that the effects of lifestyle intervention were less clear in research in

which subjects were diagnosed with Non Alcoholic Fatty Liver Disease (NAFLD) or an impaired liver function. [47, 57, 67, 75, 76] Only in the study of Oza et al., the lifestyle modification program resulted in a significant decrease of IHL after 6 months. [67] This finding confirmed the hypothesis that subjects with a higher amount of hepatic fat have a lower chance of profiting from lifestyle interventions and intensified strategies or pharmacological approaches may be required. [52]

The high number of studies reporting on liver adiposity allowed for subgroup analysis and meta-regression. Since numerous trials described the existence of a J-shaped relationship between BMI and the risk on morbidity or mortality [9, 83], the influence of BMI reduction on hepatic fat was examined. Meta-regression result suggested a dose-response relationship between BMI reduction and decreased hepatic adiposity.

This confirms existing knowledge that a clinically important weight loss (5-10% of baseline weight) may proceed in an improvement of cardiovascular risk factors. [84, 4] However, it should be mentioned that in a few studies a reduction of IHL was obtained, without a decrease in BMI. [55, 69]

A subgroup analysis based on the type of intervention, concluded that all interventions significantly reduced liver adiposity but the effect size was larger in the diet-only studies. This could be explained by the fact that a hypocaloric diet remains the most important lifestyle factor for weight loss and amelioration of cardiovascular risk and thereby could mask additional effects of exercise therapy. [42, 8]

In the studies or subgroups in which participants were exposed to a "exercise only"-intervention with a moderate training intensity, the training volume almost never exceeded 180 min/week and could therefore be insufficient to achieve a modest weight reduction. [85] Maybe an greater decrease of ectopic fat reduction was achieved when exercise training volume was higher.

In addition, results could be biased since adherence to exercise programs was only scarcely measured and physical activity levels of participants in the diet-only groups or control groups were rarely measured.

As expected, the effect size of the reduction of hepatic fat was higher in uncontrolled studies compared to the effect size in the controlled studies. [86]

In seven out of eight studies in which the effect of an intervention on heart adiposity was evaluated, a reduction of ectopic fat was found. Over the last decade, it was repeatedly confirmed that cardiac steatosis is a hallmark of obesity and diabetes mellitus type 2 (DMII). [87-89] A reduction of cardiac lipid content could be associated with an improvement of the left ventricular ejection fraction. [80] This could be explained by the fact that cardiac adiposity has a lipotoxic effect on heart (muscle) function. [90] In this analysis, the reduction in cardiac lipid content was not significant and thereby contradictory to the results concerning epicardial and pericardial fat. Since there was a very high heterogeneity between studies and cardiac lipid content was only evaluated in 25 patients, cautiousness is needed when drawing conclusions.

Only one intervention study was found in which pancreatic adiposity was measured.[63] In this study a training program of six months resulted in a non-significant reduction of pancreas fat. Clearly, more studies are necessary to look at the effect of lifestyle interventions on pancreatic adiposity.

The change in skeletal muscle adiposity after an intervention was twofold. In most studies, a trend towards decrease of IMCL was seen, but in some studies there was a trend towards increase. Keeping in mind "the athlete's paradox" [91, 92], a possible explanation could be that IMCL accumulation is only related to insulin resistance when accompanied by a sedentary lifestyle because of low oxidative capacity.[93] The latter findings indicate that a correct interpretation of IMCL could only be made by evaluating the mitochondrial capacity or oxidative enzyme activity. In none of the included articles for this meta-analysis, an evaluation of oxidative capacity was done.

One of the strengths of this literature study is the extensive systematic review allowing for meta-analysis of the effects of lifestyle interventions on all well documented regions of ectopic fat deposition. Hence, this paper gives a comprehensive description on different anatomical sites of ectopic fat and provides information about the relationship between ectopic adiposity and BMI or insulin resistance.
Moreover, the results of this meta-analysis were made clinically interpretable by re-expressing Hedges' g as % IHL in the liver. Finally, the correlation coefficient between pre

and post-values of fat in the liver and heart were real values based on the raw data given by the research team of Kritchevsky. [46]

Despite of all efforts, there are some limitations to this review and meta-analysis.
In the quality assessment, the majority of included studies received only a moderate quality score. In most studies, adequate reporting of applied methodology was lacking. It was rarely mentioned which percentage of people eligible for inclusion effectively agreed to participate in the study.
Only a few studies reported on a power measurement of the study sample. Blinding of the assessors was often described, however it mostly was not clear if study participants were aware of the research question
There were only a few studies that objectively assessed patient adherence to the dietary or exercise program.
In some studies, results were not reported transparently. Repeatedly, outcomes were presented in figures without exact mean values, standard deviations or p-values and medians and interquartile ranges were used. Receiving additional information of the authors, suggested that this presentation of results sometimes masked the underlying skewness.

It should be noted that most patients regain body weight after an intervention. [94] Since in this meta-analysis the intervention period of the majority of the included studies was relative short (<1 year) and a motivational aspect was rarely reported, it was not possible to determine the long-term effect of an intervention. Only one of all included studies described a follow up period. This study indicated that the reduction in pericardial fat volume obtained by the hypocaloric diet remains stable after 14 months, despite a regain in body weight, visceral abdominal fat, and hepatic triglyceride content. [74]
It should be a challenge to design future intervention studies in a manner that a long-term improvement in lifestyle habits could be obtained and results of ectopic fat could be documented.

Given the observed high inter-study heterogeneity due to differences in population (age, gender, comorbidities, degree of overweight, ethnicity), nature and duration of the intervention (diet, exercise, combination of diet and exercise) and the assessment method of

adiposity, conclusions should be drawn with care. More long term follow-up research is needed to investigate the effect of lifestyle interventions on ectopic fat deposits (e.g. pancreatic, renal and perivascular tissues) . This is the only manner to get a more in-depth knowledge on the relation between body weight, body fat distribution and the reversibility of a metabolic risk profile. Future studies should be designed carefully to avoid bias such as differences between intervention groups at baseline. Indeed, factors associated with body fat topography (e.g. age, sex, ethnicity, menopausal status, etc.) may confound the response to lifestyle modification programs. [95, 96]

Also, new studies in this field should plan and embed economic evaluations such as cost-effectiveness and cost-utility analyses. Such information may be important for a patient, a cost-payer or a societal perspective and may help policy makers in their decision making process.

Towards clinical practice, it is certain that a non-invasive intervention that aims to decrease body weight leads to a decrease in ectopic fat, particular in the liver and the heart. Since a significant decrease in body weight is most easily achieved by a diet intervention, caloric restriction is a good basis for obtaining a significant decrease in ectopic adiposity.[97, 85, 8] The effect of exercise training on ectopic fat seems to be rather small. However, clinicians should emphasize that effects of physical activity might be underestimated in most studies because of methodological shortcomings. Also, it must be recognized that numerous health benefits result from exercise programs (e.g. decrease in blood pressure, amelioration of the blood lipid profile and amelioration of insulin sensitivity). [8]
Since sedentarism is correlated with higher dietary intake and other health implications, physical activity can play a key role to achieve a healthier lifestyle.[98, 99] From a public health perspective it is important that overweight and obese people are getting aware of their unhealthy nutritional habits and physical sedentary behavior and to empower them to acquire a healthier lifestyle. For that purpose, it seems clear that physical activity is of uppermost importance.

Conclusion

This meta-analysis shows evidence to conclude that lifestyle interventions including hypocaloric diet, physical activity or a combined intervention have the potential to decrease ectopic adiposity in the liver and the heart in overweight and obese adults. While preliminary results of an intervention on pancreatic adiposity are promising, the results on IMCL after lifestyle interventions should be interpreted with care accounting for the athlete's paradox. A reduction of BMI seems to accompany the loss of ectopic adiposity; however it does not seem to be a prerequisite. Lifestyle interventions should be considered in treatment programs for obesity aiming further than weight loss because of their potential to reduce excess ectopic fat deposition, thereby improving metabolic and cardiovascular risk factors.

Tables and figures

Outcome (region of ectopic fat)	Used key words
Liver	(“Fatty Liver” OR “hepatic lipid” OR “hepatic fat” OR “IHTG” OR “IHL” OR “intra hepatic lipid” OR “intra hepatic fat” OR “intra hepatic triglyceride” OR “hepatic lipid content” OR “hepatic fat content” OR “hepatic fat fraction” OR “hepatic lipid fraction” OR “liver lipid content” OR “liver fat content” OR “hepatic fat accumulation” OR “hepatic lipid accumulation”)
Muscle	("muscle fat" OR “muscular fat” OR “skeletal muscle fat” OR "muscle lipid" OR “muscular lipid” OR “skeletal muscle lipid” OR "intramyocellular fat" OR "intramyocellular lipid" OR "intramyocellular triglycerides" OR “muscular triglycerides” OR "muscle fat fraction" OR "muscle lipid fraction" OR "muscle lipid content" OR "muscle fat content" OR "IMCL" OR "IMTG")
Heart	("cardiac lipotoxicity" OR "peri coronary fat" OR "peri coronary lipid" OR "pericardial fat" OR "lipotoxic cardiomyopathy" OR "pericardial fat" OR "pericardial lipid" OR "cardial fat" OR "cardial lipid" OR "epicardial fat" OR "epicardial lipid" OR "peri aortic fat" OR "peri aortic lipid" OR "peri coronary epicardial adipose tissue" OR "peri coronary epicardiac adipose tissue" OR "cardiac fat" OR "cardiac lipid" OR "heart fat" OR "heart lipid" OR "cardiac steatosis" OR "lipotoxic cardiomopathy" OR "lipotoxic heart" OR "heart steatosis" OR "heart lipotoxicity" OR "epicardial fat thickness" OR "myocardial triglyceride" OR "myocardial triglyceride accumulation" OR "myocardial TG levels" OR "myocardial TG accumultion" OR "epicardial wall thickness" OR "cardial fat" OR "cardiac fat" OR "epicardiac fat" OR "epicardial fat")
Pancreas	(“lipid pancreas” OR “fat pancreas” OR "lipid accumulation pancreas" OR "fat accumulation pancreas” OR "lipid accumulation pancreatic islets" OR "pancreatic fat accumulation" OR "pancreatic fat fraction" OR "pancreatic fat content")
Kidney	("renal fat accumulation" OR “renal lipid accumulation” OR "renal fat fraction" OR “renal lipid fraction” OR "renal steatosis" OR "renal lipid content" OR "renal fat content" OR "retroperitoneal" OR "kidney fat" OR "kidney lipid" OR "renal fat" OR "renal lipid")
Blood vessel	((("blood vessels" OR “perivascular fat” OR “vascular”) AND “fat”) OR ("adventitia" AND “fat”) OR ("adventitia" AND ("lipids" OR “lipid”)) OR “perivascular fat”)

Table 1: key words of outcome parameters (different regions of ectopic fat)

INCLUSION	EXCLUSION
Design: Clinical trial	Design: Review, meta-analysis, case study, letter to editor
Population: -humans -adults -BMI>26.5 kg/m²	Population: -patients with pathologies and conditions different from the regular comorbidities of overweight and obesity
Intervention: -minimum 4 weeks of duration -must be non-invasive and at least hold diet or exercise therapy or the combination of both -diet must be hypocaloric or aimed at reducing body weight -exercise therapy must be coordinated or supervised aimed to reduce body weight or body fat	Intervention: -in case of "advise only" -overfeeding studies -invasive intervention: bariatric surgery -medication studies: in case diet or exercise therapy was combined with a medication intervention
Outcome: -primary parameters (ectopic fat) must be measured: IMCL, hepatic, pancreatic, renal, vascular and cardial fat deposition -measurement of ectopic fat through non-invasive valid and reliable imaging techniques (CT, MRI, MRs)	Outcome: -the use of echography to make an assumption of liver fat or pancreas fat -the use of indirect markers to make an assumption of liver fat -the use of invasive techniques (biopsy)

Table 2: Inclusion and Exclusion criteria for selection of studies

Author (year) and Country/ethnicity participants	Known comorbidities and medication	Intervention period	Groups	PA modality and Training volume/week	Diet	n (M/F)	Age (y)	BMI (kg/m²)	Assessment ectopic fat	Result ectopic fat	Result BMI (kg/m²)	Assessment IR	Result IR
ALBU[53] (2010) 74%Caucasian, 21%African American, 5%hispanic	DMII few taking oral medication	12 months	D+E	aerobic, mod. I, 180 min/w	target energy intake = 1200-1500 kcal/day or 1500-1800 kcal/d	male: 25 (25/0)	61.6±1.5	32.4±0.5	CT T12-L1: liver attenuation (HU)	↑ from 51.2±2.1 to 59.7±1.8 (S)	↓ from 32.4±0.5 to 28.4±0.5 (S)	Hyperinsulinemic euglycemic clamp: glucose disposal rate (mg/kgFFM*min)	↑ from 5.7±0.4 to 8.9±0.5 (S)
						female: 29 (0/29)	58.9±1.3	34.8±0.6		↑ from 46.5±1.9 to 54.6±1.7 (S)	↓ from 34.8±0.6 to 32.0±0.6 (S)		↑ from 6.2±0.4 to 8.4±0.5 (S)
BOSY WESTPHAL[54] (2010) Caucasian	/	14.2±2 weeks	D	/	target energy intake = 800-1000 kcal/d	22 (0/22)	31.4±6.0	35.5±4.9	H-MRS liver: IHL (ref to water-%)	↓ from 0.055±0.063 to 0.032±0.030 (p<0.05)	↓ from 35.5±4.9 to 32.5±4.6 (p<0.001)	hyperinsulinemic euglycemic clamp: glucose disposal rate (mg/kg*min)	↑ from 5.08±2.28 to 5.83±2.57 (p<0.05)
BOZZETTO[55] (2012) Italy	DMII oral medication	8 weeks	D	/	high carb/high fiber/ low GI	45 (37/8)	58±5	30±2	H-MRS liver: IHL (ref to water-%)	↓ from 17.7±9.7 to 16.1±6.8 (NS)	no change in each group	fasting blood sample: HOMA-IR (µU/ml*mg/dl)	↑ from 4.8±1.8 to 5.2±1.2 (NS)
			D	/	high MUFA		57±8	28±3		↓ from 7.4±2.8 to 5.2±2.7 (p<0.005)			↑ from 3.6±1.5 to 3.9±2.0 (NS)
			D+E	aerobic, 70%VO2peak, 90 min/w	high carb/high fiber/ low GI		63±5	31±3		↑ from 8.8±4.9 to 8.9±5.7 (NS)			↑ from 5.1±1.6 to 5.4±2.9 (NS)
			D+E	aerobic, 70%VO2peak, 90 min/w	high MUFA		57±9	30±4		↓ from 11.6±8.0 to 9.1±7.4 (p<0.05)			↓ from 4.2±1.2 to 4.0±1.6 (NS)
CHAN[50] (2010) Australia	insulin resistance	22 weeks	D	/	low fat	10 (6/4)	57±8	33±1	H-MRS liver: IHL (ref to water-%)	↓ from 22±5 to 16±4 (p<0.01)	↓ from 33±1 to 31±1 (p<0.01)	fasting blood sample: HOMA-IR (µU/ml*mg/dl)	↓ from 3.1±0.5 to 2.6±0.4 (p<0.05)
DEVRIES[56] (2008) Canada	/	3 months	E	aerobic, male: 50-70%VO2peak, 60-180 min/w	/	male: 9 (9/0)	39±3	39±3	CT liver: liver attenuation (HU)	no changes	↓ from 34±2 to 33±2 (NS)	fasting blood sample: HOMA-IR (µU/ml*mg/dl)	↓ from 3.3±0.5 to 3.0±0.5 (NS)
				aerobic, female: 50-65%VO2peak, 30-180 min/w		female: 11 (0/11)	40±3	40±3			from 34±2 to 34±2 (NS)		↓ from 2.3±0.2 to 2.1±0.4 (NS)
ELIAS[57] (2010) Brasil	NAFLD	6 months	D	/	energy deficit of 500-1000 kcal/d	adherent: 17 (both)	47.6±12.9	32.8±4.0	CT liver: liver attenuation (HU)	↑ from 41.6±11.6 to 47.8±15.0 (p<0.05)	↓ from 32.8±4.0 to 29.8±4.0 (p<0.001)	fasting blood sample: HOMA-IR (µU/ml*mg/dl)	↓ with 4.2±2.9 to 2.4±1.5 (p=0.001)
						non-adherent: 10 (both)	47.4±10.0	34.5±5.4		↑ from 45.1±15.0 to 47.8±15.9 (p=0.507)	↓ from 34.5±5.4 to 33.9±5.2 (p<0.05)		↑ from 3.7±1.2 to 4.1±1.5 (p=0.470)
FINUCANE[58] (2010) United Kingdom	/	3 months	E	aerobic, 50-70% Wattmax, 180 min/w	/	48 (overall 44%woman)	71.4 (67.4–76.3)	27.4±4.9	H-MRS liver: IHL (ref to water-%)	↓ from 3.7 (1.8-10.2) to 2.4 (1.0-6.6) (S)	↓ from 27.4±4.9 to 27.3±4.8 (S)	OGTT: HOMA-2S (%)	↑ from 105.7 (63.2-139) to 128.4 (78-165.2) (NS)
			CON	/		48 (overall 44%woman)		26.9±3.6		↓ from 3.6 (1.4-8.3) to 3.5 (1.0-12.6) (NS)	↓ from 26.9±3.6 to 26.8±3.6 (NS)		↑ from 102.4 (67.8-122) to 110.5 (73.1-149) (NS)
GOODPASTER[59] (2010) 60%Caucasian, 40%African American	/	6 months	D	/	target energy intake = 1200-2100 kcal/d	63 (both)	47.5±6.2	43.7±5.9	CT liver: liver-spleen attenuation (HU)	↑ from 1.09 (1.03-1.15) to 1.15 (1.11-1.20)	↓ from 43.67 (42.33-45.02) to 40.59 (39.32-41.87) (S)	fasting blood sample: HOMA-IR (µU/ml*mg/dl)	↓ from 3.68 (3.07-4.28) to 2.65 (2.23-3.07)
			D+E	aerobic, mod. I, 300 min/w		66 (both)	46.1±6.5	43.5±4.8		↑ from 1.06 (1.00-1.12) to 1.18 (1.14-1.22)	↓ from 43.51 (42.21-44.81) to 39.62 (38.37-40.87) (S)		↓ from 4.03 (3.47-4.60) to 2.70 (2.30-3.11)
HALLSWORTH[47] (2011) United Kingdom	NAFLD	8 weeks	E	strength, 2-3 circuits, 8 exercises, 50-70%1RM, 135-180 min/w	/	11 (both)	52±13.3	32±4.9	H-MRS liver: IHL (ref to water-%)	↓ from 14.0±9.1 to 12.2±9.0 (p=0.01)	↓ from 32.3±4.9 to 32.3±4.5 (p=0.89)	fasting blood sample: HOMA-IR (µU/ml*mg/dl)	↓ from 5.9±5.9 to 4.6±4.6 (p=0.05)
			CON	/		8 (both)	62±7.4	32.3±4.8		↑ from 11.2±8.4 to 11.5±7.4 (p=0.80)	↑ from 32.3±4.8 to 32.5±4.2 (p=0.42)		↑ from 4.7±2.1 to 5.1±2.5 (p=0.57)
HAUFE[60] (2011) Germany	/	6 months	D	/	reduced fat	normal IHL: 50 (10/40)	44±9	31.9±3.9	H-MRS liver: IHL (%)	↓ from 9.6±9.8 to 5.6±6.4 (p<0.001)	↓ with 2.4±0.2 (p<0.01)	fasting blood sample: HOMA-IR (µU/ml*mg/dl)	↓ with 0.43±0.11 (p<0.01)
					reduced carbs	high IHL: 52 (8/44)	45±8	35.6±4.7		↓ from 7.6±8.2 to 4±4.6 (p<0.001)	↓ with 2.7±0.2 (p<0.01)		↓ with 0.61±0.18 (p<0.01)

JOHNSON[61] (2009) Australia	/	4 weeks	E	aerobic, 50-70%VO2peak, 90-135 min/w	/	12 (both)	49.1±2.3	32.2±0.8	H-MRS liver: IHL (ref to water-%)	↓ from 8.55±2.49 to 6.79±1.90 (S)	↓ from 32.2±0.8 to 32.1±0.8 (NS)	fasting blood sample: HOMA-IR (µU/ml*mg/dl)	↓ from 4.59±0.69 to 4.40±0.76 (NS)
			CON	home-based stretching, 90 min/w		7 (both)	47.3±3.6	31.1±1.1		↑ from 9.18±2.49 to 9.44±1.90 (NS)	↓ from 31.1±1.1 to 31.0±1.2 (NS)		↑ from 4.75±1.09 to 4.81±0.93 (NS)
KIRK[62] (2009) USA	63% impaired glucose tolerance	±11 weeks	D	/	low carbs (<60g/d)	22 (4/18)	41.8±3.1	36.1±1.0	H-MRS liver: IHL (%)	↓ (p<0.05)	7.5±0.4 % weight loss (p<0.0001)	euglycemic hyperinsulinemic clamp: HOMA-IR (mU/L*mM)	↓ with 44.0±4.7 (p<0.001)
					high carbs (>180g/d)		45.4±4.0	36.9±1.2		↓ (p<0.05)	7.5±0.4 % weight loss (p<0.0001)		↓ with 27.1±5.1 (p<0.001)
KUK[63] (2007) Caucasian	/	6 months	E	aerobic, 50%VO2max, 3-4/w- until energy expenditure of 4kcal/kg/w was reached	/	28 (0/28)	58.8±6.9	31.8±3.8	CT liver: liver attenuation (HU)	↑ with 0.60±6.33	↓ in bodyweight	/	/
				aerobic, 50%VO2max, 3-4/w- until energy expenditure of 8kcal/kg/w was reached		19 (0/19)	58.5±6.2	32.2±3.9		↑ with 1.60±7.32	↓ in bodyweight		
				aerobic, 50%VO2max, 3-4/w- until energy expenditure of 12kcal/kg/w was reached		29 (0/29)	58.1±6.3	30.7±3.3		↑ with 0.79±6.39	↓ in bodyweight		
			CON	/		11 (0/11)	57.1±4.7	32.1±4.2		↓ with 2.9±7.00	↓ in bodyweight		
LARSON MEYER[64] (2006) 65% Caucasian, 33% African American, 2% Asian	/	6 months	D	/	25% calorie restriction	12 (both)	male: 37±2 female: 37±1	male: 27.8±0.2 female: 27.2±0.2	H-MRS liver: IHL (ref to oil phantom-%)	↓ from 1.66±0.56 to 0.69±013 (S)	↓ (S)	intravenous glucose tolerance test (µU/l*min)	↑ from 3.3±0.5 to 4.2±1.0 (NS)
			D+E	aerobic, ↑ energy expenditure with 12.5%, 5 sessions/w	12.5% calorie restriction	12 (both)				↓ from 1.17±0.21 to 0.65±0.10 (S)	↓ (S)		↑ from 3.4±0.4 to 5.3±0.8 (S)
			D (low calorie)	/	target energy intake = 980 kcal/d	11 (both)				↓ from 1.16±0.29 to 0.55±0.09 (S)	↓ (S)		↑ from 3.1±0.6 to 4.7±0.9 (S)
			CON	/	/	11 (both)				↑ from 1.60±0.39 to 2.36±0.81 (S)	no changes		↑ from 2.8±0.4 to 2.5±0.4 (NS)
LAZO[65] (2010) 60% Caucasian, 32% African American, 5% Other, 2% Hispanic	DMII 12% taking insulin, 50% taking oral medication	12 months	D+E	aerobic, moderate I, 175 min/w	target energy intake = 1200-1500 kcal/day or 1500-1800 kcal/d	46 (overall 49% female)	61.6±6.7	34.9±5.0	H-MRS liver: IHL (ref to water-%)	↓ from 4.2 to 2.9	↓ from 34.7±5.4 to 32.1±5.2 (S)	/	/
			CON	/	/	50 (overall 49% female)				↓ from 6.3 to 4.9	from 35.3±4.7 to 35.3±4.8 (NS)		
MACHANN[66] (2010) Germany	impaired glucose tolerance and/or gestational diabetes	9 months	D+E	aerobic, mod. I, min. 180 min/w	low fat, aimed to reduce body weight with 5%	236 (96/140)	male: 47.3±11.4	30.2±4.2	H-MRS liver: IHL (ref to water-%)	↓ from 8.6±9.1 to 5.4±5.6 (p<0.0001)	↓ from 30.2±4.2 to 29.1±4.1 (p<0.0001)	OGTT method Matsuda and DeFronzo: insulin sensitivity (AU)	↑ from 11.3±6.8 to 14.6±8.0 (p<0.0001)
							female: 44.5±10.9	29.2±5.5		↓ from 5.1±7.6 to 4.3±9.5 (NS)	↓ from 29.2±5.5 to 28.3±5.2 (p<0.0001)		↑ from 13.6±6.8 to 14.6±7.8 (p=0.031)
OZA[67] (2009) Japan	NAFLD	3 months	D+E	aerobic, 27 METS hours/w	target energy intake = body weight*25-30 kcal/d	31 (15/16)	55.4±13.7	26.7±3.6	CT liver: liver/spleen attenuation	↑ from 1.030±0.200 to 1.162±0.184 (p<0.01)	↓ from 26.6±2.5 to 25.5±2.5 (p<0.01)	fasting blood sample: HOMA-IR (µU/ml*mg/dl)	↓ from 2.28±1.43 to 2.10±2.02 (NS)
		6 months								↑ from 1.030±0.200 to 1.140±0.120 (p<0.05)	↓ from 26.6±2.5 to 24.9±2.7 (p<0.01)		↓ from 2.28±1.43 to 2.55±1.11 (p<0.05)
PETERSEN[68] (2005) USA	DMII 5pt taking SU derivatives	3-12 weeks (until achieving normoglycemia)	D	/	low fat, energy intake = 1200 kcal/d	8 (5/3)	47±3	30.1±0.9	H-MRS liver: IHL (ref to water-%)	↓ with 81±4 (p=0.009)	↓ from 30.1±0.9 to 27.5±0.8 (p=0.002)	hyperinsulinemic euglycemi clamp: clamp glucose oxidation (mg/kgFFM*min)	↓ from 3.85±0.55 to 3.06±0.68 (NS)
SANCHEZ MUNOZ[69] (2013) Mexico	/	3 months	E	aerobic, 60-85% HRreserve, 100-300 min/w	/	8 (0/8)	25-60	32.20 (27.4-38.1)	CT liver: liver attenuation (HU)	↑ from 36.80 (27.3-46.4) to 46.53 (25.0-59.4)	á from 32.20 (27.4-38.1) to 33.35 (26.7-37.1)	fasting blood sample: HOMA-IR	↓ from 3.75 (2.0-13.3) to 3.61 (1.4-7.6)
SATO[70] (2007) Japan	/	3 months	D	/	target energy intake = ideal body weight*35kcal/d	13 (13/0)	?	32.5±2.2	H-MRS liver: IHL (ref to water-%)	↓ from 12.9±6.2 to 8.2±4.6 (p=0.023)	↓ from 32.5±2.2 to 30.5±1.6 (p<0.01)	OGTT: HOMA-IR	↓ from 4.9±1.8 to 3.3±1.3 (p<0.03)

SHAH[71] (2009) USA	/	until 10% weight loss	D	/	energy deficit of 500-1000 kcal/d	18 (6/13)	68.6±1.1	≥30	H-MRS liver: IHL (%)	↓ from 7.1±1.1 to 3.4±0.6 (p=0.01)	↓ (S)	fasting blood sample: HOMA-IR (µU/ml*mg/dl)	↓ from 5.5±1.3 to 4.0±1.2 (p=0.047)
			D+E	aerobic: 70-85%HRpeak, 90 min/w strength: 1-3 sets, 65-80%1RM, 90min/w balance: 45 min/w flexibility: 45 min/w	energy deficit of 130 kcal/d		68.5±1.3			↓ from 8.6±2.2 to 4.6±1.6 (p=0.02)	↓ (S)		↓ from 4.1±1 to 2.9±1 (p=0.02)
SHEA[46] (2011) >80% Caucasian	/	16 weeks	D+E	strength, 2-3 sets, 8-10 reps, 40–70% 1RM, 3 sessions/w	energy deficit of 500 kcal/d	20 (11/9)	male: 69.5±3.9	33.2±5.1	CT liver: liver attenuation (HU)	↓ with 1.3 (1.4)	↓ with 5.6 (0.9) kg	/	/
							female: 71.3±4.9	31.1±3.1		↑ with 4.8 (1.5)	↓ with 6.1 (0.7) kg		
SHOJAEE MORADIE[72] (2007) United Kingdom	/	6 weeks	E	aerobic, 60-85% VO2max, 60 min/w	/	10 (10/0)	47±3	27.6±0.6	H-MRS liver: IHL (ref to water)	↑ from 4.0 to 4.3 (NS)	↓ from 27.6±0.6 to 27.5±0.6 (NS)	hyperinsulinemic euglycemic clamp: glucose infusion rate (µmol/kg*min)	↑ from 46.3±3.7 to 53.1±3.2 (p=0.016)
			CON	/		7 (7/0)	55±3.4	27.6±0.9		↑ from 3.9 to 5.2 (NS)	↓ from 27.6±0.9 to 27.4±0.9 (NS)		↑ from 42.8±4.4 to 44.2±3.8 (NS)
SLENTZ[73] (2011) >80% Caucasian, <20% African American participants	/	8 months	E	strength, 1-3 sets, 8–12 reps, 8 exercises, 2-3 sessions/w	/	36 (both)	49.7±11.4	30.5±3.4	CT liver: liver attenuation (HU)	↑ with 0.4±4.9 (p=0.64)	↓ (S)	fasting blood sample: HOMA-IR (µU/ml*mg/dl)	↓ with 0.09±1.3 (p=0.63)
				aerobic, 75% VO2peak, 2-3 sessions/w		36 (both)	49.5±9.8	30.4±3.2		↑ with 2.5±5.7 (p=0.012)	↓ (S)		↓ with 0.40±0.8 (p=0.004)
				strength and aerobic, 2-3 sessions/w		35 (both)	46.9±10.0	30.7±3.4		↑ with 1.8±5.9 (p=0.079)	↓ (S)		↓ with 0.50±0.9 (p=0.002)
SNEL[74] (2012) The Netherlands	DMII insulin, metformin or SU derivatives	16 weeks	D	/	target energy intake = 1350 kcal/d	14 (8/6)	53±2	35±1.1	H-MRS liver: IHL (ref to water-%)	↓ from 22.8±3.9 to 3.6±1.0 (p<0.05)	↓ from 35.3±1.1 to 27.5±1.1 (p<0.05)	/	/
SULLIVAN[75] (2012) USA	NAFLD	16-18 weeks	E	aerobic, 45-55% VO2max, 5*30-60 min/w	/	12 (4/8)	48.6±2.2	37.1±1.1	H-MRS liver: IHL (ref to water-%)	↓ with 10.3±4.6 (NS)	from 37.1±1.1 to 37.1±1.1 (NS)	/	/
			CON	/		6 (1/5)	47.5±3.1	40.0±2.2		↑ (NS)	↑ from 40.0±2.2 to 40.1±2.1 (NS)		
THOMAS[76] (2006) United Kingdom	abnormal liver function	6 months	D	/	energy deficit of 500 kcal/d	10 (10/0)	?	31.3 (28.3-34.9)	H-MRS liver: IHL (ref to water-AU)	↓ from 13.3 (5.8-30.9) to 8.0 (2.4-26.4) (p=0.12)	↓ from 31.3 (28.3-34.9) to 30.2 (27.2-33.9) (p=0.006)	fasting blood sample: HOMA-IS	↓ from 424.04 (225.2-737.5) to 540.1 (38.0-1400.8) (p=0.33)

Table 3: Overview of all studies included in the meta-analysis, with outcome of liver adiposity

Note: DMII = diabetes mellitus type 2, PA = physical activity, NAFLD = non-alcoholic fatty liver disease, DM 2 = diabetes mellitus type 2, SU = sulphonylurea, D = diet, E = exercise, D+E = diet and exercise, CON : control group, Wattmax = maximum wattage, HRreserve = heart rate reserve, HRmax = maximum heart rate, VO2max = maximal oxygen uptake, VO2peak = peak oxygen uptake, reps = repetitions, 1RM = 1 repetition maximum, I = intensity, mod. I = moderate intensity, carbs = carbohydrates, MUFA = monounsaturated fatty acid, GI = glycemic index, IR = insulin resistance, HOMA-IS = homeostatic model assessment insulin sensitivity, HOMA-IR = homeostatic model assessment insulin resistance, QUICKI = quantitative insulin sensitivity check index, OGTT = oral glucose tolerance test, S = significant, NS = non-significant, adherent: 5% weight loss or more after the intervention, non-adherent = weight loss less than 5% bodyweight after the intervention, AU = arbitrary unit, SOL = soleus muscle, TA = tibialis anterior muscle, vast. lat = quadriceps vastus lateralis muscle, MRI = magnetic resonance imaging, CTscan = computed tomography, H-MRS = hydrogen based magnetic resonance spectroscopy

Author (year) and Country/ethnicity participants	Known comorbidities	Intervention period	Groups	PA Modality and Trainingload/week	Diet	n (M/F)	Age (y)	BMI (kg/m²)	Assessment ectopic fat	Result ectopic fat	Result BMI (kg/m²)	Assessment IR	Result IR
BOSY WESTPHAL[54] (2010) Caucasian particpiants	/	14.2±2 weeks	D	/	target energy intake = 800-1000 kcal/d	22 (0/22)	31.4±6.0	35.5±4.9	MRI: pericardial fat (g)	↓ from 20.14±9.61 to 17.83±8.13 (p<0.01)	↓ from 35.5±4.9 to 32.5±4.6 (p<0.001)	hyperinsulinemic euglycemic clamp: glucose disposal rate (mg/kg*min)	↑ from 5.08±2.28 to 5.83±2.57 (p<0.05)
BRINKLEY[78] (2011) 78%Caucasian, 25%African American participants	/	20 weeks	D+E(mod.I)	aerobic, 45-50%HRR, 165 min/w	equal hypocaloric for all groups, corrected for pa, energy deficit of 2800 kcal/w	15 (0/15)	57.3±5.7	33.6±4.5	CT scan: pericardial fat (cm³)	↓ with 17% (p<0.0001)	↓ with 15% (p<0.05)	/	/
			D+E(high I)	aerobic, 70-75%HRR, 90 min/w		9 (0/9)	59.4±4.9	34.4±4.7		↓ with 17% (p<0.0001)	↓ with 15% (p<0.05)		
			D	/		8 (0/8)	57.6±4.8	32.2±4.0		↓ with 17% (p<0.0001)	↓ with 15% (p<0.05)		
IACOBELLIS[40] (2008) Caucasian participants	/	6 months	D	/	target energy intake = 900kcal/d	20 (8/12)	35±10	45±5	echocardio: epicardial fat thickness (mm)	↓ from 12.3±1.8 to 8.3±1 (p<0.001)	↓ from 45±5 to 38±5 (p<0.001)	/	/
KIM[79] (2009) Japan	/	12 weeks	E	aerobic, 50 - 70% HRmax, borg scale: 11-13, 180 min/w	/	24 (24/0)	49.4±9.6	30.7±3.3	echocardio: epicardial fat thickness (mm)	↓ from 8.1±1.64 to 7.39±1.54 (p<0.001)	↓ from 30.7±3.3 to 29.3±2.9 (p<0.01)	QUICKI (µU/ml+mg/dl)	↑ (NS)
SCHRAUWEN HINDERLING[80] (2010) The Netherlands	/	12 weeks	E	*aerobic: 55%Wattmax, 3 sessions/w *strength: 1x8reps55% 1RM and 2x8reps75% 1RM, 3 sessions/w	/	14 (14/0)	58.4±0.9	29.9±0.01	H-MRS: cardiac lipid content (ref to water-%)	↓ from 0.99±0.15 to 0.54±0.04 (p=0.02)	↓ from 29.9±0.01 to 29.5±0.01 (p=0.1)	/	/
SCHRAUWEN HINDERLING[81] (2011) The Netherlands	DMII metformin and SU derivatives	12 weeks	E	*aerobic: 55%Wattmax, 3 sessions/w *strength: 1x8reps55% 1RM and 2x8reps75% 1RM, 3 sessions/w	/	11 (11/0)	59.5±0.9	30.5±1.4	H-MRS: cardiac lipid content (ref to water-%)	↑ from 0.80±0.22 to 0.95±0.21 (p=0.15)	↓ from 30.5±1.4 to 30.4±1.4 (p=0.8)	hyperinsulinemic euglycemic clamp: glucose infusion rate (µmol/kg*min)	↑ from 5.8±1.9 to 10.3±2.0 (p=0.02)
SHEA[46] (2011) >80%Caucasian participants	/	16 weeks	D+E	strength, 2-3 sets, 8-10 reps, 40–70%1RM, 3 sessions/w	energy deficit of 500 kcal/d	male: 11 (11/0)	69.5±3.9	33.2±5.1	CT scan: pericardial fat (cm³)	↓ with 5.7 (S)	bodyweight: ↓ with 5.6kg (S)	/	/
						female: 9 (0/9)	71.3±4.9	31.1±3.1		↓ with 6.6 (S)	bodyweight: ↓ with 6.1kg (S)		
SNEL[74] (2012) The Netherlands	DMII insulin, metformin or SU derivatives	16 weeks	D	/	target energy intake = 1350 kcal/d	14 (8/6)	53±2	35±1.1	MRI: pericardial fat (ml)	↓ from 39±4 to 31±2 (p<0.05)	↓ from 35.3±1.1 to 27.5±1.1 (p<0.05)	/	/

Table 4: Overview of all studies included in the meta-analysis, with outcome of heart adiposity

Note: DMII = diabetes mellitus type 2, SU = sulphonylurea, D = diet, E = exercise, D+E = diet and exercise, CON : control group, Wattmax = maximum wattage, VO2max = maximal oxygen uptake, reps = repetitions, 1RM = 1 repetition maximum, HRR = heart rate reserve, HRmax = maximum heart rate, I = intensity, mod.I = moderate intensity, carbs = carbohydrates, MUFA = monounsaturated fatty acid, GI = glycemic index, QUICKI = quantitative insulin sensitivity check index, NS = non-significant, adherent: 5% weight loss or more, adherent = weight loss: 5% bodyweight after the intervention, non-adherent = weight loss less than 5% after the intervention, SOL = soleus muscle, TA = tibialis anterior muscle, vast. lat = quadriceps vastus lateralis muscle

Author (year) and Country/ethnicity participants	Known comorbidities	Intervention period	Groups	PA Modality and Training load/week	Diet	n (M/F)	Age (y)	BMI (kg/m²)	Assessment ectopic fat	Result ectopic fat	Result BMI (kg/m²)	Assessment IR	Result IR
KUK [63] (2007) Caucasian participants	/	6 months	E	aerobic, 50%VO2max, 3-4/w- until energy expenditure of 4kcal/kg/w was reached	/	28 (0/28)	58.8±6.9	31.8±3.8	CT pancreas: pancreas attenuation (HU)	↑ with 0.60±6.33 (p>0.05)	↓in bodyweight	/	/
				aerobic, 50%VO_2max, 3-4/w- until energy expenditure of 8kcal/kg/w was reached		19 (0/19)	58.5±6.2	32.2±3.9		↑ with 1.17±5.43 (p>0.05)	↓in bodyweight		
				aerobic, 50%VO_2max, 3-4/w- until energy expenditure of 12kcal/kg/w was		29 (0/29)	58.1±6.3	30.7±3.3		↑ with 1.70±4.82 (p>0.05)	↓in bodyweight		
			CON	/		11 (0/11)	57.1±4.7	32.1±4.2		↑ with 0.25±7.55 (p>0.05)	↓in bodyweight		

Table 5: Overview of all studies included in the meta-analysis, with outcome of pancreatic adiposity

Note: PA = physical activity, E = exercise, CON = control group, VO2max = maximum oxygen uptake, IR = insulin resistance

Author (year) and Country/Ethnicity participants	Known comorbiditi es	Intervention period	Groups	PA modality and Training load/week	Diet	n (M/F)	Age (y)	BMI (kg/m²)	Assessment IMCL	Result IMCL	Result BMI (kg/m²)	Assessment IR	Result IR
GAN[77] (2003) Caucasian participants	/	6 weeks	E	aerobic, 55-70%VO2max, 160 min/w	/	18 (18/0)	37.4±1.3	30.9±0.7	H-MRS SOL (ref to creatine-AU)	↑ from 11.1±0.8 to 11.6±0.8 (p=0.50)	↓ from 30.9±0.7 to 30.4±0.8 (NS)	hyperinsulinemic - eugenic clamp: glucose infusion rate (mmol/min/kgFFM)	↑ from 35.4±2.8 to 41.0±2.9 (p=0.01)
									H-MRS TA (ref to creatine-AU)	↓ from 6.1±0.8 to 5.5±0.7 (p=0.66)			
JOHNSON[61] (2009) Australia	/	4 weeks	E	aerobic, 50-70%VO2peak, 90-135 min/w	/	12 (both)	49.1±2.3	32.2±0.8	H-MRS vast.lat (ref to water-%)	↑ from 1.39±0.19 to 1.64±0.16 (NS)	↓ from 32.2±0.8 to 32.1±0.8 (NS)	fasting blood sample: HOMA-IR	↓ from 4.59±0.69 to 4.40±0.76 (NS)
			CON	home-based stretching, 90 min/w	/	7 (both)	47.3±3.6	31.1±1.1		↑ from 1.20±0.19 to 1.36%±0.27 (NS)	↓ from 31.1±1.1 to 31.0±1.2 (NS)		↑ from 4.75±1.09 to 4.81±0.93 (NS)
LARSON MEYER[64] (2006) 65%Caucasian, 33%African American, 2%Asian participants	/	6 months	D	/	25%calorie restriction	12 (both)	male: 37±2 female: 37±1	male: 27.8±0.2 female: 27.2±0.2	H-MRS SOL (ref to oil phantom-%)	↓ from 3.79±0.33 to 3.54±0.44 (NS)	↓ (S)	intravenous glucose tolerance test (µU/l*min)	↑ from 3.3±0.5 to 4.2±1.0 (NS)
			D+E	aerobic, ↑ energy expenditure with 12.5% 5 sessions/w	12.5%calorie restriction	12 (both)				↓ from 1.17±0.21 to 0.65±0.10 (S)	↓ (S)		↑ from 3.4±0.4 to 5.3±0.8 (S)
			D (low calorie diet)	/	target energy intake = 980 kcal/d	11 (both)				↓ from 1.16±0.29 to 0.55±0.09 (S)	↓ (S)		↑ from 3.1±0.6 to 4.7±0.9 (S)
			CON	/	/	11 (both)				↑ from 4.05±0.5 to 4.20±0.92 (NS)	no changes		↑ from 2.8±0.4 to 2.5±0.4 (NS)
MACHANN[66] (2010) Germany	impaired glucose tolerance and/or gestational diabetes	9 months	D+E	aerobic, mod. I, min. 180 min/w	low fat, aimed to reduce body weight with 5%	SOL: 70 (70/0)	47.3±11.4	30.2±4.2	H-MRS SOL (ref to creatine-AU)	↓ from 17.7±7.8 to 15.4±8.8 (p=0.0004)	↓ from 30.2±4.2 to 29.1±4.1 (p<0.0001)	OGTT method Matsuda and DeFronzo: insulin sensitivity (AU)	↑ from 11.3±6.8 to 14.6±8.0 (p<0.0001)
						TA: 99 (99/0)			H-MRS TA (ref to creatine-AU)	↓ from 3.8±1.6 to 3.3±1.4 (p=0.0004)			
						SOL: 101 (0/101)	44.5±10.9	29.2±5.5	H-MRS SOL (ref to creatine-AU)	↓ from 14.2±7.5 to 12.4±4.2 (p=0.0009)	↓ from 29.2±5.5 to 28.3±5.2 (p<0.0001)		↑ from 13.6±6.8 to 14.6±7.8 (p=0.031)
						TA: 131 (0/131)			H-MRS TA (ref to creatine-AU)	↓ from 4.2±1.9 to 3.9±1.6 (NS)			
PETERSEN[68] (2005) United States of America	DMII 5pt taking SU derivatives	3-12 weeks (until achievement of normoglycemia)	D	/	low fat, target energy intake = ±1200 kcal/d	8 (5/3)	47±3	30.1±0.9	H-MRS SOL (ref to water-%)	↓ with 81±4% (p=0.009)	↓ from 30.1±0.9 to 27.5±0.8 (p=0.002)	hyperinsulinemic euglycemic clamp: clamp glucose oxidation (mg/kgFFM/min)	↓ from 3.85±0.55 to 3.06±0.68 (NS)
SATO[70] (2007) Japan	/	3 months	D	/	target energy intake = ideal body weight *35kcal/d	13 (13/0)	?	32.5±2.2	H-MRS TA (ref to creatine-AU)	↑ from 2.95±1.29 to 3.11±1.71 (p=0.70)	↓ from 32.5±2.2 to 30.5±1.6 (p<0.01)	OGTT: HOMA-IR	↓ from 4.9±1.8 to 3.3±1.3 (p<0.03)
SHOJAEE MORADIE[72] (2007) United Kingdom	/	6 weeks	E	aerobic, 60-85%VO2max, 60 min/w	/	10 (10/0)	47±3	27.6±0.6	H-MRS TA (ref to water-AU)	↓ from 10.3 to 7.7 (NS)	↓ from 27.6±0.6 to 27.5±0.6 (NS)	hyperinsulinemic euglycemic clamp: glucose infusion rate (µmol/min/kg)	↑ from 46.3±3.7 to 53.1±3.2 (p=0.016)
			CON	/		7 (7/0)	55±34	27.6±0.9		↓ from 10.0 to 9.6 (NS)	↓ from 27.6±0.9 to 27.4±0.9 (NS)		↑ from 42.8±4.4 to 44.2±3.8 (p=0.08)
THOMAS[76] (2006) United Kingdom	abnormal liver function	6 months	D	/	energy deficit of 500 kcal/d	10 (10/0)	?	31.3	H-MRS SOL (ref to creatine-%)	↓ from 19.6 (10.3-37.2) to 17.2 (10.8-27.3) (p=0.65)	↓ from 31.3 (28.3-34.9) to 30.2 (27.2-33.9) (p=0.006)	HOMA-IS	↓ from 424.04 to 540.1 (p=0.33)
									H-MRS vast.lat (ref to creatine-%)	↓ from 11.6 (7.6-17.7) to 8.3 (5.4-12.8) (p=0.05)			

Table 6: Overview of all studies included in the meta-analysis, with outcome of IMCL

Note: DMII = diabetes mellitus type 2, PA = physical activity, DM 2 = diabetes mellitus type 2, SU = sulphonylurea, D = diet, E = exercise, D+E = diet and exercise, CON : control group, VO2max = maximal oxygen uptake, VO2peak = peak oxygen uptake, I = intensity, mod. I = moderate intensity, IR = insulin resistance, HOMA-IS = homeostatic model assessment insulin sensitivity, HOMA-IR = homeostatic model assessment insulin resistance, OGTT = oral glucose tolerance test, S = significant, NS = non-significant, SOL = soleus muscle, TA = tibialis anterior muscle, vast. lat = quadriceps vastus lateralis muscle, H-MRS = hydrogen based magnetic resonance spectroscopy, AU = arbitrary unit

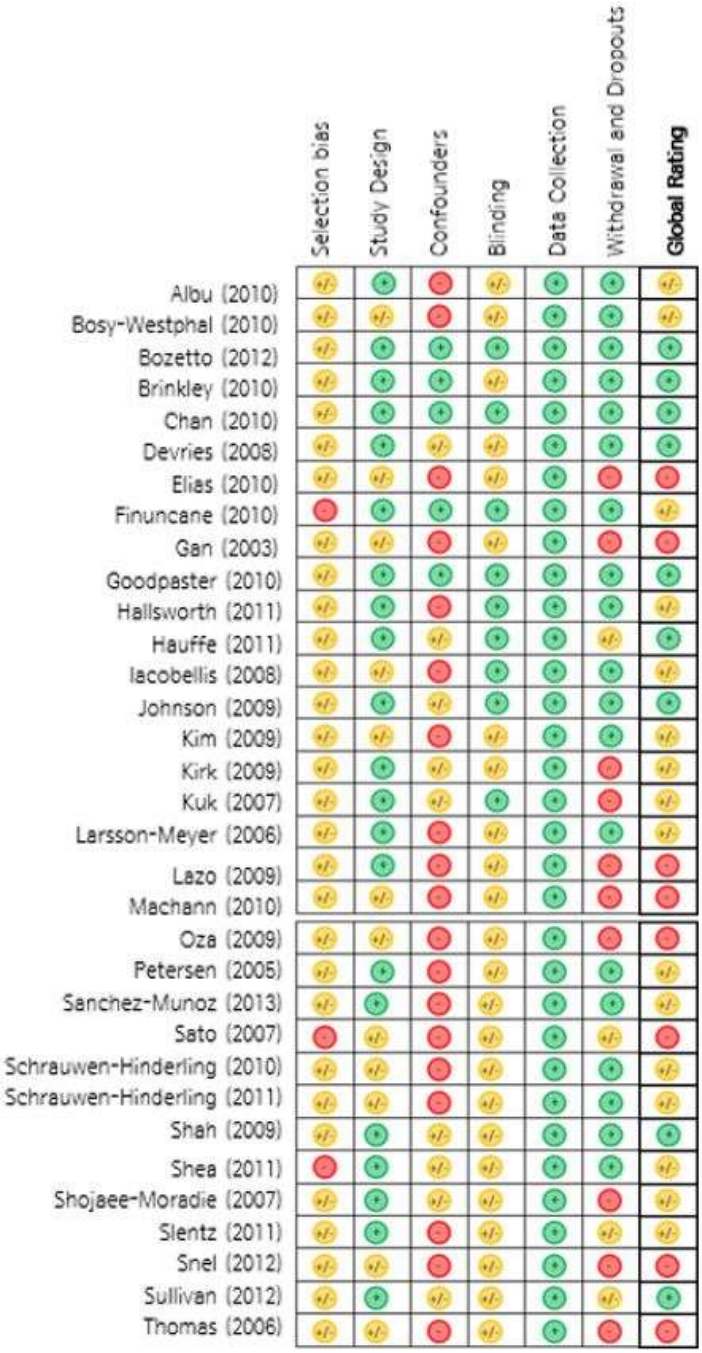

Table 7: Methodologic quality of each included article, scored with the Quality Assessment Tool for Quantitative Studies (Effective Public Health Practice Project)

Note: +: high quality; +/-: moderate quality; -: low quality

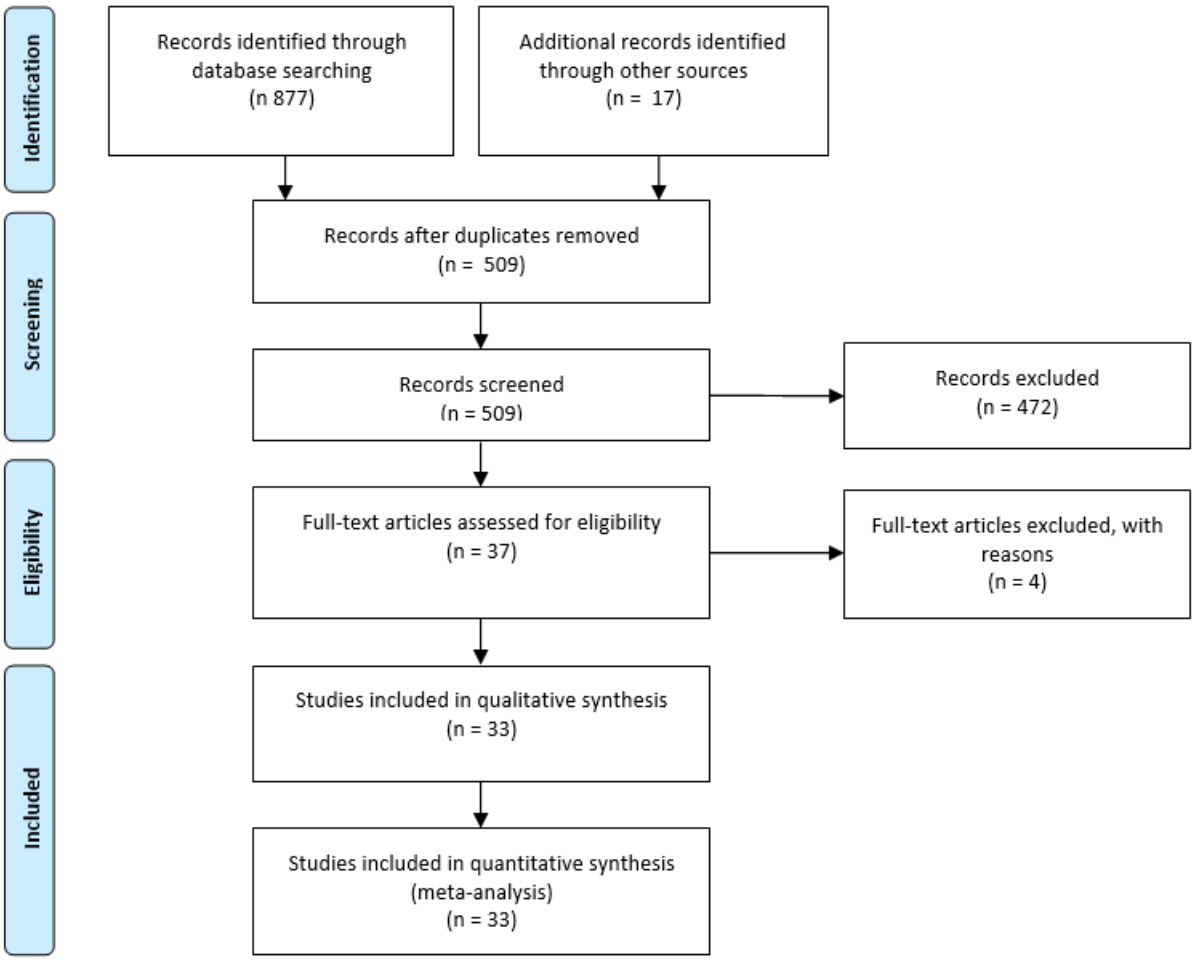

Figure 1: Flow chart following PRISMA guidelines

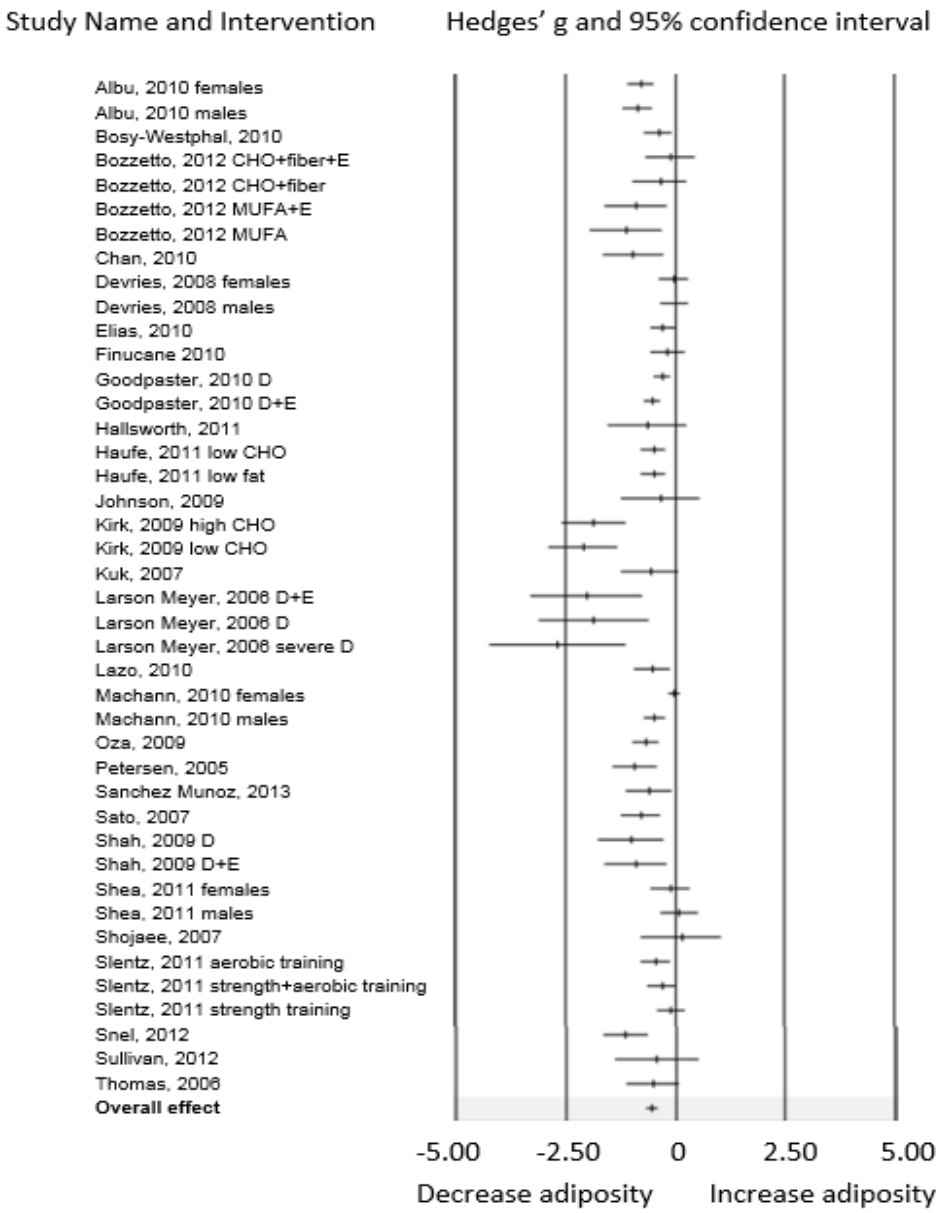

Figure 2: Forest plot of the effects found in the individual studies and intervention groups and the overall effect in hepatic adiposity

Note: Note: CHO = carbohydrates, MUFA = monounsaturated fatty acids, D = diet, E = exercise, D+E = diet and exercise

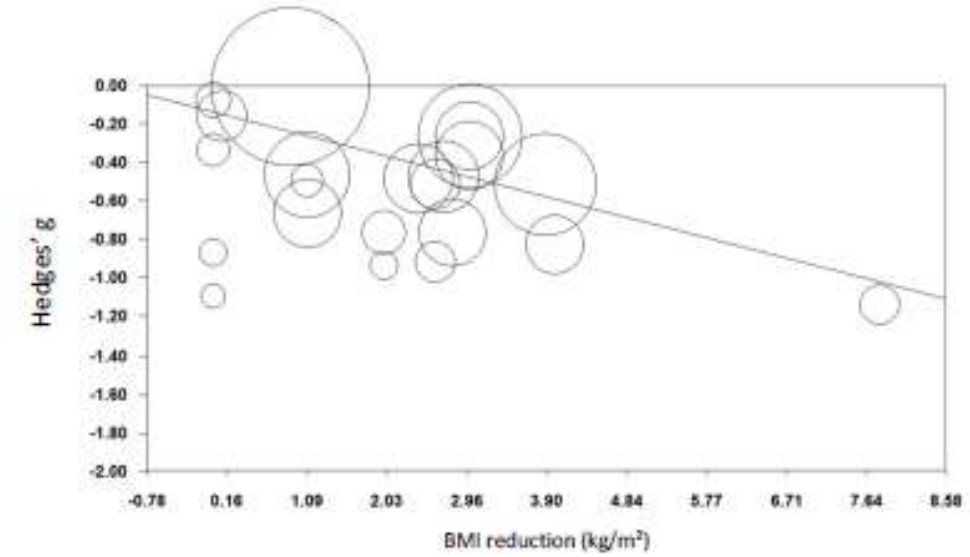

Figure 3: Meta-regression of change in hepatic adiposity (Hedges'g) with BMI reduction

Note: The circles on the graph represent the included studies; the size of the circle indicates the weight given to the study, which is proportionate to the number of participants

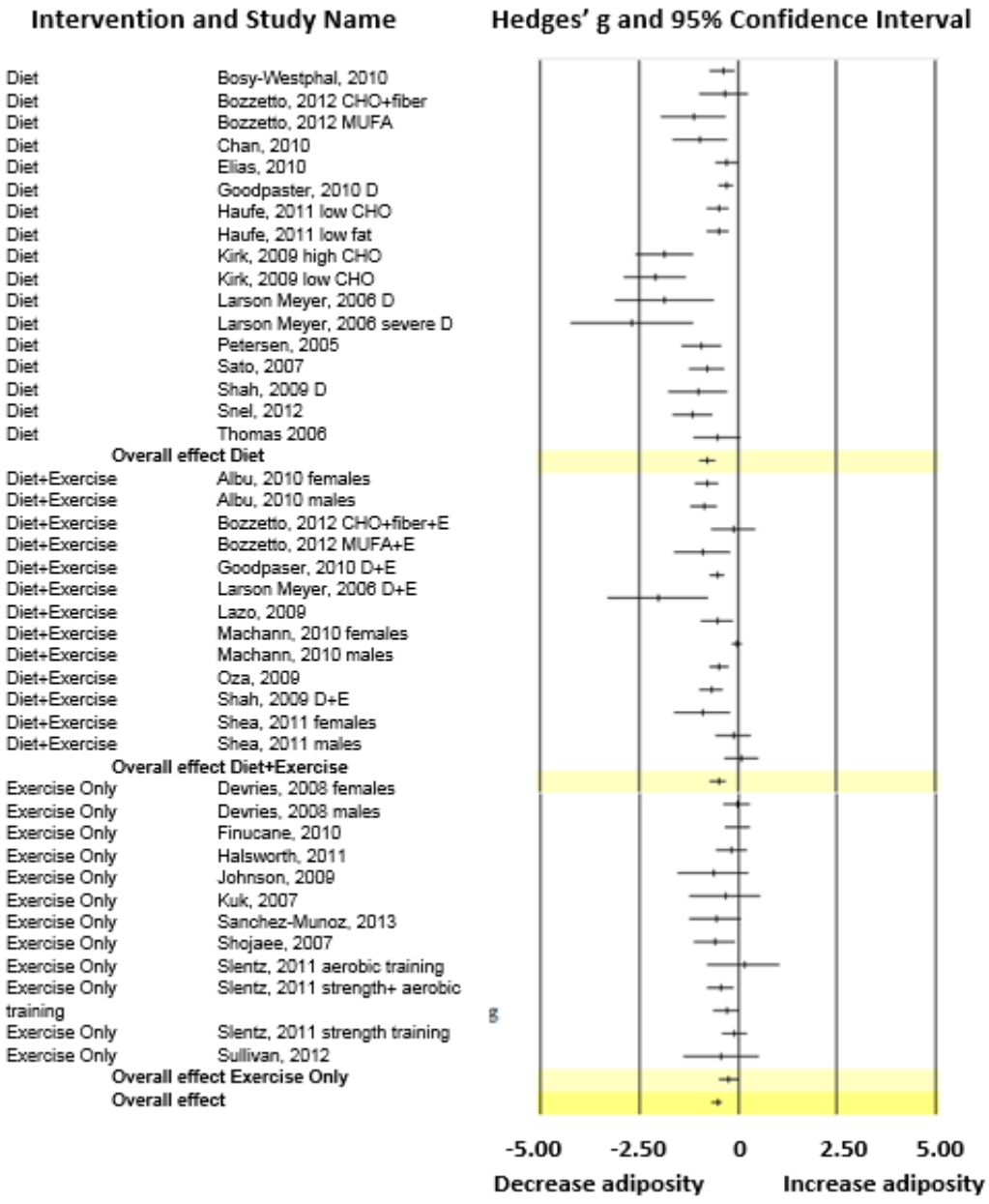

Figure 4: Forest plot of the effects found in the different treatment modalities and the overall effect of an intervention with the outcome of hepatic adiposity

Note: with CHO = carbohydrates, MUFA = monounsaturated fatty acids, D = diet, E = exercise, D+E = diet and exercise

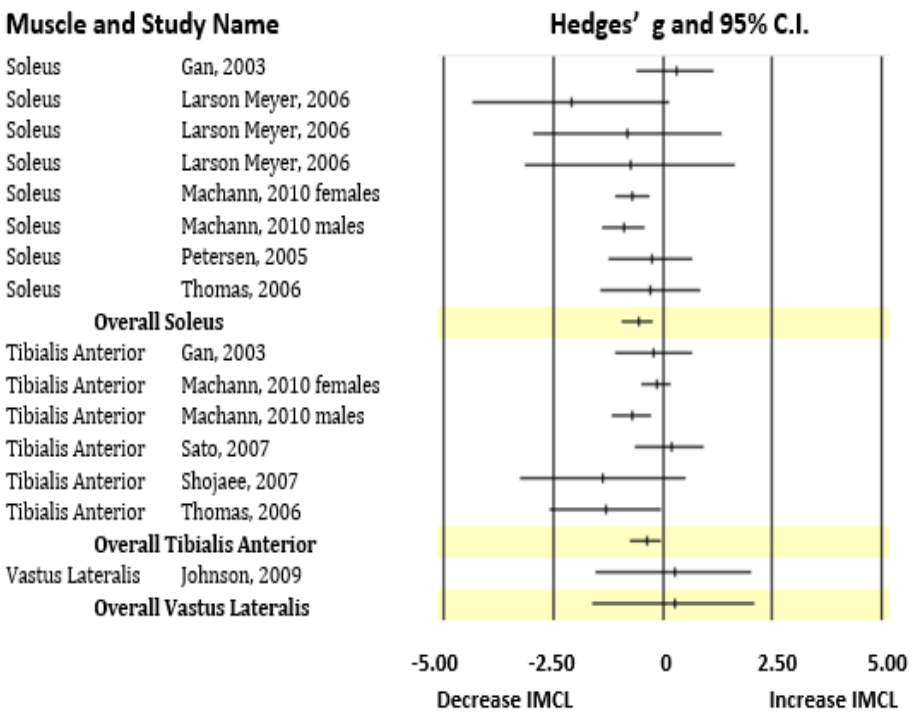

Figure 5: Forest plot of the effects found in the different skeletal muscles (outcome IMCL)

Note: D = diet, E = exercise, D+E = diet and exercise

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2.2 Exercise and ectopic fat deposition in overweight and obese children

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. [3, 1] Since disease progression into adulthood is plausible, this current situation constitutes a challenge for future demands on health services. [4, 3, 5-7]

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 storage and 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 pediatric 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 pediatric 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 evaluated 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 pediatric 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 cardio metabolic comorbidities were examined in most studies. In three studies, (part of the) subjects were diagnosed with NAFLD or NASH. [48, 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 [50, 44] 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. [51, 46, 47]

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%).

Adiposity of the liver: 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)

Adiposity of the liver: 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, p=0.17].

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. [55-57, 24, 58, 37, 59, 38] 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 (1H-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. [50, 44, 70] It can be explained by the fact that physical activity sensitizes muscles to insulin and modifies hepatic lipids. [71-73] Furthermore, 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. [74, 68, 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. [48, 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. [50, 44]

One of the most challenging aspects for healthcare professionals in pediatric 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 behavior change, it is important that adherence to the prescribed intervention protocol is adequately assessed and reported. A comparison with habitual diet and exercise behavior 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 pediatric obesity. Although there were significant ameliorations in insulin sensitivity in all intervention groups, no significant changes in IMCL were found.

Tables and figures

PATIENT	INTERVENTION	OUTCOME
"Diabetes Mellitus, Type 2"[Mesh] OR “Overweight"[Mesh] OR “Insulin Resistance"[Mesh]) OR "Metabolic Syndrome X"[Mesh] OR	"Sports"[Mesh] OR "Exercise Therapy"[Mesh] OR "Diet"[Mesh] OR "Exercise"[Mesh] OR "lifestyle intervention" OR “anaerobic training" OR "aerobic training" OR	SEARCH STRATEGY A: IntraMyocellular Lipid (IMCL) "muscle fat" OR "muscle lipid" OR "intramyocellular fat" OR "intramyocellular lipid" OR “intramyocellular triglycerides" OR “muscular triglycerides" OR "skeletal muscle fat" OR "muscle fat fraction" OR "muscle lipid fraction" OR "muscle lipid content" OR "muscle fat content" OR "IMCL" OR “IMTG" SEARCH STRATEGY B: ECTOPIC FAT LIVER "hepatic lipid" OR "Fatty Liver"[Mesh] OR "hepatic fat” OR “IHTG” OR “IHL” OR "hepatic fat fraction" OR "liver lipid content" OR "liver fat content" OR "hepatic liver content” OR "hepatic fat content" OR "hepatic fat accumulation" SEARCH STRATEGY C: ECTOPIC FAT PANCREAS "lipid accumulation pancreas" OR "fat accumulation pancreas cells” OR "fat accumulation beta cells” OR "lipid pancreas" OR "pancreas lipid" OR "lipid accumulation pancreatic islets" OR "pancreas fat" OR "pancreatic fat accumulation" OR "pancreatic fat fraction" OR "pancreatic fat content" OR "pancreatic lipid" or "pancreatic fat" OR "pancreatic lipotoxicity" OR “pancreatic fat fraction” SEARCH STRATEGY D: ECTOPIC FAT HEART "cardiac lipotoxicity" OR "peri coronary fat" OR "pericardial fat" OR "lipotoxic cardiomyopathy" OR "peri coronary lipid" OR "pericardial fat" OR "pericardial lipid" OR "cardial fat" OR "cardial lipid" OR "epicardial fat" OR "epicardial lipid" OR "peri arotic fat" OR "peri aortic lipid" OR "peri coronary epicardial adipose tissue" OR "peri coronary epicardiac adipose tissue" OR "cardiac fat" OR "cardiac lipid" OR "heart fat" OR "heart lipid" OR "cardiac steatosis" OR "lipotoxic cardiomopathy" OR "lipotoxic heart" OR "heart steatosis" OR "heart lipotoxicity" OR "cardial lipotoxicity" OR "epicardial fat thickness" OR "myocardial triglyceride" OR "myocardial triglyceride accumulation" OR "myocardial TG levels" OR "myocardial TG accumultion" OR "epicardial wall thickness" OR "cardial fat" OR "cardiac fat" OR "epicardiac fat" OR "epicardial fat" SEARCH STRATEGY E: ECTOPIC FAT KIDNEY’S "renal fat accumulation" OR "renal fat fraction" OR "renal steatosis" OR "renal lipid content" OR "renal fat content" OR "retroperitoneal fat" OR "kidney fat" OR "kidney lipid" OR "renal fat" OR "renal lipid"

Table 1: Search strategy

Author (Year) Country/Ethnicity participants	Design	Reported comorbidities	Intervention period	Intervention	Exercise modality	Training load/week	Diet	n (M/F)	Age (y) and/or Pubertal Status	BMI (kg/m²)	Assessment Liver Fat	Baseline values liver adiposity	Reported change liver fat (absolute value)	Calculated or reported change in liver fat (%)	Result BMI (kg/m²)	Result fat mass - FM (%) fat-free mass –FFM (kg)
HASSON (2012) 50%African American 50%Latino	CON	/	4 months	Exercise + Dietary Advice (E+DA)	strength training (ST) - supervised	2 sessions/w	Dietary Advice (DA)	ST+DA: 31 (mixed)	15.4 ± 1.1 (tanner 4-5)	>95th percentile	multislice MRI: hepatic fat fraction (%)	unknown	ST+DA: reduction	ST+DA: ↓ of 27.3% (=reported)	unknown	unknown
				Dietary Advice (DA)	/	/		DA: 39 (mixed)					DA: no change	0		
				Control (CON)	/	/	/	CON: 30 (mixed)					CON: reduction	↓ of 4.3% (=reported)		
LEE (2012) 50%African American 50%White	CON	/	3 months	Exercise (E)	aerobic training (AT) - supervised	180 min/w 50-75%VO2 peak 180 min/w	Weight Maintenance	AT: 15 (15/0)	AT: 15.2 ± 1.9 (pubertal)	AT: 36.6±5.9 (>95th percentile)	1H-MRS liver: IHL (%) measured in 10 subjects	4.7±4.0	AT: ↓ with 1.1±0.7 (p=0.061)	↓ of 23%	AT: ↓ with 0.3±0.3 (p=0.179)	FM: ↓ with 2.7±0.6 (p=0.04) FFM: ↑ with 1.25±0.33 (p=0.748)
					strength training (ST) - supervised	180 min/w 50%1RM 10 exercises 1-2 sets 8-12 reps		ST: 16 (16/0)	ST: 14.6 ± 1.5 (pubertal)	ST: 34.5±2.4 (>95th percentile)	^{1}H-MRS liver: IHL (%) measured in 9 subjects	2.9±2.4	ST: ↓ with 1.2±0.6 (p=0.042)	↓ of 41%	ST: ↓ with 0.5±0.3 (p=0.047)	FM: ↓ with 2.5±0.6 (p=0.04) FFM: ↑ with 2.13±0.32 (p=0.160)
				Control (CON)	/	/		CON: 12 (12/0)	CON: 14.8±1.4 (pubertal)	CON: 33.9±4.2 (>95th percentile)	^{1}H-MRS liver: IHL (%) measured in 10 subjects	2.2±2.2	CON: ↑ with 0.9±0.7	↑ of 41%	CON: ↑ with 0.3±0.3	FM: ↑ with 0.02±0.6 (p=0.046) FFM: ↑ with 1.42±0.38 (p=0.834)
LEE (2013) 55-75%Black 25%White	CON	/	3 months	Exercise (E)	AT: aerobic training (AT) - supervised	180 min/w 50-75%VO2 peak 180 min/w	Weight Maintenance	AT: 16 (0/16)	AT: 14.6 ± 1.9 (tanner 4-5)	AT: 32.9±3.8 (>95th percentile)	^{1}H-MRS liver: IHL (%)	2.2±3.3	AT: ↓ with 1.70±0.74 (p=0.022)	↓ of 77%	AT: ↓ with 0.46±0.58 (p=0.430)	FM: ↓ with 1.70±0.85 (p=0.046) FFM: ↑ with 0.13±0.63 (p=0.834)
					strength training (ST) - supervised	180 min/w 60%1RM 10 exercises 1-2 sets 8-12 reps		ST: 16 (0/16)	ST: 14.8 ± 1.9 (tanner 4-5)	ST: 36.4±3.8 (>95th percentile)		2.0±1.3	ST: ↓ with 0.70±0.49 (p=0.308)	↓ of 35%	ST: ↓ with 0.28±0.54 (p=0.608)	FM: ↓ with 1.63±0.78 (p=0.035) FFM: ↑ with 0.61±0.61 (p=0.317)
				Control (CON)	/	/		CON: 12 (0/12)	CON: 15.0 ± 2.2 (tanner 4-5)	CON: 35.3±4.0 (>95th percentile)		3.0±5.4	CON: ↑ with 1.75±0.57	↑ of 58%	CON: ↓ with 0.03±0.44	FM: ↑ with 0.02±0.6 FFM: ↑ with 1.42±0.38

MAURAS (2012) 42%African American 39%White 19%other	UC	/	6 months	Exercise + Diet (E+D)	aerobic training (AT) in fitness center	90 min/w	Supervised Hypocaloric Diet (D: decrease of 250-500 cal/d)	19 (mixed)	12.0±0.4 (pre-pubertal and pubertal)	33.2±0.7 (>95th percentile)	^{1}H-MRS liver: IHL (%)	10.1±0.8	↓ from 10.1±0.8 to 8.7±1.0 (p=0.09)	↓ of 14%	↓ from 10.1±0.8 to 8.1±1.0 (p=0.09)	FM: ↓ with 2.0±0.8 (p=0.02) FFM: unknown
PACIFICO (2013) Italy	UC	NAFLD	12 months	Exercise Advice + Diet (EA+D)	Exercise advice (EA)	min 300 min/w moderate intensity	Supervised Hypocaloric Diet (D: 25-30 cal/kg/d)	120 (mixed)	11.9 (11.5-12.2) (pre-pubertal and pubertal)	>95th percentile	multislice MRI: hepatic fat fraction (%) measured in 52 subjects	15.2	↓ from 15.2 to 6.4 (p=0.001)	↓ of 58%	↓ from 26 to 24 (p=0.001)	FM: ↓ from 39.0 to 36.9 (p=0.001) FFM: unknown
POZZATO (2010) 100%Caucasian	UC	/	12 months	Exercise Advice + Diet (EA+D)	Exercise advice (EA)	210-315 min/w moderate intensity	Dietary Advice and records showed Hypocaloric Diet	26 (11/15)	6-14	Obesity following Cole curves	multislice MRI: hepatic fat fraction (%)	7.76±13.03	↓ from 7.76±13.03 to 2.83±6.79 (p<0.0001)	↓ of 64%	BMI z-score: ↓ from 2.28±0.46 to 1.99±0.61 (p<0.001)	unknown
RAMON (2013) 76%White 24%other	UC	hepatic steatosis (here ≥ 9% liver fat)	6 months	Diet + Behavioral Counseling (D+BC)	unknown	unknown	Low Fat Diet (LFD)	9 (7/2)	LFD: 11.8±3.0	LFD: 34.0±6.1 (>95th percentile)	^{1}H-MRS liver: IHL (%)	29.3±14.1	LFD: ↓ from 29.3±14.1 to 18.7±11.1 (p=0.01)	↓ of 36%	LFD: ↓ from 34.0±6.1 to 32.8 (p=0.0004)	unknown
							Low Glycemic Load Diet (LGLD)	8 (1/7)	LGLD: 13.8±3.2 (pre-pubertal and pubertal)	LGLD: 31.3±5.4 (>95th percentile)		23.8±12.2	LGLD: ↓ from 23.8±12.2 to 15.4±5.6 (p=0.05)	↓ of 35%	LGLD: ↓ from 31.3±5.4 to 29.9±6.5 (p=0.0007)	unknown
VAN DER HEIJDEN (2010) 100%Hispanic	UC	/	3 months	Exercise (E)	aerobic training (AT)	120 min/w 70%VO2peak	/	15 (7/8)	15.6±0.4 (tanner 4-5)	33.7±1.1 (>95th percentile)	^{1}H-MRS liver: IHL (%)	8.9 ±3,2	↓ from 8.9 ±3.2 to 5.6 ±1.8 (p=0.04)	↓ of 43%	↓ from 33.7±1.1 to 33.4±1.1 (NS)	FM: ↓ from 35.2±2.0 to 34.2±2.1 (p<0.05) FFM: ↑ from 54.6±2.7 to 55.3±2.8 (NS)
VITOLA (2009) 25%African American 75%Caucasian	UC	4 subjects with NAFLD	until decrease of 5% body weight	Exercise Advice + Diet (EA+D)	Exercise advice (EA)	unknown	Hypocaloric Diet (D: 1200-1500 kcal/d)	8 (7/1)	15.3±0.6 (tanner 4.4±0.3)	35.7±1.4 (>95th percentile)	^{1}H-MRS liver: IHL (%)	17.5 ±6.0	↓ from 17.5±6.0 to 5.3±2.3 (p<0.01)	↓ of 61.6%±8.5% (=reported)	↓ from 35.7±1.4 to 32.2±1.0 (p=0.01)	FM: ↓ with 8.5 ±1.8 (p=0.01) FFM (%): ↓ with 3.1 ±1.9 (NS)

Table 2: Overview of all studies included in the meta-analysis, with outcome of liver adiposity

Note: CON=controlled; UC= uncontrolled; NAFLD=Non-Alcoholic Fatty Liver Disease; RM=Repetition Maximum; MRI=Magnetic Resonance Imaging; ^{1}H-MRS=Magnetic Resonance Spectroscopy

Author (Year) Country/Ethnicity participants	Design	known co-morbidities	Intervention period	Intervention	Exercise modality	Training load/week	Diet	n (M/F)	Age (y) and/or Pubertal Status	BMI (kg/m²)	Assessment IMCL	Result IMCL	esult BMI (kg/m²	Result fatt mass - FM (%) fat-free mass - FFM (kg)	Result Insulin resistance or sensitivity
LEE (2012) 50%African American 50%White	CON	/	3 m	Exercise (E)	aerobic training (AT) - supervised	AT: 180 min/w 50-75% VO2peak	Weight Maintenance	AT: 15 (15/0)	AT: 15.2 ±1.9 (pubertal)	AT: 36.6±5.9 (>95th percentile)	1H-MRS tibialis anterior: IMCL (mmol/kg ww) measured in 10 subjects	↑ with 1.1±0.5 (p=0.639)	AT: ↓ with 0.3±0.3 (p=0.179)	FM: ↓ with 1.70±0.85 (p=0.046) FFM: ↑ with 0.13±0.63 (p=0,834)	IS: ↑ with 0.4±0.2 (NS)
					strength training (ST) - supervised	ST: 180 min/w 50%1RM 10 exercises 1-2sets 8-12reps		ST: 16 (16/0)	ST: 14.6 ±1.5 (pubertal)	ST: 34.5±2.4 (>95th percentile)	1H-MRS tibialis anterior: IMCL (mmol/kg ww) measured in 9 subjects	↓ with 0.03±0.4 (p=0.231)	ST: ↓ with 0.5±0.3 (p=0.047)	FM: ↓ with 1.63±0.78 (p=0.035) FFM: ↑ with 0.61±0.61 (p=0.317)	IS: ↑ with 0.8±0.2 (p=0.0009)
				Control (CON)	/	/		CON: 12 (12/0)	CON: 14.8±1.4 (pubertal)	CON: 33.9±4.2 (>95th percentile)	1H-MRS tibialis anterior: IMCL (mmol/kg ww) measured in 10 subjects	↑ with 0.7±0.5	CON: ↑ with 0.3±0.3	FM: ↑ with 0.02±0.6 FFM: ↑ with 1.42±0.38	IS: ↓ with 0.1±0.3 (NS)
McCORMACK (2013) more than 50%white	CON	/	2 m	Exercise + Lifestyle Advise (E+LA)	aerobic training (AT) - supervised	E+LA: 60-105 min/w 60-80% HRreserve	Dietary Advise (DA)	E+LA: 10 (2/8)	E+LA: 13.8±2.2 (tanner 4-5)	E+LA: 39.1±6.5 (≥95th percentile)	E+LA: 1H-MRS tibialis anterior: IMCL (au) measured in 6 subjects	E+LA: ↑ of 15%(NS)	E+LA: ↑ with 0.36 (NS)	FM: ↓ with 1%(NS) FFM: ↑ with 2.0	HOMA-IR: ↓ with 2.43 (S)
				Lifestyle Advise (LA)	Exercise Advise (EA)	unknown		LA: 8 (3/5)	LA: 12.1±1.2, (tanner 4-5)	LA: 34.3±7.4 (≥95th percentile)	LA: 1H-MRS tibialis anterior: IMCL (au) measured in 5 subjects	LA: ↓ of 22%(NS)	LA: ↓ with 0.19 (NS)	FM: ↓ with 1%(NS) FFM: ↑ with 0.6	HOMA-IR: ↓ with 1.21 (S)
VAN DER HEYDEN (2010) 100%Hispanic	UC	/	3 m	Exercise (E)	aerobic training (AT)	120min/w, 70%VO2peak	/	15 (7/8)	15.6±0.4 (tanner 4-5)	33.7±1.1 (>95th percentile)	1H-MRS soleus: IMCL (%ref to water)	no sigificant change	↓ from 33.7±1.1 to 33.4±1.1(NS)	FM: ↓ from 35.2±2.0 to 34.2±2.1 (p<0.05) FFM: ↑ from 54.6±2.7 to 55.3±2.8 (NS)	HOMA-IR: ↓ (S)

Table 3: Overview of all included studies with outcome of IMCL

Note: CON=controlled; UC= uncontrolled; RM=Repetition Maximum; ^{1}H-MRS=Magnetic Resonance Spectroscopy

Author (year)	Adequate sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessement	Incomplete outcome data addressed	Free of selective outcome reporting	Free of other bias	In- and exclusion criteria allocation are described
HASSON (2012)	Unclear	Unclear	Unclear	Unclear	YES	NO	NO	NO
LEE (2012)	YES	Unclear	Unclear	Unclear	YES	YES	NO	YES
LEE (2013)	YES	Unclear	Unclear	Unclear	YES	YES	NO	YES
MAURAS (2012)	NO	NO	Unclear	Unclear	Unclear	NO	NO	YES
McCORMACK (2013)	Unclear	Unclear	Unclear	Unclear	YES	NO	NO	YES
PACIFICO (2013)	NO	NO	Unclear	Unclear	YES	NO	NO	YES
POZZATO (2010)	NO	NO	YES	YES	YES	NO	NO	YES
RAMON (2013)	Unclear	Unclear	Unclear	Unclear	YES	YES	YES	YES
VAN DER HEIJDEN (2010)	NO	NO	Unclear	Unclear	Unclear	NO	NO	YES
VITOLA (2009)	NO	NO	Unclear	Unclear	YES	YES	NO	YES

Table 4: Risk of Bias assessed with the Cochrane tool

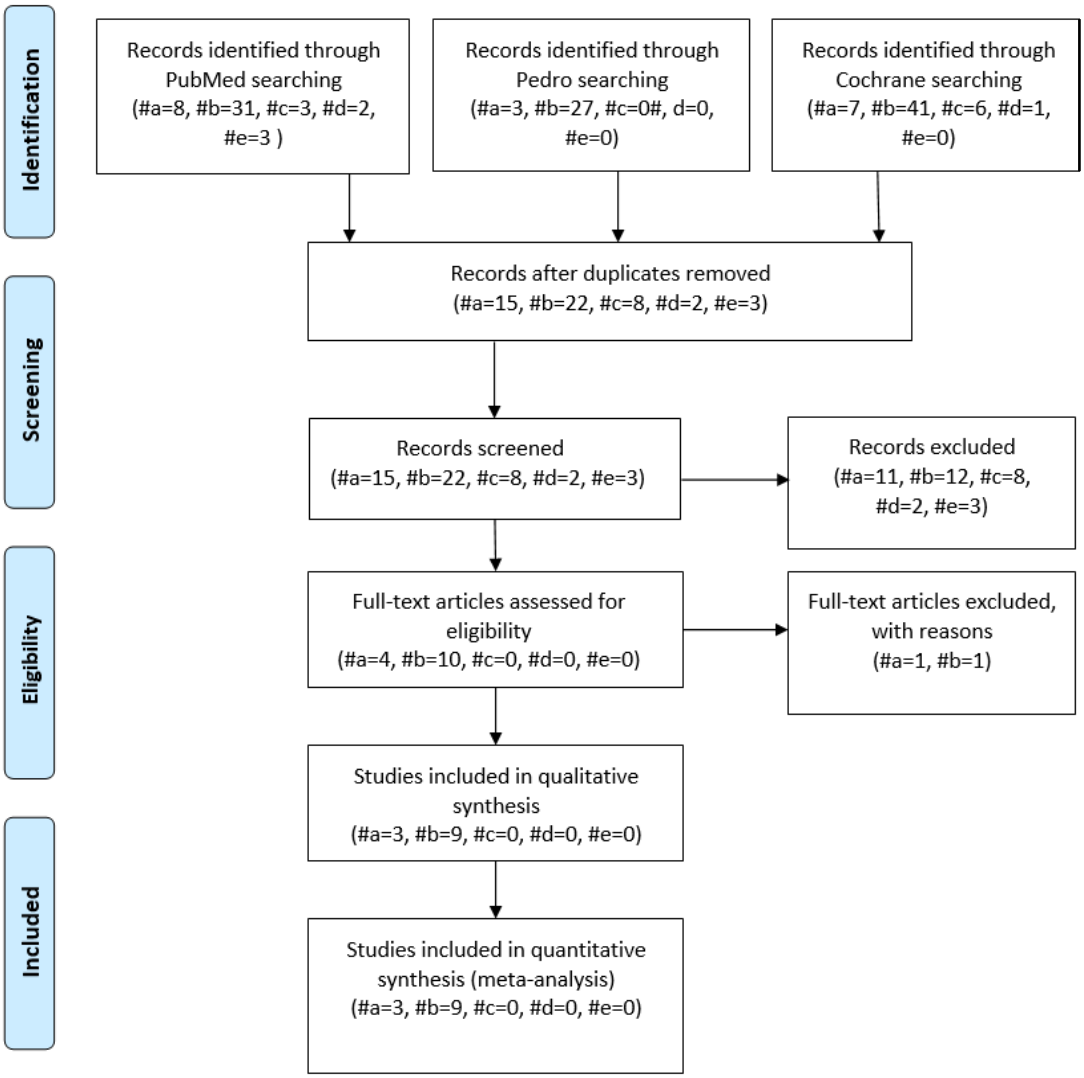

Figure 1: Flow chart following PRISMA guidelines

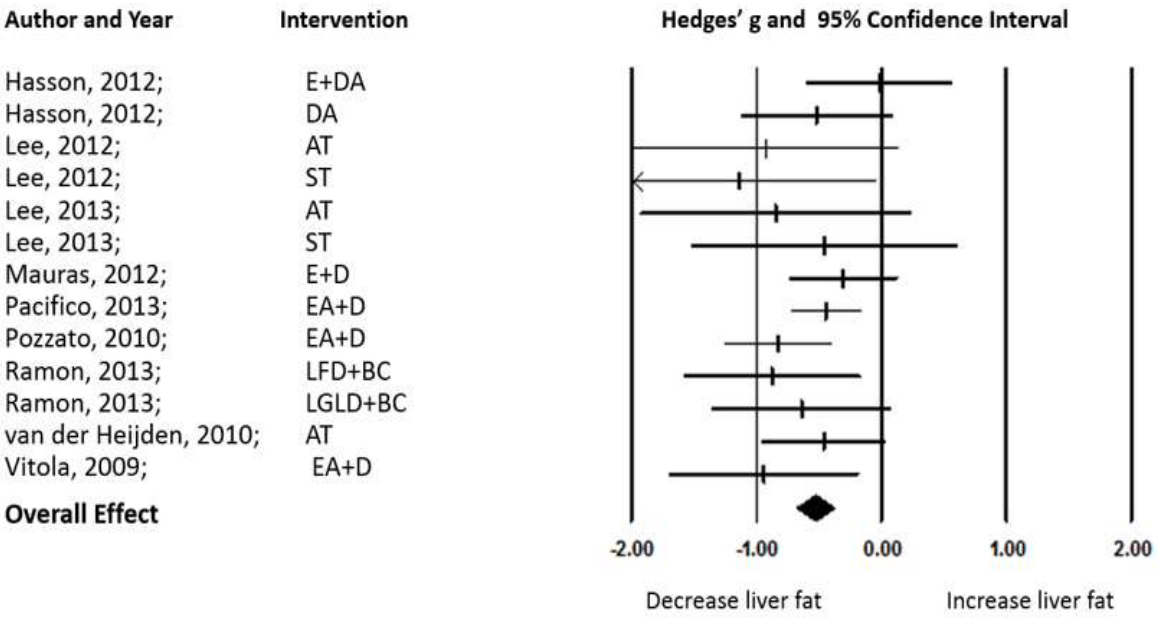

Figure 2: Forrest Plot liver adiposity

Note: E=Exercise; DA=Dietary Advise; AT=Aerobic Training; ST=Strength Training; D=Diet; EA=Exercise Advise; LFD=Low Fat Diet; BC=Behavior Counseling; LGLD=Low Glycemic Load Diet

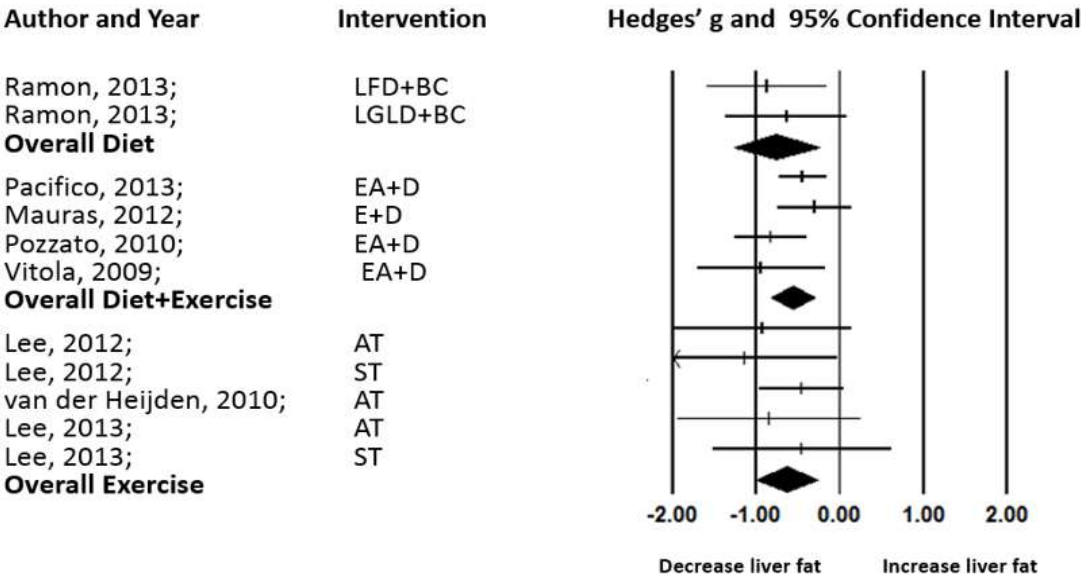

Figure 3: Forest plot liver adiposity, subgroup intervention modality.

Note: LFD=Low Fat Diet; BC=Behavioral Counseling; LGLD=Low Glycemic Load Diet; EA=Exercise Advice; D=Diet; E=Exercise; AT=Aerobic Training; ST=Strength Training

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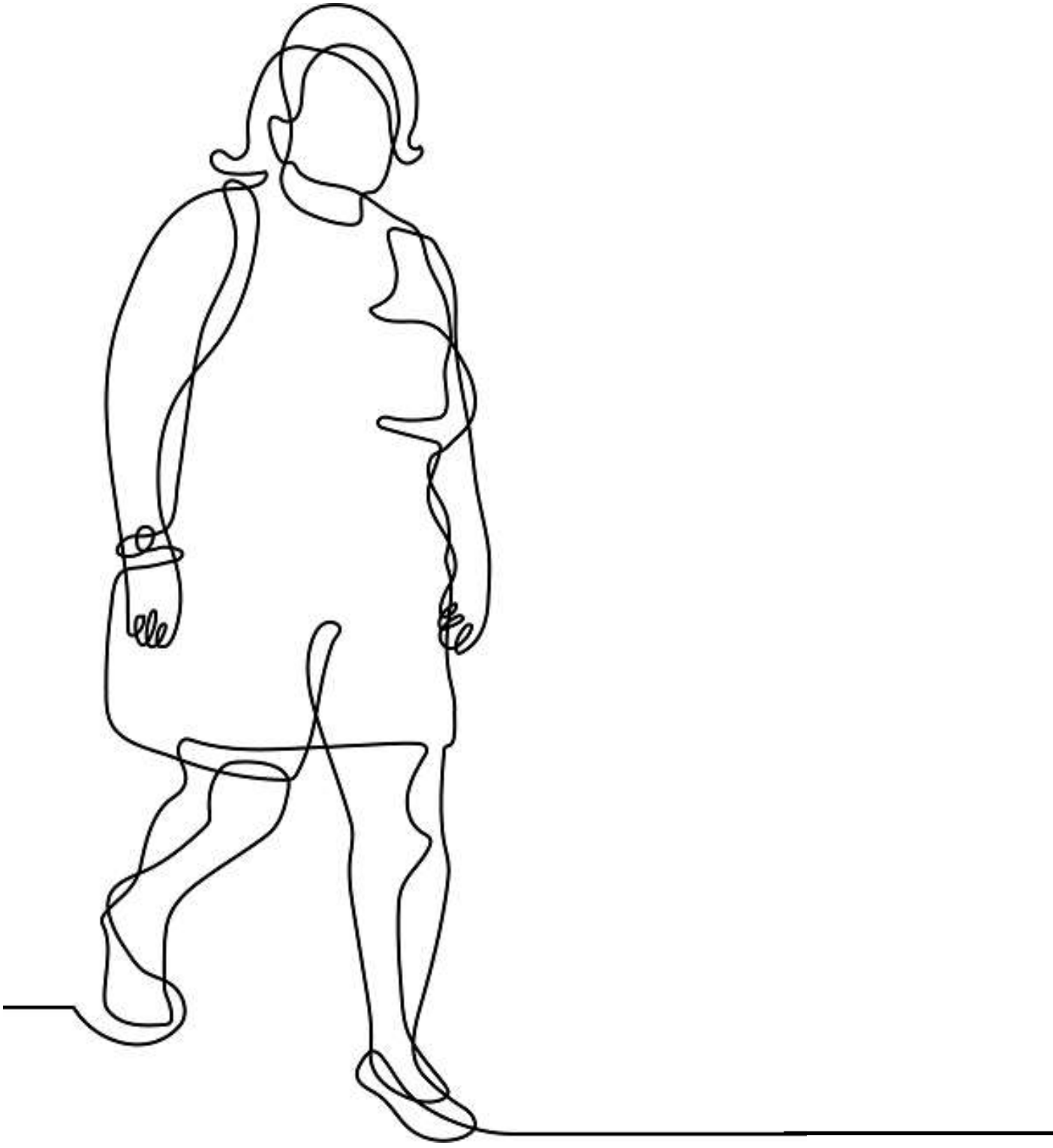
CHAPTER 3

Methodology of the clinical study



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Study protocol of the clinical study

Introduction

The current epidemic of type 2 diabetes can be attributed to the increased incidence of obesity. However, since not all people with overweight develop insulin resistance or type 2 diabetes, excess ectopic fat deposition could be a differentiating factor. [1] Ectopic fat is fat that accumulates in places that are normally not associated with adipose tissue and is considered as a major driver of insulin resistance and the development of diabetes type 2, metabolic and cardiovascular complications. [2-5] The accumulation of visceral fat is known to play a pathological role in metabolic complications and seems to be a marker of ectopic fat accumulation in other locations such as in or around the liver, the heart and skeletal muscles. [1,6,7]

According to the guidelines, behavioral weight management programs is the cornerstone of long-term obesity management because this result in greater weight reduction than programs involving only-exercise or only-diet. [8,9]

Recently, some meta-analyses showed a decrease of visceral and ectopic fat in different locations after lifestyle interventions (diet and/or exercise) in adults and adolescents. [10-12] However, to the best of our knowledge, no RCT studied concurrent changes in multiple places of ectopic fat deposition during a weight loss intervention. At the same time, the additional value of exercise to supplement a hypocaloric diet is unknown.

In this RCT, visceral fat, intra hepatic lipids (IHL), intra myocellular lipids (IMCL) and pericardial fat are assessed before, during and after a weight loss intervention of six months. Hereby, the clinical and economic value of exercise to supplement a hypocaloric diet is investigated.

Research Questions: Does a combined therapy group of prescribed exercise and hypocaloric diet results in significant greater reductions of ectopic fat compared to a control group of hypocaloric diet? Are extra costs that are associated with this intensive lifestyle treatment justified regarding quality of life and economic outcomes? It is hypothesized that a combined therapy according to established guidelines will be superior to hypocaloric dietary therapy, taken all clinical and economic outcomes in account.

Methods

Study Design

An overview of the design is depicted in figure 1. Approval of protocol and consent forms by the ethical committee of the University Hospital Antwerp is obtained (approval number: 14/17/205).

Study Participants

Firstly, study information is given by endocrinologists of the obesity clinic of the Antwerp University Hospital (tertiary referral facility) to their patients. Secondly, poster recruitment is done in the University of Antwerp and in the staff dressing room at the Antwerp University Hospital. An attempt is made to include 60 premenopausal women with overweight or obesity.

An overview of all inclusion and exclusion criteria can be found in table 1.

In brief, participants are included when they have a BMI of at least 27 kg/m² and want to participate in a lifestyle based weight loss intervention. Body weight must be stable during the past six months and individuals who have a clinical history of type 2 diabetes, prediabetes with the use of medication, or hypothyroidism are excluded. However, de novo diagnosed (pre-) diabetes or Non-Alcoholic Fatty Liver Disease (NAFLD) is allowed when there is no urge to start up medication therapy during the study. Participants taking medication that influences body weight or metabolism (e.g. tricyclic antidepressants) are excluded. The use of blood pressure lowering medication or lipid-lowering agents is allowed but changes in the medication regimen cannot be made during the study. Premenopausal state is verified by hormonal data (follicle-stimulation hormone (FSH) > 25 mU/ml and estradiol < 20 pg/ml). Potential participants are excluded when pregnancy is planned, when physical activity is not possible due to problems of the musculoskeletal system, when smoking, when drinking more than two alcoholic consumptions/day or binge drinking (self-reported).

Since all participants undergo medical imaging, exclusion criteria related to MRI and CT scans are also applicable.

Intervention

The lifestyle-based intervention takes six months. Participants are randomized in a dietary intervention group (Usual Care at the Antwerp University Hospital) or a Combined Therapy

group (dietary intervention + increased physical activity). Minimpy 0.2, an open-source minimization program [13], is used to allocate participants to comparable groups regarding mean age, presence of the metabolic syndrome and quantity of visceral fat. Before the start of the study, an interview (+/- 1 hour) with each participant takes place. Barriers to lifestyle change are discussed respecting principles of motivational interviewing and realistic goals are set.

Usual Care

Each participant receives a hypocaloric diet based on the individual resting metabolic rate. Resting metabolic rate (RMR) is estimated using the WHO formula[14] or measured using indirect calorimetry. Total energy expenditure is calculated by multiplying RMR with a physical activity level (PAL) of 1.3. A hypocaloric diet with an energy deficit of 500 kcal/day is prescribed.

Individual counselling sessions will be conducted by a skilled dietitian two-weekly the first month and on a monthly basis the next five months. The first consultation has a duration of 60 minutes and is used to identify the current dietary patterns and recommend specific changes. To introduce the principles of balanced meals, energy restriction and portion sizes, an individualized hypocaloric dietary scheme is explained which can be followed during the first two weeks of the intervention.

The next consultations have a duration of approximately 30 minutes and are used to identify barriers and provide appropriate coping strategies.

At each visit, nutritional compliance is recorded on a 0 to 10 numeric rating scale.

The Usual Care group is asked to continue with their normal physical activity pattern during the six-month intervention period.

Combined Therapy

In the Combined Therapy group, Usual Care is supplemented with a prescribed exercise program (table 2). This exercise program is designed and instructed by the research physiotherapist. The program was personalized by taken all physical inconveniences to account. Standardization is done by applying same exercise volume (intensity, frequency and duration) and progression in each participant. Aerobic training and strength training is performed in a fitness or health center near home.

At the beginning of the study, subjects who are allocated to the intervention group perform a maximal CPET with breath-by-breath measurement to define the training zone. In this test, the first ventilatory threshold (VT1) and respiratory compensation point (RCP) is defined to set up a proper training schedule. The symptom-limited CPET is performed on an electronically bicycle ergometer (LODE, Corival) using a computerized gas analyzer system (JAEGER, Oxycon Pro) and a 12-lead ECG (GE, MAC 5500). The exercise test consists of (i) a five-minutes resting period, (ii) starting at 0 Watt, there is an incremental increase in workload with 15 Watt every minute until exhaustion and (iii) a 3-min recovery. Blood pressure (BP), is measured using a standard cuff sphygmomanometer and perceived exertion (Borg scale) is measured at rest at the end of the test. Participants are actively encouraged to achieve their limit of tolerance. All CPET's are done by the same qualified personnel and supervised by a physician.

The aerobic exercise intensity is set at 90-95% of the heart rate achieved at the RCP (with intensity > first Ventilatory Threshold). Aerobic training has a duration of 30 to 45 minutes and is performed on three or four different cardio devices depending on patients' abilities. Each training day, core stability training is completed with three strength exercises on isotonic strength training devices for large muscle groups. All exercises are done in two sets of 15 repetitions with the goal to achieve better muscular strength endurance. The initial exercise intensity is set at 50% of the baseline one repetition maximum (1RM) and progression is built in the training scheme. This combined training is performed individually during six months, three times/week. One session takes approximately 50-65 minutes.

A major objective in the development of this training scheme was to obtain balance between exercise physiological principles (e.g. dose responsiveness) and possible barriers to physical activity (e.g. risk of injuries, lack of time). [15-18] Also, an effort is made to reach a uniform manner of guidance to the physical activity program. The participants are encouraged to discuss with the physiotherapist any problems of discomfort. Adaptations to the training program can be made, with respect to training volume. Adherence is checked by registration systems specific for each training center.

Data collection and outcome measurements

Participants will be assessed at baseline, at three months and after completing of the intervention (6 months) by blinded outcome assessors.

Primary Outcome: A CT-scan (GE, Lightspeed VCT) of the abdomen and the thorax is performed at the start, after three and six months. A single-slice CT of the abdomen is used to evaluate cross-sectional abdominal visceral adipose tissue areas (cm^2) at the L4-L5 region according previously described methods. [19]

Multi-slice ECG-triggered CT is used to measure pericardial and epicardial fat areas (cm^3). All scans are performed at end-diastole and slice thickness of 2.5 mm. No contrast liquid is used. Data are analyzed using the corresponding workstation (GE, AW Volumeshare 2). Epicardial and pericardial fat areas are manually delineated slice by slice starting at the pulmonary artery and ending at the apex of the heart. The number of slices are inventoried to facilitate reproducibility of the analysis procedure for the following measurements. Tissue with attenuation values in the interval of -30 to -190 Hounsfield units are considered to be fat. [20] 1H-magnetic resonance spectroscopy (1H-MRS) is used to quantify Intra MyoCellular Lipids (IMCL) and liver lipids of each participant before, after three months and after six months. To measure IMCL (%, in ref. to water), the right calf of each participant is positioned near isocenter surrounded by a body coil within a 3T MRI scanner (SIEMENS MAGNETOM Prisma, Siemens Healthcare AG, Zürich, Germany). To ensure maximum field homogeneity, manual shimming on the volume of interest is done. The 1H-MRS data are acquired by a single-voxel Point-Resolved Spectroscopy (PRESS) acquisition. Imaging parameters are set as follows; repetition time (TR)/echo time (TE) 2000/33 ms, voxel size 15 mm^3, and weak water suppression. During the first scan, a homogenous part of the tibialis anterior is searched, avoiding visible interfascial adipose tissue and blood vessels. Repeatability of the voxel placement is facilitated with anatomic landmarks, by measuring the distance of the localization of the voxel to the tibial plateau.

To define the hepatic lipid content (% in reference to water), the body coil is positioned around the chest, as the participants lay supine. MRS data are acquired using STEAM, with the parameter setting as follows: 3.0 cm^3 voxel in the right liver, TR/TE 3000/20 ms, and no water suppression.

All spectra are exported and reconstructed in the jMRUI software version 5.2 for quantitative analysis of the lipid peaks, using the Amares quantification method. [21] Preprocessing was standardized using the jMRUI2XML software. [22]

Secondary Outcome: Each participant undergoes a standard metabolic screening (clinical examination and anthropometric measurement) at the beginning of the study, at three and at six months.

All anthropometric measurements are performed in fasting conditions and undressed. Height is measured to the nearest 0.5 cm and body weight is measured to the nearest 0.2 kg. BMI is calculated as weight (kilograms) over height (meters) squared. Waist circumference is measured at the midlevel between the lower rib margin and the iliac crest. Body composition is determined by bio-impedance analysis as described by Lukaski et al., using a AKern SRL-BIA 101. [23] Body fat mass percentage is calculated using the formula of Sun et al. [24] Systolic and diastolic blood pressure is determined on the right arm of the participant, after at least five minutes lying down, using a mercury sphygmomanometer.

A 3-hour oral glucose tolerance test (OGTT) with 75 g of glucose with sampling at 0, 15, 30, 60, 90, 120, 150 and 180 minutes is carried out at the start of the study. Insulin and C-peptide are determined at 0, 30, 60, 120 and 180 minutes. Homeostasis model assessment (HOMA-IR) is used to estimate insulin resistance as described by Matthews et al. and is calculated as [insulin (mU/l) * glucose (mmol/l)]. [25]

A fasting blood analysis (from an antecubital vein) is taken at the start of the study and after six months intervention. This analysis includes assessment of the lipid profile (total cholesterol, HDL-C, TG, LDL-C). LDL-C is calculated using the Friedewald formula. [26]

All blood samples are analyzed in the University Hospital of Antwerp. Plasma glucose, total cholesterol and TG will be measured on Vitros 750 XRC (Ortho Clinical Diagnostics, Johnson & Johnson, UK). HDL-C will be measured on Hitachi 912 (Roche Diagnostics, Germany). Insulin levels will be measured with the Medgenic two-site IRMA assay (BioSource, Belgium).

Health economic evaluation alongside the trial

Currently, patients with overweight visiting the Department of Endocrinology at the Antwerp University Hospital receive a prescription for a session with a professional dietitian. Hence, diet (caloric restriction) is the primary strategy to tackle the problem of overweight in this institution. However, the department is contemplating to adapt this strategy towards a combined diet and exercise intervention (Combined Therapy). This may be important to tackle the problem of obesity and related comorbidities. Therefore, the Antwerp University

Hospital wants to assess the effectiveness of this Combined Therapy on the reduction of weight and ectopic fat deposition in such patients with overweight.

This section presents the protocol of a within trial and long term (by extrapolation) cost-utility analysis based on the discussed trial. The final report is presented following the Consolidated Health Economic Evaluation Reporting Standards (CHEERS). [27]

This economic evaluation is conducted from the societal perspective. Table 3 depicts different costs that are considered. Direct costs related to the problem of overweight are analyzed (e.g., physician visits and dietitian visits, but - in the long term analysis - also costs of obesity related complications such as cardiovascular and metabolic disease). In addition, also direct nonmedical costs (e.g. fitness center membership fees, sports clothing, transport) and indirect non-medical costs (e.g., absenteeism and leisure time cost) are calculated. Costs to assure sustainability of the intervention are included while start-up costs of the intervention (e.g. costs of research) are excluded to ensure that the alternative interventions are evaluated and compared as if under real world conditions. Intangible costs are also be excluded. For the within trial cost-utility analysis a time horizon of one year is chosen, whilst for the long term cost-utility analysis a time horizon of ten years is considered. [28] Costs and quality of life measures are measured after three, six, nine and twelve months using self-reported cost diaries and the EuroQol EQ-5D questionnaires. [29]

For the within trial analysis, no discounting is needed (time horizon twelve months). However, for the long term analysis a discount rate for costs and for outcomes is set on 3% and 1.5% respectively. [30]

Besides the natural units of ectopic fat deposition reduction (expressed as ppm, cm^2 or cm^3) and overweight/obesity (Body Mass Index expressed as kg/m2) health outcome is also evaluated in Quality Adjusted Life Years (QALYs) as QALY is often used in cost-utility analyses of physical activity interventions. [31] The EQ-5D is used to assess the individual participant's health profile at baseline and at three, six, nine and twelve months after the beginning of the intervention. Utilities, needed to calculate QALYs are delineated using the preference algorithm or social tariff estimated from a representative sample of the Belgium population. [30]

Direct medical costs are calculated by combining data from the hospital files and the participant's self-reported cost diaries whereas direct nonmedical and indirect costs are derived from the self-reported cost diaries. Cost for sustainability of the program is reported

by the researchers. Cost calculation is based on routine Belgian insurance prices by multiplying the units consumed times the unit price. Both (units consumed and unit price) is presented separately to improve objectivity and comparability of the final report. Costs of productivity loss and absenteeism are valued by the reported societal cost of one day of absenteeism in Belgium by Securex (www.securex.be). Opportunity cost of leisure time invested in fitness activity is valued as a shadow price, by multiplying the cost of absenteeism with a factor. Transportation cost is valued by standard tariffs for public transport and cost per km in case of transport by car. Transportation time is valued in the same way as leisure time lost. All resource (unit) costs are expressed in 2016 Euros. Decision modelling of the within trial cost-utility analysis is based on a decision tree.

For the decision modelling of the extrapolation an existing Markov model for overweight based on BMI data is used. [32] In addition, ectopic fat surfaces (cm^2) or volumes (cm^3) or ppm are used as intermediate outcomes for (cardio-) vascular health and diabetes mellitus type 2. A systematic review of epidemiologic studies are used to set extract correlations between such ectopic fat accumulations and health status (over a longer term). These data are be imputed in a new Markov model for extrapolation. The Markov model shows good characteristics for decision modelling under conditions of chronic disease (such as overweight and obesity). [33,34] The models used in this economic evaluation, including the description of the stadia, the length of one cycle and the transition probabilities, is presented to the reader in the final report.

In case of dropout, the researchers will contact the participants to ask for reasons of non-adherence and to ask to continue to fill out and return their cost diaries and EQ-5D questionnaires.

In the results section of the final report, mean values and 95% Confidence Intervals for all parameters are presented. For each intervention (Combined Therapy and Usual Care), resource units and unit prices are depicted separately. The mean values for the different cost categories and outcomes of interested are presented. Similarly, the mean differences between the groups receiving the Combined Therapy and Usual Care is shown. For the within-trial analysis, the Incremental Cost-Utility Ratio (ICUR) is calculated and reported. This leads to information in terms of 2016 Euros per Quality Adjusted Life Years gained. For the extrapolation analysis using Markov decision modelling, for each organ under investigation,

Incremental Cost-Utility Ratios (ICUR) are calculated and reported. This leads to information in terms of 2016 Euros per gained QALY. Cost-effectiveness planes are presented.
To characterize uncertainty in both the within-trial analysis and extrapolation analysis of this trial embedded health economic evaluation, univariate sensitivity analyses are conducted (for each organ under investigation) to describe possible effects of the most influential variables on the ICURs (e.g. price of fitness center membership fee, discount rate). The result of the sensitivity analyses is presented using a Tornado diagram. Each bar of the diagram represents the impact of uncertainty in an individual variable on the ICUR.

Discussion

At the moment, recruitment is ongoing. Our primary outcomes are anticipated to be available during 2017.
In this study, effects of lifestyle interventions in women with overweight are evaluated on the reduction of ectopic fat. Our secondary outcomes can correlate ectopic fat deposition with metabolic parameters, anthropometrics and lifestyle factors. Moreover, the added value of an individual prescribed training program to hypocaloric diet is discussed.
It is seen that the treatment of overweight in primary healthcare practice is not consistent with the established guidelines of behavioral weight management programs. Moreover, treatment in primary healthcare practice is mainly conducted by nutritionists and physicians and thereby consists rarely of exercise training. [9,35] This can be considered a missed opportunity because the effect of advise-only is very limited and literature shows that exercise in addition to diet leads to greater weight reduction. [18,36]
Based on correlations between ectopic fat deposition and cardiovascular outcomes, the impact of the intervention on future morbidity, quality of life and economic outcomes are analyzed. The impact of exercise in addition to diet is expressed in projected healthcare costs and Quality-adjusted life years.
Through the final paper with results, novel evidence will be provided for the use of exercise interventions besides energy restriction in people with overweight.
In summary, this study will provide information to the added value of exercise to hypocaloric diet on different health parameters.

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We want to express our thanks to the collaborating fitness and health centers. All cooperating clubs are interested in improving health in people with overweight. Our study participants can profit of competence of fitness instructors and equipment in the following health centers:

BodyWorld, Putte, Belgium; www.bodyworld.be

EasyFit Ranst, Ranst, Belgium; www.easyfitpremium.be

Eco Gym, Nijlen, Belgium; www.ecogym.be

Fanatics Sports Factory, Mortsel, Belgium; www.fanatics.be

Fitality Club Deurne, Deurne, Belgium; http://www.fitalityclubs.be/vestigingen/deurne

Fitality Club Aartselaar, Aartselaar, Belgium; www.fitalityclubs.be/vestigingen/aartselaar

Fitnesscenter Schilde, Schilde, Belgium; www.fitnesscenterschilde.be

Fitopia, Edegem, Belgium; www.fitopia.be

Go Fit Mechelen, Mechelen, Belgium; www.gofit.nu

Gymtonic, Reet, Belgium; www.gymtonic.be

Het Atelier, Sint-Niklaas, Belgium; www.het-atelier.be

I-Fitness Turnhout, Turnhout, Belgium; i-fitness.be/nl/club/turnhout

I-Fitness Berchem, Berchem, Belgium; i-fitness.be/nl/club/berchem

Jims Fitness, Antwerpen, Belgium; www.jimsfitness.be

Martinique Fitness, Wuustwezel, Belgium; www.fitnessmartinique.be

Meirdamsport Beveren, Beveren, Belgium; www.meirdamsport.be

NRG Fitness Kapellen, Kapellen, Belgium; www.nrgfitness.be

Synergie Wellness Point, Wommelgem, Belgium; www.synergie.be

Wezenberg Fitness, Antwerpen, Belgium; http://www.wezenbergfit.be

Tables and figures

INCLUSION	EXCLUSION
women	planned pregnancy within one year
BMI > 27 kg/m²	hypothyroidism
age > 18 years	diabetes type 2 or prediabetes with medication use
stable body weight, i.e., not varying by >3% for at least 6 months prior to the first consultation	changes in medication regimen which can affect study outcomes (e.g. of lipid-lowering or antihypertensive agents)
premenopausal state defined by hormonal data; FSH > 25 mU/ml and estradiol < 20 pg/ml	using drugs known to affect body weight and lipid distribution including tricyclic antidepressant agents
willing to participate in a lifestyle based weight loss intervention (diet or exercise)	abuses alcohol or has a history of alcohol abuse, i.e., more than 2 alcoholic consumptions/day or binge drinking
no physical dysfunctions which makes increased physical activity impossible	exclusion criteria related to MRI and CT
able to read and understand the guidelines given by the dietician and sign the informed consent	

Table 1: inclusion and exclusion criteria for the selection of participants

WARMING UP

treadmill 4 minutes

CORE STABILITY

At least 4 exercises: planking, bridging, back and abdominal exercises using a fitness ball or exercise mat; Progression by: increasing time, increasing reps, decreasing support surface

STRENGTH TRAINING

Week	*Training load*	*Day 1*	*Day 2*	*Day 3*
Week 1 - Week 4	50-60% 1RM	total abdominal	shoulder press	chest press
	2*15 reps	low row	lat machine	vertical traction
		multi hip	leg press	glute
Week 5 - week 8	Increased load	total abdominal	shoulder press	chest press
	2*15 reps	low row	lat machine	vertical traction
		multi hip	leg press	glute
Week 9 - week 13	Increased load	total abdominal	shoulder press	chest press
	2*15 reps	low row	lat machine	vertical traction
		multi hip	leg press	glute
Week 14 - week 18	Increased load	total abdominal	shoulder press	chest press
	2*15 reps	low row	lat machine	vertical traction
		multi hip	leg press	glute
Week 19 - week 24	Increased load	total abdominal	shoulder press	chest press
	2*15 reps	low row	lat machine	vertical traction
		multi hip	leg press	glute

AEROBIC FITNESS TRAINING					
Week	*Training Load*	*Machine 1*	*Machine 2*	*Machine 3*	*Machine 4*
Week 1 - week 6	90% of HR at RCP 8 minutes/exercise	Run (Treadmill)	Step or Bike or Recline	Rowing machine or Top	Synchro or Vario or Crosswalker
Week 7 - week 12	90% of HR at RCP 10 minutes/exercise	Run (Treadmill)	Step or Bike or Recline	Rowing machine or Top or Synchro or Vario or Crosswalker	/
Week 13 - week 18	90% of HR at RCP 12 minutes/exercise	Run (Treadmill)	Step or Bike or Recline	Rowing machine or Top or Synchro or Vario or Crosswalker	/
Week 19 - week 24	90% of HR at RCP 15 minutes/exercise	Run (Treadmill)	Step or Bike or Recline	Rowing machine or Top or Synchro or Vario or Crosswalker	/

Table 2: Exercise training schedule

Note: Participants will be encouraged to exercise three times/week (day 1, day 2, day 3)
All strength and cardiovascular exercises described are from Technogym Series: Selection, Excite, Element+;
1RM = 1 Repetition Maximum; HR = heartrate, RCP = respiratory compensation point

Costs	Examples
Direct medical costs	Health care provider fees
	(general practioner, medical specialists, dieticians, …)
	Medication
	Medical costs of obesity related complications
Direct non-medical costs	Transportation
	Fitness centre membership fees
	Sports clothing
	Sustainability (reboots, incentives)
Indirect non-medical costs	Productivity loss (absenteeism, presenteeism)
	Loss of leisure time
	Informal care

Table 3: Different costs under consideration in this health economic evaluation

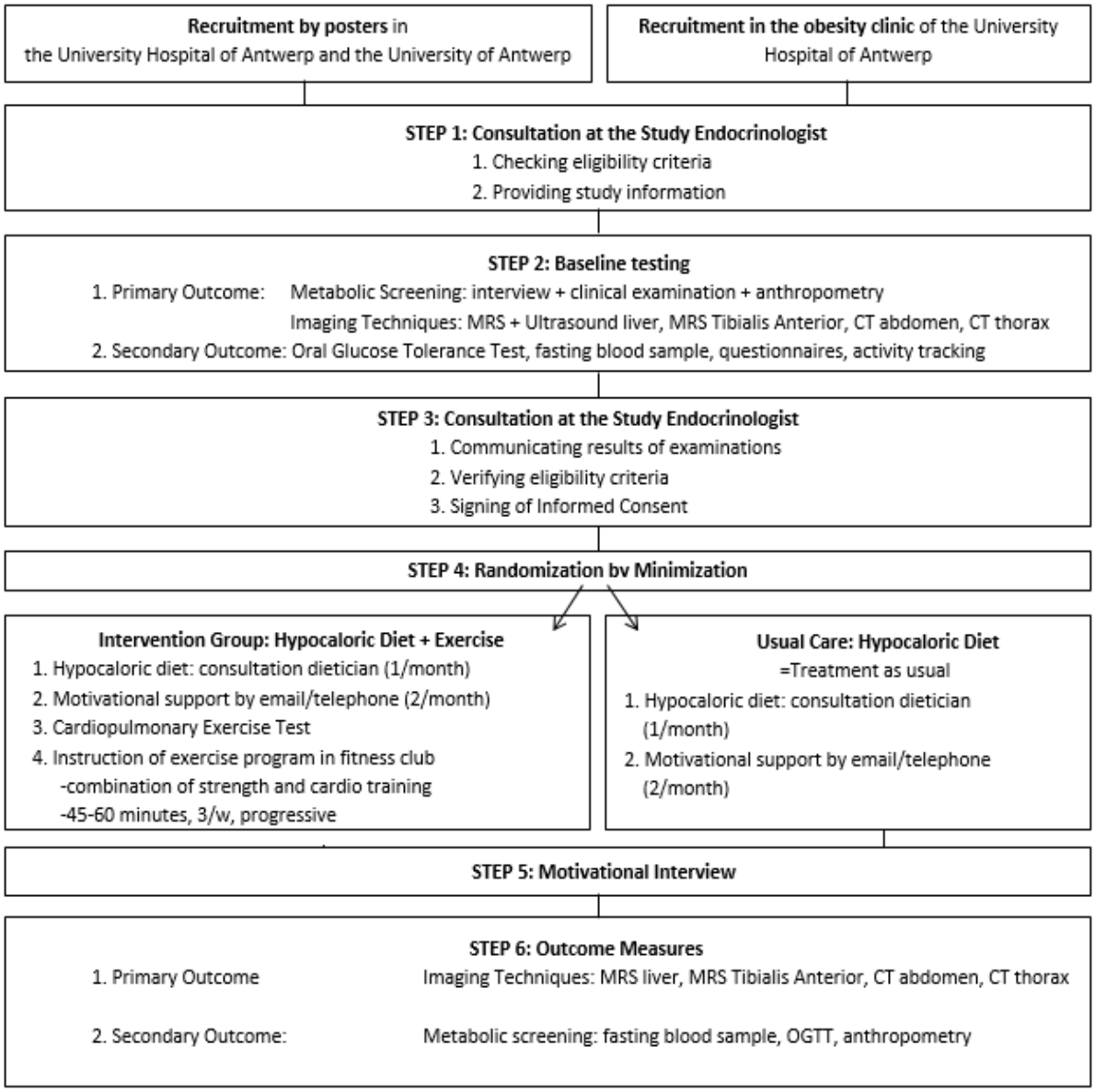

Figure 1: Flow Chart Study Design

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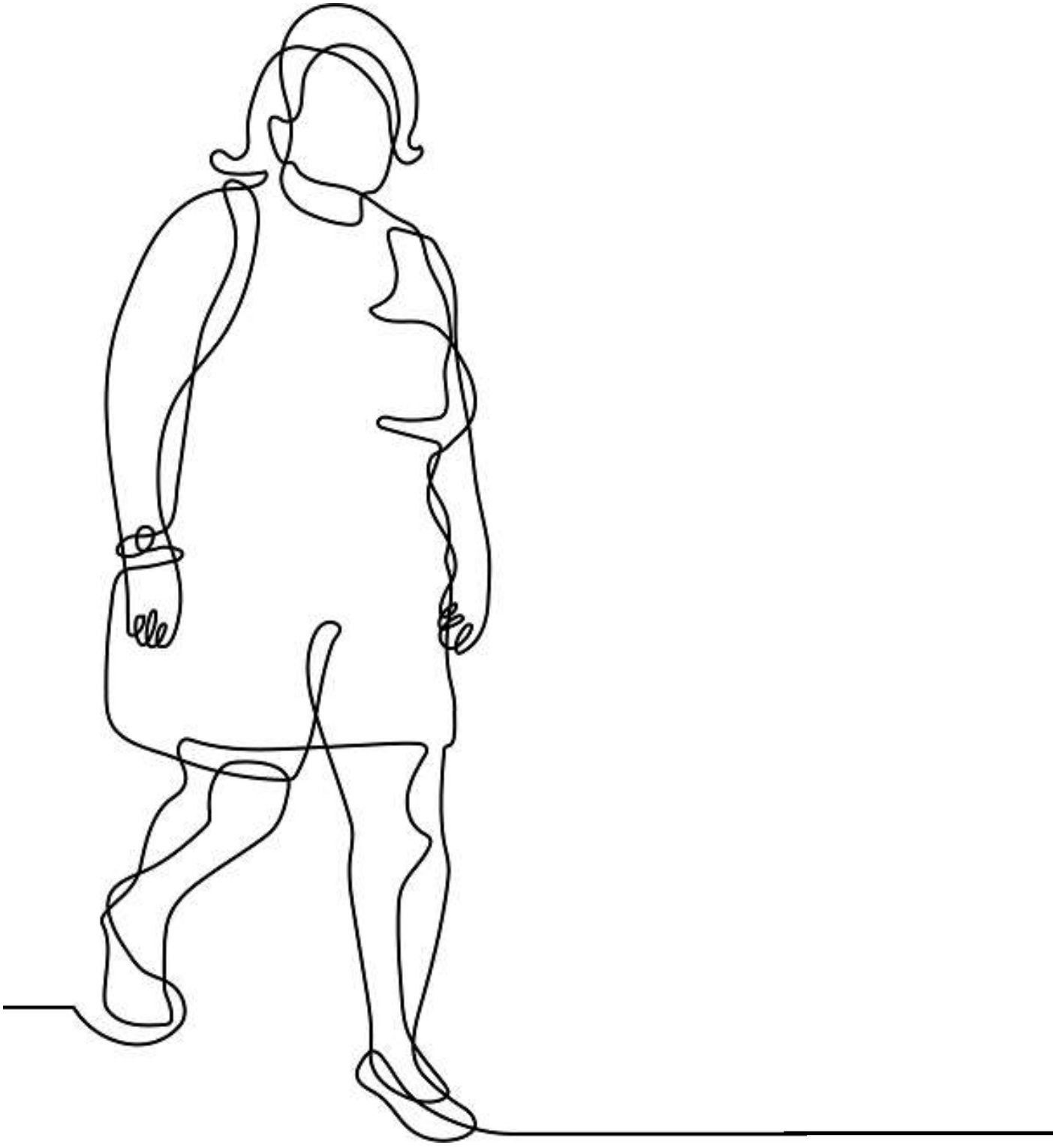
CHAPTER 4

Results



4.1 Repeatability of 1H-MRS

Introduction

In people with overweight and obesity, much attention has been given to the distribution of body fat as it is an important characteristic associated with metabolic and clinical alterations like hypertension and dyslipidemia.

Fat storage in insulin-sensitive tissues like skeletal muscles or the liver is called "ectopic fat" accumulation and is associated with insulin resistance, independent of BMI (1). Since this kind of fat accumulation seems to disrupt metabolic processes and impair organ function (2), it is hypothesized that it is especially seen in obese subjects with a "metabolically unhealthy" phenotype leading to cardiovascular disease (3, 4). The pathophysiological link between ectopic lipids and cardiovascular morbidity is caused by inflammation and an impaired insulin action on target tissues e.g. in the hepatocytes or skeletal muscles (5).

In recent years, the role of fat accumulation in the liver is confirmed, leading to the development of cardiovascular disease (6, 7). Nowadays, ultrasound is used as a screening technique for liver diseases in people with overweight and obesity. Since the sensitivity for detecting mild degrees of steatosis is low in ultrasound techniques, other non-invasive techniques have gained clinical importance. Next to Computed tomography (CT) and Magnetic Resonance Imaging (MRI), Magnetic Resonance Spectroscopy (1H-MRS) is a good alternative giving quantitative information. Moreover, when intrahepatic lipid accumulation (IHL) exceeds 5.5% measured by 1H-MRS, hepatic steatosis is diagnosed and rigorous weight loss is prescribed (8-10).Besides fat accumulation in the liver, fat storage in skeletal muscle (IMCL) disturbs the insulin metabolism. IMCL accumulation leads to an excess of harmful lipid metabolites such as triglycerides and ceramide and thereby could cause insulin resistance (11). Summarizing, IMCL and IHL are correlated with insulin resistance in inactive individuals and can be used to make a correct diagnosis of obesity related comorbidities and define the treatment effects in people with overweight and obesity. However, there is a growing interest in the use of 1H-MRS to quantify ectopic fat, variability in outcome is plausible. After all, changes in medication, diet or physical activity can cause variability in local lipid distribution (12, 13).

Unfortunately, performance of the 1H-MRS, determining the voxel position and success of the spectral fitting (data analysis) may affect the repeatability of 1H-MRS.

In order to interpret the clinical relevance of changes in ectopic fat accumulation during a weight loss intervention, it is important to know which variability can be expected.
The purpose of the present study was to determine the repeatability and variability of IMCL and IHL accumulation in premenopausal women with overweight and obesity measured by 1H-MRS in a clinical context. If the measurement variation is small, treatment results can be tracked by 1H-MRS.

Methods

An overview of the methods used, can be found in a summarizing protocol paper (14). This study is part of a weight loss intervention study. The purpose of the intervention study was to evaluate changes in ectopic adiposity in or around the heart, abdomen, liver and skeletal muscle during and after lifestyle intervention in premenopausal women with overweight or obesity. In this sub study, repeatability of the 1H-MRS measurement methods is evaluated. All participants underwent a baseline and second MRI measurements before starting the intervention.

Participant recruitment

All subjects were recruited by endocrinologists of the Antwerp University Hospital (Obesity clinic) to participate in a lifestyle intervention study of six months. All participants provided their written informed consent before participating. This study was approved by the ethical committee of the Antwerp University Hospital (approval number: 14/17/205). The study protocol conformed to the ethical guide lines of the 1975. Declaration of Helsinki.

Inclusion and exclusion criteria

Since the response to a weight loss intervention is different between women and men and the proportion of women is significantly greater in the obesity clinic, only women were recruited. All included subjects had to be weight stable (≤ 3% change in weight) for at least 6 months before enrollment with a BMI of at least 27 kg/m² and premenopausal state was checked through follicle stimulating hormones and estradiol. Individuals who took medication that influenced body weight or metabolism (tricyclic antidepressants etc.), who smoked or had a clinical history of type 2 diabetes or hypothyroidism were excluded. However, de novo diagnosed (pre-) diabetes, impaired glucose tolerance or non-alcoholic fatty liver disease was

not excluded when there was no urge to start up medication therapy. The use of blood pressure lowering medication was allowed. However, changing of medication regimen was an exclusion criteria. Since all participants underwent medical imaging, exclusion criteria related to MRI and CT scan were applicable.

Magnetic Resonance Spectroscopy Procedure

1H-magnetic resonance spectroscopy (1H-MRS) was used to quantify Intramyocellular Lipids (IMCL) and liver lipids (IHL) of each participant using a 3T MRI scanner (SIEMENS MAGNETOM Prisma, Siemens Healthcare AG, Erlangen, Germany). Before the start of the intervention study, each participant underwent twice a Magnetic Resonance Spectroscopy exam with a time interval of 12 days. By carefully choosing the region of interest during the second measurement, an attempt was made to measure in the same voxel position as in the first 1H-MRS. Each MRI exam was performed in the evening between 19:00 and 24:00. To ensure maximum field homogeneity, manual shimming on the volume of interest was done. Total time in the scanner room, including repositioning of subjects between liver and muscle measurement, was between 45 and 55 minutes for each subject. Subjects were asked to avoid consuming high fat loaded food and doing any sports or excessive physical effort three days prior to the 1H-MRS exam.

To make a quantitative analysis of obtained spectra, the jMRUI software was used (version 5.2; http://www.jmrui.eu) (15). Metabolite signals were analyzed by using the non-linear least-squares algorithm AMARES with prior knowledge technology. Preprocessing was standardized using the jMRUI2XML software (16). When the quality of the spectrum was doubtful or no acceptable fitting was obtained, the spectrum was excluded from statistical analysis. Hereby, repeatability could not be defined in that subject.

To define the hepatic lipid content, the body array coil was positioned around the chest, as the participants lay supine. The region of interest was positioned in the right liver (segment 6 or 7), avoiding diaphragm, edges of the liver, vascular and biliary structures. Breath-hold (16s) single-voxel 1H-MRS data were acquired using STEAM, with the parameter setting as follows: 3.0*3.0*3.0 cm^3 voxel in the right liver, TR/TE 3000/20ms, no water suppression. For the quantification of IHL, a ratio from the 1H-MRS spectra was calculated and defined as the methylene peak (1.3ppm) versus the H2O peak (4.7ppm) and the following formula was used:

IHL = lipid signal / (water signal + lipid signal).(10) An example of a 1H-MRS measurement in the liver and its quantification is shown in Figs. 1a and 1b.

To define the intramyocellular lipid content, the region of interest was set in the Tibialis Anterior muscle. This muscle was chosen because of its parallel fiber arrangement with optimal lipid peak separation. During the first visit a homogenous part of the Tibialis Anterior was searched, avoiding visible interstitial tissue, fat and blood vessels. Voxel placement during the second visit was facilitated with anatomic landmarks, by measuring the distance of the localization of the voxel to the tibia plateau. Imaging parameters were set as follows; repetition time (TR)/echo time (TE) 2000/33ms, voxel size 15*15*15 mm^3, six averages and weak water suppression.

For the fitting of muscle spectra, seven resonances were defined: (7) the IMCL methyl protons peak at a resonance of 0.9 ppm, (6) the ExtraMyoCellular Lipid (EMCL) methyl protons peak at a resonance of 1.1 ppm, (5) the IMCL methylene protons peak at a resonance of 1.3 ppm, (4) the EMCL methylene protons peak at a resonance of 1.5 ppm, (3) the total creatine (i.e., free creatine plus phosphocreatine) methyl peak at a resonance of approximately 3.0 ppm, and (2) the trimethylamines (i.e., choline plus carnitine) peak at a resonance of 3.2 ppm and the water peak (1). For the quantification of IMCL, the lipid peak at 1.3 ppm was scaled to the total creatine peak.

An example of a 1H-MRS measurement in the Tibialis Anterior muscle and its quantification is shown in Figs. 2a and 2b

Statistical analysis

All analyses were performed with SPSS 24.

Results of the measurements of the two visits were tested for normal distribution and compared with a paired t-test. Repeatability was expressed by the Bland-Altman statistic and the coefficient of variation (CV). The CV was calculated by dividing the standard deviation (sd) of the mean difference between two visits by the mean values measurements on both days. The Bland-Altman plot analysis was used to evaluate the agreement between the measurements of two visits. Hereby, the mean of the measurements during both visits was plotted on the x-axis against the difference between the two measurements on the y-axis. In this plot, the agreement interval is visible and is calculated by using the mean and the

standard deviation of the differences between two measurements (mean value ± 1.96 standard deviation of the difference between two visits).

Moreover, the repeatability coefficient (RC) and the intraclass correlation coefficient (ICC) were calculated. The RC represents a value, which is higher than the absolute difference between two measurements, with a probability of 95%.

The ICC expresses the correlation between measurements at two visits. An ICC superior to 0.80 was considered as excellent and between 0.60 and 0.79 as good. All data are presented as means +/- sd. A p-value ≤ 0.05 was considered to be statistically significant.

Results

Subjects

Sixty-two subjects were found. One of them was excluded based on menopausal state (results of blood analysis). Sixty-one subjects completed the entire MR protocol, with 2x61 spectra from Tibialis Anterior muscle and 2x61 spectra from the liver.

The repeatability of IHL measurements could not be defined in three since at least one of their spectra was of poor quality due to motion artefacts. The repeatability of IMCL measurements could not be defined in six participants since at least one spectrum could not be used in these participants. Common problems were poor quality of obtained spectra or excessive overlapping of lipid peaks.

The flow chart of obtained data can be found in Fig. 3.

Since 17 women had an IHL content of more than 5.5%, they could be defined as having hepatic steatosis.(10) The ethnicity of our study population was predominantly Caucasian (54 subjects) with seven and two women from Africa and Asia resp. An overview of all patient characteristics can be found in table 1.IMCL accumulation and IHL content was individual widespread and thereby skewed in our population (Kolmogorov-Smirnov and the Shapiro-Wilk Test ($p<0.01$).

Intrahepatic lipids

Repeatability of 1H-MRS to quantify IHL was defined based on two spectra in 58 patients. No significant differences in mean IHL values between first and second visit were detected (5.62 ± 5.33% vs 5.62 ± 8.08%, with p = 0.99). A Bland Altman plot and Scatter plot are shown in

Figs. 4 and 5. The ICC showed an excellent correlation between measurements of both visits (ICC = 0.97) with a RC of 5.86. The CV between the two visits was 37.0%. Calculations of the CV were repeated in a subgroup with lower IHL content and a subgroup with higher IHL content or NAFLD. A lower variation was seen in 17 women with NAFLD (IHL>5.5%). These results showed a lower variability with an ICC of 0.934, a RC of 9.95 and a CV of 24.10%. In this subpopulation, IHL content ranged between 6.01 and 37.55%. In our subgroup of women without NAFLD, the ICC was 0.72 with a RC of 2.67% and a CV of 59.73%. In this subpopulation, IHL content ranged between 0.02 and 4.75%.

Intramyocellular lipids

Repeatability of 1H-MRS to quantify IMCL was defined based on two spectra in 55 subjects. The mean IMCL values from the second visit were not significantly different from the values from the first visit (7.28 ± 4.28 vs. 7.28 ± 4.44 with p = 0.43).

A Bland Altman plot is shown in Fig. 6. The ICC showed an excellent correlation between measurements of both visits (ICC=0.803) and RC was 10.5. The CV was 51.2%.

Discussion

In this study, the repeatability of Magnetic Resonance Spectroscopy for use for the evaluation of liver fat content (IHL) and muscular fat content (IMCL) was investigated. The outcome measures cover both technical variability in performance of the 1H-MRS (equipment, repositioning) and physiologic variability.

It is remarkable that IHL and IMCL content are very individually different in a population of premenopausal women with overweight and obesity. This can be explained by the fact that our population was selected based on BMI cutoffs and not on the appearance of liver diseases or amount of ectopic fat. The distribution of IMCL and IHL data is skewed in our study with a long tail to higher ectopic fat content. Skewness of data and high individual differences amongst subjects is in line with previously reported data from cross-sectional and intervention studies but not described in other repeatability studies (17). Although the coefficient of variation is widely used to test repeatability in measuring IHL, this is harder to interpret in a skewed data set. However, in order to meet the clinical interpretation of obtained results, the use of parametric statistics was preferred over logarithmic transformation of the data set.

Since variability outcomes depend on the used experimental methodology, caution is needed when study outcomes are compared. The results of our repeatability study concerning IHL show a higher variation than the results from a similar research by Van Werven et al. (37% versus 9.5%) (18). In this last study, repeatability was assessed in a mixed population of healthy people and people with obesity and hypobetalipoproteinemia. All our subjects have overweight or obesity and this seems to be an important confounding factor in the acquisition of 1H-Magnetic Resonance Spectroscopy data. The higher the BMI of a subject, the greater the risk on disturbed and poor quality of liver spectra (19).

Since previous research showed that repeatability to measure IHL depends on the degree of fat accumulation, an extra analysis was done in a subgroups of people with hepatic steatosis (18, 20). A lower variation between two measurements is obtained in repeatability studies with a smaller time interval between measurements. The variability between two measurements at the same day vary between 0.3% and 20.5%. Since rather limited or ignorable physiological variation of liver fat is expected, these big differences cannot be explained (21, 22).

1H-MRS of IHL could have an important clinical value since it is non-invasive and could be used to screen a large number of individuals at risk of NAFLD and to monitor NAFLD treatment. However 1H-MRS is stated to be the gold standard, there are modern imaging techniques (e.g. Dixon-based multi echo imaging) allowing quantification of proton density fat fraction in the entire liver. Moreover, there are some limitations associated with the 1H-MRS technique. The measurement is time consuming, requires complex data analysis, and the presence of inflammation and other abnormalities cannot be determined.(1)

Despite the fact that there are studies with an acceptable repeatability in the IMCL quantification by 1H-MRS (1.5 T scanners), the results of this study showed a higher variability. Torriani et al. found an acceptable intervisit repeatability with a CV of 18.9% for the jMRUI-quantification of IMCL in the Tibialis Anterior muscle (ref. to creatine) on a 3T scanner (23). A better repeatability (CV=10.26%) was obtained quantifying IMCL content in the Tibialis Anterior muscle on a 1.5T (ref. to water) in a larger study with overweight and obese individuals (24). Literature suggests that a better distinction of IMCL should be obtained with 3.0T scanners although it is possible that measurement precision and repeatability is not improved because of linewidth broadening due to susceptibility effects and lower peak separation (25, 26).

Generally, coefficients of variations were smaller in intraday repeatability studies (CV about 13%)(27-29) than in intervisit repeatability studies (CV between 6.1% and 21%) (23, 27).

An other possible explanation for the aberrant findings in this study can be a voxel displacement of a few millimeters. This can change the extramyocellular Lipid content by an order of magnitude and hereby decreasing reliability of IMCL measurement (27). A better repeatability could also be obtained when voxel size was smaller or high resolution chemical shift imaging was used (25). At last, a time interval of 12 days between the measurements may be too long as IMCL show a very fast regulation (30, 31). As mentioned before, it is difficult to compare studies because of differences regarding the used MRI device, coil or sequence protocols. This study focused on IMCL variation in the Tibialis Anterior muscle, which consists of a high content of fast twitch glycolytic fibers (type IIb). Therefore, results could not be generalized to other skeletal muscles. In order to detect changes in IMCL content, a muscle with predominantly slow twitch (type I fibers e.g. the soleus muscle) might be more applicable since it has a higher insulin sensitivity (32).

There are some important strengths. Since fat distribution is not completely equally divided in the liver or muscle (10, 18, 21), an effort was made to position the voxel during the second measurement identically to the first 1H-MRS measurement. In the Tibialis Anterior, this was obtained by measuring precisely the distance from the voxel to the tibial plateau during the first 1H-MRS. During the second 1H-MRS, the MRI slices in which the voxel was set were carefully chosen based on distance from the tibial plateau. Voxel position in the liver was reproduced using MR images of the first exam.
Moreover, no efforts were spared to perform time-consuming manual shimming adjustments. It is likely that they have improved the performance of 1H-MRS.

However, it has to be acknowledged that there are also some limitations to this study.
Firstly, an additional assessment of intra-session repeatability would permit comparison of the contributions of physiologic variability (which would be minimized within the same scan session) and technical variability.
Secondly, notwithstanding the instruction regarding high fat loaded food and excessive physical effort, it is possible that patients did not obey which caused a bias. Dietary macronutrients and physical activity influences fat distribution, particularly in skeletal muscle (12, 33). Since this research was performed in a clinical hospital setting and research time on the MRI device took place after clinical routine, it was not possible to perform the 1H-MRS assessments in fasting conditions. It is likely that a standardized protocol regarding physical activity and diet would have resulted in lower variability (34). Although, this kind of standardization is often not realistic in clinical context.

Thirdly, jMRUI software was used. It is possible that results can differ when using other software e.g. LCModel algorithm. In this study, we had some difficulties to quantify spectra with low IHL content. This problem was solved with appropriate prior knowledge. This shows that the use of prior knowledge is an important confounding factor in the quantification of IHL.
Fourthly, we quantified only the methylene peak (CH2) of triglycerides, which only presents a limited amount of the total fat content in patients with severe steatosis. Finally, we used relative quantifications of lipids instead of absolute quantifications. Hereby it is assumed that the reference metabolite (water or creatine) does not changes over time, although this is still unclear in literature.

In conclusion, we proved that that it is feasible to assess IHL and IMCL in a non-invasive way in women with overweight or obesity by using 1H-MRS. The repeatability of 1H-MRS to quantify IHL content is acceptable in people with NAFLD, making it a technique that is suitable for the quantification of liver fat in people at risk for liver fibrosis.
The repeatability of 1H-MRS to estimate IMCL was less convincing to use in a clinical setting. An understanding of the variability that characterizes this technique is important to interpret changes due to clinical interventions. Since the degree of ectopic fat deposition differs from one to another, dispersion of IHL and IMCL values is possible.

Acknowledgments
I want to thank Dr. Marinette Van der Graaf, medical physicist and senior scientist magnetic resonance at the Radboud University Medical Center. She was involved for finetuning the scanning protocol. Afterwards, she helped fitting spectra with low IHL content by applying appropriate prior knowledge to the JMRUI software and gave extensive feedback on the manuscript.

Tables and figures

	Subgroup Reliability Study IMCL		Subgroup Reliability Study IHL		Subgroup subjects with steatosis	
	n=55		*n=58*		*n=17*	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
Age (y)	36.8	8.8	36.8	8.3	37.7	8.6
BMI (kg/m²)	32.3	3.0	32.6	3.5	33.1	3.2
Body Fat (%)	38.9	3.6	39.2	3.9	39.4	2.8
Waist (cm)	100.3	8.8	100.4	8.8	101.0	7.0
Visceral fat (cm²)	115.9	44.8	116.3	43.8	127.1	44.3
HDL (mg/dL)	57.6	11.5	57.8	11.3	53.7	8.7
LDL (mg/dL)	119.7	37.5	118.1	37.0	116.2	38.5
Triglycerides (mg/dL)	104.4	56.3	100.2	47.6	118.9	64.2
HOMA-IR	3.2	1.5	3.2	1.6	3.7	1.5

Table 1: Descriptive statistics of study participants

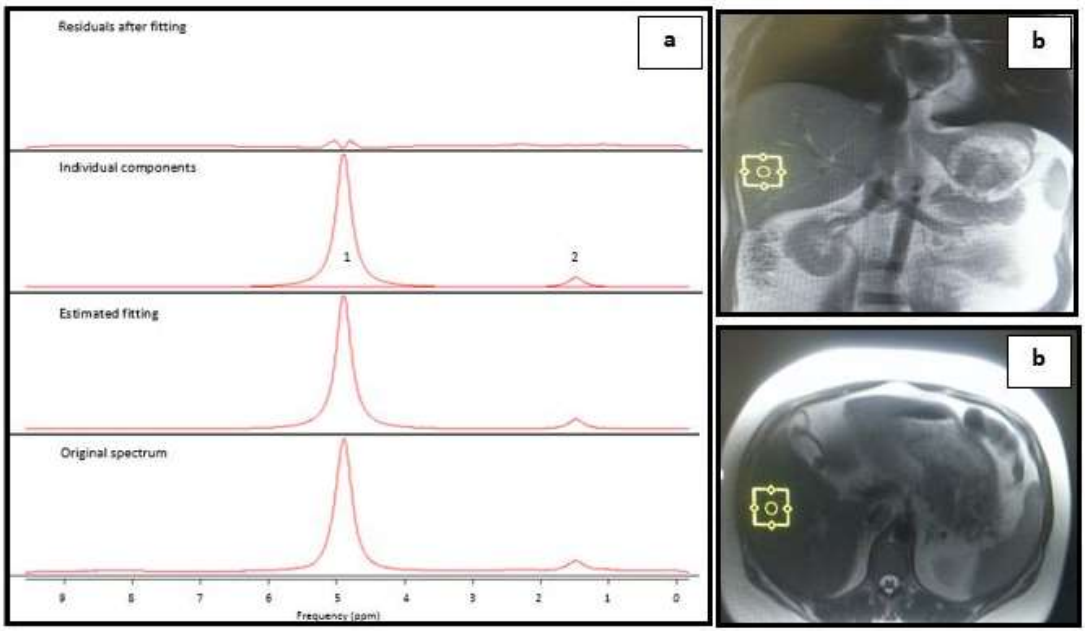


Figure 1a; Example ^{1}H-MRS spectrum of the liver fitted with the use of the Java-based MR user interface (jMRUI) and Java-based MR user interface 2XML (jMRUI2XML).

The individual fitted components are 1-water at 4.7 ppm; 2-IHL metylene peak at 3.2 ppm

Figure 1b; voxel positioning in the liver in the coronal and axial plane

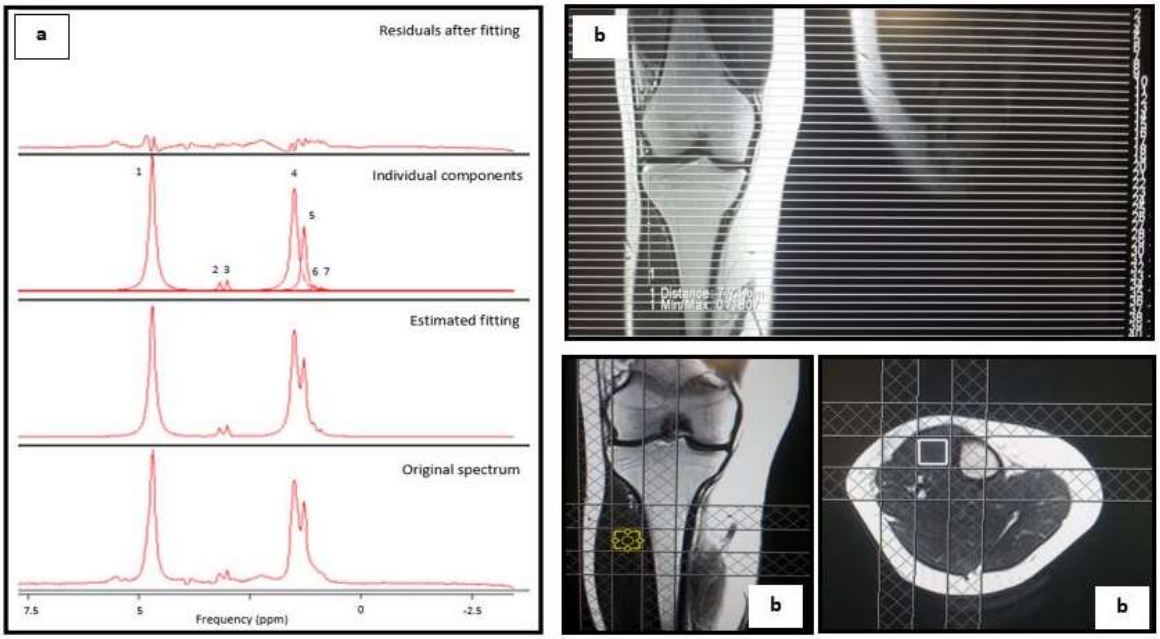


Figure 2a. Example ^{1}H-MRS measurement in the Tibialis Anterior Muscle, fitted with the use of the Java-based MR user interface (jMRUI) and Java-based MR user interface 2XML (jMRUI2XML).
The individual fitted components are *1-water at 4.7 ppm; 2-TMA: trimethylamines peak at 3.2 ppm; 3-TCr: total creatine methyl peak at 3.0 ppm; 4-EMCL(-CH2): EMCL methylene proton peak at 1.5 ppm; 5-IMCL(-CH2): IMCL methylene proton peak at 1.3 ppm; 6-EMCL(-CH3): EMCL methylene proton peak at 1.1ppm*
Figure 2b. voxel positioning in the coronal and axial plane and measurement of the distance between the voxel and the tibial plateau.

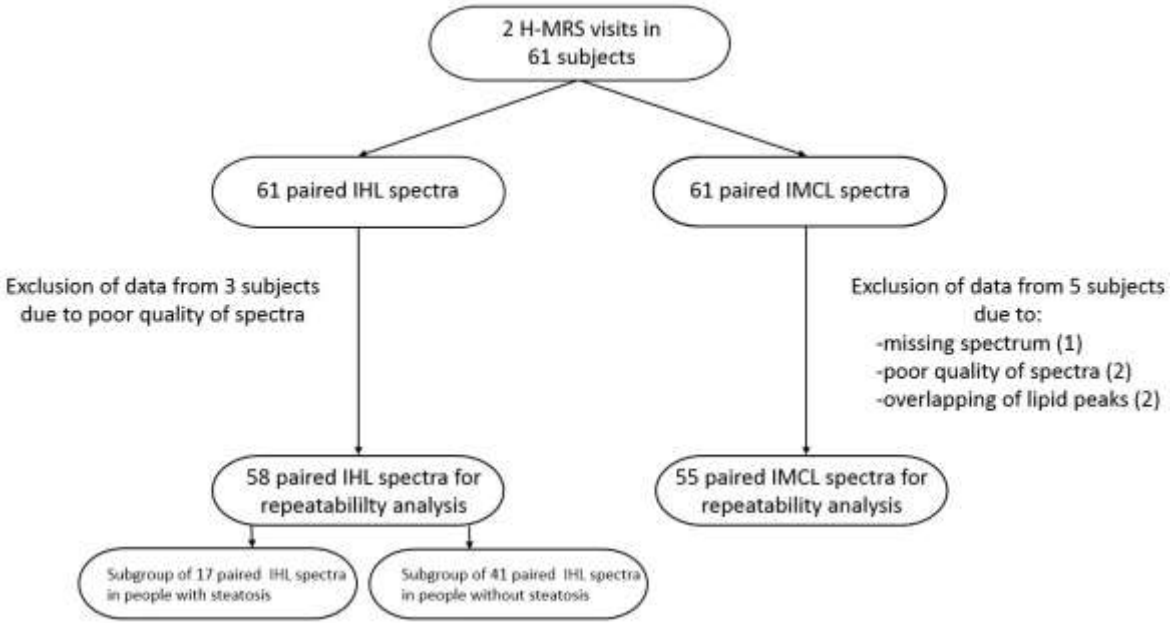


Figure 3. Flow Chart of obtained data

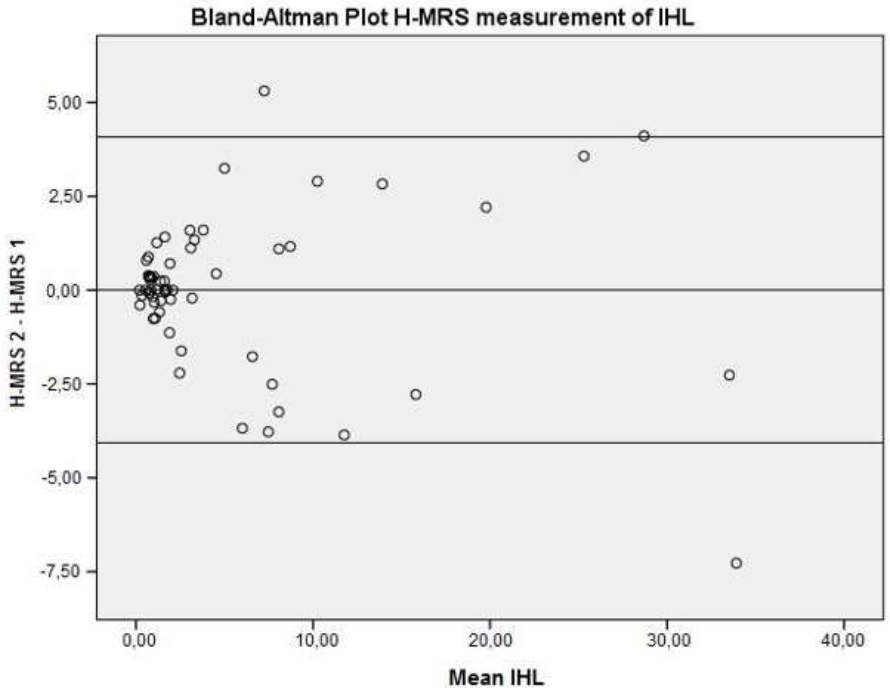


Figure 4. Bland-Altman plot of repeatability of ^{1}H-MRS to quantify IHL content

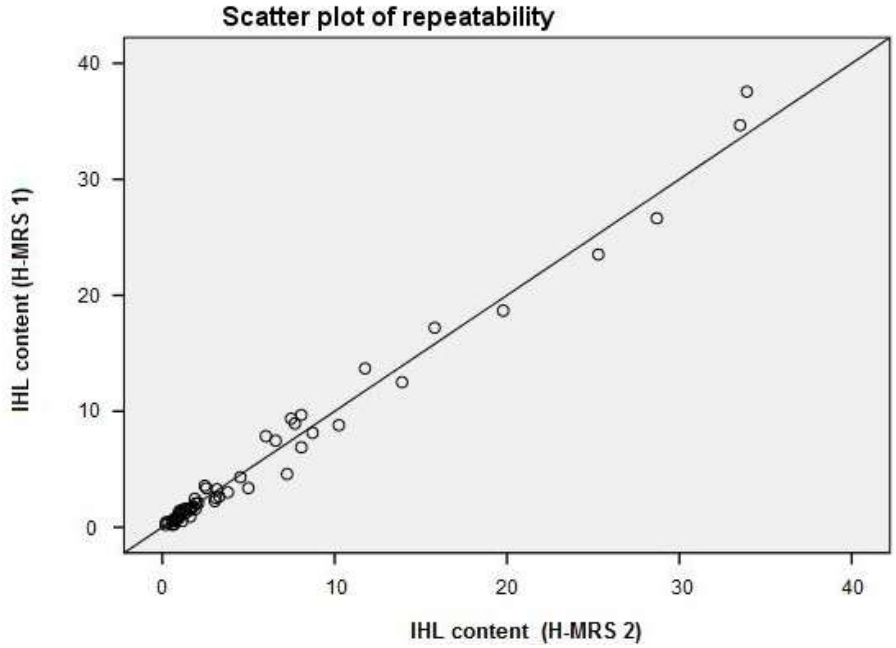


Figure 5. Scatter Plot repeatability of ^{1}H-MRS to quantify IHL content

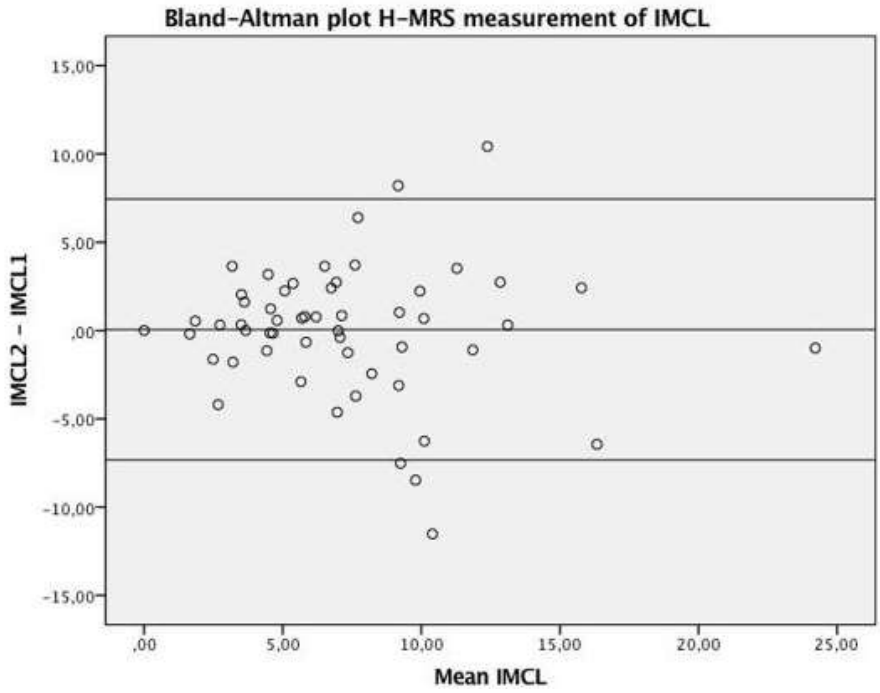


Figure 6. Bland-Altman plot of repeatability of ^{1}H-MRS to quantify IMCL content

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4.2 Cost-of-illness analysis

Introduction

The increasing prevalence of overweight and obesity during the last decades has become a serious public health concern and has placed a financial burden on the wider economy. [1, 2] The association between overweight or obesity and health disorders such as the metabolic syndrome has been well established.

In Flanders, the northern Dutch speaking part of Belgium, the prevalence of overweight (BMI $\geq$ 25 kg.m-2) and obesity (BMI $\geq$ 30 kg.m-2) in the female population (aged 35 to 44 years) is estimated at 42.5% and 11.4% respectively. [3]

The extent to which overweight and obesity related metabolic abnormalities are seen defines the patient's global cardio metabolic risk profile. [4] In this regard, a distinction is made between metabolically healthy obesity (MHO) and obese people suffering from the metabolic syndrome. [5] The prevalence of the metabolic syndrome in a population increases in people with higher BMI and age and is a marker for high health care utilization and costs in people with overweight. [6, 7]

In general, overweight and obesity are associated with high direct and indirect costs. Direct costs include the costs of the diagnosis and treatment of overweight and obesity and especially their associated diseases and complications, while the indirect costs are those resulting from productivity loss such as work absenteeism, early retirement, and the lost value of life due to premature mortality. [8, 9] In European countries, this cost may range from 0.7% to 0.8% of the total annual expenses of the health insurers. [10]

Although, overweight resulted in 7.4% of the Disability-Adjusted Life Year (DALY) in Belgium in 2004, the cost of overweight or obesity is as yet not well understood. [10] Especially information on the health costs in the overweight or obese population without major comorbidities is still lacking. The economic burden of a disease or condition can be estimated by cost-of-illness studies. [11] In general, two approaches are used in cost-of-illness studies, namely the prevalence-based and the incidence-based approach. Prevalence-based studies estimate the costs associated with past and current consequences of the disease or condition in a given time period, typically a year. The incidence-based approach estimates the costs and consequences associated with new cases of the disease or condition

in the current and future years. [12]
Insights into the economic impact of overweight and obesity are important to strengthen the knowledge of the current burden associated with this condition and may inform decision makers to understand the scale of overweight or obesity related problems. This may help them to establish evidence-based public health policies to tackle the overweight and obesity problem.

The aim of this paper was to estimate the health status (i.e. the comorbidities) and the corresponding costs in a sample of 62 premenopausal non-diabetic overweight or obese females in Flanders. This paper was the first step towards a complete cost-effectiveness and cost-utility analysis of an intervention (diet and physical activity) to reduce body weight, abdominal and ectopic adipose tissue in this sample.

Methods

Theoretical framework

Figure 1 depicts the theoretical framework that was followed in this study. A bottom-up cost-of-illness approach was applied to estimate the prevalence of different comorbidities and the related direct and indirect costs in this sample of overweight and obese females. Direct medical costs include expenses for visits to physicians or medication. Direct non-medical costs include for example the use of braces if needed in conditions of morbid obesity. Indirect costs encompass productivity loss (e.g. premature mortality, absenteeism or presenteeism) or leisure time loss. Intangible costs refer to costs related to pain or psychological suffering (e.g. stigmatization), however the latter is difficult to measure and will not be analyzed in this present study.
A prevalence-based approach was used to examine the costs of overweight and obesity in premenopausal women in the Flanders region. A prevalence-based study estimates the economic burden of a disease or condition over a well-defined time period, in this case three months. [13]

Study participants

The study was conducted in the larger Antwerp metropolitan area in Flanders.

Study participants were recruited among patients visiting endocrinologists at the obesity clinic of the University Hospital of Antwerp between May 2016 and August 2016. In addition, poster recruitment was performed in the University Hospital of Antwerp and the University of Antwerp. A total of 120 participants responded and wanted to volunteer in this study. Table 1 shows the inclusion and exclusion criteria for the selection of the study participants. All included participants were non-diabetic premenopausal overweight or obese females. Since fitness training was part of the intervention and an individually but standardized exercise scheme was followed, women were excluded when suffering from cardiovascular diseases or musculoskeletal disorders that made strength or endurance training impossible. This selection procedure resulted in a sample size of 62 participants.

All included participants (n = 62) were randomized to a hypocaloric dietary intervention group or a combination group of hypocaloric diet and increased physical activity and they were measured at baseline, in between (3 months) and at the end of the intervention (6 months).

This study was the first step of a trial-embedded health economic evaluation. Its protocol has been described and published elsewhere. [14] The protocol and consent forms were approved by the ethical committee of the University Hospital Antwerp (approval number: 14/17/205). All participants gave prior written consent.

Data collection

Data were gathered by means of self-reported questionnaires (cost diary). In this paper, results from baseline questionnaires (3) are discussed. Questionnaires were based on similar questionnaires used in a study from the University of Ghent in which costs in an obese population in Flanders were reported. [15] In this way, our results could be compared with a reference group of obese people.

One questionnaire with closed questions aimed at assessing the type of comorbidities over the last three months. A set of overweight-related (e.g. hypertension, cardiovascular disorders, gall bladder; cancer; arthritis or osteoarthritis, depression) and pathologies unrelated to overweight (e.g. allergy, asthma, chronic obstructive pulmonary disease (COPD), skin disorders, hypo/hyperthyroidism, stomach ulcer, migraine headache) was listed.

Respondents could answer “yes”, “no” or “previously”. One open question offered the opportunity to add “other disorders”. A final question asked if the physician gave advice to start a treatment for overweight or obesity. This could be answered by “yes” or “no” while mentioning a date.

Another questionnaire focused on the assessment of direct and indirect costs. Units consumed over the last three months as well as prices were asked for visits to health care providers such as physicians, specialists, nurses, dieticians and physiotherapists. Consumption units and prices of medication were also asked retrospectively over the same period. Furthermore, consumption and prices of hospitalization, day hospitalization, surgery and special diagnostics or medical examinations were assessed over the same period together with the number of days absent from work due to any health problems.

The third questionnaire was the European Quality-of-Life-5D (EQ-5D-5L). The EQ-5D consists of five dimensions (mobility, self-care, usual activity, pain/discomfort, anxiety/depression) on a five level scale (ranging from 1 = no difficulties to 5 = extreme difficulties) allowing to define a health state and the calculation of a corresponding utility index (as a proxy of quality of life). A utility of 1 is equal to perfect health, while 0 stands for death. [16]

Questionnaires were given to the volunteers during the first meeting in the laboratory. Participants were asked to fill out the questionnaires retrospectively (for the last three months) and to return the completed questionnaires when visiting the laboratory for a second time one week later.

Data analysis

Data management was conducted in three steps. In a first step one researcher (JB) manually imputed the results from the questionnaires into a spreadsheet (Microsoft Excel 2013). In a second step two researchers (WH, JT) checked each cell value of the spreadsheet with the questionnaire and corrected the value if needed. In a third step, one researcher (JT) conducted a final round of ad random data checking.

Data from the comorbidity questionnaire were analyzed using a frequency analyses.

Direct medical cost data were presented in 2017 Euros and all units consumed and unit prices were reported in a non-aggregated form. If participants did not mention unit prices of care providers, the fees determined on 01/01/2017 were searched on the website of the National Institute for Health and Disability Insurance (NIHDI). [17-19]

It was assumed that patients consulted accredited physicians. For assessing the costs of a specialist doctor, the billing service tariff of the University Hospital of Antwerp was applied. According to the NIHDI, the federal institution that organizes the mandatory health insurance, the price for a dietician consultation amounts to 19.66 Euro. In the present study, this is an out-of-pocket expense for the patient, because such a visit is reimbursed by Flemish health insurers only in case of diabetes. It was assumed that the participants visited an accredited physiotherapist for a 30 minutes treatment. This price per visit amounts to 22.26 Euro of which 16.37 Euro is reimbursed from the insurer (in case the therapist joined the NIHDI convention and the patient has right to the normal reimbursement rate), resulting in a cost for the patient of 5.89 Euro per visit. [20] Respondents mentioned no nursing costs, hence these were not valued.

Medication costs were calculated based on the units consumed through the Belgian commented online drug compendium. [21] Costs for hospitalization, surgery, day-care hospitalization and medical examinations or diagnostic testing were based on the billing service 2017 tariff of the University Hospital of Antwerp. A mean cost of one day in a hospital in the Antwerp region was set at 673.00 Euro (medical services not included). No direct non-medical costs were reported, hence these were not valued.

Absenteeism was analyzed as an indirect cost and valued as 288.00 Euro per day following the whitepaper of SECUREX, a company providing social secretary services in Flanders. [22] The costs were reported over a three months time span. Costs were analyzed from the perspective of the patient, the health insurer and the society respectively.

Finally, the EQ-5D questionnaire was analyzed using the EQ-5D-5L crosswalk index value calculator with social UK tariff. [16]

Statistical analysis was performed using SPSS Version 22.0 (IBM SPSS Statistics V22.0). As could be expected, cost data in this study were not normally distributed. For pragmatic reasons however, results were presented as means and the corresponding 95% confidence intervals. To analyze the association between BMI, utility score, age, number of comorbidities and the cost, Spearman rank order correlation coefficients were calculated. Statistical significance was set at 5%.

Results

A total of 59 out of the 62 females returned the questionnaires, which were analyzed for this study. Their mean age was 37 years (ranging from 19 to 53 years) with a mean body mass index of 32.6 kg.m-2 (measured values, range from 27 to 44 kg.m-2). Most of the participants were obese (86%) and were residents of the Antwerp metropolitan region (82%) in Flanders living outside of the city (62%). About 47% of the participants hold a higher education (no university) degree while 18% were university degree holders. In the total sample, four participants (7%) were unemployed at baseline but two of them found a job during the course of this intervention study. Also, four students were included in this study. On average, a participant suffered from about three comorbidities. Only four responders were free of any comorbidity while six volunteers showed six to eight comorbidities. Besides diabetes, which was an exclusion criterion, the following comorbidities of the questionnaire were not reported: any form of cancer, COPD, liver cirrhosis, chronic hepatitis, Parkinson disease and HIV. Since only three subjects had the metabolic syndrome, the majority of our sample (95%) were metabolically healthy overweight or obese women. [5] Table 2 presents the relative prevalence of the reported comorbidities in this sample. Musculoskeletal disorders and allergies seemed to be the most prominent comorbidities, but also depression, hypertension, skin problems and migraine headache were highly prevalent. Also "other disorders" were mentioned: reflux oesophagitis, hypercholesterolemia, lupus erythematosus, low limb lipoedemia, glaucoma, eczema (all disorders mentioned once). With the exception of thyroid disorders, all listed diseases showed higher prevalence in the sample under investigation as compared to a reference sample of 1547 representative for the adult Flemish female population. [23] In 59% of the cases it was the general practitioner who suggested to start a treatment against the patient's overweight or obesity.

On average, respondents consulted a physician once over the last three months [95%CI : 0.7 to 1.3]. Average number of visits to a specialist doctor were also reported to be once in three months [95%CI : 0.4 to 1.6]. In this three-month period before the start of the intervention, only three respondents went to a dietician. Of those, two participants went twice while one person went once. No visits to a nurse were reported. During this three-month period the average number of visits to a physiotherapist was two times [95%CI : 0.8 to 3.5].

Medical examination and diagnostic testing over the last three months was reported by 29%

of the respondents. A second examination or test was consumed by 5% of the participants. Hospitalization was needed for 7% of the study participants. For those, length of stay in the hospital was one (n = 3) to two (n = 1) nights. Two patients needed surgery, both for shoulder problems. Three volunteers reported a visit to a day-care hospital for sleep disorder, liver biopsy and shoulder problems.

About 65% of the participants were on medication during the past three months. When contraceptives were not taken into account the prevalence of drug consumption was reduced to 55%. Two different drugs were consumed by 42% of the respondents while 5% of the volunteers consumed seven different drugs. Table 3 shows the indications and the relative prevalence of drug consumption in the sample. Pain reducing drugs were consumed by 53% of the sample while medication against depression and hypertension ranked on the second place (both 17%).

Absenteeism was reported by 13.6% of the participants. The average number of days absent from work in this sample over the past three months was 5.5 days [95% CI: 0.3 to 10.7]. Three participants reported 90 days absenteeism for reasons of depression while one person remained absent from work during 30 days because of low back pain.

Table 4 shows the average direct and indirect costs over the past three months from the perspective of the patient, the insurers and the society. On average, a participant of this sample has spent 62.60 Euro for health care while health insurers paid 280.20 Euro over the past three months period. If absenteeism is taken into consideration, the total societal cost for this sample of premenopausal non-diabetic females over the last three months was 2239.7 Euro.

The calculated average utility index was 0.83 [95% CI : 0.79 to 0.87]. About 27% of the participants reported to be "in full health" (i.e. utility index = 1) while 13.5% had a utility index score lower than 0.70. One person reported a utility index of 0.34.

Table 5 depicts the Spearman rank order correlation coefficients matrix between costs, utility index, BMI, age and number of comorbidities. The results suggested that societal cost in the sample is negatively but significantly related to utility scores (i.e. self-reported health status) while a positive association (albeit not statistically significant) between costs for the society and the number of self reported comorbidities, age and body mass index were found. Utility was negatively and significantly associated with comorbidity, cost of absenteeism as well as costs from the different perspectives and BMI, a negative association ($p > 0.05$) was

observed with age. The number of comorbidities was positively related with cost for the patient ($p < 0.05$) and cost for the insurer and society ($p > 0.05$) as well as cost from absenteeism ($p > 0.05$). The correlation between number of comorbidities and BMI was positive and statistically significant.

Discussion

Overweight and obesity are important public health issues and may impose a significant health economic burden to a society. In this study health status and related costs of 59 premenopausal non-diabetic overweight or obese (BMI > 27 kg.m-2) females in Flanders were assessed using a three months self-reported recall questionnaire. All results are compared with reference data of the general female population in Flanders [3, 22, 23] or reference data from a COI in obese men and women in the larger Ghent area. [15]

Although the majority of the sample (95%) encompasses the so-called MHO, the average number of the self-reported comorbidities was striking. Compared to the general female population in Flanders, prevalence of all listed disorders was a factor 0.1 to 10 higher in the sample under investigation except for thyroid disorders which was an exclusion criterion. (Table 1). [23] The most prevalent complaints were back and neck disorders (62.7%) and joint problems (40.7%). The findings in this study corroborate the results of a U.S. study in which 312 obese adults reported to suffer mainly from low back pain (50%) and joint pain (28%). [24]

The fact that depressed mood status was about 5 times more prevalent (39%) in the sample under investigation than in the general adult female population (7.6%) [23], supports the results of a national survey of obese persons in Sweden. This survey concluded much higher levels of anxiety and depression in overweight and obese, as well as poorer perceived health compared with a healthy, non-obese reference group. [25] Thus, such sample of overweight and obese females shows higher comorbidity prevalence compared to the general female population, which may incur higher health costs. [7]

Although diabetes type 2 and cardiovascular diseases were excluded, the prevalence of other overweight and obesity related comorbidities in this sample was associated with increased drug consumption (Table 3). Our findings suggest an even more important medication use in people with overweight and obesity than was reported by Raebel et al. who concluded that

on average a person with obesity compared to a non-obese person consumes 1.81 times more prescription drugs over a one-year time period. [26] Yearly costs for medication in the sample was about 96.00 Euro.

The costs from institutionalized care such as hospitalization and diagnostic examinations totalled on average 774.80 Euro in this study which are higher than those in the healthy reference population (571.00 Euro). [23] From a societal perspective, the yearly costs for visiting health care professionals in this study was 504.40 Euro. This amount is similar as compared to the same cost in the general Flemish population (527.00 Euro), but the contribution of consultations to a specialist doctor was higher in our sample in comparison with the general female population in Flanders. [23]

The average yearly direct medical costs from the societal perspective totalled 1374.80 Euro in the present sample of non-diabetic women with overweight or obesity which is only about 30% of the total direct costs reported in the Ghent study. [15] In the latter study, performed in 62 obese diabetic and non-diabetic patients, similar cost diaries were used. In the Ghent study, yearly costs visiting health care providers (822.00 Euro), yearly medication costs (300.00 Euro) and yearly costs of institutionalized care (3200.00 Euro) were much higher than in our study sample. [15] Main reason for the aberrant differences between both studies may be the strict inclusion and exclusion criteria in our study that prevented people with serious health problems (e.g. ischaemic heart disease and diabetes) to participate in this study. It has been described that ischaemic heart disease and diabetes are the main contributors to the high costs of obesity, probably because ischaemic heart disease treatment is expensive and diabetes is a disease that has the highest incidence risk in people with obesity. [27, 28] Besides this, the mean BMI and age in the Ghent study was higher and might have lead to higher costs. [29]

Based on extrapolation of data of three months, participants of this study were on average 22 days absent from work per year. This absenteeism rate is much higher than that in the general population (14 days). [22] The simple extrapolation from three months of data towards twelve months may have led to a bias of the yearly absenteeism. However, this finding is consistent with the findings of Seidell et al. who found increased absenteeism in obese females because of medical problems such as mental health disorders or musculoskeletal problems. [30] Both types of disorders were also prominent in the sample of the present study.

As the correlation matrix showed (Table 5), productivity-loss may be an important cost driver for the society and resulted in this sample in a yearly average indirect cost of 6208.00 Euro. Participants in the present study showed a mean utility index score of 0.83 (SD = 0.141). This value is similar to the utility score of the general population in Flanders [3] and somewhat higher than the pilot study of the larger Ghent area which showed a mean utility index score of 0.82 (SD = 0.12). [15] About 13.5% of the sample showed low utility index scores (< 0.70). Such scores are comparable with utility index scores reported by patients with Parkinson disease (utility index = 0.58) [31] or severe rheumatic disorder (utility index = 0.57). [32]
The sample in the present study consisted predominantly of the so-called MHO [33] which resulted in average utilities and direct costs that were very similar to the general population but importantly different from those of the obese reference sample. [15] This can be illustrated by the fact that people were excluded when they had musculoskeletal problems that made strength or cardiovascular training impossible leading to exclusion of severe osteoarthritis. Also, it is stated that an increased BMI is associated with reduced health-related quality of life, even in the absence of metabolic comorbidities. [34] Four out of 59 participants reported high productivity losses that resulted in yearly indirect costs of more than 103680.00 Euro per person. Nine out of the 59 participants showed five or more comorbidities. In this subsample average costs increased to 360.00 Euro (patient's perspective), to 1256.00 Euro (insurer's perspective) and 10160.00 Euro (societal perspective) while average utility was decreased to 0.73 (ranging from 0.45 to 1.00). In this regard, it can be confirmed that MHO do have an increased risk of diabetes type 2 and coronary diseases in later life which indicates that healthy obesity can be described as an intermediate stage of disease progression accompanied with important health costs in later life. [35, 36]
In this sample, a higher rate of unemployment was seen in comparison with general female unemployment rates in Flanders (7% vs. 4.5%). Although it is assumed that there is a negative effect of obesity on employment, mediated through disability resulting from the accumulation of chronic conditions, there is also a possibility that pre-existing conditions contribute to obesity at baseline. [37, 38]
Health-related costs in overweight or obese residents in Flanders are as yet no well documented. This study adds information to the findings of a pilot study that was previously conducted using similar methods in a sample of 62 obese males and females of the Ghent area in Flanders. [15] Combined data and results of both studies may help Flemish policy

makers during their decision-making processes when planning actions to contain the burden of overweight and obesity. For example, the findings of this study may be used by health economists as input data when modelling is needed in cost-effectiveness studies.

According to a recently conducted research in Europe, overweight is responsible for 20-26% of the direct medical costs. [28] Research in various countries showed that 2-5% of annual healthcare costs are attributable to overweight. [8, 39-43]

This study was conducted on a sample that was recruited to participate in a prospective randomized controlled trial. Hence it was a captive sample, which may explain the high response rate (59 out of 62) and helped the researchers to ask participants if they had doubts about the reporting or in case of missing values. Despite this advantage, under reporting or over reporting can never be excluded. Although it is reported in literature that the reconstruction of total costs based on self-reported costs (cost diary) shows good agreement with data from other sources such as from insurance companies.[44, 45]

Another limitation of this study is the fact that intangible costs were omitted. For example the use of pain medication was considerable in this sample, but the subjectively felt "pain and suffering" was not calculated. Similarly, tangible and intangible costs due to discrimination, bullying or stigmatization were not calculated. [46] Since welfare losses were not valued, the total economic burden of overweight and obesity may be underestimated. [47] It is recommended that future studies examining the economic impact of overweight or obesity also examine the intangible costs to catch the full economic burden. [48] It can be suggested to determine non-financial welfare costs using disability adjusted life years (DALYs). [49, 50]

In the sample under investigation, the average yearly total cost from a societal perspective was 8958.8 Euro. The fact that data from three months were extrapolated to a whole year, might have led to a bias. Since no appropriate recall periods are determined in cost-of-illness studies, it is hard to identify whether it is better to collect a short-term snapshot of resource use and extrapolate to a longer period or to ask questions covering a broader period that potentially increases recall bias or leads to more uncertainty and hence higher variability of results. [51, 52]

Often, costs are calculated based on the reported rates of comorbidities, or data from an insurance company. [40, 53] In this study, a method was used in which all costs were reported by participants over a short period of three months. Extrapolation of the short-term

data (three month) to one year was used because of its patient-friendliness and proven validity. [44]

Finally, this sample consisted MHO females without diabetes or any other major comorbidity. Thus, the cost results in this study are likely to underestimate the true costs on population level of adult women with overweight and obesity.

Conclusion

On average, "healthy overweight or obese" non-diabetic premenopausal females (mean age 37 years) of the Antwerp metropolitan area showed similar direct health costs and health utility as compared to the general Flemish female population. The observed high number of comorbidities and drug intake did not primarily lead to an increased average direct medical cost. In contrast, average absenteeism was high in this sample and resulted in important total costs from the societal perspective.

Overweight or obese persons are at increased risk for disease such as diabetes mellitus type 2 later in life, which may then cause even more important health costs. A subsample of nine participants with five or more comorbidities showed already high costs and low health utility as compared to the general population. Thus, secondary prevention to avoid health deterioration in MHO females is needed to contain health care and social costs.

Declarations

This study was approved by the ethics committee of the University Hospital of Antwerp (number: 14/17/205 -ref: 7543075363)

Tables and figures

INCLUSION	EXCLUSION
women	planned pregnancy within one year
BMI > 27 kg/m²	hypothyroidism
age > 18 years	diabetes type 2 or prediabetes with medication use
stable body weight, i.e., not varying by >3% for at least 3 months prior to the first consultation	changes in medication regimen which can affect study outcomes (e.g. of lipid-lowering or antihypertensive agents)
premenopausal state defined by hormonal data; FSH > 25 mU/ml and estradiol < 20 pg/ml	using drugs known to affect body weight and lipid distribution including tricyclic antidepressant agents
willing to participate in a lifestyle based weight loss intervention (diet or exercise)	abuses alcohol or has a history of alcohol abuse, i.e., more than 2 alcoholic consumptions/day or binge drinking
no physical dysfunctions which makes increased physical activity impossible	exclusion criteria related to MRI and CT
able to read and understand the guidelines given by the dietician and sign the informed consent	Serious problems or diseases limiting the performance of a standardized training protocol, for example serious osteoarthritis, heart ischemia, …

Table 1: Inclusion and exclusion criteria of participants

COMORBIDITY	STUDY SAMPLE	REFERENCE OF FLEMISH FEMALES [23]
	Prevalence (%)	Prevalence (%)
Hypertension	22.0	18.6
Cardiovascular problems	1.7	1.3
Gall bladder problems	6.8	1.0
Arthritis or osteoarthritis	16.9	10.6
Depression	39.0	7.6
Incontinence	8.5	6.2
Back and neck problems	62.7	25.2
Joint problems	40.7	21.1
Ovarian cysts	6.8	*
Allergy	47.5	16.6
Asthma	3.4	3.1
Skin	28.8	3.3
Thyroid problems	3.4	6.9
Hernia inguinal	10.2	*
Stomach ulcer	13.6	1.4
Kidney stones	5.1	0.5
Migraine	27.1	14.0
Epilepsy	1.7	0.9

Table 2: Relative prevalence (%) of reported comorbidities in this sample in reference to the prevalence (%) in the general female population in Flanders. [23]

Note: * = unknown

INDICATION	STUDY SAMPLE	POPULATION [23]
	Prevalence (%)	Prevalence (%)
Pain	53.3	3.5
Depression	16.7	3.1
Cardiovascular problems	16.7	11.9
Allergy	13.3	2.4
Stomach problems	11.7	2.7
Infections	6.7	1.6
Endocrine problems	3.3	2.7
Dietary Supplements	3.3	*
Birth control	10.0	4.2
Inflammation	13.3	3.6

Table 3: Indications and the relative prevalence (%) of drug consumption in this sample in reference to the general female prevalence (%)in Flanders. [23]

Note: * = unknown

COSTS	UNITS CONSUMED	PRICE PATIENT	PRICE INSURER	PRICE SOCIETY
DIRECT COSTS				
Physician	1.0	4.1	21.4	25.5
Specialist doctor	1.0	11.8	38.9	50.7
Dietician	0.1	2.7	0.0	2.7
Physiotherapist	2.1	12.5	34.7	47.2
Medication	*	9.7	14.3	23.9
Hospitalization and surgery	0.1	14.5	52.9	68.4
Day-care hospitalization	0.05	3.0	66.0	69.0
Medical examination	0.3	4.3	52.0	56.3
Subtotal direct costs		*62.6*	*280.2*	*343.7*
INDIRECT COSTS				
Absenteeism	5.5	0.0	0.0	1552.3
TOTAL DIRECT + INDIRECT COSTS		**62.6**	**280.2**	**2239.7**

Table 4: Average direct and indirect costs (€) over 3 months from the perspective of the patient, the insurers and the society measured by recall (3 months) cost diaries.

Note: * = unknown, all costs are expressed in Euro (€)

	Utility	Cost absenteeism	Total cost patient	Total cost insurer	Total cost society	BMI	Age	Number comorbidities
Utility	1.000	-0.395**	-0.532**	-0.501**	-0.550**	-0.303*	-0.249	-0.443**
Cost absenteeism	-0.395**	1.000	0.359**	0.371**	0.585**	0.140	0.178	0.201
Total cost patient	-0.532**	0.359**	1.000	0.895**	0.886**	0.197	.291*	0.260*
Total cost insurer	-0.501**	0.371**	0.895**	1.000	0.929**	0.222	0.194	0.201
Total cost society	-0.550**	0.585**	0.886**	0.929**	1.000	0.182	0.189	0.190
BMI	-0.303*	0.140	0.197	0.222	0.182	1.000	0.017	0.394**
Age	-0.249	0.178	0.291*	0.194	0.189	0.017	1.000	0.168
Number comorbidies	-0.443**	0.201	0.260*	0.201	0.190	0.394**	0.168	1.000

Table 5: Spearman rank order correlation coefficients (Rho) matrix between costs, utility index, BMI, age and number of comorbidities in this study sample.

Note: *: correlation is significant at the 0.05 level; **: correlation is significant at the 0.01 level

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4.3 The additional value of unsupervised exercise training over a 6-month dietary intervention

Introduction

Weight loss and management is the core objective in people with overweight and obesity. This goal can be reached by different strategies going from non-invasive forms of behaviour therapy, pharmacotherapy or invasive approaches weight loss surgery. Although a comprehensive lifestyle change approach with reduced calorie diet and increased physical activity to achieve weight reduction is supported in obesity guidelines, this combined therapy is not always prescribed. Diet is considered to be the cornerstone of obesity management and media's messages about the futility of exercise have lead to confusion to the usefulness of exercise training. It is often seen, also in patient-centred care in which treatment recommendations are customized in response to patients' preferences and beliefs, that the role of exercise training is not emphasized. Frequently, a more general public health approach of physical activity promotion ("You should exercise more regularly") is used instead of individualised exercise prescription and training.[1,2]

Information on the effectiveness of exercise training seems insufficient for policy making. Exercise training does not contribute to initial weight reduction when combined with a hypocaloric diet. However, exercise training has the potential to optimize body composition, enhance cardiovascular fitness, consolidate achieved weight loss, increase cardiorespiratory fitness and reduce metabolic risk.[3] Policy makers are facing the problem how to set priorities in the allocation of limited health care resources to medical or public health interventions. Knowledge on this can be obtained by performing health economic evaluations of treatments providing payers and governments with better insights how to spend the available resources in the most efficient way.

It is well known that suffering from obesity is not healthy and is associated with obesity related comorbidities and an increased risk of nearly every chronic condition.[4] A recent cost-of-illness analysis revealed the same pattern in metabolically healthy women with overweight and obesity.[5] This population shows higher overall and overweight-related comorbidities resulting in higher average (healthcare) costs and lower average health utility as compared to the general population. Regarding public health, every person with obesity should be motivated to achieve a normal weight in the long term. In the meantime, the

reduction of overweight related comorbidities can slow down disease progression.[6] In this point of view, exercise training influences disease development by reducing cardiovascular and metabolic risks. Possibly, this risk reduction is the result of reduced ectopic fat at different locations.[7]

Research is needed to provide the scientific, economic and patient-oriented rationale regarding the impact of exercise prescription on metabolic risk in people with overweight or obesity.
Therefore, the research question of this Randomized Controlled Trial (RCT) was:
What is the effectiveness and cost-effectiveness of a hypocaloric diet intervention alone or in combination with a free but unsupervised exercise training program to the reduction of ectopic fat and amelioration of the metabolic risk in women with overweight and obesity?

Methods

The CONsolidated Standards of Reporting Trials (CONSORT) guidelines 2010 and the Consolidated Health Economic Evaluation Reporting Standards (CHEERS) statement were used to report about this single-blinded randomized clinical trial.[8,9] The study protocol was described extensively in a previous publication.[10]

Participants

Women with overweight and obesity were recruited by endocrinologists at the obesity clinic of the Antwerp University Hospital (tertiary referral facility). Additionally, poster recruitment was used at the University of Antwerp and the Antwerp University Hospital.
In brief, premenopausal women without cardiovascular consequences (diabetes type 2 or cardiac diseases) with a stable BMI of at least 27 kg/m² were included. Non-Alcoholic Fatty Liver Disease (NAFLD) and prediabetes were allowed when no medication therapy was needed. Participants who took medication that influenced body weight or metabolism (e.g. tricyclic antidepressants) were excluded. The use of cardiovascular medication was allowed but it was agreed that changes in the medication regimen could not be made during the study. Since medical imaging techniques were used, general exclusion criteria to Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) were applicable.

Intervention

In this six-month intervention study, participants were assigned to a Usual Care Group or an Intervention Group. To minimize the imbalance between baseline values in each group, a minimization technique was used based on age, occurrence of the Metabolic Syndrome and the amount of Visceral Adipose Tissue (VAT).

Usual Care Group (UC Group): A hypocaloric diet with an energy deficit of 500 kcal/day based on the individual resting metabolic rate was prescribed. Subjects participated in seven dietary individual counselling sessions. These consultations had a duration between 30 and 60 minutes and were used to introduce the principles of balanced meals, energy restriction and portion sizes, to discuss dietary patterns and recommend specific changes. At each visit, nutritional compliance was estimated by the dietician on a 0 to 10 numeric rating scale and was based on the feedback of the participant. Possible weight loss was not taken into account. Subjects were labelled as non-adherent when adherence score was lower than 6.5. The participants of the UC Group were asked to continue with their normal physical activity pattern. Participants received motivational support by mail or telephone twice every month following a fixed protocol.

Intervention Group (I Group): In the Intervention Group, the hypocaloric diet of the UC Group was supplemented with a free individualized training program in a fitness or health centre near home. The prescribed program was a combination of aerobic training and strength training to perform three times/week. (supplementary file; appendix 1- training scheme) Exercise sessions were non-supervised but the exercise scheme was instructed by the research physiotherapist and was designed with respect to exercise physiological principles, personal physical inconveniences and possible individual barriers to physical activity and exercise.[11,12] The aerobic exercise scheme was standardized by applying the same exercise volume and progression in each participant based on the results of a maximal cardio pulmonary exercise test (CPET; electronic bicycle ergometer: LODE, Corival; gas analyser system: JAEGER, Oxycon Pro). The exercise intensity of aerobic training was heart rate-driven and set at 90-95% of the heart rate achieved at the respiratory compensation point. Aerobic training was performed on three to four different cardio devices during 30 to 45 minutes in total. Each training day, aerobic training was completed with core stability

training and three strength exercises on isotonic strength training devices for a total of 20 minutes. After 6 months, compliance was scored on a scale from 0 to 9 depending on session duration, exercise intensity and weekly training frequency. Subjects were labelled as non-adherent when compliance score was lower than 6.
Participants received motivational support by mail or telephone twice every month following a fixed protocol.

Outcome Measures

Primary Outcome: Primary clinical outcome measures were the changes in ectopic fat deposition from baseline to three months and six months. The instruction was given to avoid consuming high fat loaded food and doing excessive physical effort three days prior to the clinical tests.
A single-slice CT of the abdomen (GE, Lightspeed VCT) was used to evaluate VAT (cm^2) and SAT at the L4-L5 region according previously described methods.[13]
Multi-slice end-diastolic ECG-triggered CT was used to measure pericardial fat areas (PCF, cm^3) and epicardial fat areas (ECF, cm^3). PCF and ECF were manually delineated slice-by-slice starting at the pulmonary artery and ending at the apex of the heart using the workstation (GE, AW Volumeshare 2), according to previously described methods.[14] Tissue with attenuation values in the interval of -30 to -190 Hounsfield units were considered to be fat. 1H-Magnetic Resonance Spectroscopy (1H-MRS, SIEMENS MAGNETOM Prisma 3T) was used to quantify ectopic fat in the right Tibialis Anterior muscle (Intra MyoCellular Lipids or IMCL - /creatine) and the liver (Intra Hepatic Lipdis or IHL - % in ref. to water). Pre-processing of all spectra was standardized using the jMRUI2XML software and quantification was done using Amares in the jMRUI software version 5.2.[15,16]

Secondary Outcome: All anthropometric measurements were performed in fasting conditions. Waist circumference (WC) was measured at the midlevel between the lower rib margin and the iliac crest. Body composition was determined by bio-impedance analysis (BIA) as described by Lukaski et al.[17] and body fat mass percentage was calculated using the formula of Sun et al.[18] Systolic and diastolic blood pressure (SBP and DBP) was determined after lying down for at least 5 minutes, using a mercury sphygmomanometer at the right arm.

A blood analysis (from an antecubital vein) was taken at the start of the study and after six months and analysed for fasting glucose (FG) and the lipid profile (total cholesterol, High-density Lipoprotein (HDL-C), Triglycerides (TG))

Costs and quality of life measures were assessed at baseline, after three and six months using self-reported cost diaries and the EuroQol (EQ-5D-5L) questionnaires.[19] The EQ-5D-5L algorithm with the UK - Tariff was used to transform the individual health profiles into utility indices (i.e. a health-related quality of life weight ranging between 0 and 1with "1" indicating perfect health and "0" dead). Direct medical costs (e.g. medication use, (para)medical consultations and assessments, hospitalization) direct non-medical costs (e.g. use of braces, incontinence pads) and indirect non-medical costs (absenteeism from work, unemployment) were calculated. Costs of health care consumption were calculated using the following formula: units consumed * unit price and were reported in a non-aggregated form in 2017 euros. No discounting was needed given the time horizon of six months. If participants did not mention unit prices of care providers, the fees determined on 01/01/2017 were searched on the website of the National Institute for Health and Disability Insurance (NIHDI). [20-22] Hereby, it was assumed that patients consulted accredited health care workers and the patient had right to the normal reimbursement rate. If it was not possible to reconstruct the true healthcare cost, the billing service tariff of the University Hospital of Antwerp was applied. Medication costs were calculated based on the units consumed through the Belgian commented online drug compendium. Absenteeism was analyzed as an indirect cost and valued as 288.00 euro per day following the whitepaper of SECUREX, a company providing social secretary services in Flanders.[23] The societal cost of unemployment was estimated to be 92.62 euro per day, based on a report from the European Federation for Services to Individuals.[24] This study was conducted from a societal perspective. Quality adjusted life years (QALYs) were calculated by multiplying the utility index level for a given condition with the time period an individual lived with the condition.

Differences in costs and QALYs between UC and I Group, would permit to calculate the Incremental Cost-Effectiveness Ratio (ICER) and hereby define the cost-effectiveness of the intervention compared with the control group.

The ICER is calculated as follows: ICER= $Cost_I$ - $Cost_{UC}$ / $QALY_I$ - $QALY_{UC}$. In case no

significant between-group differences are found in the primary and secondary outcomes, the Average Cost-Utility Ratios (ACUR) will be calculated by dividing the societal direct medical, direct non-medical and indirect costs by the utility score for each group (I and UC) separately. ACURs are reported as euro per QALY. Changes in costs and QALY between baseline and six months were calculated.

Notwithstanding the fact that the dietary and exercise treatments were free of charge for participants in this study, the real cost was estimated. By questioning travel costs and extra costs related to the treatment (f.i. sporting gear), the total cost for the participant could be estimated. This was added to the mean cost of seven dietary counselling sessions and the mean cost of a fitness membership over six months.

Data Analysis

Assuming a two-sided α of 0.05 and a power of 0.95, a sample size of 39 women was required to detect IHL changes. The effect size was calculated based on IHL data from the exercise group in the Hallsworth et al. study.[25] Since dropout rate in lifestyle intervention studies can be up to 35% in people with overweight, we concluded to include 60 women at baseline.[26,27]

A repeated measures ANOVA was used to detect changes in the total group over three time points (T0, T3 and T6 months) and a paired samples T-test for parameters measured only at baseline and after six months, costs and QALYs. Sphericity was checked with Mauchly's Test of Sphericity. When sphericity was violated a Greenhouse-Geisser correction was used. Relationships between ectopic fat changes and changes in other metabolic parameters were assessed. All values are expressed as means ± standard deviations (SD).

Linear mixed models were fitted to test if the change in ectopic fat over the three time points was different between the UC and I Group. For each ectopic fat parameter, a separate model was fitted, with ectopic fat as the dependent (outcome) variable. The independent variables (fixed effects) included time, treatment and their interaction. To account for the non-independence between observations within the same individual, a random intercept term for individual was added to the model. The significance of the interaction term, that tests whether the effect of the UC Group was different from the I Group, was assessed by comparing the model with the group*time interaction term to a model that only included the main effects of time and treatment, using an F-test with a

Kenward-Roger correction for degrees of freedom. Furthermore, it was tested whether there was a difference in outcome (fat) between the three time points. Pairwise differences between time points were tested using a Tukey correction for multiple comparisons.

Results

Flow of participants

The study participants flow chart is shown in figure 1.

Dropout rate was 15 %. There were five "early dropouts" (before measurements at month three) and four "late dropouts" (before measurements at month six). Reasons for leaving the study were similar in both groups and were mostly due to lack of time.

There was no significant difference in baseline values between UC and I Group regarding primary and secondary outcomes (table 1). NAFLD and Metabolic Syndrome were seen in seventeen (28%) and three (5%) women in the total sample respectively.

Changes in ectopic fat and metabolic risk factors in the total sample

Figure 2 shows the changes in the total sample in ectopic fat deposition sites. Ectopic fat content decreased in all places after 6 months, except in the case of PCF and IMCL. VAT and SAT significantly decreased over the intervention period of six months ($F(2,92)=18.190$, $p<0.001$; resp. $F(2,92)=36.362$, $p<0.001$). Also ECF ($F(2,94)=32.736$, $p<0.001$) and intrahepatic lipid content ($F(2,96)=9,098$, $p<0.001$) decreased significantly after six months in the total sample.

Figure 3 shows the changes in the total sample in parameters of the Metabolic Syndrome. WC in the total group changed significantly after six months ($F(2,98)= 33.143$, $p<0.001$). Also TG decreased significantly after six months ($p=0.003$). No significant changes were found for HDL-C ($p=0.664$), FG ($p=0.633$), DBP ($F(2,74)=0.445$, $p=0.605$) and SBP ($F(2,76)=1.195$, $p=0.308$).

Few correlations were found for the total sample between the changes in parameters of the Metabolic Syndrome and changes in ectopic fat: only change in SBP after three months was correlated with change in VAT after three months ($R^2=0.427$, $p=0.003$). Furthermore, changes in WC were weakly to moderately and positively correlated with changes in VAT ($p<0.05$).

Differences between the UC Group and the I Group

No significant differences were found in changes in mean ectopic fat or mean metabolic parameters over six months between the two groups. In table 2, the clinical changes over time are presented in each group. In table 3, the interaction terms can be found.
In addition, changes and 95% confidence intervals of the change in the primary output parameters are presented in figure 4. Excluding non-adherent participants for the analysis of differences between both groups revealed no new results.

Cost-effectiveness

One participant of the UC Group was excluded from this analysis since it was an outlier regarding direct medical and non-medical costs. This woman showed direct medical and non-medical costs which were factor 10 of the mean cost in the UC Group. The indirect cost of productivity loss was assessed and it was seen that the societal cost of unemployment and absenteeism was a major cost driver in this study. Since the costs of unemployment and absenteeism were twice as high in the UC Group (in comparison with the I Group), these costs were not included. After correction for these variables, there were no differences between medical and non-medical costs and thus total health care costs in both groups at each time point. Table 4 depicts an overview of direct medical costs, direct non-medical costs, indirect costs and QALY's.
The UC Group had a mean real intervention cost of 219.46 ± 61.79 euro while in the I Group a mean cost of 688.70 ± 299.84 euro was found. Since no between-group differences were observed in mean ectopic fat or mean metabolic parameters, the ACURs were calculated. There was a decrease of the ACUR after six months in the UC and I Group. In the UC Group the ACUR decreased from 486.19 euro to 351.50 euro. In the I Group, the ACUR decreased from 876.23 euro to 598.76 euro.

Discussion

Ectopic fat deposition is more strongly associated with type 2 diabetes and cardiovascular disease risk than generalized obesity.[28-30] This study is, to our knowledge, the first one that examined the additional value of an individualized exercise scheme to a hypocaloric diet regarding decreases of ectopic fat in multiple regions.

A significant reduction of ectopic fat of the heart, liver and abdomen was seen after six months hypocaloric diet whether or not combined with unsupervised exercise training. Previous studies were inconsistent regarding the changes of IMCL after lifestyle interventions and their relation with metabolic risk. [31,32] A decrease of IMCL would be expected in accordance with weight loss. Although, an increase in metabolic flexibility after exercise training can result in IMCL increase together with high oxidative capacity and enhanced insulin sensitivity.[31,33,34] In this regard, it would be interesting if data from IMCL could be compared with insulin action and oxidative capacity.[35]
Ectopic fat can also be a flexible fat depot that can increase or decrease after a single bout of exercise or a short-term dietary intervention.[36] Since instructions were given to avoid high fat loaded food and exercise training prior to the clinical exams, we suggest that the obtained results are the effect of the treatment.

The reduction of ectopic fat deposition can yield important clinical benefits. Particularly in the liver, a mean reduction of 3.5% IHL was found. Hepatic steatosis was diagnosed in 17 women at baseline because IHL content exceeded 5.5%.[37] After the treatment period, the hepatic liver content was normalized (<5.5%) in 11 of these women. In five women, hepatic liver content was still higher than 5.5%. (There was 1 missing measurement of IHL due to dropout) Greater reductions of IHL (to 5%) were found in a meta-analysis of the literature.[31]

No differences between diet only and the combined therapy (diet + exercise) were seen. The reduction of ectopic fat and amelioration of the metabolic risk was similar in the usual care group and the intervention group. It is possible that a hypocaloric diet remains the most important lifestyle factor and thereby mask additional effects of exercise therapy.[38,39] Although, the rather small effect of exercise training was also concluded in the meta-analysis of the literature, there are some RCT's in which exercise training show an additional value over diet in the reduction of abdominal and hepatic fat content.[40,41] In the study of Goodpaster et al., compliance was promoted and controlled using pedometers and exercise videos and participants could receive small financial incentives for adherence to the program. Possibly, this could have lead to better exercise compliance and lower dropout rate (9%).

The used training volume was also high in both studies (5-7 exercise sessions/week and a possible higher training intensity). There is good evidence that a higher exercise intensity yields greater reduction of ectopic fat and health benefits.[42] Despite the fact that dose responsiveness between exercise and health effects is well established, the question should be if unsupervised exercise training (with training volume based on current exercise guidelines) is achievable by patients themselves. In this study, half of the fitness centers had a digital registration system in which training intensity, frequency and duration was checked afterwards. It was seen that the prescribed exercise intensity during aerobic training (target heart rate) was sometimes not reached during training because participants had lowered the load by themselves.

Maybe, it is more realistic and encouraging for sedentary people to focus on taking little steps, e.g. by getting less sedentary and a bit more active in daily life. This is in line with current Flemish guidelines, summarized in the Physical Activity Triangle.[43] Maybe, small incremental increases in physical activity may pave the road to more physical activity and exercise training? Whenever the goal is to achieve the training volume of current exercise guidelines, exercise prescription might not be the best tool. Exercise should be guided and supervised by exercise professionals. In this way, an environment is created in which people with overweight feel safe, accepted and encouraged to exercise, taken barriers about exercise training, negative beliefs and expectations into account.[44,45] In this regard, a controlled and encouraging exercise program with higher training volume in a supervised setting seems a better choice in sedentary patients with overweight or obesity. This can lead to better exercise compliance with low dropout rate.

The changes in ectopic fat were not linear between baseline, three and six months (table 2, figure 4). A strong decrease was seen after three months of diet or diet and exercise. No significant changes were seen between time points three and six months. The (non-)linearity of ectopic fat decrease is not discussed in literature. Only one other study was found in which ectopic fat was measured in between the treatment period of six months with similar non-linear decreases of liver fat and VAT.[46] In numerous shorter intervention studies, changes in ectopic fat occured after a three weeks to three months diet or exercise program.[47-50] According to multiple intervention studies, minimal study duration to decrease ectopic fat in the liver, the abdomen and the heart is four weeks. In this regard,

long-term (6-12 months) studies of ectopic fat reduction might be combinations of ectopic fat reduction followed by variable maintenance of ectopic fat.[51-54]

In this RCT, strict inclusion and exclusion criteria were used resulting in a homogeneous population of young women with overweight or obesity. Major comorbidities were excluded resulting in benign metabolic profiles (see table 1). Since mean metabolic parameters were within normal ranges, only few changes in Metabolic Syndrome parameters happened after six months treatment. The significant reduction of the waist could be expected because of the decrease of VAT and SAT. Furthermore, there was a significant decrease of triglycerides after six months.

Besides an assessment of clinical outcomes, an adapted economic analysis was performed. As expected, healthcare costs observed in this study were skewed.[55] Some participants showed direct medical and non-medical costs that were up to a 5-fold of the mean costs (from the societal perspective). No significant differences in costs and QALYs between UC and I Group were found. These results should be interpreted with care, taken the skewness of the data into account.
Because there were no clinical differences in outcome between treatment groups, the Incremental Cost-Effectiveness Ratio (ICER, the ratio of the difference in costs to the difference in effectiveness) was not calculated. In addition to the ICER, the ACUR expressing the ratio of the average cost spent per QALY in a single study group (i.e. no incremental analyses between the two study groups) has served as another important measure in cost-effectiveness analyses.[56] In both groups there was a decrease of the ACUR. This means that the average cost per QALY decreased which can be explained by a decrease of societal direct costs after 6 months. The decrease in direct costs was attributed to a decrease in medical costs. Based on the ACUR results, no statement about the cost-effectiveness (i.e. a strategy that costs less and generates more effect than another one) can be made, since no incremental analyses between the UC Group and the I Group are performed. The price of the treatment was three times higher in the I Group as compared to the UC Group. This is the cost of a fitness club membership, patient transport costs and sport gear. Since there was no difference between the results of the UC Group and the I Group, the combination of diet and exercise can not be defined to be cost-effective in the reduction of ectopic fat.

Some limitations of our study need to be acknowledged. Firstly, all participants were women, and because of rigorous eligibility criteria, only premenopausal women without diabetes or thyroid problems were included. Hereby, results might not be generalized. On the other side, since menopausal state was checked, the influence of estrogen levels on fat accumulation was negligible. Secondly, activity of daily living was not controlled and thus total energy balance was not calculated. This might have influenced the results. Thirdly, a full economic analysis was not possible since there were no changes in primary and secondary outcomes between UC and I Group. Since the limited amount of participants for economic analysis and the dropout rate of 15%, results should be interpreted with care.

In summary, a multidisciplinary lifestyle approach seems successful in reducing ectopic fat deposition and improving the metabolic risk profile in women with overweight and obesity. The addition of free but unsupervised exercise training cannot further improve the metabolic health or phenotype over a six-month period.

Tables and figures

	Usual Care: Diet (n = 30)	Intervention Group: Diet + Exercise (n = 32)	*P-value*
General characteristics			
Age (y)	36.11 ± 8.94	37.64 ± 8.53	*0.49*
BMI (kg/m²)	32.27 ± 3.50	32.98 ± 3.60	*0.51*
Participants with NAFLD	n = 5 (17%)	n = 12 (37.5 %)	
Participants with Metabolic Syndrome	n = 1 (3%)	n = 2 (6.25 %)	
Fat parameters			
Intra hepatic lipid content (%)	4.37 ± 7.49	7.08 ± 8.95	*0.20*
Intra myocellular lipid content (/creatine)	7.37 ± 3.82	8.10 ± 5.99	*0.57*
Visceral fat (cm²)	118.78 ± 48.18	115.27 ± 44.89	*0.77*
Subcutaneous abdominal fat (cm²)	493.46 ± 112.07	493.87 ± 76.20	*0.99*
Pericardial fat (cm³)	159.47 ± 55.58	150.81 ± 46.94	*0.51*
Epicardial fat (cm³)	53.73 ± 30.32	65.36 ± 54.65	*0.31*
Body fat (%)	39.20 ± 4.21	39.18 ± 3.83	*0.98*
Fat free mass (kg)	54.70 ± 5.23	54.11 ± 6.26	*0.69*
Metabolic syndrome parameters			
Waist (cm)	100.09 ± 10.13	101.28 ± 8.35	*0.61*
Triglycerides (mg/dL)	105.22 ± 69.19	99.13 ± 43.19	*0.68*
Systolic Blood Pressure (mm Hg)	121.81 ± 14.78	120.00 ± 10.34	*0.58*
Diastolic Blood Pressure (mm Hg)	72.48 ± 9.94	73.93 ± 9.18	*0.55*
HDL-cholesterol (mg/dL)	57.19 ± 10.72	58.63 ± 11.35	*0.55*
Fasting Glucose (mg/dL)	84.56 ± 7.23	86.30 ± 8.57	*0.29*

Table 1: Descriptive statistics of study participants (mean ± SD)

CLINICAL OUTCOME PARAMETERS	BASELINE	MONTH 3	MONTH 6	*change (over time)*		
				Month 3 vs. Baseline	Month 6 vs. Baseline	Month 3 vs. Month 6
BMI (kg/m²)						
UC Group: Diet	32.27 ± 3.50	30.27 ± 3.97	29.91 ± 4.44	*<0.01*	*<0.01*	*0.35*
I Group: Diet + Exercise	32.98 ± 3.60	31.01 ± 3.48	31.06 ± 3.79	*<0.01*	*<0.01*	*0.59*
Intra Hepatic Lipid Content (/water)						
UC Group: Diet	4.37 ± 7.49	1.79 ± 2.93	1.87 ± 2.74	*0.70*	*0.04*	*0.33*
I Group: Diet + Exercise	7.08 ± 8.95	4.66 ± 8.01	3.61 ± 3.63	*0.05*	*0.06*	*0.73*
Intra Myocellular Lipid Content (/creatine)						
UC Group: Diet	7.37 ± 3.82	7.31 ± 4.36	7.04 ± 4.53	*0.93*	*0.67*	*0.67*
I Group: Diet + Exercise	8.10 ± 5.99	7.67 ± 6.99	8.57 ± 7.23	*0.78*	*0.75*	*0.25*
Visceral fat (cm²)						
UC Group: Diet	118.78 ± 48.18	96.83 ± 44.77	98.91 ± 48.05	*<0.01*	*<0.01*	*0.78*
I Group: Diet + Exercise	115.27 ± 44.89	99.63 ± 38.50	99.21 ± 47.78	*<0.01*	*<0.01*	*0.36*
Subcutaneous abdominal fat (cm²)						
UC Group: Diet	495.11 ± 113.86	439.75 ± 130.13	438.65 ± 136.52	*<0.01*	*<0.01*	*0.51*
I Group: Diet + Exercise	490.87 ± 65.62	445.33 ± 91.58	452.11 ± 99.16	*<0.01*	*<0.01*	*0.18*
Pericardial fat (cm³)						
UC Group: Diet	159.47 ± 55.58	148.28 ± 48.49	153.65 ± 56.57	*<0.001*	*0.07*	*0.15*
I Group: Diet + Exercise	150.81 ± 46.94	140.59 ± 43.09	150.59 ± 55.72	*0.17*	*0.83*	*0.39*
Epicardial fat (cm³)						
UC Group: Diet	53.73 ± 30.32	44.00 ± 25.25	43.16 ± 26.30	*0.001*	*0.002*	*0.50*
I Group: Diet + Exercise	65.36 ± 54.65	49.76 ± 45.00	49.64 ± 45.89	*<0.01*	*<0.01*	*0.20*
Body fat (%)						
UC Group: Diet	39.16 ± 4.28	37.52 ± 5.71	37.61 ± 5.54	*0.01*	*0.15*	*0.64*
I Group: Diet + Exercise	39.00 ± 3.76	37.23 ± 4.71	37.95 ± 5.49	*<0.01*	*0.14*	*0.31*
Fat free mass (kg)						
UC Group: Diet	54.78 ± 5.31	52.92 ± 5.23	51.93 ± 5.49	*<0.01*	*<0.01*	*0.34*
I Group: Diet + Exercise	54.11 ± 6.26	52.45 ± 6.28	51.46 ± 6.39	*<0.01*	*<0.01*	*0.14*
Waist (cm)						
UC Group: Diet	100.09 ± 10.13	95.23 ± 8.80	91.43 ± 9.96	*<0.01*	*<0.01*	*0.003*
I Group: Diet + Exercise	101.28 ± 8.35	96.73 ± 9.34	94.88 ± 9.07	*<0.01*	*<0.01*	*0.09*
Triglycerides (mg/dL)						
UC Group: Diet	105.22 ± 69.19	-	84.14 ± 46.88	-	*0.05*	-
I Group: Diet + Exercise	99.13 ± 43.19	-	87.32 ± 41.84	-	*0.03*	-
Systolic Blood Pressure (mmHg)						
UC Group: Diet	121.81 ± 14.78	120.86 ± 14.10	118.05 ± 8.37	*0.68*	*0.15*	*0.82*
I Group: Diet + Exercise	120.00 ± 10.34	118.20 ± 8.20	118.58 ± 8.79	*0.25*	*0.62*	*0.86*
Diastolic Blood Pressure (mmHg)						
UC Group: Diet	72.48 ± 9.94	74.43 ± 8.55	72.86 ± 6.30	*0.50*	*0.71*	*0.52*
I Group: Diet + Exercise	73.93 ± 9.18	74.40 ± 6.05	75.58 ± 6.01	*0.93*	*0.32*	*0.82*
HDL-C (mg/dL)						
UC Group: Diet	57.19 ± 10.72	-	57.24 ± 13.35	-	*0.56*	-
I Group: Diet + Exercise	58.63 ± 11.25	-	58.78 ± 10.26	-	*0.93*	-
Fasting Glucose (mg/dL)						
UC Group: Diet	84.56 ± 7.23	-	83.25 ± 8.90	-	*0.47*	-
I Group: Diet + Exercise	86.30 ± 8.57	-	86.50 ± 15.93	-	*0.85*	-

Table 2: Change in clinical outcome parameters in each group (mean ± SD) and the *P-value*

	p_Interaction	p_Time
Visceral Adipose Tissue (VAT)	0.59	<0.01
Pericardial fat areas (PCF)	0.25	<0.01
Epicardial fat areas (ECF)	0.72	<0.01
Intramyocellular lipids (IMCL)	0.74	0.89
Intrahepatic lipids (IHL)	0.74	<0.01

Table 3: Interaction terms from the linear mixed model analyses

with p_Interaction: interaction term Time*Treatment and p_Time: main effect of time

Economic outcome parameters	**Baseline**	***p-value (group)***	**Month 6**	***p-value (group)***
Societal direct medical and non-medical costs				
UC Group: Diet	405.48 ± 845.97	*0.40*	301.94 ± 535.74	*0.48*
I Group: Diet + Exercise	731.65 ± 1669.53		518.53 ± 1359.65	
Quality-Adjusted Life Years (QALYs)				
UC Group: Diet	0.83 ± 0.16	*0.81*	0.86 ± 0.14	*0.79*
I Group: Diet + Exercise	0.84 ± 0.14		0.87 ± 0.13	

Table 4: Change in direct costs and QALY (mean euro ± SD)

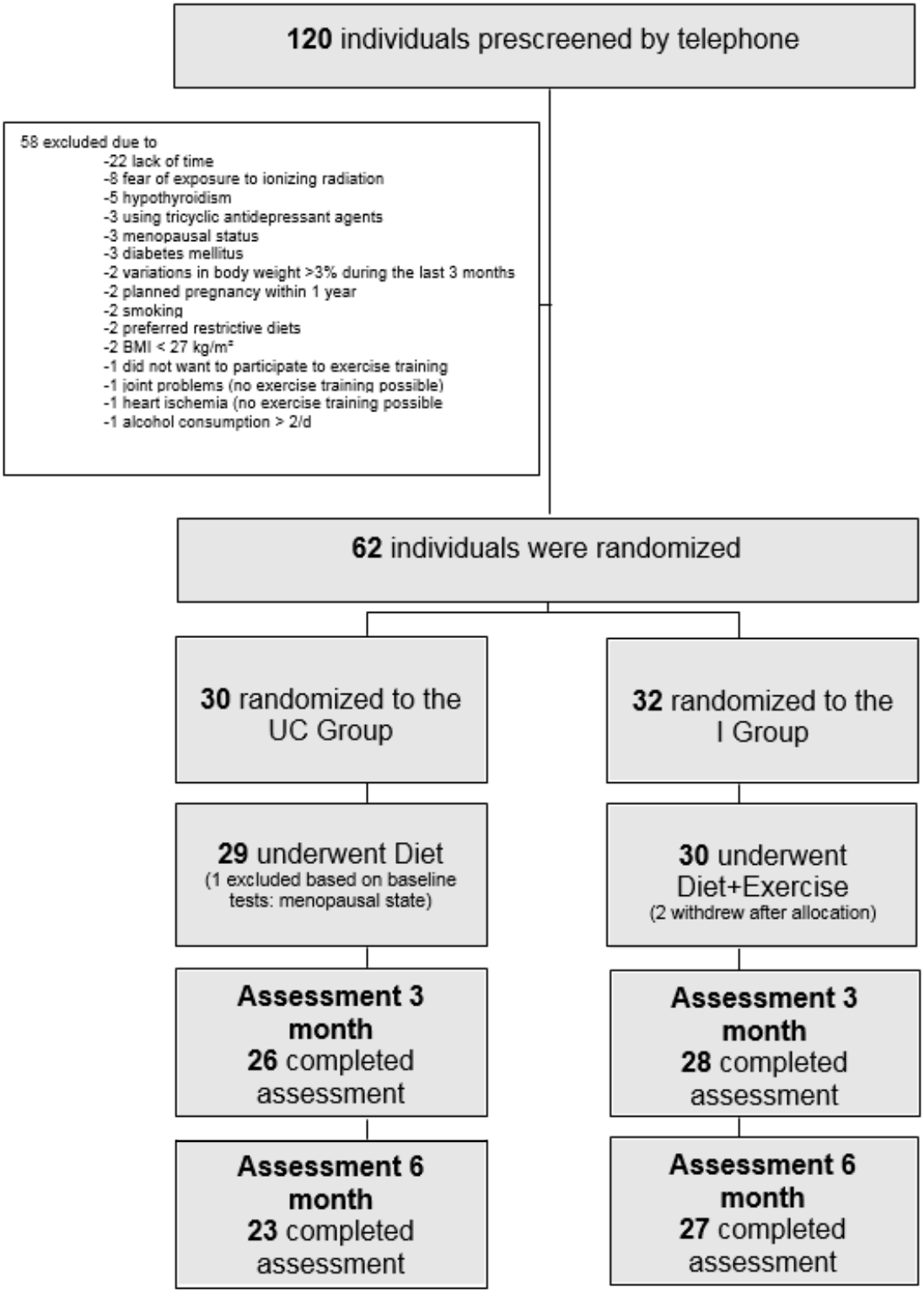

Figure 1: Descriptive statistics of study participants (mean ± SD)

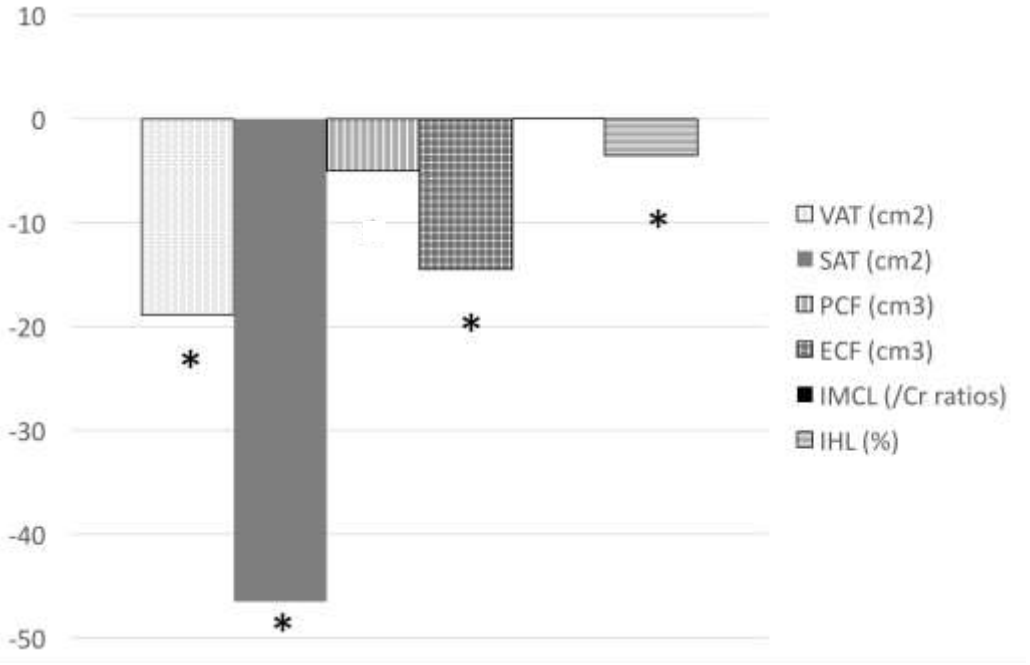

Figure 2: Changes in ectopic fat deposition after an intervention duration of 6 months in the total study sample

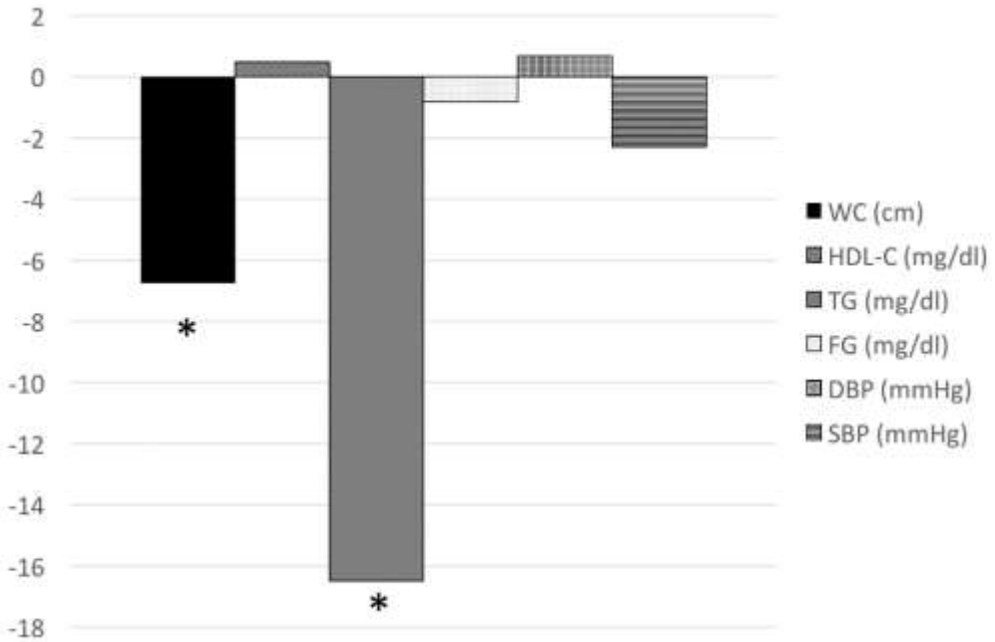

Figure. 3: Changes of the Metabolic Syndrome parameters after an intervention duration of 6 months in the total study sample

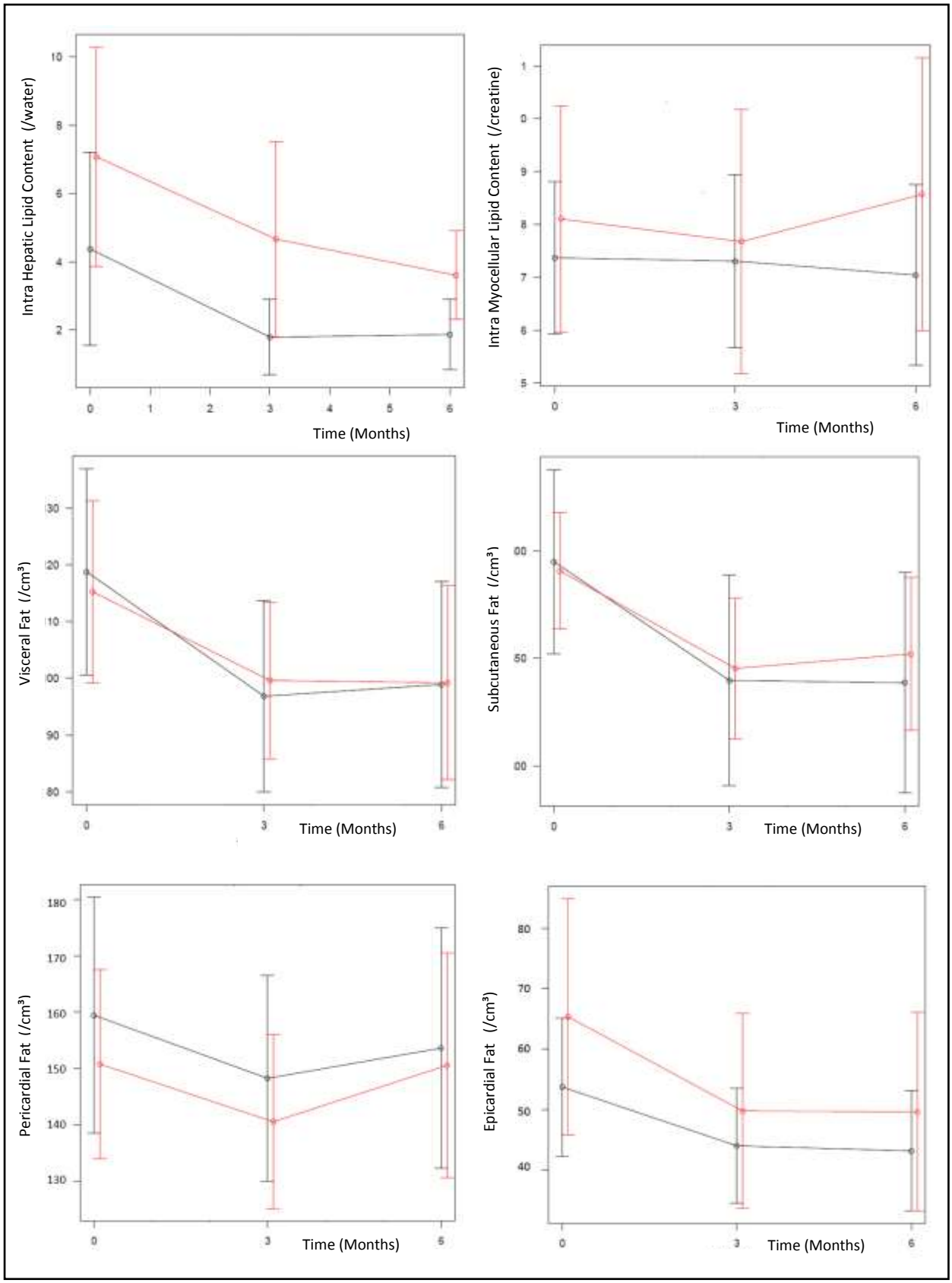

Figure 4: Mean values of ectopic fat deposition and subcutaneous fat at each time point. The error bars represent the 95% confidence interval around the mean.

With **black: Usual Care Group (Diet)**; red: Intervention Group (Diet+Exercise)

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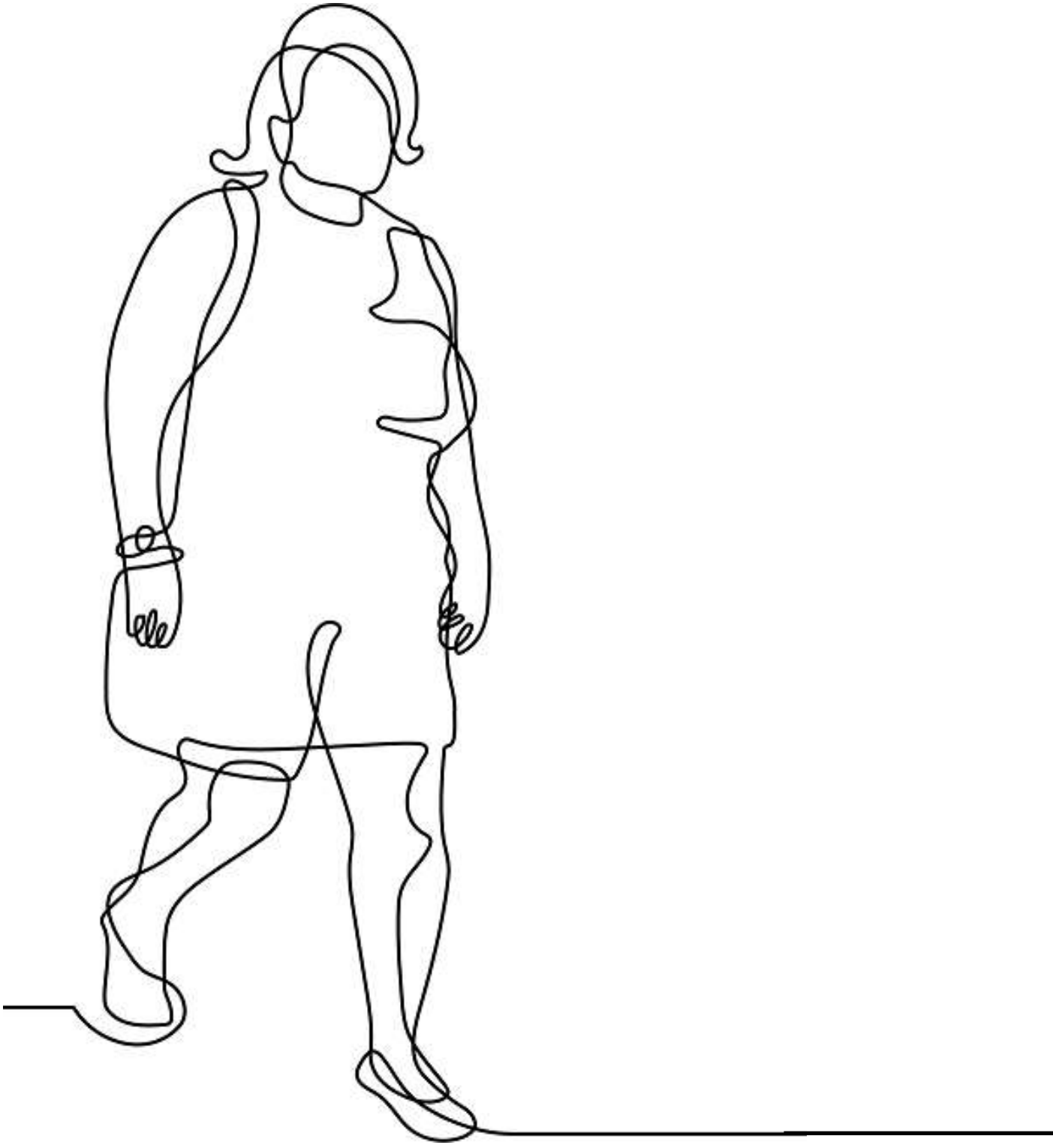
CHAPTER 5

General discussion and conclusion



In **Chapter 2**, the evidence for the use of a non-invasive weight loss intervention (diet and/or exercise) in adults (Part 2.1) or children and adolescents (Part 2.2) with overweight or obesity based on its effect on ectopic adiposity was presented.

In **Chapter 3**, the methods of a randomized controlled trial and cost-effectiveness analysis were described.

In **Chapter 4**, original research intervention results were presented.
In part 4.1, the repeatability and variability of 1H-MRS to quantify IntraMyoCellular Lipids (IMCL) and Intra Hepatic Lipid (IHL) accumulation was assessed in a clinical context.
In part 4.2, the health status (i.e. the comorbidities) and the corresponding costs in a sample of premenopausal non-diabetic overweight or obese females were described in a Cost-Of-Illness analysis (COI).
In part 4.3, the effectiveness and cost-effectiveness of a hypocaloric diet whether or not in combination with an unsupervised exercise training program was presented, based on its effect on ectopic adiposity.

5.1 Summary of findings

This thesis focused on lifestyle interventions in women with overweight and obesity.
It was hypothesized that ectopic fat deposition is the driver to the development of metabolically unhealthy obesity. Since ectopic fat deposition can be influenced by lifestyle, the added value of exercise over a hypocaloric diet was assessed.

Chapter 2 described the results of two rigorous meta-analyses.
Both studies showed that lifestyle interventions including hypocaloric diet, physical activity or a combined intervention had the potential to decrease ectopic adiposity in the liver in overweight and obese children, adolescents and adults. A dietary intervention was dominant and resulted in the greatest decrease in hepatic fat content in adults with overweight and obesity (in comparison with an exercise-only intervention or a combined intervention of diet and exercise). A decrease in IHL of 2% could be obtained in children and adolescents and a decrease of 5% could be obtained in adults. A decreasing of cardiac adiposity was seen in adults and preliminary results on pancreatic adiposity looked promising.
The results on IMCL after lifestyle interventions were, as expected, not consistent in children, adolescents and adults.

Chapter 3 described the methods of a randomized controlled trial, designed to evaluate the cost-of-illness of overweight and obesity as well as the effectiveness and cost-effectiveness of a hypocaloric diet intervention whether or not in combination with an unsupervised exercise training program.

Chapter 4 described results of the RCT.
Part 4.1 showed that it is possible to assess IHL and IMCL in a non-invasive way in women with overweight or obesity using the 1H-MRS infrastructure at the Antwerp University Hospital. IHL and IMCL content were very individually different in a rather homogenous population of premenopausal women with overweight and obesity, resulting in a skewed dataset with a long tail to higher ectopic fat content.
Although the coefficient of variation (CV) is widely used to test repeatability in IHL assessment by H-MRS, this might not be correct to use in a skewed data set. In order to

meet the clinical interpretation of obtained results, parametric statistics were preferred over logarithmic transformation of the data.
The CV of IHL assessment was 37.0% in the total sample and 24.10% in women with NAFLD. These results showed that the technique is more suitable for the quantification of liver fat in people at risk for liver fibrosis, than in an overall obese population.
The CV of IMCL assessment was 51.2% in the total population.

In part 4.2 the health status and health-related costs of study participants was assessed using a three months self-reported recall questionnaire. All results were compared with reference data of the general female population in Flanders and reference data from a COI in obese men and women in the larger Ghent area. [1-4]
Notwithstanding the majority of our study participants (95%) could be categorized as metabolically healthy obese (MHO), the average number of the self-reported comorbidities was striking. Compared to the general female population in Flanders, prevalence of all listed disorders was a factor 0.1 to 10 times higher in the study, leading to increased drug consumption. Nevertheless, average utility scores (representing Quality of Life) and direct costs were similar to those of the general Flemish population.
Productivity-loss or absenteeism was an important cost driver for the society in this study sample.

Part 4.3 showed that a multidisciplinary lifestyle approach was successful in reducing ectopic fat deposition (liver, heart and abdomen) and improving the metabolic risk profile in women with overweight and obesity. Changes in WC were weakly to moderately correlated with changes in visceral adipose tissue (VAT).
There were no significant differences between the diet-only group (Usual Care Group - UC) and the combined group of diet and exercise (Intervention Group - I).
The Average Cost Utility Ratio (ACUR) decreased after six months in both groups. This means that the average cost per QALY decreases and this is mainly the result of a decrease of societal direct (medical) costs after 6 months.

5.2 Discussion and conclusion

In this final discussion and conclusion, some themes are explored in-depth.

Both meta-analyses showed that lifestyle interventions including hypocaloric diet, physical activity or a combined intervention had the potential to decrease ectopic adiposity in the liver in overweight and obese children, adolescents and adults. [5, 6] In most included studies, training volume failed to meet exercise recommendations and was therefore insufficient; This can be an explanation why the effect of diet was equal to or higher than the combination of diet and exercise.

The COI revealed that the average number of the self-reported musculoskeletal problems was much higher than in general population leading to significant more medication use. Taking the concept of load and tolerance into account, exercise prescription should be tailored and exercise professionals should be involved. [7] Physiotherapy staff might be best placed to perform pre-participation screening, prescribe exercise as part of a structured, safe and effective program, supervise and make adjustments when needed. [8]
Moreover, depressed mood status was about 5 times more prevalent than in the general adult female population. [4] Literature confirms that higher levels of anxiety and depression are seen in people with overweight and obesity. [9] Despite relative normal average utility scores (representing Quality of Life), 13.5% of our participants showed utility scores comparable with those reported by people suffering from Parkinson disease or severe rheumatic disorders. [10]
These results confirm that medical, psychological and social problems are associated with overweight or obesity and emphasize the importance of a comprehensive multidisciplinary approach to obesity management. [11] Different aspects of behavior therapy should be managed by professionals taken psychological and physical (dis)abilities into account.
It is seen that unemployment and work absenteeism were cost drivers regarding the indirect costs. The extrapolation from three months of data towards twelve months may have led to an overestimation of absenteeism and unemployment. However, the high absenteeism rate is consistent with the findings of Seidell et al. who described increased absenteeism in obese females and related those to medical problems such as mental health disorders or

musculoskeletal problems. [12] Because intangible costs were omitted, the total economic burden of overweight and obesity may have been underestimated. [13] Also, this COI was conducted in a sample of mostly metabolically healthy obese women without diabetes or any other major comorbidities and therefore may have underestimated the true costs of overweight and obesity on a population level.

Data from the meta-analysis in adults were comparable with data from our RCT study. It could be concluded that lifestyle interventions have the potential to decrease ectopic adiposity in the liver, the heart and abdomen in overweight and obese adults.
A mean absolute reduction of IHL between 2.5% and 3.5% was seen in the RCT, which was somewhat lower than the reduction of 5%, described in our meta analysis. [5] Since the coefficient of variation of IHL and IMCL assessment was rather high in the studied sample, 1H-MRS results of the intervention should be interpreted with care.
Besides this, the mean reduction of visceral fat between 16.0 cm^2 and 20.0 cm^2 was also lower than the 30 cm^2 described in our previous meta-analysis on visceral fat. [14]
Next to a decrease in IHL and visceral fat, a decreasing ectopic adiposity of the heart was seen. Over the last decade, it was repeatedly confirmed that cardiac steatosis is a hallmark of obesity and diabetes mellitus type 2 (DMII). [15-17] A reduction of cardiac lipid content could be associated with an improvement of the left ventricular ejection fraction. [18]
In de meta analyses of adults and children as well in the data from the RCT study, no effects of lifestyle interventions on IMCL were found. IMCL is discussed in literature about the association with metabolic health. [19, 20] A correct interpretation of IMCL could only be made by evaluating the mitochondrial capacity or oxidative enzyme activity. [21-24]
Unfortunately, only overall (and not local) insulin sensitivity could be assessed (by an oral glucose tolerance test) and this was only done at baseline. Regarding reliability of the MRS technique, additional assessment of intra-session repeatability might have given insight in the contribution of the physiologic variability in IMCL.

Following the literature research, a dietary intervention was dominant and resulted in the greatest decrease in hepatic fat content in adults and children with overweight and obesity. [5, 6] This might be a first reason why there were no differences between clinical results of

the Usual Care Group and the Intervention Group. The huge impact of a hypocaloric diet could easily have masked additional short-term effects of exercise therapy. [25, 26]
Since some randomized controlled trials conclude that physical activity has an additional value over diet in reducing abdominal and hepatic fat content, we expected to find an additional value of exercise training over a hypocaloric diet. [27, 28] The training volumes used in literature met exercise guidelines and were comparable with our exercise prescription. Even though, by not controlling activities of daily living in the RCT, total energy balance could not be calculated and may have led to a bias. It is possible that patients "compensated" for their training by being more physically inactive afterwards. [29]
By controlling patient training adherence, it was seen that mean training frequency was generally only twice per week, mainly due to (self-reported) lack of time. Hereby, the training volume was lower (120 min/week) than prescribed.

Although dose responsiveness between exercise and health effects is well established, It could be argued that current exercise guidelines are not always feasible for sedentary patients with negative beliefs about physical activity and exercise. Maybe it is more realistic and encouraging for unfit people to focus on taking little steps, e.g. by getting less sedentary and a bit more active in daily life. This is in line with current Flemish prevention guidelines, summarized in the so-called Physical Activity Triangle. [30] Also, new Physical Activity Guidelines for Americans highlight that any amount of physical activity has some health benefits. [31] Maybe, small incremental increases in physical activity may pave the road to more physical activity and exercise training?
Next to this, the concept of high-intensity training (HIT) is gaining interest in patient settings. HIT is characterized by relatively short (45s to 4 min) bursts of repeated vigorous activity or sprint sequences (in case of sprint interval training, SIT), interspersed by periods of rest or low-intensity exercise for recovery. [32, 33] The greatest advantage of this training might be the time-efficiency. [34] As little as 3 HIIT sessions per week, involving $\leq$10 min of intense exercise within a time commitment of $\leq$30 min per session, has been shown to have training effects in patients with cardio metabolic disorders, [35] A meta-analysis showed comparable results between HIT and moderate-intensity continuous training regarding adiposity reductions. [36] On the other hand, HIT training might not always be suitable for sedentary patients. Since this training form feels hard, this can lead to avoidance of adoption of the

activity, or drop out due to the associated negative feelings. [37, 38] Poor implementation and maintenance in daily life might be associated with HIT. [39]
Regarding this intervention study, HIT training might have been feasible in some patients without musculoskeletal disorders. [40] Although, I strongly believe that the non-supervised setting might not have been ideal for any kind of training in which a certain training volume (intensity and/or frequency) is expected. Training in people with overweight and obesity should be initially guided and strictly supervised. Literature shows that even after a five month supervised training program, sedentary healthy adults are unable to meet the recommendations of minimal exercise by unsupervised exercise prescription. [41] In this regard, it seems essential that a supervised environment is created in which people feel safe, accepted and encouraged to be physically active, taking barriers about exercise training, negative beliefs and expectations into account. This can lead to better exercise compliance with low dropout rate. [42, 43]

This doctoral thesis knows some limitations.
An exhaustive economic analysis of the RCT study results was not possible since there were no significant differences between Usual Care and the Intervention Group. Since the limited amount of participants for economic analysis and the dropout rate of 15%, results should be interpreted with care.
Only women were included in the RCT. Since body fat distribution shows gender differences, results might not be generalizable. [44] These differences include not only the amount and distribution of adipose tissue, but also differences in its metabolic capacity and functions. [45, 46]
A power analysis was performed, based on existing IHL data from Hallsworth et al. [47] This study was chosen because the measurement of IHL by MRS is more "mainstream" than measurements of other ectopic fat regions. [5] It would have been more appropriate if a power analysis of each outcome parameter was done and the largest sample size was picked out.
Although a CPET was performed in baseline measurements of participants of the Intervention Group, cardiorespiratory fitness was no outcome measure in this study. Regardless of there was a decrease of ectopic fat or ameliorations of the metabolic risk factors, exercise training increases cardiorespiratory fitness. This leads to reduced mortality and morbidity. [48]

Long-term follow-up studies are needed to investigate the effect of lifestyle interventions on ectopic fat deposits (e.g. pancreatic, renal and perivascular tissues). These studies should provide supervised exercise training by exercise professionals at a sufficient training volume. It is important that regular physical activity levels of participants are controlled, e.g. by activity trackers. This is the only manner to get a more in-depth knowledge on the relation between exercise, body fat distribution and the reversibility of a metabolic risk profile.

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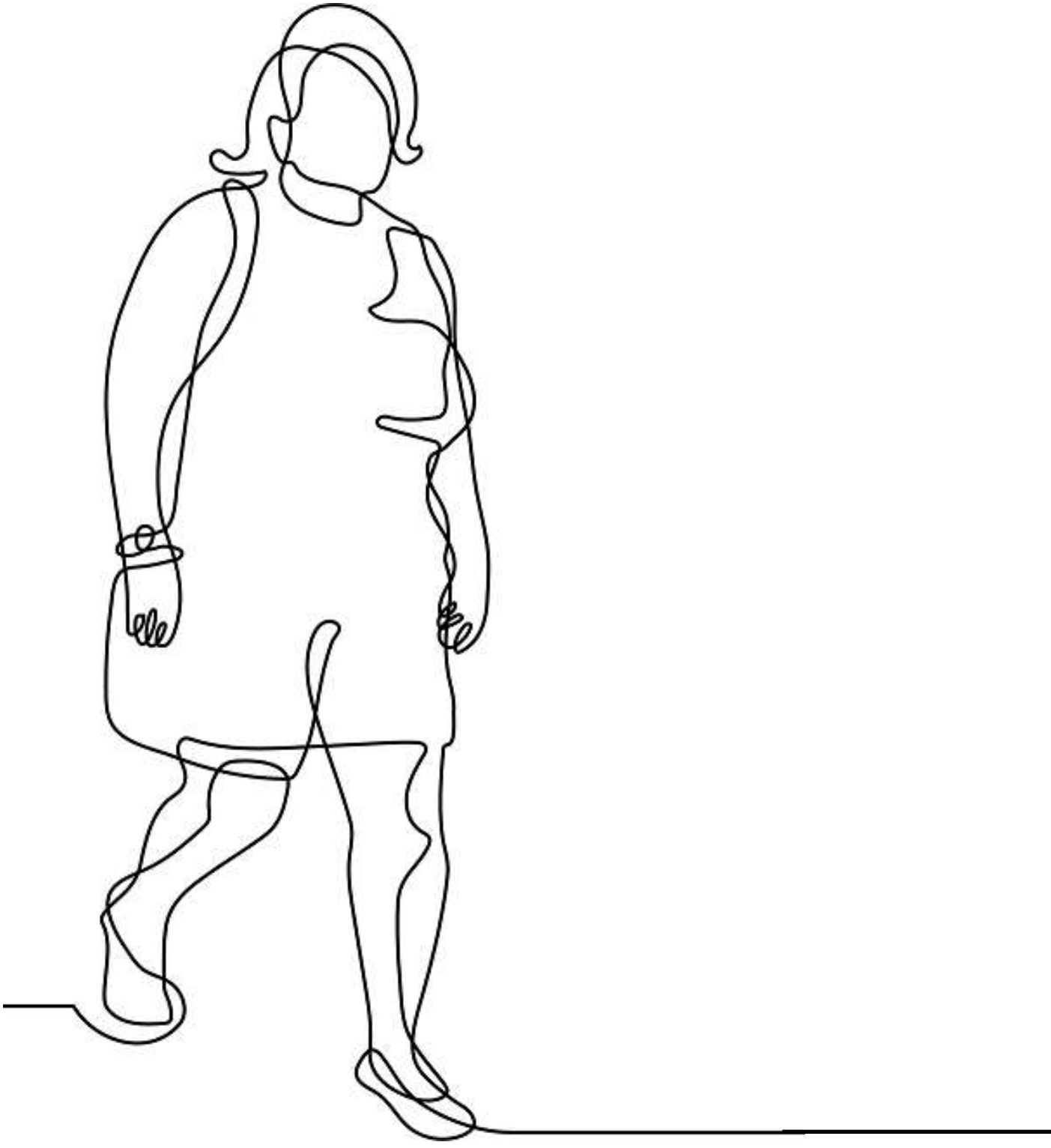
CHAPTER 6



Synopsis

The high prevalence of overweight and obesity and its comorbidities puts a financial burden on health services. In order to slow down disease progression, the reduction of overweight related comorbidities is a primary target in a patients' treatment.
Although a comprehensive lifestyle change approach with reduced calorie diet and increase physical activity is supported in obesity guidelines, this is often not followed in clinical practice.
In general, a hypocaloric diet (supervised by a dietician) is considered to be the cornerstone of obesity management. A more general public health approach of physical activity promotion ("You should exercise more regularly") is used instead of individualised exercise prescription and (supervised) training.

Since ectopic fat deposition can be the driver to the development of overweight related comorbidities, multiple places of ectopic fat deposition were the primary outcome measures in this doctoral thesis. It was hypothesized that exercise training contributes to the reduction of overweight related comorbidities that is preceded by the reduction of ectopic fat deposition.

Although the majority of the sample (95%) encompasses the so-called metabolically healthy obese, the average number of the self-reported comorbidities was striking. The most prevalent complaints were back and neck disorders and joint problems.
Both the rigorous performed literature study and clinical study showed that lifestyle changes have beneficial effects on ectopic fat deposition in the abdomen, liver and the heart.
Since there was no difference between the 'Usual Care Group' (Hypocaloric Diet) and the 'Intervention Group' (Hypocaloric Diet + Exercise prescription), the combination of diet and exercise prescription could not be defined to be cost-effective in the reduction of ectopic fat.
Reason of this is twofold. Firstly, the huge impact of a hypocaloric diet can easily mask additional short-term effects of exercise therapy. Secondly, achieved training volume might be too low in this clinical study. Although, exercise guidelines were respected in the individualized exercise prescription, it was generally seen that mean performed training frequency and intensity was lower than prescribed.

Because people with overweight and obesity might suffer from different (musculoskeletal) comorbidities and have possibly negative beliefs and perceptions about exercise training, it is suggested to create a (supervised) environment in which people feel safe, accepted and encouraged to exercise at a prescribed training volume.

Lekensamenvatting

Het veelvuldig voorkomen van overgewicht/obesitas en overgewicht gerelateerde aandoeningen is een maatschappelijk probleem en drukt op de gezondheidsuitgaven. De afname van overgewicht gerelateerde aandoeningen is een eerste doelstelling in de behandeling van patiënten met overgewicht en obesitas.
Hoewel obesitas richtlijnen pleiten voor een geïntegreerde aanpak van o.a. een hypocalorisch dieet en verhoogde fysieke activiteit, wordt dit vaak niet gevolgd in de klinische praktijk. Over het algemeen wordt een hypocalorisch dieet (gesuperviseerd door een diëtist) beschouwd als de basis binnen de behandeling van overgewicht en obesitas. Een meer algemene benadering van bewegingsadvies ("Je moet regelmatig bewegen") wordt gegeven i.p.v. geïndividualiseerde (gesuperviseerde) oefentherapie. Vetopstapeling op plaatsen die dit niet als primaire functie hebben (m.n.ectopische vetopstapeling) kan de aanleiding zijn tot overgewicht gerelateerde problemen en is een belangrijke uitkomstmaat in dit doctoraatsonderzoek. Het werd verondersteld dat oefentherapie bijdraagt tot de afname van overgewicht gerelateerde aandoeningen en wordt voorafgegaan door een afname van ectopische vetopstapeling.

Hoewel de meerderheid van de deelnemers van de klinische studie voldeed aan het profiel van "metabool gezonde obesen", werden verrassend veel overgewicht gerelateerde aandoeningen gerapporteerd (vnl. rug- en nekklachten en gewrichtsklachten).
Uit de literatuurstudie en de klinische studie werd geconcludeerd dat levensstijlveranderingen een positief effect hebben op ectopische vetopstapeling in de buik, lever en het hart. Omdat er geen verschil gevonden werd tussen de resultaten van de 'Usual Care Group' (hypocalorisch dieet) en de 'Intervention Group' (hypocalorisch dieet + geïndividualiseerde oefentherapie), kon er niet geconcludeerd worden dat de combinatie van een hypocalorisch dieet en geïndividualiseerde oefentherapie kostenefficiënt was in de behandeling van overgewicht of obesitas. Mogelijks is de impact van een hypocalorisch dieet dusdanig groot dat korte-termijn effecten van oefentherapie gemaskeerd worden. Bovendien was de trainingsomvang mogelijks te laag in deze studie. Hoewel de richtlijnen gerespecteerd werden in de voorgeschreven oefentherapie, werd er gezien dat deelnemers dit oefenprogramma niet strikt volgden en zowel de trainingsfrequentie als intensiteit lager waren dan vooropgesteld.
Omwille van het feit dat mensen met overgewicht mogelijks een negatieve perceptie hebben m.b.t. oefentherapie en kampen met diverse overgewicht gerelateerde aandoeningen, wordt er aanbevolen om een (gesuperviseerde) omgeving te creëren in welke mensen zich veilig, geaccepteerd en aangemoedigd voelen om te bewegen volgens een bepaalde intensiteit en trainingsfrequentie.

List of abbreviations

ACUR: Average Cost Utility Ratio

AT: Aerobic Training

AU: Arbitrary Unit

BC: Behavior Counseling

BIA: Bio-Impedance Analysis

BMI: Body Mass Index

BP: Blood Pressure

Carbs.: carbohydrates

CHEERS: Consolidated Health Economic Evaluation Reporting Standards

CON: Control (group)

CONSORT: CONsolidated Standards of Reporting Trials

COPD: Chronic Obstructive Pulmonary Disease

CPET: Cardio Pulmonary Exercise Test

CT: Computed Tomography

CV: Coefficient of Variation

D: Diet

DA: Dietary Advise

DALY: Disability-Adjusted Life Year

DBP: Diastolic Blood Pressure

DMII: Diabetes Mellitus type 2

DXA: Dual-energy X-ray Absorptiometry

E: Exercise

EA: Exercise Advise

ECF: EpiCardial Fat

EMCL: ExtraMyoCellular Lipid

EOSS: Edmonton Obesity Staging System

EPHPP: Effective Public Health Practice Project

EQ-5D-5L: European Quality-of-Life (5 Dimensions) Questionnaire

FFAs: Free Fatty Acids

FG: Fasting Glucose

FSH: Follicle-Stimulation Hormone

GI: Glycemic Index

HDL-C: High Density Lipoprotein

^{1}H-MRS: hydrogen based Magnetic Resonance Spectroscopy

HOMA-IR: Homeostatic Model Assessment Insulin Resistance

HOMA-IS: Homeostatic Model Assessment Insulin Sensitivity

HRmax: Maximum Heart Rate

HRR: Heart Rate Reserve

I: Intensity

ICC: Intraclass Correlation Coefficient

ICUR: Incremental Cost-Utility Ratio

I Group: Intervention Group

IHL: Intra Hepatic Lipids (%)

IMCL: Intra MyoCellular Lipids (/creatine)

IR: Insulin Resistance

jMRUI: java-based Magnetic Resonance User Interface

LFD: Low Fat Diet

LGLD: Low Glycemic Load Diet

MeSH: Medical Subject Headings

MetS: Metabolic Syndrome

MHO: Metabolically Healthy Obesity

Mod.I: Moderate Intensity

MRI: Magnetic Resonance Imaging

MUFA: MonoUnsaturated Fatty Acid

NAFLD: Non-Alcoholic Fatty Liver Disease

NASH: Non-Alcoholic Steatohepatitis

NCEP-ATP III: third report of the National Cholesterol Education Program (Adult Treatment Panel)

NIH: National Institutes of Health (U.S.A.)

NIHDI: National Institute for Health and Disability Insurance (Belgium, RIZIV)

NS: not-significant or non-significance

OGTT: Oral Glucose Tolerance Test

PA: Physical Activity

PAL: Physical Activity Level

PCF: PeriCardial Fat

ppm: parts per milion

PRESS: Point-Resolved Spectroscopy

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analysis

QALY's: Quality Adjusted Life Years

QUICKI: Quantitative Insulin Sensitivity Check Index

RC: Repeatability Coefficient

RCP: Respiratory Compensation Point

RCT: Randomized Controlled Trial

Reps: Repetitions

RMR: Resting Metabolic Rate

1RM: 1 Repetition Maximum

S: significant or significance

SBP: Systolic Blood Pressure

SD: Standard Deviation

SES: Social Economic Status

SOL: Soleus Muscle

ST: Strength Training

SU: SulphonylUrea

TA: Tibialis Anterior Muscle

TE: Echo Time

TG: Triglycerides

TR: Repetition Time

UC Group: Usual Care Group

Vast. Lat: M. Quadriceps Vastus Lateralis Muscle

VAT: Visceral Adipose Tissue (cm^2)

VO2max: Maximal Oxygen Uptake

VO2peak: Peak Oxygen Uptake

VT1: first Ventilatory Threshold

Wattmax: Maximum Load (Watt)

WC: Waist Circumference

Curriculum Vitae

PERSONAL INFORMATION

Name	HENS, Wendy
Date of Birth	26/06/1983

AFFILIATIONS

Antwerp University Hospital (UZA)	Cardiac Rehabilitation Center Wilrijkstraat 10, B-2650 Edegem E-mail: wendy.hens@uza.be
University of Antwerp (UA)	Department of Rehabilitation Sciences and Physiotherapy (REVAKI) Universiteitsplein 1, B-2610 Wilrijk E-mail: wendy.hens@uantwerpen.be

EDUCATION

Master in Physiotherapy	Hogeschool Antwerpen, Merksem Cum Laude, 2006
Languages & Economics	Sint Ursula Instituut, Onze Lieve Vrouw Waver 2001

THESES

PhD Thesis	Exercise, ectopic fat and the metabolic profile in women with overweight and obesity Promotor: Prof. Dr. J. Gielen, Prof. Dr. L. Van Gaal, Prof. Dr. D. Vissers Guidance: Prof. Dr. J. Taeymans
Master Thesis	Het effect van oefenen op een vibratieplatform (Power Plate ®) op lichaamssamenstelling en metabole parameters bij mensen met overgewicht of obesitas die een hypocalorisch dieet volgen. Promotor: Prof. Dr. L. Van Gaal, Prof. Dr. D. Vissers

EMPLOYMENTS

2019-present	Coordinator Physiotherapist Cardiac Rehabilitation, Antwerp University Hospital, Edegem
2008-present	Assistant Department of Rehabilitation Sciences and Physiotherapy, University of Antwerp, Wilrijk
2017-2018	Fellow worker Flemish Institute for Healthy Living, projects: "Liever Actiever" and "Health & Environment", Brussels
2007-2008	Physiotherapist - private practice, Putte
2007-2008	Fitness coach - Synergy Wellness Point, Wommelgem
2006-2008	Physiotherapist - Cardiac Rehabilitation Center - Imeldaziekenhuis, Bonheiden

SCIENTIFIC ACTIVITIES

Articles in international peer-reviewed scientific journals

Hens Wendy, Vissers Dirk, Annemans Lieven, Gielen Jan, Van Gaal Luc, Taeymans Jan, Verhaeghe Nick
"Health-related costs in a sample of premenopausal non-diabetic overweight or obese females in Antwerp region : a cost-of-illness analysis", Archives of public health - ISSN 0778-7367 - 76(2018), 42

Hens Wendy, Vissers Dirk, Annemans Lieven, Gielen Jan, Van Gaal Luc, Taeymans Jan
"Cost-effectiveness analysis of exercise in addition to diet on the reduction of ectopic fat in women with overweight : study protocol for a randomised controlled trial", Physioscience - ISSN 1860-3092 - 13:2(2017), p. 1-8

Hens Wendy, Vissers Dirk, Hansen Dominique, Peeters Stefaan, Gielen Jan, Van Gaal Luc, Taeymans Jan
"The effect of diet or exercise on ectopic adiposity in children and adolescents with obesity : a systematic review and meta-analysis", Obesity reviews - ISSN 1467-7881 - Hoboken, Wiley, 18:11(2017), p. 1310-1322

Hansen Dominique, **Hens Wendy**, Peeters Stefaan, Wittebrood Carla, Van Ussel Sofi, Verleyen Dirk, Vissers Dirk
"Physical therapy as treatment for childhood obesity in primary health care : clinical recommendation from AXXON (Belgian Physical Therapy Association),Physical therapy / American Physical Therapy Association - ISSN 0031-9023 - 96:6(2016), p. 850-864

Vissers Dirk, **Hens Wendy**, Hansen Dominique, Taeymans Jan
"The effect of diet or exercise on visceral adipose tissue in overweight youth", Medicine and science in sports and exercise - ISSN 0195-9131 - 48:7(2016), p. 1415-1424

Cornelis Justien, Taeymans Jan, **Hens Wendy**, Beckers Paul, Vrints Christiaan, Vissers Dirk
"Prognostic respiratory parameters in heart failure patients with and without exercise oscillatory ventilation : a systematic review and descriptive meta-analysis", International journal of cardiology - ISSN 0167-5273 - 182(2015), p. 476-486

Hens Wendy, Taeymans Jan, Cornelis Justien, Gielen Jan, Van Gaal Luc, Vissers Dirk
"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"
Journal of Physical Activity and Health - ISSN 15433080 - (2015), p. 1-19

Cornelis Justien, Taeymans Jan, **Hens Wendy**, Beckers Paul, Vrints Christiaan, Vissers Dirk
Response to letter to the editor: Exercise oscillatory ventilation: perfusion abnormality in heart failure
International journal of cardiology - ISSN 0167-5273 - 187(2015), p. 103-103

Vissers Dirk, **Hens Wendy**, Taeymans Jan, Baeyens Jean-Pierre, Poortmans Jacques, Van Gaal Luc
"The effect of exercise on visceral adipose tissue in overweight adults : a systematic review and meta-analysis", PLoS ONE - ISSN 1932-6203 - 8:2(2013), e56415

Articles in national peer-reviewed scientific journals

Hansen Dominique, **Hens Wendy**, Peeters Stefaan, Wittebrood Carla, Van Ussel Sofi, Verleyen Dirk, Zwaenepoel Bruno, Vissers Dirk
"Kinesitherapie als onderdeel van eerstelijnsbehandeling voor obesitas bij kinderen en adolescenten : klinische aanbeveling van AXXON (Belgische vereniging voor kinesitherapie) in primaire gezondheidszorg" Sport en geneeskunde: the Flemish/Dutch journal of sports medicine and sports science / Vlaamse Vereniging voor Sportgeneeskunde [Gent]; Nederlandse Vereniging voor Sportgeneeskunde [Bilthoven] - ISSN 1874-6659 - 1(2016), p. 1-21

Articles under consideration

Hens Wendy, Vissers Dirk, Vanhevel Floris, Van Gaal Luc, Gielen Jan
"Repeatability of 1H-MRS to quantify ectopic lipid content in the liver and the Tibialis Anterior in women with overweight and obesity", submitted

Hens Wendy, Vissers Dirk, Taeymans Jan, Verhaeghe Nick, Gielen Jan, Van Gaal Luc
"Unsupervised exercise training cannot improve the metabolic health or phenotype over a 6-month dietary intervention: a randomized controlled trial with an embedded economic analysis.", submitted

Oral presentations

Hens Wendy
"Unsupervised exercise training cannot improve the metabolic health or phenotype over a 6-month dietary intervention: a randomized controlled trial with an embedded economic analysis" - Belgian Association for the Study of Obesity; Free Communications Meeting, Belgium (2019)

Hens Wendy
"Health-related costs in a sample of premenopausal non-diabetic overweight or obese females in Antwerp region : a cost-of-illness analysis" - European Congress on Obesity (ECO), Vienna (2018)

Hens Wendy
"Cost-of-illness in people with overweight and obesity" - UA Symposium: Health Economics in Clinical Research, Belgium (2018)

Hens Wendy
"Health-related costs in overweight and obesity" - Belgian Association for the Study of Obesity; Free Communications Meeting, Belgium (2018)

Hens Wendy
"Repeatability of 1H-MRS to quantify ectopic lipid content" - Belgian Association for the Study of Obesity; Free Communications Meeting, Belgium (2017)

Hens Wendy
"Exercise in children and adolescents with obesity" - Public Policy Exchange Meeting "Tackling Childhood Obesity in Europe through awareness and prevention, Belgium (2017)

Hens Wendy
"The use of imaging techniques to quantify ectopic lipid content in people with overweight and obesity" - Proteomics Research Day, Belgium (2017)

Hens Wendy
"The use of imaging techniques to quantify ectopic lipid content in people with overweight and obesity" - Proteomics Research Day, Belgium (2017)

Hens Wendy
"Healthy school environment and walkability" - Octopusplan, Belgium (2017)

Hens Wendy
"Ectopic fat in people with overweight and obesity: rationale and study protocol for a randomized controlled trial"- European Congress of Sports Medicine (EFSMA), Belgium (2015)

Poster presentations

Hens Wendy, Taeymans Jan, Cornelis Justien, Gielen Jan, Van Gaal Luc, Vissers Dirk
"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" - International Congress on abdominal obesity, South Korea (2013)

Vissers Dirk, **Hens Wendy**, Taeymans Jan, Baeyens Jean-Pierre, Poortmans Jacques, Van Gaal Luc
"The effect of exercise on visceral adipose tissue in overweight adults : a systematic review and meta-analysis" - European congress of obesity, France (2012)

Scientific related activities and courses

Symposium "Health economics in clinical research" - UA (2018)
Seminar "Local government: structures and functions" - Vlaams Instituut Gezond Leven (2018)
Training "Health promotion" - Vlaams Instituut Gezond Leven (2018)
Seminar "Sport Inclusion and G Sport" - Kinekring Noordrand Brussel (2018)
Symposia "Cardiopulmonary Exercise Testing" - Acertys (2018, 2017, 2015)
Seminar "School environment 2.0" - Octopusplan (2017)
Seminar "Sport Inclusion" - Sport Vlaanderen (2017)
Seminar Cardiology "Tailored exercise rehabilitation" - UZA (2017)
Training "Logistic Regression" - StatUA (2015)
Symposium "35 years cardiac rehabilitation" - UZA (2014)
Workshop "Publishing connect" - Elsevier, UA (2014)
Training "Microsoft Access" - Bit By Bit (2014)
Summer School "Qualitative Research Methods in Health Care: a basic course" - UA (2013)
Training "Basic Statistics" - StatUA (2013)
Training "Giving presentations in English" - Linguapolis (2012)
Training "Academic writing" - Linguapolis (2012)
Seminar "Diabetes" - Euromut (2011)
Congress "Food and health" - Nutrimedes (2011)
Congress "Geneeskundige dagen Antwerpen: obesitas" - KARVA, UA (2010)
Seminar "Cardiovascular regeneration: fact or fiction" - UZA (2010)
Sportmedical congress: "Start to run, start to suffer, start to treat" - Tiense kinesitherapeutenkring (2009)
Conference: "International education and research conference for occupational therapy and physiotherapy" - Artesis Hogeschool (2009)
Certificate "Electrocardiography in Clinical Practice"- UA (2008)
Training "ECG basics" - Imeldaziekenhuis (2007)

Other Scientific contributions

Travel Grant FWO for the European Congress of Obesity Vienna
Peer review contributions for Physical & Occupational Therapy in Pediatrics, Physical Therapy Journal
Member of the working group KINECOACH "internal diseases" - collaboration UA, Hasselt University and AXXON
Member of the working group HEALTH POLICY in Sint Katelijne Waver - collaboration with local government and Logo Mechelen
Member of the working group EXERCISE IN CHILDREN WITH OBESITY, Eetexpert - Guideline for health care providers (2015)

EDUCATIONAL ACTIVITIES

Teaching activities

Lector "Cardiology" - REVAKI, UA (3rd bachelor and 1st master)
Lector "Exercise Physiology" - REVAKI, UA (3rd bachelor)
Lector KINECOACH - collaboration UA, Hasselt University and AXXON (Belgian physical therapy association)
"Kinecoach" Basics Exercise Physiology"
"Kinecoach: children and adolescents with overweight and obesity"
"Kinecoach: adults with overweight and obesity"-
"Kinecoach: cardiac rehabilitation" (under construction)

Coaching and supervising activities

Guidance internships- REVAKI, UA
Co-supervisor bachelor and master theses - REVAKI, UA
Co-supervisor bachelor theses - Nutrition and dietetics program., Artesis Plantijn (2015)
Co-supervisor bachelor thesis - Applied Psychology, Thomas More Hogeschool (2015)
Student coaching and study career counseling - REVAKI, UA (2008-2017)

Didactic courses

Workshop ECHO UA: Individualized blended learning path (2018)
Workshop ECHO UA: Flipped classroom (2016)
Workshop CIKO UA: Teaching large groups (2014)
Workshop ECHO UA: Blended learning (2014)
Workshop ECHO UA: Peer assessment (2014)
CVO Crescendo: Certificate Specific Teacher Training Program (2012-2014)
Workshop ECHO UA: Guiding master theses (2011)
Training for assistants, ECHO UA (2011)
Congress: Hoger onderwijs in de gezondheidszorg, Lunteren (2009)

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Prof. Dr. Hansen, Dominique - het is enorm fijn om met jou samen te werken. Hoewel ik tijdens mijn masteropleiding nooit les van je kreeg, heb ik dit ruimschoots ingehaald tijdens de KINECOACH opleidingen. Je inspireert en begeestert!

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