# LEISURE TIME PHYSICAL ACTIVITY, LIVER FAT AND CARDIO-METABOLIC RISK FACTORS: A MONOZYGOTIC CO-TWIN CONTROL AND AN OBSERVATIONAL STUDY

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# ABSTRACT

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**Introduction**: Obesity has a strong link with cardio-metabolic risk factors. This fact has raised the necessity to understand the underlying causes of obesity for efficient prevention and treatment strategies. It has been demonstrated that the distribution of the fat in ectopic fat depots, such as liver and viscera, associates with features of the metabolic syndrome (MS) and an increased risk for manifesting cardiovascular diseases. Interestingly, MS can be present regardless obesity-defined anthropometric measurements. Hence, abnormal fat content in ectopic fat depots is deemed to increase the risk for developing the cardio-metabolic risk factors in non-obese population. A non-pharmacological method for maintaining physiological quantity of fat in ectopic fat depots is physical activity. Based on the aforementioned, the aim of this study was initially to examine the independent effect of chronic leisure-time physical activity (LTPA) on the intrahepatic triglyceride (IHTG) content. The second aim was to test the association of the IHTG with variables of LTPA, anthropometrics, blood lipids, blood pressure, glucose homeostasis as well as abdominal adipose tissue in healthy non-obese young adults prior to overt cardio-metabolic diseases.

**Methods:** Twenty-three apparently healthy monozygotic (MZ) twin pairs (N=46, mean age= 34 years, range 32-36) had been assessed retrospectively regarding their LTPA level in the FITFATTWIN study. All the subjects were tested for the IHTG content by analyzing magnetic resonance images (MRI) of the liver, the MS criteria as well as variables of the abdominal adipose tissue. For the first aim of the study, ten of those MZ twin pairs (N=20) with the greatest LTPA discordance were divided into two groups of "active" and "inactive" co-twins. We performed a comparison of difference in the IHTG content between the "active" and "inactive" group. In the latter analysis, due to the twinship, both the genetic background and the childhood environment were controlled. Therefore, we examined the independent effect of LTPA on IHTG content. In regard to the second aim, an individual-based correlation analysis of 22 pairs (46 volunteers) was conducted between the IHTG content and retrospectively-tested LTPA indexes, the MS criteria and the abdominal adipose tissue.

**Results:** The comparison between the two groups, concerning the IHTG content, exhibited no statistically significant difference although the average IHTG content tended to be lower in the "active" co-twin group. The individual-based association analysis revealed statistically significant correlations between IHTG content and anthropometrics, abdominal adipose tissue, and glucose homeostasis variables. More specifically, IHTG content correlated with BMI ($r=0.313$, $P=0.006$), waist circumference and ($r=0.396$, $P=0.034$), and total fat percent ($r=0.397$, $P=0.006$). Furthermore, IHTG content associated significantly with the visceral adipose tissue (VAT) ($r=0.465$, $P=0.001$) and with the subcutaneous adipose tissue (SAT) $r=0.350$ ($P=0.017$). The value of the 30min time point of the oral glucose tolerance test was also related with the IHTG content ($r=0.348$, $P<0.05$). IHTG content did not associate neither with LTPA indexes nor with blood pressure.

**Conclusion:** Previous findings have supported the notion that chronic LTPA can act as an independent effect on health variables which associate with cardio-metabolic lesions. The MZ co-twin study aimed to examine the potential causal association between the liver fat and LTPA. The findings of this did not show significant difference on the IHTG content between the groups with the LTPA discordance potentially because subjects were healthy young adults with averagely normal values of IHTG deposition. In the individual-based association analysis, the IHTG deposition correlated with some cardio-metabolic risk factor. It was demonstrated that the higher the IHTG content the higher the values of the measured factors. Even though these associations do not imply causality the results agree with the literature concerning the role of liver fat on the exacerbation of the cardio-metabolic risk factors.

**Keywords:** non-alcoholic fatty liver disease, hepatic steatosis, metabolic syndrome, exercise

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**ABBREVIATIONS**

| | |
|---|---|
| BMI | Body mass index |
| BP | Blood pressure |
| ChREBP | Carbohydrate-responsive element-binding protein |
| CVD | Cardiovascular diseases |
| DBP | Diastolic blood pressure |
| FFA | Free fatty acids |
| HDL-c | High density lipoprotein cholesterol |
| HOMA | Homeostatic model assessment |
| HS | Hepatic steatosis |
| IHTG | Intra-hepatic triglycerides |
| LDL-c | Low density lipoprotein cholesterol |
| LTPA | Leisure-time physical activity |
| MET | Metabolic equivalent |
| MRI | Magnetic resonance imaging |
| MRS | Magnetic resonance spectroscopy |
| MS | Metabolic syndrome |
| MUFA | Mono unsaturated fatty acids |
| MZ | Monozygotic |
| NAFLD | Non-alcoholic fatty liver disease |
| OGTT | Oral glucose tolerance test |
| PUFA | Poly-unsaturated fatty acids |
| SAT | Subcutaneous adipose tissue |
| SBP | Systolic blood pressure |
| SFA | Saturated fatty acids |
| SREBP-1c | Sterol-regulatory element-binding protein 1c |
| TG | Triglycerides |
| VAT | Visceral adipose tissue |
| VLDL | Very low density lipoprotein |
| WC | Waist circumference |

**CONTENT**

# 1 INTRODUCTION

Epidemiological data has shown that prevalence of type 2 diabetes has been increased the last decades in Europe. The estimated proportion of diabetes in 2013, among people between 20 and 79 years of age, was 8.5%. (Tamayo et al., 2014). Likewise, European Society of Cardiology reports that cardiovascular diseases (CVD) remain one of the major cause of morbidity and leading cause of mortality in Europe (European Cardiovascular Disease Statistics 2017). It has been supported that metabolic syndrome (MS) increases the risk for both CVD and type 2 diabetes by 2-fold and 5-fold respectively (Grundy 2008).

Furthermore, obesity and mainly the androgenic type has been associated with the manifestation of the MS, abnormal glucose tolerance and chronic low-grade inflammation. More specifically, the cardio-metabolic risk factors' prevalence of MS, which includes the high waist circumference (WC), the elevated blood glucose, the increased plasma triglycerides (TG), the low levels of high density lipoprotein cholesterol (HDL-c), and the elevated blood pressure are higher in obese subjects. However, it should be noted that there is an inhomogeneity of those abnormalities among obese. In other words, controlled data from population-based study for age and sex of non-Hispanic white subjects have shown that approximately 60% of obese individuals have abnormal glucose and lipid metabolism while 31% of them are metabolically healthy. (Wildman et al., 2008). These results show that obesity, when is defined with traditional anthropometric criteria, might not solely cause MS. Therefore, after the development of the imaging methods, research has focused on the evaluation of the specific body fat distribution rather than just the anthropometrics in order to understand the connection of excess adiposity with metabolic lesions (Després, 2012). Evidence from cohort study has supported the major association of visceral and liver fat deposition in the manifestation of cardio-metabolic abnormalities even when body mass index (BMI) is controlled (Speliotes et al., 2010).

Concerning the liver, as one of the ectopic fat depot, research has shown that hepatic steatosis (HS) has a pivotal role in metabolic health. Findings from a cross-sectional study evidence that the absence of substantial TG accumulation into hepatocytes of extreme obese individuals did not correlate excess adiposity to metabolic dysfunctions (Magkos, et

al., 2010). Furthermore, cohort studies confirm the association between HS and abnormal metabolic traits as well as with other high risk abdominal fat partitions such as the visceral adipose tissue (VAT) and the subcutaneous adipose tissue (SAT) (Liu et al., 2011; Speliotes et al., 2010). Furthermore, hepatic insulin resistance as a result of fat accumulation in the liver has been proposed as one of the dominant factors for developing of hyperglycemia and increased serum TG irrespectively of obesity in healthy men (Seppala-Lindroos et al., 2002). Thus, it is worth testing the potential association of intrahepatic triglycerides (IHTG) with variables of metabolic traits and fat divisions in healthy non-obese sample.

Furthermore, western society lifestyle habits comprise reduced physical activity, enhanced sedentariness, and abnormal food intake. As a result, individuals usually face a positive energy balance. Thus, it has been argued that the substantial lack of physical activity has partly induced an increasing rate of metabolic dysfunctions (Després, 2012). Among others lifestyle modification factors, physical activity and nutrition has been proved to be protected components of MS development mediated by improvements in IHTG and hepatic insulin resistance (Keating et al., 2012). In addition, leisure time physical activity has proven to be an indented factor for preventing the increased magnitude of high risk fat areas and total fat percentage (Leskinen et al., 2009; Rottensteiner et al., 2016).

The beneficial effects of physical activity and exercise on excess IHTG, the high risk fat areas, and the metabolic variables have been tested by both observational and control trials (Goodpaster et al., 2010; Keating et al., 2012; Perseghin et al., 2007). However, both types of studies have the limitations of genetic selection bias. The genetic background affects the distribution of body fat accumulation and the participation in physical activity, hence unrelated individuals might bias the studies' results (Rottensteiner et al., 2016). In addition, few studies have focused on the prophylactic role of physical activity on cardio-metabolic risk factors prior to any apparent chronic disease. Therefore, monozygotic (MZ) co-twin control study is the optimal study type to examine the effect of leisure time physical activity on IHTG difference between MZ co-twins with leisure time physical activity discordance.

Thus, there are two purposes of this work. The first aim is to observe whether there are differences of the IHTG between MZ co-twin subjects with physical activity discordance in the absence of any chronic disease and abnormal values of metabolic variables. The

second aim is to examine the cross-sectional association of IHTG with high-risk fat abdominal compartments, body composition, lipid profile, insulin resistance and physical activity index in active healthy adults at their middle thirties.

## 2 NON ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

Non-alcoholic fatty liver disease (NAFLD) defines the abnormal IHTG accumulation in the absence of excess ethanol consumption with cut off values <20g/day for women and <30g/day for men. Moreover, the inflammatory non-alcoholic steatohepatitis (NASH), liver cirrhosis and carcinoma are under the most severe spectrum of the NAFLD. It has not yet elucidated whether excess IHTG can develop NASH. The estimated prevalence of excess IHTG is 20-30% in general population. Also, the most important risk factors for abnormal IHTG are male sex, higher age, obesity, insulin resistance, low-grade inflammation and MS. Obesity is strictly connected with NAFLD, with 80-90% of obese having NAFLD. (Bellentani, 2010). Fatty liver has thoroughly been supported as a central component of MS because it is associated closely with the cardio-metabolic risk factors (Kotronen & Yki-Järvinen, 2007). Regarding the less severe spectrum of the NAFLD, fatty liver is presented when the proportion of IHTG accumulation into the cytoplasm of hepatic cells -in the form of lipid droplets- exceeds the 5% of hepatocytes. Referring to the severity classes of NAFLD, steatosis of 5% - 33%, 34% - 65% and >66% defines the moderate, middle and severe grade respectively (Mennesson et al., 2009). In this study the terms liver fat, IHTG and HS are used interchangeably.

Liver is an essential organ for the human energy metabolism. It has a pivotal homeostatic role between the other high metabolic sites such as muscle and adipose tissue. For this purpose, the liver exports substrates, nutrients and hormones. In the fed state it metabolizes mainly fatty and amino acids as well as glucose to provide energy fuel for the extrahepatic tissues. (Rui, 2014). The HS is caused when the increased IHTG formation exceeds chronically the fatty acids oxidation and secretion rate. The sources of necessary free fatty acids (FFA) that contribute to the TG esterification include the diet, de novo lipogenesis and the adipose tissue. The purposes of FFA within the liver are either to be oxidized in the mitochondrion or esterified in TG. The TG particles are separated in the storage TG pool and in the secretory TG pool that hold by the very low density lipoprotein (VLDL)-TG (Cohen, Horton, & Hobbs, 2011). The following subchapters consist of the physiological mechanisms that underlie the primary stage of NALFD, HS.

## 2.1 Hepatic Steatosis and Adipose Tissue

In a general overview, it has been supported that in the basis of an unfavorable genotype obese people accumulate fat in subcutaneous area due to the chronic positive energy balance and the diminished energy expenditure. It is presumed that when SAT reaches the available capacity that can store, FFA are accumulated in the non-traditional fat storage areas such as viscera, liver and muscle. Therefore, these areas are known as ectopic fat depots. Moreover, the fact that SAT is incapable to store extra FFA may stem from the failure of the tissue to differentiate FFA and proliferate the adipocytes (hyperplasia). Because of this incompetency the adipocytes are enlarged (hypertrophy). Furthermore, adipose tissue has been defined as an endocrine gland. Due to the defective adipose tissue, its endocrine function may also be affected. Hence, the obesity related metabolic lesions may be caused by the abnormal secretion of adipokines and pro-inflammation cytokines. This abnormality may also impair insulin action in adipose tissue and the ectopic fat areas and may promote the abnormal fat accumulation in the latter fat depots. (Cornier et al., 2011)

In regards to the liver as an ectopic fat depot and its contributors for IHTG, the circulating FFA derived from adipose tissue is the major source of the HS in patients with NAFLD and attributes approximately 59% of the total IHTG. In reference to the de novo lipogenesis and the diet, the contribution to hepatic FFA approximates the 26% and 15% respectively (Donnelly et al., 2005). During the fasting state and the low blood glucose levels, the lipolysis of the adipose tissue increases the circulating FFA for the provision of energy fuel. In case of obesity related insulin resistance the anti-lipolytic action of insulin in adipose tissue fails. Thus, circulating FFA remains high even in the postprandial phase and may potentially exacerbate HS (Petta et al., 2016, 2).

Furthermore, obesity and particularly the intra-abdominal one has been associated with cardio-metabolic abnormalities. More specifically, VAT, on the contrary to SAT, is more prone to induce abnormal adipose tissue accumulation and metabolic lesions. The FFA enters the liver through the hepatic artery and portal vein circulation. Regarding the contribution of the adipose tissue fat compartments on HS, VAT in comparison to SAT, drains more FFA directly to the liver through the portal vein because of its anatomical

difference. Additionally, due to cellular difference contrasted to SAT, VAT has larger adipocytes which can become more insulin resistant, hyperlipolytic and resistant to anti-lipolytic effect of insulin. (Ibrahim, 2010, 12).

There is a hypothesis that the involvement of adipose tissue in the pathogenesis of HS is partly based on the secretion of adipokines and pro-inflammatory cytokines. As mentioned earlier, the hypertrophic adipocytes seem to be in charge of the adipokines secretion that are drained into the liver and interfere in the hepatic insulin pathway. The liver insulin resistance has also been assumed to be the corner stone for the obesity-associated increased cardio-metabolic risk factors. (Brown & Goldstein, 2008). In this regard, evidence from the study of Jarrar and coworkers (2008) shown that extremely obese participants with NAFLD had lower adiponectin levels, higher interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-a) when they compared with matched obese control group. The hypo-adiponectinemia and the presence of these pro-inflammatory cytokines have been associated with impaired insulin sensitivity. Nevertheless, as the authors concluded, these findings indicate only the association between potential risk factors and imply the complexity of the NAFLD pathogenesis. (Jarrar et al., 2008).

### 2.2 Hepatic Steatosis and Dietary Nutrients

The third most important source of FFA for TG esterification within the hepatic cells is the dietary fats. The TG-rich chylomicrons are those that package and transport the postprandial fats in the liver. Both observational and intervention studies have examined the association between the dietary fat and its effect on HS.

Kuk et al., (2008) tested the habitual and acute effect of dietary fat consumption associated with liver fat score in overweight and obese senior men whose ethanol consumption was within normal range. The habitual dietary fat was calculated by the average fat intake of a 10-day food frequent questionnaire while the liver fat was estimated by computed tomography. Regarding the results, liver fat score had a significant positive and moderate correlation with both total dietary fat percentage and saturated fat percentage. Notable, there was not any significant relationship between liver fat score and acute dietary fat consumption. (Kuk et al., 2008).

Regarding the contribution of specific dietary fatty acids on the IHTG deposition, Pacifico et al., (2011) demonstrated that a hypo-caloric diet for 12 months with 23-30% of the total energy derived from fats (one third was saturated fatty acids (SFA), and the rest mono (MUFA) and poly-unsaturated fatty acids (PUFA) with ratio 4:1 ω6 to ω3) induced a reduction of 13% in the liver fat score. In conjunction with the diet, participants participated in one hour of moderate intensity physical activity per day, at least 5 days per week. Eleven out of 25 children and adolescent who experienced substantial weight loss were included in this measurement. Notwithstanding, due to the small sample size the results cannot be extrapolated to the population. (Pacifico et al., 2011).

Bjermo et al., (2012) tested the exclusive effects of PUFA and SFA on HS by applying an iso-caloric diet intervention. Sixty seven obese subjects were divided into two groups. The participants asked to maintain their calorie intake for a 10 week period and replace fats only with PUFA for the first group and only with SFA for the second one. The findings demonstrated significantly higher liver score in the SFA diet group compared to the PUFA in the follow up measurement, regardless the weight changes and the total fat intake. (Bjermo et al., 2012).

The potential underlying mechanisms that explained the differences in HS as a result of different FFA composition on a diet, it is presumed that the PUFA utilization is preferred by the liver β-oxidation in comparison to SFA. Furthermore, the later FFA has been a cause of the endoplasmic reticulum stress which has been associated with NAFLD. (Green & Hodson, 2014, 5026).

### 2.3 Hepatic Steatosis and De novo Lipogenesis

De novo lipogenesis is referred to the mechanism in which carbohydrates and insulin contribute to the hepatic TG composition. The gastrointestinal tract absorbs the postprandial nutrients which thereafter are transported to the muscles, adipose tissue and liver. The nutrients drained into liver through the portal vein circulation system. Concerning the glucose, while its level rises in the blood induces a concomitant increment on insulin level. The insulin triggers the glucose uptake by the hepatocytes. The glucose

molecules that reach the liver have two functions. They will be either converted into glycogen, when glycogen depots have been depleted, or contribute to the TG synthesis from acetyl-CoA subunits of the glycolysis. (Rui, 2014).

The de novo lipogenesis is induced by two different pathways. During fed state, the increased levels of insulin leads to the transcriptional upregulation of the sterol regulatory element binding protein 1c (SREBP1c) which through different pathways increases the enzymes that promotes lipogenesis via FFA which are derived from the acetyl-CoA. Also, insulin diminished lipolysis by suppressing the activity of the adipocyte TG hydrolase (ATGL). On the other hand, glucose upregulates the transcription factor carbohydrate responsive element-binding protein (ChREBP) that also promote de novo lipogenesis. In addition, ChREBP contributes to availability of FFA by increasing the liver-type pyruvate kinase. Insulin resistance and its subsequent hyperglycemia that related to obesity and the NAFLD, can additionally stimulate de novo lipogenesis. (Fabbrini, Sullivan, & Klein, 2010, 6)

In addition to glucose, fructose, which is mainly metabolized in the liver, has been associated with the increased prevalence of the diseases non-alcoholic steatohepatitis, obesity and type 2 diabetes mellitus. In the liver metabolism point of view, it has been proved that fructose forwards glycogenesis when liver glycogen depots are depleted independently of the action of insulin. However, in the absence of hepatic glycogen synthesis demands, fructose molecules promote de novo lipogenesis through increasing the acetyl-CoA and via the upregulation of SREBP1c that further augments the lipogenic enzymes. It is deemed that the excess fructose consumption in the western type diet induces augmented prevalence of NAFLD regardless the manifestation of obesity. (Sanders & Griffin, 2016, 463).

Additionally, in the fed state the hepatic FFA that are not metabolized in the β-oxidation to contribute to gluconeogenesis or do not form TG and other lipids are secreted into blood via very low-density lipoprotein (VLDL). It has been observed that obese subjects with IHTG demonstrate high VLDL-TG secretion. However, the progression of NAFLD did not promote extra VLDL-TG secretion that seemed reaching a plateau. Thus, impaired VLDL-TG secretion may be a cause for IHTG modification. Correspondingly to VLDL-TG

secretion factor, impaired intrahepatic FFA oxidation may also subscribe to IHTG. (Fabbrini et al., 2008).

In conclusion, the abnormal flux rate of FFA to the liver through diet and the adipose tissue as well as the de novo lipogenesis are the main causes of increase in IHTG. The reduction of SFA with the increase of MUFA and PUFA may facilitate the modulation of the liver fat score although this has not fully elucidated. When it comes to abdominal obesity, the adipose tissue contribute to increase in HS through the reduction of adiponectin and the increase in the anti-inflammatory cytokines. This phenomenon may provoke local and systemic insulin resistance. In a long-term basis, the subsequent hyper-insulinemia can determine directly the level of IHTG via the de novo lipogenesis and indirectly by impairing its anti-lypolitic effect in the adipose tissue. The mechanistic processes of IHTG deposition are presented briefly in Figure 1.

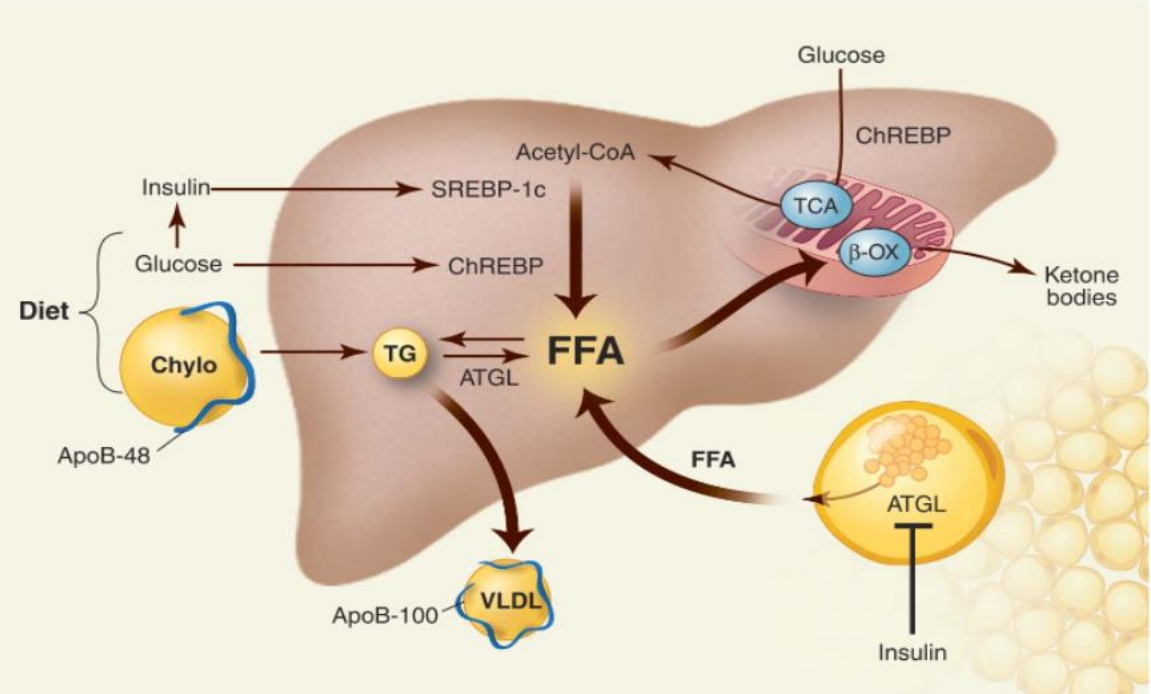


*Figure 1. Liver TG metabolism. The major sources of FFAs are dietary fats, endogenous de novo synthesis and peripheral adipose tissue. FFAs have four possible fates. They can be metabolized beta oxidation (β-OX), stored as TG or other forms of lipids and secreted in the blood stream through VLDL. The imbalance between FFA and TG input causes HS. (Cohen, Horton, & Hobbs, 2011).*

## 3 INTERACTION BETWEEN HEPATIC STEATOSIS AND METABOLIC SYDNROME

The MS is a cluster of risk factors attributed to metabolic dysfunctions such as dyslipidemia, central obesity, hypertension, as well as insulin resistance. According to different guidelines (Table 1), the increased abdominal obesity measured with WC, the high TG, the low HDL-c, the abnormal blood pressure (BP) and the elevated fasting glucose are the clinical criteria for detecting MS. The high importance of MS relies on the fact that when is present increases the risk for the manifestation of the cardiovascular diseases and type 2 diabetes by 2-fold and 5-fold, respectively. (Grundy et al., 2004).

*Table 1. Proposed criteria for clinical diagnosis of the metabolic syndrome. (Grundy et al., 2008)*

| Clinical Measure | WHO (1998) | NCEP (2001) | IDF (2005) |
|---|---|---|---|
| Insulin resistance | IGT, IFG, T2DM or ↓ insulin sensitivity* plus any two of the following | None but any three of the following five features | None |
| Body weight | Males: waist to hip ratio >0.90; females: waist to hip ratio >0.85 and/or BMI >30 kg/m$^2$ | WC ≥102 cm in men or ≥88 cm in women† | Increased WC (population specific) plus any two of the following |
| Lipid | TG ≥150 mg/dL and/or HDL-C <35 mg/dL in men or <39 mg/dL in women | TG ≥150 mg/dL | TG ≥150 mg/dL or on TG Rx |
| | | HDL-C <40 mg/dL in men or <50 mg/dL in women | HDL-C <40 mg/dL in men or <50 mg/dL in women or on HDL-C Rx |
| Blood pressure | ≥140/90 mm Hg | ≥130/85 mm Hg | ≥130 mm Hg systolic or ≥85 mm Hg diastolic or on hypertension Rx |
| Glucose | IGT, IFG, or T2DM | ≥110 mg/dL (includes diabetes)‡ | ≥100 mg/dL (includes diabetes) |
| Other | Microalbuminuria | | |

WHO indicates World Heath Organization; NCEP, National Cholesterol Education Program Adult Treatment Panel III; IDF, International Diabetes Federation; IGT, impaired glucose intolerance; IFG, impaired fasting glucose; T2DM, type 2 diabetes; WC, waist circumference; BMI, body mass index; TG, triglycerides; HDL-C, HDL cholesterol.

*Insulin sensitivity measured under hyperinsulinemic euglycemic conditions, glucose uptake below lowest quartile for background population under investigation.

†In Asian populations, the WC threshold for abdominal obesity is ≥90 cm in men or ≥80 cm in women.

‡The 2001 definition identified fasting plasma glucose of ≥110 mg/dL (6.1 mmol/L) as elevated. This was modified in 2004 to be ≥100 mg/dL (5.6 mmol/L), in accordance with the American Diabetes Associations updated definition of impaired fasting glucose (IFG).

The ectopic fat deposition in non-traditional fat storing sites has been associated with cardio-metabolic risk factors and MS. Once liver plays a pivotal role in the glucose, amino acid and fat metabolism, abnormal HS has been considered MS manifestation on the liver. It is not known whether MS is a cause or an effect of fatty liver. Nevertheless, in a prospective study the metabolically unhealthy and obese subjects exhibited higher prevalence of NAFLD compared to the metabolically healthy non-obese, the metabolically unhealthy non-obese and the metabolically healthy obese subjects after 4 years of follow-

up. Thus, it can be presumed that is more likely fatty liver to coexist with obesity and MS rather than only with one of these two dysfunctions. (Lee et al., 2015).

The following subchapters consist studies that have examined the association between HS and each of MS factors as well as some other surrogates markers, in both healthy and unhealthy human subjects.

### 3.1. Hepatic Steatosis and Abdominal obesity

Abdominal obesity determined by WC is one of the identification criteria for determining MS. However, the advantage of developing the imaging methods, such as computer tomography, ultrasound, magnetic resonance imaging (MRI) and spectroscopy (MRS) has facilitated the understanding of how the distribution of adiposity in the body is related to cardio-metabolic risk factors. This subchapter includes studies that has tried to address the question of how the ectopic IHTG is associated with the risk factor of abdominal obesity.

It has been observed that VAT has been the strongest explanatory factor for IHTG, which also remained significant even after statistical control of SAT. Westerbacka and colleagues (2004) in a cross-sectional study demonstrate that young overweight male and obese female adults had significant similarities in the magnitude of VAT and IHTG, although females had higher SAT. Presumably, the higher amount of SAT in women group was not responsible to promote further IHTG. Additionally in this study, IHTG had a medium positive correlation with VAT, whilst the correlation between IHTG and SAT was significant for both women and men but less strong. Notable, VAT was the only independent predictor of liver fat after adjustment for age sex and BMI. (Westerbacka et al., 2004).

On the contrary, Seppala-Lindroos and colleges (2002) did not find any association between IHTG and the intra-abdominal fat in healthy non-obese young adults. However, researchers reported that this result did not exclude the possibility of the association between these two ectopic fat depots, once in this study there was not any distinction between retroperitoneal and intraperitoneal adiposity. (Seppala-Lindroos et al., 2002).

Likewise, Burgert et al., (2006) by approaching differently the relation of intra-abdominal and intra-hepatic fat, demonstrated that the higher the content of VAT the more augmented IHTG was. More specifically, although obese adolescents did not manifest significant difference between the amount of SAT and the total body fat percentage, those who had concomitantly NAFLD expressed also higher visceral adiposity compared with those without NAFLD. Furthermore, although the two groups were comparable for total body fat percentages, significant differences were also exhibited for deep to superficial SAT. Finally, higher proportion of obese adolescents with NAFLD diagnosed with MS in contrast to solely obese counterparts. (Burgert et al., 2006).

In the study of Pietilainen et al., (2005) young MZ twins participants with obesity discordance were evaluated on how acquired obesity, BMI, WC and percentage of total fat-determined, regulated fat deposition in ectopic sites. The 'lean' co-twins were in the normal weight to overweight borderline while the 'heavy' co-twins were obese. Regarding the between pair analysis, the heavier group had significantly more liver fat score compared to leaner group. The intra-pair analysis demonstrated that the differences of VAT, SAT, body fat percentage and BMI correlated significantly with differences in IHTG of MZ twin pairs. (Pietilainen et al., 2005). Alike to previous study, Leskinen and colleagues (2009) demonstrate that in both the individual and the intra-pair MZ twin analysis the visceral and the hepatic fat associated significantly. (Leskinen et al., 2009).

Moreover, in the Jackson Heart study, individuals in the tertile with the higher magnitude of IHTG had significant higher VAT compared with those in the lowest tertile. In an age-adjusted correlation coefficient analysis, VAT had a medium positive correlation with IHTG (Liu et al., 2011). In comparable results to the latter study, VAT correlated positively with IHTG content in the Framingham Heart study. Also, in those participants with fatty liver the mean differences of VAT were significant higher than in those without fatty liver. (Speliotes et al., 2010).

Regarding the attributing factors and the magnitudes in which VAT and IHTG are regulated, Pietilainen and coworkers (2005) demonstrate that the BMI, the percentage of total body fat and SAT determined the variation of intra-abdominal adiposity by 67, 42 and 56% respectively. However, for the IHTG the contribution was 33, 12, and 29% respectively (Pietilainen et al., 2005). Also, the findings of the identical twin-based study

showed the contribution of acquired obesity - defined as increased SAT - in visceral and liver fat existed independently of genetic background. Interestingly, in this study SAT seemed to contribute roughly twice more to VAT compared to the contribution to the liver fat content. Researchers discussed that HS can be present regardless the abnormal magnitude of the SAT. Furthermore, although obesity-related genetic factors contribute to the IHTG deposition, there are additional acquired factors such as the total and the saturated fat consumption can simultaneously increase IHTG. (Leskinen et al., 2009; Pietilainen et al., 2005).

Similarly, another cross-sectional study tried to address the different prevalence of NAFLD in women with excess visceral type MS and subcutaneous type MS. As it is mentioned in the study, previous research have shown that prevalence of NAFLD was higher in those men with MS who had more VAT than subcutaneous. Sogabe and colleagues (2014) showed that there were more cases of NAFLD in those women with visceral type MS compared to those with subcutaneous type MS. The VAT type MS was an independent predictor of NAFLD in women with MS. (Sogabe et al., 2014).

In conclusion, although there is unequivocal association between IHTG and VAT regardless of the presence of substantial increased SAT no causal relationship can be established between the fatty liver and the abnormal VAT, due to the observational form of these studies. Moreover, the study limitations such as low validity and reliability of liver fat quantification tools as well as subjects' selection bias do not permit extrapolation of the result and clear conclusion drawing. (Liu et al., 2011; Speliotes et al., 2010).

### 3.2. Hepatic Steatosis, Glucose Tolerance and Insulin Resistance

Regarding the glucose homeostasis, the hepatocytes play a major role in glucose and glycogen metabolism in both the fed and the fasted state. Regarding the postprandial state, the glucose molecules enter the liver through the membrane glucose transporter GLUT2 after the insulin secretion of the pancreatic β-cells. Also, insulin stimulates the precursors for hepatic glycogen synthesis. Regarding the short-term fasted periods, liver produces and secretes glucose to the circulation through the mechanism of glycogenolysis, while in prolong fasting periods and during aerobic exercise hepatocytes uses molecules of lactate,

pyruvate, glycerol and amino acids for gluconeogenesis and glucose secretion to the circulation. (Rui, 2014, 2-4).

Furthermore, hepatic gluconeogenesis is downregulated by the transcription factor FoxO1 in the presence of insulin. On the other hand, the expression of transcription factors carbohydrate-responsive element-binding protein (ChREBP) and sterol-regulatory element-binding protein 1c (SREBP-1c) upregulate de novo lipogenesis in the presence of insulin. Insulin signaling dysfunctions in the liver has been associated with impaired gluconeogenesis that is translated to excess glucose secretion potentially due to the failure of insulin to upregulate the transcription factor FoxO1. That phenomenon contributes to a consequent vicious cycle of excess insulin secretion by the pancreas for the constant elevated glucose secretion by the liver. Both hyperglycemia and hyperinsulinemia are precursors of type 2 diabetes. The lesions in hepatic insulin signaling pathway induce abnormal liver fat deposition and secretion. In contrast to healthy patients whose liver metabolism is normal the patients with type 2 diabetes exhibit the damaging hyperglycemia alongside with hyperinsulinemia and hypertriglyceridemia that in turn increases the risk for CVD (Figure 2). (Brown and Goldstein 2008).

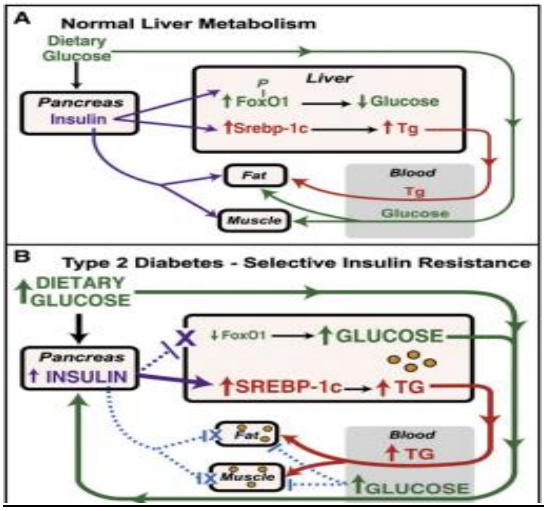


*Figure 2. A) Normal liver function for a given glucose load. Liver downregulates the gluconeogenesis and promotes the triglycerides (TG) secretion to the rest metabolic sites in normal insulin function.*

*B) Liver insulin resistance evokes failure of the gluconeogenesis downregulation and augment of the IHTG formation and secretion to the periphery. That promotes the*

*detrimental combination of hyperglycemia and hypertriglyceridemia. (Brown and Goldstein 2008).*

Although HS and insulin resistance coexist in patients with NAFLD it has not been yet elucidated whether this disease is a cause, an effect or both on malfunctions of hepatic insulin action (Fabbrini et al., 2010). However, HS has been associated with features of hepatic insulin resistance even in non-diabetic normal weight participants independent of the BMI (Seppala-Liindroos et al., 2002). Therefore, this subchapter consist of studies which have attempted to explore the association of HS with features of impairments in glucose tolerance in both subjects with normal and abnormal distribution of adiposity.

In more details, Seppala-Liindroos and coworkers (2002) examined the effects of hyperinsulinemia on the endogenous glucose production and suppression between two groups who differ in liver fat. All the subjects were healthy normal-weight young adults with normal waist to hip ratio but they differed statistically on the liver fat. The findings demonstrate that both endogenous glucose production and suppression in the last hour of hyperinsoulinemia (240-300 min) were statistically different and unfavorable for the high liver fat group compared to the low liver fat group. Moreover, fasting insulin levels were positively correlated with liver fat scores in individual correlation analysis. However, insulin sensitivity, which is determined by hepatic glucose utilization, did not differ between the groups. Nevertheless, although some of the facets of insulin resistance might be associated with hepatic insulin resistance these findings cannot be solely attributed to higher liver fat because of the observational nature of the study. (Seppala-Lindroos et al., 2002).

Regarding the notion that ectopic adiposity is associated closer with metabolic abnormalities than anthropometric measurements which do not take into account the diversity of fat allocation, previous studies have shown that IHTG is an independent determinant factor for obesity-related metabolic dysfunctions. Magkos et al., (2010) supported this concept by demonstrating the lack of differences in metabolic traits between moderate and severe obese subjects who had equal IHTG content. More specifically, hepatic insulin sensitivity, VLDL-TG and VLDL-apollipoprotein B-100 did not differ significantly between sever and moderate obese individuals although BMI and VAT was statistically higher in severe obese. (Magkos et al., 2010).

What is more, Speliotes and colleagues (2010) evaluated the association of insulin function with excess IHTG in a cross-sectional study with community-based sample. When fatty liver used as a dichotomous variable, those with presence of HS had significantly higher prevalence of type 2 diabetes, fasting glucose, insulin resistance and decreased adiponectin levels compared to those without fatty liver. Also, when the variable of fatty liver was used as a continuous variable for examining the association with other metabolic variables results a significant positive correlation with fasting glucose and the homeostatic models assessment- insulin resistance and a significant negative relationship with adiponectin levels. Interestingly, insulin resistance remained significantly a predicted variable of the fatty liver when BMI, WC and VAT were adjusted. (Speliotes et al., 2010).

Likewise, the results from similar big cohort cross-sectional study revealed that the fasting glucose values as well as the prevalence of diabetes and the impaired glucose tolerance were statistically higher in the subjects with the highest scores of fatty liver compared to the subjects who were in the category with lower scores of fatty liver. When the variable of fasting plasma glucose was used as an outcome variable both liver fat score and VAT were accounted as significant predictors after BMI and reciprocal adjustments of IHTG and VAT. However, the differences of regression coefficients between fatty liver score and VAT were not significant. In other words, although both VAT and liver fat score predicted changes in fasting glucose neither one could be found stronger independent variable for fasting glucose than the other (Liu et al., 2011, 6).

In addition to observational studies, control trials have been conducted to address the relationship between the hepatic steatosis and features of dysglycemia. Non-pharmacological approaches have been designed for the scope of improving the liver fat content, and the glucose tolerance including lifestyle changes such as physical activity and diet. Concerning the diet, the methods that have been manipulated have shown that the caloric restriction, the reduction of saturated fat and sugars intake as well as the increase in mono and poly-unsaturated fats might facilitate

Thamer and coworkers (2008) in a non-randomized control trial test the effect of a 6-month dietary lifestyle intervention with weekly exercise on insulin sensitivity in non-diabetic subjects that had increased risk for diabetes type 2. Post-intervention measurements

revealed improvements in the body mass, VAT and IHTG as well as in the insulin sensitivity and 2-hour oral glucose tolerance test. In this study, amelioration in VAT and IHTG predicted the improvements in insulin sensitivity, independently of sex, age, baseline values of these variables and the time to followed-up. Although the decline of the IHTG and the VAT associated with an increased insulin sensitivity even after the small reduction of BMI it is claimed that the subjects with initial high liver and VAT, insulin impairment was unchanged. Thus, researchers support a potential threshold of ectopic fat level after which improvements in metabolic traits are revealed. (Thamer et al., 2007).

In the same context, overconsumption of fructose can contribute to the ectopic fat accumulation. Similarly, sucrose-sweetened beverages have been associated with indices of cardio-metabolic defects. Thus, Maersk et al., (2012) in a randomized control trial examined the effect of sucrose-sweetened regular cola, iso-caloric semi skim milk, aspartame- sweetened diet cola and water consumption on ectopic fat depots. Non-diabetic overweight participants took part and were randomized in four groups. Participants of each group consumed one liter per day out of those drinks for 6 month. Regarding the findings, sucrose-sweetened cola consumers raised statistical significant both the visceral and the liver fat. Notable, body composition changes were not apparent between pre and post measurement. Additionally, when it comes to glucose metabolism, neither fasting glucose and insulin nor the homeostatic model assessment (HOMA) differed statistically among group after the intervention. (Maersk et al., 2012).

Finally, Musso and colleagues (2012) meta-analyze randomized control trials that have examined the effect of weight loss, physical activity and specific nutrient intake on liver histology and cardio-metabolic risk factors on patients with NAFLD. Regarding weight reduction, findings revealed that the threshold of 7% weight loss evoked improvement in hepatic histological scores. In some cases 5% weight loss was substantial enough as well. Notable, weight loss >10% did not seem to have additional reduction of histological scores. Moreover, weight loss ameliorates plasma lipids, glucose tolerance and plasma adiponectin. In reference to the quantity of specific macronutrients in diet, either lower fat or carbohydrates diets had similar extend of IHTG reduction. More specifically, lower carbohydrate diet favored glucose metabolism and plasma TG, while the improvements in HDL-c and low density lipoprotein cholesterol (LDL-c) as well as in adiponectin levels were in favor of lower fat diets. (Musso et al., 2012).

In summary, both HS and VAT has been found to connect with cardiac and metabolic risk factor and, especially, to dysglycemia. However, whether VAT or fatty liver contribute more to this relationship remains controversial. Both observational and control trials have shown that the ectopic fat in both sights affects the glucose tolerance regardless the presence of significant BMI reduction. Nevertheless, as the researchers discussed, these results do not imply that adipose tissue is not a contributor of cardio-metabolic risk factors per se, but ectopic fat accumulation might be the linkage of obesity-related metabolic disease (Magkos et al., 2010). These results assist the understanding of how ectopic fat associated with features of abnormal glucose metabolism and the development of targeted therapeutic methods for high risk individuals. (Speliotes et al., 2010).

### 3.3 Hepatic Steatosis, Blood Lipids and Blood Pressure

Among other cardio-metabolic risk factors dyslipidemia and elevated BP has been associated with fatty liver. Patients with NAFLD have features of dyslipidemia that translated as increased serum TG and small dense LDL-c particles as well as decreased HDL-c. Although the underlying mechanisms of the IHTG association with dyslipidemia remain unknown, the over-secretion of VLDL particles from the liver and the diminished clearance of lipoproteins from the circulation is assumed to be the cause of these defects. (Chatrath, Vuppalanchi, & Chalasani, 2012).

Concerning the association between high BP and HS, epidemiological findings show that hypertension is more prevalent in those with HS (Speliotes et al 2010). Nevertheless, it is presumed that this relationship is attributed to the coexistence of insulin resistance and MS, rather that the direct causal association of fatty liver and abnormal BP (Brookes & Cooper, 2007). The following subchapter consists of studies that have examined the association between dyslipidemia, BP and HS in both healthy and unhealthy subjects.

Starting with results from the Framingham Heart Study, Speliotes et al., (2010) demonstrated a significant positive association of fatty liver with serum TG, systolic BP (SBP) and diastolic BP (DBP) and negative correlation with HDL-c in a middle age sample. Moreover, the lipid profile as well as SBP and DBP were significant different against those

with fatty liver. However, in a multivariate adjusted analysis with the fatty liver score as independent variable, only the HDL-c and the TG and not BP remained dependent ones after adjusting for obesity traits. Interestingly, the variable of hypertension prevalence remained a significant effect of fatty liver after adjustments for obesity traits. (Speliotes et al., 2010).

According to a similar study-design but in a cohort of adolescents, Patel and colleagues (2015) tested the relationship of NAFLD and BP. Between subject with presence of NAFLD and subjects with absence of the disease the mean difference of SBP was 4 mmHg when DBP had also mean difference of 4mmHg after age and sex adjustments. Nevertheless, after adjusting in addition for body fat the mean difference in both SBP and DBP did not remain significant. The authors report that because of the small prevalence of NAFLD in this cohort it is impossible to generalize the findings. (Patel et al., 2015).

In reference of the interaction among lipid profile, body fat distribution, Westerbacka and coworkers (2004) report that there were no significant differences neither in LDL-c and HDL-c nor in serum TG between overweight females and normal weight males who did not have any difference in HS and VAT. Both groups were comparable about the age while female's group had significantly more SAT and total body fat in comparison to the male one. The findings of these study could support the importance of the ectopic fat on the effect of lipid profile. (Westerbacka et al., 2004). Additionally, Seppala-Lindroos et al., (2002) include normal weight individuals of the similar age and categorize them in those with high HS and low HS in order to examine their lipid profile among the other cardio-metabolic risk factors. The high HS group had statistically less HDL-c and more TG compared to the group with the low HS. Last but not least, in an individual correlation analysis, the liver fat score associated positively with 24-hour SBP. (Seppala-Lindroos et al., 2002).

Summarizing, dyslipidemia and increase BP is more prevalent in those with elevated IHTG. Regarding the lipid profile, it is deemed that the obesity traits does not influence the association with the liver fat. On the contrary, although the prevalence of the increase BP is associated with the HS, the association is deserted when the total body fat is not taken into account.

## 4 PHYSICAL ACTIVITY, EXERCISE AND IHTG

As previous mentioned, abnormal fatty liver content is considered the hepatic manifestation of MS. Physical activity and exercise are basic components of the non-pharmacological regimens for reducing cardio-metabolic risk factors, enhancing health and preventing exacerbation of existing diseases. The American College of Sport Medicine (ACSM) defines physical activity as "any bodily movement produced by the contraction of skeletal muscles that results in a substantial increase in caloric requirements over resting energy expenditure" (Pescatello 2014, 2). Regarding the exercise, it is a form of planned and structured physical activity that is held under specific intensity, duration, time and mode for the purpose of improving physical fitness. The components included in physical fitness are cardiorespiratory endurance, body composition, muscular strength and endurance as well as flexibility. The federal physical activity guidelines for aerobic activities provide general guidelines that every healthy adult, who wants to experience the benefits of physical activity, should meet (Table 2).

*Table 2. DHHS and USDA recommendations for physical activity in adults. (Kistler et al., 2011).*

| | Moderate physical activity (minutes a week) | Vigorous physical activity (minutes a week) |
|---|---|---|
| Minimum targets | ≥150 | ≥75 |
| Targets for more extensive health benefits | ≥300 | ≥150 |

DHHS, Department of Health and Human Services; USDA, US Department of Agriculture.

DHHS and USDA recommendations for physical activity in adults can be met by achieving ≥150 min a week of moderate physical activity or ≥75 min a week of vigorous physical activity. To achieve additional and more extensive health benefits, the guidelines recommend increasing time spent in moderate physical activity to ≥300 min a week or increasing time spent in vigorous physical activity to ≥150 min a week.

Evidence has shown a modulation on insulin resistance, low grade inflammation and VAT as a result of participating in exercise. There is a big amount of studies that have researched the effect of physical activity on liver fat, which is considered the mediator between insulin resistance and cardio-metabolic risk factors. It is claimed that aerobic exercise-mediated liver histology improvements stem from the increased hepatic non- esterified fatty acids oxidation, decreased postprandial hepatic lipogenesis as well as reduced hepatic pro-inflammatory adipokines flux that derived from VAT (Mussoet al., 2012). Interestingly, even though IHTG is highly related with BMI, exercise-derived fatty liver and metabolic

risk factors reduction can be significant regardless the magnitude of weight loss in obese subjects (Sullivan et al., 2012; Thamer et al., 2007). This chapter includes studies, both observational and intervention trials, which have examined the independent effect of exercise and physical activity on fatty liver and metabolic features that has been associated with it.

### 4.1 Observational Studies

Starting with the big cohort of Framingham Heart Study, high fatty liver score, which used as a dichotomous variable, associated significantly with prevalent of MS, insulin resistance, plasma TG and reduced HDL-c after statistical adjustments of BMI, WC and VAT compared to the group with low fatty liver scores. Similarly, presence of fatty liver was associated with low levels of physical activity. However, when physical activity was adjusted from the association between cardio-metabolic risk factors and fatty liver scores, the results remained consistent. In other words, although physical activity levels might be a factor that contributes to the maintenance HS did not seem to influence the relationship with dyslipidemia and dysglycemia (Speliotes et al., 2010).

Moreover, Nguyen-Duy and coworkers (2003), in a cross-sectional study demonstrate that cardiorespiratory fitness retained a significant independent negative correlator of circulating TG, total cholesterol/HDL-c and a positive one with liver fat after controlling for SAT and the alcohol consumption, but the significance did not remain after controlling for VAT. It is worth mentioning that the association between VAT and HS remained significant regardless SAT and the cardiorespiratory fitness. Regarding the fitness level and the liver fat, the unadjusted correlation showed that the higher the fitness of the middle age overweight and/or obese subjects the lower IHTG was. The researcher by assuming that cardiorespiratory fitness depicts the regular participation in physical activity, suggested that any improvements in lipid profile might be attributed to the effect of exercise on the visceral and hepatic fat content, regardless concomitant reduction in SAT and total body weight. However, the ability of exercise to decrease the liver fat seems to be dependent on the attenuation of visceral fat. (Nguyen-Duy et al., 2003).

Correspondingly, Leskinen and colleagues (2009) in a prospective study find that more physically active subjects, who also manifested higher cardiorespiratory fitness, had lower liver and VAT compared to those who were inactive and less fit. It should be pointed out that the active and inactive subjects were co-twins of MZ and dizygotic twin pairs who did not have discrepancies in BMI, WC, SAT or daily energy intake. Indeed, the subjects had physical activity discordance both in the first time point of the assessment as well as in the 32 year-follow up evaluation. The assessment of fat compartments were measured only at the end time point. The strength of this study was the MZ twin participants once twin pairs share either whole or part of their genes, as well as childhood environment. Considering these factors, the results can support the notion that chronic adherence to physical activity may influence independently the high risk adiposity compartments. Therefore, researchers conclude that although the observational nature of the study, physical activity may have a causal reverse relationship with cardio-metabolic risk factors. (Leskinen et al., 2009).

Findings from the study of Perseghin and coworkers (2007) revealed a reverse association between physical activity and fatty liver. Participants in this study were apparently healthy nonalcoholic individuals who were divided into subjects with fatty liver and subjects without fatty liver. The assessment of subjects' total physical activity index were calculated by summing the physical activity of individual's work, sport and leisure time activity. Between the two groups the prevalence of higher total physical activity was statistically different in favor of the non-fatty liver group. Also, the insulin sensitivity measured by HOMA index was statistical worse for fatty liver group compared to the non-fatty liver group. In an individual based correlation analysis, the total physical activity index correlated inversely with the IHTG content. The correlative results between physical activity and IHTG did not alternate after controlling for age, sex, BMI, insulin sensitivity and adiponectin. (Perseghin et al., 2007).

Furthermore, in a retrospective observational study Kistler and colleagues (2011) examine the association between meeting the physical activity recommendations and the histological severity of NAFLD. The disease severity was evaluated by liver biopsy and was determined by the presence of borderline or definite NASH. In regards to the level of physical activity, researchers determined retrospectively the physical activity volume by using self-reported questionnaires. The findings demonstrated that only the subjects who had met the vigorous intensity physical activity recommendations had significantly lower

odds of revealing NASH. On the other hand, subjects who had not met at all or met only the moderate intensity physical activity recommendations did not decrease significantly the odds for exhibiting the same disease. Importantly, neither the subjects who had met the moderate physical activity recommendations for additional benefits had decreased the odds of having NASH. Last but not least, findings show that adherence on the vigorous physical activity recommendations for additional benefits lowered further the probability of having NASH. (Kistler et al., 2011).

### 4.2 Intervention Studies

Interventional studies are considered methodologically optimal for examining the effects of physical activity and/or exercise regimens as non-pharmacological therapies in metabolic abnormalities. Nevertheless, this type of studies have practical limitations such as the narrow intervention duration and small sample sizes. Furthermore, another consideration is the magnitude of the independent effect of exercise on high risk compartment once the majority of the studies include diet interventions in conjunction with the exercise programs. Notwithstanding, this subchapter includes some representative control trials which have examined the causal relationship between physical activity and/or exercise and the IHTG content.

In regards to the effect of dietary intervention on the hepatic fat content with or without parallel physical activity regimen, Goodpaster and coworkers (2010) split obese individuals into two groups and examine whether exercise additionally to the diet would reduce further the hepatic fat. The findings from this randomized study revealed that after a period of 6 months following the exercise recommendations alongside with diet, non-diabetic severe obese individuals experienced substantial more IHTG content's reduction compared to the initial measurement.The group that followed only the dietary intervention demonstrated a reduction, but significantly lower than the first group. These results depicted the additional effect of exercise on the liver fat scores for a short intervention period in severe obese group. (Goodpaster et al., 2010).

Furthermore, as far as findings from two meta-analysis of randomized control trials are concerned, physical exercise has been proven to be an efficient method to decrease HS in

obese NAFLD patients. More specifically, pooled data from two studies which utilized moderate aerobic activity and one that utilized resistance exercise revealed substantial HS reduction in comparison to control groups (Musso et al., 2012). Alike results were found in the meta-analysis study of Keating and coworkers (2012). However, the latter group of researchers did not find any statistical difference on the effect size of HS reduction when they compared the exercise combined with diet programs with and the solely diet regimens. (Keating et al., 2012).

In conclusion, taking into consideration the aforementioned observational studies, physical activity has been associated with lower fatty liver deposition. This relationship has been claimed to be causal once longitudinal findings have shown that even after genetic control, the leisure time physical activity has been inversely associated with HS. Furthermore, although regular exercise participation associated with both reduced liver fat and decreased scores of dyslipidemia and dysglycemia, it has been supported that these relationships are regulated by the reduction degree of VAT. In addition, it has been supported that the vigorous intensity is more important than moderate or total volume of physical activity for modulating NAFLD severity. Regarding the randomized control trials, it can be summarized that exercise can be an independent method for reducing HS in obese patients. Last but not least, it has been presumed that diet regimens seems to be equally efficient in improving HS when compared with the combine methods of exercise and diet.

## 5 AIMS, RESEARCH QUESTIONS AND HYPOTHESES

The main purposes of this study is primarily to investigate whether chronic leisure time physical activity could mediate differences in liver fat scores, body composition and abdominal fat. Furthermore, the second aim is to examine the association of liver fat with established cardio-metabolic risk factors such as blood lipids, glucose homeostasis's markers, anthropometrics, abdominal fat and physical activity levels. Thus, the following research questions and hypotheses have been stated as follows.

Question 1: Does chronic physical activity affect the IHTG accumulation in young healthy adults regardless the genetic background?

Hypothesis 1: *Previous findings revealed that chronic leisure-time physical activity can be an independent prophylactic mode for depositing high risk fat in viscera and liver in chronically more active seniors in comparison to less active counterparts (Leskinen et al., 2009). Additionally, Perseghin and associates (2007) demonstrate that the higher liver fat the lower the physical activity index. (Perseghin et al., 2007). Hence, we expect that participants who obtain higher load of leisure-time time physical activity manifest lower liver fat volume compare to those who were less physically active.*

Question 2: Is there any relationship between IHTG and other cardio-metabolic risk factors?

Hypothesis 2: *IHTG has been significantly correlated with visceral adiposity, serum TG, insulin resistance and has been associated inversely with HDL-c in healthy subject prior any overt manifestation of metabolic disease (Speliotes et al., 2011; Seppala-Lindroos et al., 2002). Furthermore, liver fat score has significantly been associated with WC, BMI, VAT as well as SAT (Pietilainen et al., 2005). Hence, it is expected that the liver fat percentage is going to be related unfavorably with the risk factors of cardio-metabolic diseases and the physical activity indexes.*

Question 3: Is there any association between IHTG and magnitude of leisure-time physical activity (LTPA)?

Hypotheses 2: *Nguyen-Duy and colleagues (2003) has found inverse association between cardiorespiratory fitness and hepatic steatosis (Nguyen-Duy et al., 2003). Furthermore, physical activity index was also related inversely with IHTG in subjects with fatty liver and healthy ones. (Perseghin et al., 2007). Hence, we anticipate that the higher LTPA is, the less the liver fat percentage will be.*

# 6 METHODS

## 6.1 Subjects:

For this work 23 apparently healthy male MZ twin pairs were selected from the cohort FinnTwin16 of the FITFATTWIN study. All the participants were non-obese and without any overt health issues. Ten pairs among all were identified as the ones with the most potent physical activity discordant. The co-twins were divided into two groups based on the levels of LTPA (active and in-active). The subjects' characteristics can be seen in the tables 3 and 4.

The study was conducted based on the good clinical and scientific practice/guidelines and the Declaration of Helsinki. The study design was approved March 22, 2011, by the Ethics Committee of the Central Finland Health Care District (Dnro 4U/2011). All participants provided written informed consent.

## 6.2 Study design

This work is based on the FITFATTWIN study, in which the 10 MZ twin pairs have been selected as the ones with the most apparent discordant for LTPA levels. The participants have been tested also for differences in the intra-abdominal fat and glucose homeostasis (Rottensteiner et al., 2016). This study adds the variable of the liver fat percentage in the FITFATTWIN study.

Regarding the LTPA discordant twins, the more active co-twins as well as the less active co-twins were grouped into two different teams, the "active" and the "inactive" one. Firstly, we examined the deference of IHTG between the two groups. The selection of MZ twins as subjects gives us the possibility to test the independent effect of LTPA on the IHTG content. This means that the genetic background and the childhood environment, parameters which affect the fat distribution, were controlled.

The second part of the study included a cross-sectional analysis . Based on the data from all 23 pairs (46 individuals) we run an individual-based association analysis between liver fat and blood lipids (total cholesterol, HDL-c and TG), glucose homeostasis (Oral Glucose Tolerance Test), abdominal fat (VAT and SAT), anthropometrics and body composition (BMI, WC and total fat percent) and LTPA indexes. All the subjects were assessed for their past 3 years and 12 months LTPA.

### 6.3 Retrospective physical activity assessment

Regarding the detailed participant's inclusion process among the twin pairs, the reader is advised to refer to the study of Rottensteiner et al (2016). For note, here we present an overview of all subjects' LTPA assessment, based on the aforementioned original study, as well as the final criteria related to the LTPA discordance of the 10 pairs. All the subjects' selection flowchart is presented in Figure 3. (Rottensteiner et al., 2016).

All the final selected 23 twin pairs, both those who classified as LTPA discordance and the other pairs, were interviewed for recalling their LTPA and the modes of commuting to and from work the past 6 years starting from the examination year, with one-year interval. The total volume of LTPA was expressed with metabolic equivalent (MET) index (MET-hours per day; frequency (per month) x duration (min) x intensity (MET) + work journey (5 times per week x min x 4MET)). The mean MET index of the past 3 years in measurement unit of MET× h/day was one of the final criteria for identifying the 10 pairs with the maximal physical activity discordant.

The primary criteria (criteria 1 and 2 in figure 3) for determining the exercise discordance were based on answered questionnaires in which the frequency of LTPA between the co-twins was taken into account. Hence, the criteria 1 dictated that active co-twin would be considered the co-twin who participated ≥2 times per week in LTPA whereas the in-active the co-twin who participated in physical activity ≤2 times per week. In case the criteria 1 was not met the active co-twin was considered the one who participate ≥2 times per week in LTPA in a higher intensity, frequency and duration compared to their counterpart (criteria 2).

The second key point, which was also considered as a criteria for distinguishing the active from the inactive co-twin, was a more thoroughly interview of the past 12 -month daily LTPA starting from the examination year. The researchers estimated the dose of each activity by quantifying it again in units of MET× h/day. Likewise to the 3-year-LTPA, the overall volume of 12-month-LTPA index was utilized as an extra criterion for identifying the pairs with the major LTPA discordance. The last factor that was taken into account regarding the general physical activity assessment of all subjects and the estimation of the LTPA discordance was the 16-point Baecke questionnaire (Baecke et al. 1982). This item estimates the physical activity by summing 3 elements: work, sport and LTPA. The sport component, in which the volume of vigorous activity was determined, was another selection criterion for detecting the so called active from the inactive co-twins.

The four specific cut-off values from defying the discordant are quoted from the original article as follows (Rottensteiner et al., 2016, 510-511:

"1.Inclusion based on criterion 1 or 2, given previously.

2. A pairwise difference of ≥1.5 MET× h/day between active and inactive co-twins in LTPA (including work journey activity), according to the 12-month physical activity interview (12-month-LTPA index; see later portion)

3.12-month-LTPA index < 5 MET× h/day for the inactive co-twin.

4. ≥1 MET× h/day pairwise difference between active and inactive co-twins in LTPA (including work journey activity) for the past 3 years, according to the shorter physical activity interview (3-year-LTPA index)

5. A higher Baecke sport index for the active versus the inactive co-twin".

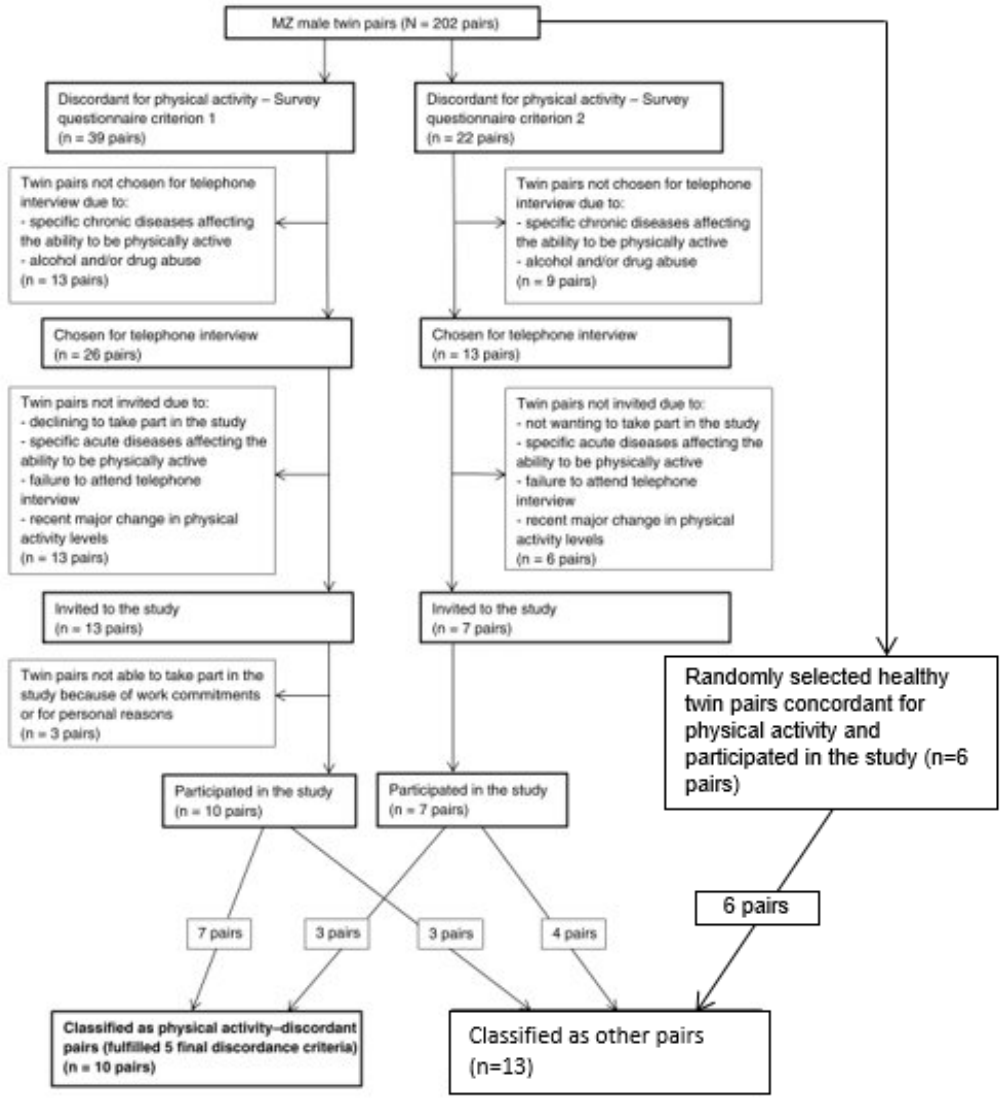


*Figure 3. Modified flowchart of subjects' selection process from* Rottensteiner et al (2016). *The data of the 10 pairs with LTPA discordance were selected to examine differences between the "active" and "inactive" groups. In addition, the data of the 13 pairs who were classified as other pairs were added to examine cross-sectional (the individual-based analysis) associations of health variables.*

## 6.4 Measurements

### *MRI of the abdomen*

A T1-weighted MR scanner (Siemens Symphony, Siemens Medical Systems, Erlangen, Germany) with a 1.5 T were used for capturing axial whole body images. The scanner

parameters were set as follows: matrix size 512 × 384, field of view 1,418 × 680, repetition time (TR) 80ms and echo time (TE) 2.20ms.

The modified Dixon technique was used for the IHTG quantification (Fishbein, Gardner, Potter, Schmalbrock, & Smith, 1997). This method consists of the acquisition of magnitude signal from single slice in-phase (IP) and out-phase (OP) images and the calculation of liver fat score from the liver fat fraction MRI ($FF_{MRI}$) equation ($FF_{MRI}$= ($Signal_{IP}$ – $Signal_{OP}$)/(2×$Signal_{IP}$)). The average signal is obtained from 2 dimensions six regions of interest (ROIs), 1$cm^2$ each in size, that are placed in different liver segments. More specifically, the ROIs were placed in five segments of liver parenchyma: lobus caudatus (segment I), superior sub-segment of the lateral segment of the left lobe (II), left medial segment of the left lobe (IV), superior sub-segment of the anterior segment of the right lobe (VIII) and superior sub-segment of the posterior segment of the right lobe (VII) (Gazelle & Haaga, 1992). All the images were analyzed in the Osirix software (OsiriX Foundation, Geneva, Switzerland). The image analysis was blinded in regards to the LTPA level. The acquired higher liver fat score depicts the higher percentage of steatotic hepatocytes within the liver. This method is considered reliable and valid when compared with the gold standard method of histological analysis of the liver ($r=0.843$, $P<0.001$) (Lee et al., 2010). Abdominal adipose tissue distribution (VAT and SAT) was quantified by analysing a single transaxial slice at the level of L2-L3 intervertebral disc using the slice-o-matic software (http://www.tomovision.com/products/sliceomatic.htm). The image analysis was blinded in regards to the LTPA level as well. (Rottensteiner et al., 2016).

***Blood samples***

Ten hour-fasting blood samples were collected by venipuncture from all the participants after being in a supine position for 10 min. Blood glucose and lipids (total cholesterol, TG and HDL-c) were defined by using the Konelab 20 XT analyzer (Thermo Fisher Scientific, Vantaa, Finland) while serum insulin was defines with an IMMULITE®1000 Analyzer (Siemens Medical Solution Diagnostics, Los Angeles, CA). After the collection of the fasting samples and for assessing the insulin resistance the oral glucose tolerance test (OGTT) was performed. Subjects receive a glucose load of 75g (GlucosePro, Comed LLC, Tampere, Finland) and blood samples were taken at 30 min, 1 h, and 2 h post the ingestion. Glucose and insulin levels at those times points were measured with the previously-

mentioned methods. The homeostatic model assessment (HOMA) index for the evaluation of the insulin resistance was calculated by the equation fasting plasma glucose × fasting plasma insulin)/22.5.

***Anthropometrics and body composition***

Height and body mass were measured to the nearest on 0.5cm and 100g respectively. BMI was determined by the equation; $kg/m^2$. WC was measured by midway between the spina iliaca superior and the lower rib margin to the nearest of 0.5 cm. Whole body composition was defined by using dual-energy x-ray absorptiometry (DEXA Prodigy; GE Lunar Corp., Madison, WI) after participants followed an overnight fast.

**6.5 Statistical Analysis**

The data is presented in mean and standard deviation (mean ± SD), as well as minimum to maximum values. The normality of the data was tested by the Sharipo-Wilk test while the equality of variance was tested by the Levene's test. The between groups comparison were performed with the parametric independent-samples t-test. Pearson's and Spearman's correlation coefficient were used for parametric and non-parametric associations respectively, by utilizing linear regression models. The level of significance was set at $P<0.05$ in all analyses. The SPSS software program (SPSS, Inc., Chicago, IL) was used for the statistical analyses.

## 7 RESULTS

The comparison between the active and inactive group showed that physical activity was significantly higher for the active co-twins. The 3-year-LTPA index in the active members of the physical activity discordant twins pairs was 5.0±2.7 MET× h/day compared to that of co-twins consumed on average 1.7±1.3 MET×h/day in the 3-year-LTPA index (P=0.001). Alike difference was shown in the 12-month-LTMET index in which the active co-twins had statistical significantly (P<0.001) higher mean scores 3.9±1.2 MET× h/day vs inactive co-twins 1.2±0.9 MET× h/day (table 3).

As far as the fatty liver score is concerned, there was no significant statistical difference between the "active" and "inactive" group of the co-twins with the physical activity discordance, although the latter group tended to have higher mean liver fat content. Likewise, neither BMI nor WC displayed statistically significant differences between groups. According to the individual-based correlation analysis IHTG scores did not associate with neither the LTPA indexes nor the Baecke sport index whilst liver fat score was found to have positive association with total body fat percentage, VAT and SAT (table 5).

In regards to the group of 46 subjects (23 pairs), there was a high standard deviation on the LTPA indexes while our participants were not obese on average, based on BMI and WC. Furthermore, the average values of lipid profile and glucose homeostasis were close to desirable levels.

Considering the variables' associations, the liver fat content had a significant positive correlation with BMI and WC with coefficient of r=0.313 (P=0.006) and r=0.396 (P=0.034), respectively. Yet, liver fat deposition associated positively with total fat percent (r=0.397, P=0.006). Additionally, abdominal fat mass was found to have a positive association with liver fat. More specifically, the correlation coefficient between the liver fat score and the VAT one was r=0.465 (P=0.001), whereas it was r=0.350 (P=0.017) between liver fat score and SAT mass. Concerning the associations between liver fat scores, blood lipids and blood pressure, there were no significant correlations. Concerning the glucose homeostasis, the correlation analysis revealed that the higher the liver fat score

the higher the insulin values on the 30min time point of the OGTT (r=0.348, P<0.05) (Table 5 and figure 4). No association was found between HOMA index and IHTG. Also, none of the LTPA variables associated with the IHTG content.

In a partial correlation analysis, liver fat score correlated positively with VAT (r=0.328, P=0.028) after adjustment for SAT. Unlikely, liver fat score did not correlate significantly with total subcutaneous fat after adjusting the variable of VAT. Last but not least, after simultaneous adjustment for BMI and WC, liver fat score associated significantly only with VAT (r=0.427, 0.003) and not with SAT.

*Table 3. Measurements of LTPA, anthropometric and abdominal fat of the 10 twin pairs with significant discordance in the LTPA (N=20).*

| | Inactive[1] (N=10) | Active[1] (N=10) | |
|---|---|---|---|
| | Mean ± SD | Mean ± SD | P value |
| **Age (years)** | 34 (range 32-36) | | |
| ***Leisure-time physical activity*** | | | |
| **3-year-LTPA index (MET× h/day )** | 1.7±1.3 | 5.0±2.7 | 0.001 |
| **12-month-LTPA index (MET× h/day )** | 1.2±0.9 | 3.9±1.2 | <0.001 |
| **Baecke sport index** | 2.2±0.4 | 3.1±0.4 | 0.005 |
| **Anthropometrics** | | | |
| **Body mass (kg)** | 77.8±5.2 | 75.8±5.4 | 0.38 |
| **Body height (cm)** | 179.1±12.7 | 179.8±5.4 | 0.21 |
| **Total body fat percentage (%)** | 24.0±4.6 | 20.7±4.0 | 0.02 |
| **BMI (kg/m²)** | 24.2±3.3 | 23.4±1.7 | 0.28 |
| **WC (cm)** | 88.6±8.2 | 85.3±6.2 | 0.09 |
| **Abdominal adipose tissue** | | | |
| **VAT (kg)[1]** | 2.21±0.7 | 1.69±0.64 | 0.01 |
| **SAT (kg)[1]** | 2.65±0.7 | 2.35±0.68 | 0.21 |
| **Liver fat score[2]** | 0.81±0.19 | 0.80±0.22 | 0.84 |

[1]Determined as in Rottensteiner et al. (2015)

[2]Estimated by: liver fat fraction=(signal in-phase - signal out-phase)/(2*Signal in-phase). Log-transformed

LTPA: Leisure-time physical activity OGTT: Oral glucose tolerance test. SAT: Total subcutaneous fat. Tg:Triglycerides.

VAT: Total visceral fat. WC:Waist circumference.

*Table 4. All subjects' characteristics and measurements (N=46).*

| | (N=46) | | |
|---|---|---|---|
| | **Mean±SD** | **Minimum** | **Maximum** |
| **Age (years)** | 34 | 32 | 37 |
| ***Leisure-time physical activity*** | | | |
| **3-year-LTPA index (MET× h/day)** | 4.6±4.6 | 0.15 | 18.33 |
| **12-month-LTPA index (MET× h/day )** | 4.2±4.5 | 0.12 | 27.67 |
| ***Anthropometrics*** | | | |
| **Body height (m)** | 1.78±0.07 | 1.5 | 1.9 |
| **Body mass (kg)** | 76.5±9.83 | 51.3 | 95.9 |
| **BMI (Kg/m²)** | 24.12±2.71 | 19.8 | 33.57 |
| **WC(cm)** | 86.2±7.78 | 70.5 | 111.5 |
| **Total body fat percentage (%)** | 21.4±6.89 | 7.6 | 36 |
| ***Blood Lipids*** | | | |
| **Fasting total colesterol (mmol/l)** | 4.79±0.96 | 2.9 | 6.4 |
| **Fasting HDL-c (mmol/l)** | 1.33±0.34 | 0.81 | 2.39 |
| **Fasting Triglycerides (mmol/l)** | 1.02±0.50 | 0.37 | 2.97 |
| **Glucose homeostasis (n=42)** | | | |
| **HOMA index** | 0.96±0.87 | 0.04 | 4.28 |
| **Fasting blood glucose (mmol/l)** | 5.5±0.5 | 4.7 | 6.6 |
| **OGTT 30 min glucose (mmol/l)** | 7.5±1.4 | 4.7 | 11.3 |
| **Fasting plasma insulin (µU/mL)** | 3.85±3.19 | 0.2 | 14.6 |
| **OGTT 30 min insulin (µU/mL)** | 47.2±28.4 | 13.3 | 123 |
| **Blood Preassure** | | | |
| **Systolic blood pressure (mmHg)** | 113±10 | 94 | 140 |
| **Diastolic blood pressure (mmHg)** | 69±8 | 50 | 80 |
| ***Abdominal adipose tissue*** | | | |
| **SAT(Kg)** | 2.37±0.91 | 0.77 | 5.36 |
| **VAT (Kg)** | 1.83±0.83 | 0.54 | 4.18 |
| **Liver fat score[2]** | 0.74±0.27 | 0.2 | 1.36 |

[2] Estimated by: liver fat fraction=(signal in-phase - signal out-phase)/(2*Signal in-phase). Log-transformed.
BMI: Body mass index. HDL-c: High density lipoprotein cholesterol. HOMA: Homeostatic model assessment. LTPA: Leisure-time phsycial activity. OGTT: Oral glucose tolerance test. SAT: Total subcutaneous adipose tissue. Tg:Triglycerides. VAT: Total visceral adipose tissue.WC: Waist circumference.

*Table 5. Statistical significant univariate and bivariate correlations.*

| | (N=46) | (N=20)[1] | (N=46) | (N=46) |
|---|---|---|---|---|
| | **Liver fat score** | | **Liver fat score [a]** | **Liver fat score [c]** |
| **WC (cm)** | r=0.396** | | | |
| **BMI (Kg/m²)** | r=0.313* | | | |
| **Total body fat percentage (%)** | r=0.397** | r=0.547* | | |
| **SAT (Kg)** | r=0.350* | r=0.477* | | |
| **VAT (Kg)** | r=0.465*** | r=0.525* | r=0.328* | r=0.427** |
| **OGTT 30min Insulin (µU/m) (n=42)** | r=0.348* | | | |

[1]Pairs with leisure-time physical activity discordance.
[a]Adjusted for SAT. [c]Adjusted for BMI and WC
BMI: Body mass index.OGTT: Oral glucose tolerance test. SAT: Total subcutaneous adipose tissue VAT: Total visceral adipose tissue. WC:Waist circumference.
*P<0.05. **P<0.01. ***P≤0.001

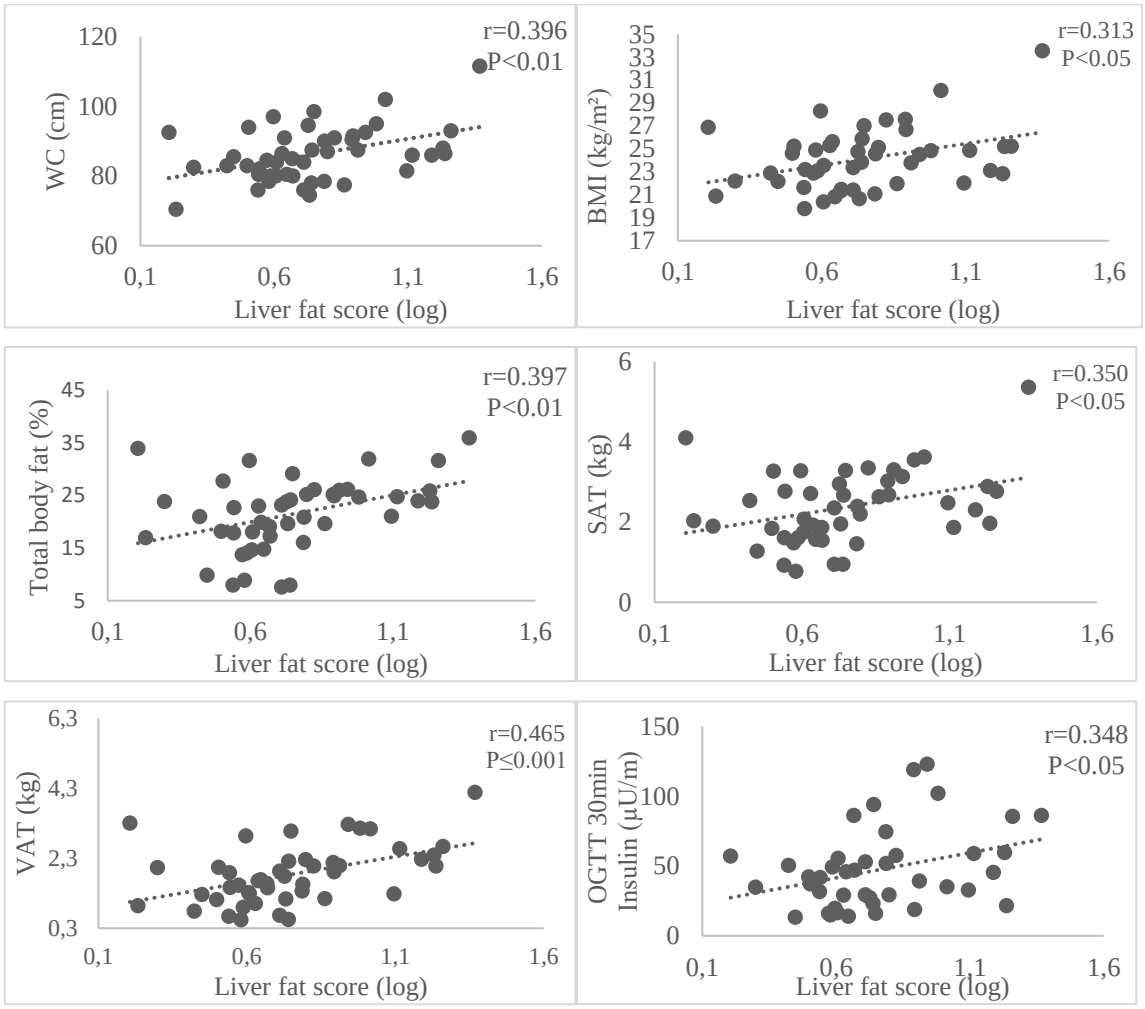


*Figure 4. Significant correlation coefficients of liver fat score with anthropometrics and abdominal adipose tissue. BMI: Body mass index. OGTT: Oral glucose tolerance test. SAT: Total subcutaneous adipose tissue. VAT: Total visceral adipose tissue. WC: Waist circumference.*

# 8 DISCUSSION

The primary findings of the study depicted that active group of co-twins collected higher LTPA levels. Although the liver fat score was higher in the inactive group the difference with their counterpart group was not significant. The correlation analysis of liver fat score with traditional and non-traditional cardio-metabolic risk factors displayed statistically meaningful associations with the abdominal fat, anthropometric measures, and glucose tolerance test. The following sub-chapters include the analysis of the results based on the previous literature as well as the limitations of the study.

## 8.1 Leisure-time physical activity discordance and liver fat in twin subjects.

The findings from the retrospective assessment of LTPA part of the study lead us to reject the alternative hypothesis. In other words, liver fat scores did not differ significantly between the active and in-active groups of co-twins. In contrast to our results, a previous study showed that older (mean 60 years of age) MZ and dizygotic twin participants who had significant discordance in physical activity for 27 years manifested significant between- group difference in the IHTG deposition. More specifically, the in-active co-twins had more liver fat percentage in comparison to the more physically active co-twins. (Leskinen et al., 2009). The inconsistency of our results with the ones of the latter study might be indirectly due to the higher age of the subjects and some methodological differences.

Regarding the explanation why the effect of physical activity on IHTTG content was not independent and significant in this study as it was found in the similar study of Leskinen and colleagues (2009), it is assumed that differences in the methodology of the latter study might have affected its results which we based on as hypothesis in this work. In other words, it might be so that other variables, which were not assessed, other than LTPA were responsible for the lower liver fat score in the group with the active co-twins. For supporting this concept it is reminded that the IHTG accumulation is a results of the derived FFA from the adipose tissue, the diet fats, the de novo lipogenesis, the rate of TG secretion and the mitochondrial fat oxidation. Twin pairs most likely share similar habits in nutrition

as well as similar childhood family environment. However, in the study of Leskinen and coworkers (2009) although total energy consumption and alcohol intake were assessed in the time point of measurements, neither serum lipids nor specific nutrients intake were distinguished. These two variables are potential influencers of IHTG content. Taking all these into consideration, we could mention that the difference on the liver fat scores might be ascribed on those factors that were not assessed retrospectively as LTPA was. Presumably, the dietary preferences and the lipid profile were different between the pairs due to the higher age and the effect of LTPA was minor in this study. Hence, these methodological differences might have given false basis for our hypothesis.

Nevertheless, the higher likely reason of the retention of the null hypothesis is the rather similar BMI between the groups in our study that subsequently affects the IHTG content. Findings from the study of Pietilainen and colleagues (2005) depicted that MZ twins with obesity discordance, defined by BMI difference over 4 $kg/m^2$, revealed considerable higher liver fat scores for obese co-twins, whilst twin pairs with BMI differences $\leq 2$ $kg/m^2$ had no difference in liver fat score. Additionally, exercise effects on liver fat deprived mainly by the reduction of hepatic fats availability and synthesis and the increase on the hepatic TG oxidation. It is mentioned though, that once the body mass kept constant the modulation of the liver fat might be modest. (Brouwers et al., 2016). Therefore, the cause of why the active subjects in this study did not manifest less liver fat might hide under the fact that they did not differ on body mass from the in-active co-twins.

On the other hand, there are findings showing that liver fat is associated more specifically to VAT than SAT or BMI (Westerbacka et al., 2004; Burgert et al., 2006), although that has not been supported by other findings (Seppala-Lindroos et al., 2002). The alternative hypothesis in the first question of this work was based on the premise that the higher contributor of the IHTG content is the circulating FFA from the adipose tissue (Donnelly et al., 2005). Also, it has been supported that VAT drains more FFA to the liver than SAT do (Ibrahim et al., 2009). Moreover, both in the work with the older twin subjects (Leskinen et al., 2009) and in our study sample, the twins with LTPA discordance had significant differences in VAT and total body fat percentage (Table 3), whilst they manifested no difference in anthropometrics (BMI, WC) and in SAT. Subsequently, it is assumed that also in my work the active co-twins would have had less ITHG once they had significant less VAT. However, Tiikkainen and colleagues (2001) revealed that the reduction of liver

fat after 8% loss in body mass in obese women associated significantly with the initial magnitude of IHTG deposition rather than the changes in VAT or SAT. The IHTG decrease was substantially higher in those women with higher initial liver fat content regardless the similar intra-abdominal and subcutaneous fat loss. Last but not least, the researchers speculated that variables other that VAT and SAT might regulate liver fat. (Tiikkainen et al., 2001). The fact that in this study both groups' fatty liver content was averagely low (non-log transformed IHTG=7.2%) might explain the reason why there was no substantial difference between the groups regardless the positive correlation of IHTG content with total body fat percentage, SAT and VAT.

The strength of this work is the MZ twin pairs as subjects who are perfectly matched in terms of the genes and most likely, they have had similar environmental influence once they shared the same family childhood environment. By choosing the MZ twins with the most apparent LTPA discordant for substantial time period we can argue that the acquired habits of being physically active will be the solely responsible factor for any changes in health features. Measurements regarding the nutrient intake between the active and in-active twin group of our study showed that there was any difference neither in macronutrients nor in total energy intake (Rottensteiner et al., 2016). Thus, with the given findings we could state that IHTG content cannot be influenced considerably by the amount of LTPA at this age group of apparently healthy males with similar nutrient intake. Compared to the general observational studies and control trials, studies with MZ twin pairs discordant in LTPA avoid the highly potential risk of individual variability on the responses to physical activity that might over or under estimate the real independent effect of exercise on health factors. Additionally, the method of MRI as a valid and reliable method of liver fat content increases the strength of our findings.

Notwithstanding, the first part of the study has some limitations. The retrospective method of assessing the LTPA indexes hides the recalling error from the subjects regarding the levels of LTPA. Furthermore, the number of our participants was rather small and inadequate to reveal any statistical significant differences. Subsequently, we cannot extrapolate the results to the population. Nevertheless, it has been clear that regular participation in physical activities induce several acute and long-standing health benefits. Thus, it goes without saying that the consistency in adherence on physical activity guidelines will be evoking beneficial health results.

## 8.2 Association between liver fat score, cardio-metabolic risk factors and physical activity.

Regarding the findings of the cross-sectional part of our study which included all the 23 MZ twin subjects (N=46) lead us to reject partly the null hypothesis which stated that liver fat would not have any association with cardio-metabolic risk factors. More specifically, we had expected that liver fat would have associated with BMI, WC, SAT, VAT and total body fat percentage and features of insulin resistance. However, there was no association among liver fat score and BP, lipid profile and the rest measurements of glucose homeostasis. Therefore, due to the lack of association of the latter variables as well as the LTPA with the liver fat score, the stated alternative hypothesis is rejected.

The adipose tissue has been found to be the major source of non-esterified fatty acids of the liver for IHTG formation (Donnelly et al., 2005). Especially, the increased abdominal adipose tissue measured by tools which take into account the circumference of waist and height (waist to height ratio), has been associated stronger with cardio-metabolic risk, even in apparently normal weight individuals  defines by BMI and WC (Li et al., 2013). Moreover, in a prospective study the hazard ratio for stroke, CVD and diabetes mellitus was bigger for the subjects with higher waist-hip-ratio compared to the increased BMI or WC even after the adjustment of BMI, age, cigarette smoking, SPB, total cholesterol, physical activity and alcohol intake (Canoy et al., 2007). Furthermore, a better predictor of cardio-metabolic risk has been deemed to be the ectopic fat that is located in the viscera and the liver (Britton & Fox, 2011). Findings from two big cohort studies depict the high association of the VAT, the SAT and the liver fat with cardio-metabolic risk and also the association between two factors (Speliotes et al., 2010; Liu et al., 2011), while the liver fat content has been proposed to be stronger determinant for coronary calcification and metabolic syndrome (Lee et al., 2012; Lee et al., 2015b). The findings of our work are consistent with these results regarding the association of the liver fat and the SAT as well as the VAT, yet in healthy individuals.

Regarding the percentage of total body fat and the liver fat score, we found that there was a positive significant correlation between these variables. Previous findings showed that

there was no association between these two variables in obese adolescents with fatty liver (Burgert et al., 2006). On the other hand, only when adult patients with simultaneous NAFLD and obesity manifested significant higher total body fat percentages in contrast to those with only present of NALFD (Lee et al., 2015).

On the association of the liver fat with glucose homeostasis and insulin sensitivity, previous finding has demonstrated that healthy subjects with liver fat had higher endogenous glucose production in the hyperinsulinemic state, as well as worst suppression of the endogenous glucose during the last 60 minutes of the 300-minute euglycemic insulin clamp, compared to the healthy individuals without fatty liver (Seppala-Lindroos et al., 2002). Likewise, the absence of the substantial liver fat failed to manifest hepatic insulin resistance in both severely obese groups with difference in the grades of obesity (Magkos et al., 2010). Findings from our study evidenced that HS associated positively only with the OGTT 30 min insulin.

As far as the blood lipids and BP association with HS, two big cohort studies depicted that there is a positive association between liver fat content, BP and TG as well as negative correlation with HDL-c regardless the age. However, when the traits of obesity were adjusted for the analysis BP stopped being related with HS (Patel et al., 2015), and only HDL-c and TG retained correlated (Speliotes et al., 2010). In line with the latter study and the association of HS with the blood lipids Magkos and colleagues (2010) found that the absence of fatty liver in obesity of class I did not evoke any difference in the VLDL-TG levels in comparison to the significantly counterpart group with obesity class III. (Magkos et al., 2010). Our results showed absence of correlation between HS, BP and blood lipids in our cross sectional analysis of the healthy young adults. Likewise, liver fat score did not correlate with the prospectively assessed LTPA. As we mentioned in the previous subchapter the absence of this association might be ascribed to the averagely normal weight and liver fat content of our subjects (Brouwers et al., 2016) .

Concerning our findings about the relationship between VAT with IHTG deposition independently of SAT and BMI, previous findings has depicted that Japanese women with MS and simultaneous elevated VAT were more prone to have fatty liver compared to those with MS and elevated SAT (Sogabe et al., 2014). Similarly, Pietilainen and colleagues (2005) have supported that the acquired obesity in MZ twins (defined predominantly by

the elevated SAT) associated with elevated IHTG content. However, this relationship does not demonstrate causality and it might be so that the VAT, which also associated with HS, mediates to this correlation. (Pietilainen et al., 2005).

In regards to the underlying physiological mechanism that explains the interaction between VAT and fatty liver have not yet been fully elucidated. However, it is supported that the link derives from the dysfunctional cross-talk between the VAT and the liver. More specifically, FFA and adipocytokines influx to the liver mediate abnormal liver fat content. Also, insulin resistance in parallel with the oxidative stress and the low grade inflammation are presumed as the main factors that connect VAT with excess IHTG. (Verrijken, Francque, & Gaal, 2011). Due to cellular differences between the SAT and VAT, the latter are more prone to have large adipocytes which are less insulin sensitive, more lipolytic and tolerant to the anti-lypolitic effect of insulin (Ibrahim, 2010, 12).

Moreover, this work's association of HS with the OGTT 30 minute insulin might be explained with caution as a sign of hepatic insulin abnormal sensitivity. Abdul-Ghani and colleagues (2007) demonstrated that an equation that takes into account the levels of glucose and insulin from 0 minute to 30 minute of the OGTT correlated significantly with the most definitive method of hyperinsulinemic-euglycemic clamp about the hepatic insulin resistance. The explanation relies on the premise that the initial response of the insulin after the rise of blood glucose aims to suppress the hepatic endogenous production. Thus, the increase in the initial glucose and insulin levels following the ingestion of glucose depicts a failure of the pancreatic β-cells for adequate insulin production or impaired hepatic insulin sensitivity. (Abdul-Ghani et al., 2007).

Although it has not yet been elucidated whether fatty liver is a cause or an effect of impaired glucose tolerance and MS, the potential association has supported to depend on the hepatic insulin resistance. In the state of elevated VAT, the hypertrophic adipocytes tend to increase the flux of adipocytokines such as resistin, adiponectin and leptin. Increased levels of resistin found in obese individuals antagonize liver insulin action, whilst, although leptin promotes insulin-sensitivity, obesity has been associated with leptin resistance. Moreover, adiponectin facilitate insulin action, which has been also found low in obesity. Hence, the maleficent secretion of those adipocytiokines might underlie the hepatic steatosis and

impaired hepatic insulin function in obesity and even non-obese with abnormally high VAT. (Buechler et al., 2011).

In regards to the strengths of this part of our work we could mention that our results are consistent with the literature and confirm the importance of liver fat score once has been related with the high risk fat compartments even in the absence of overt abnormal values of cardio-metabolic risk factors. We cannot presume whether associations of liver fat with high risk could remain and contribute to future lesions or not but based on the literature the liver fat, even in some cases regardless the increased BMI, might be the hepatic manifestation of MS.

Finally, it goes without saying that the low number of our participants is a major limitation of our work and we cannot extrapolate our result into the population. Furthermore, the nature of cross-sectional analysis does not imply causal relationships but only association. That means that other factors, which have not been taken into account, might affect our findings.

### 8.3 Conclusion

In conclusion, ectopic fat in areas such as the liver and the viscera is believed to mediate the cardio-metabolic lesions of obesity. The presence of ectopic adipose tissue has also been pointed out as the cause of metabolic syndrome manifestation in non-obese population. Physical activity and exercise have been associated reversely with obesity and with the obesity-related metabolic dysfunctions. However, there exists a challenge to test the independent contribution of physical activity on the amelioration of metabolic factors. Previous studies have found that chronic adherence on physical activity might evoke lower levels of visceral and liver fat and that effect was independent of genetic background and total energy expenditure.

In this study, the effect of physical activity was examined on intrahepatic fat in heathy young adults without any overt health issues. Results showed that even though the less active co-twins had significantly higher VAT compared to the active counter parts that did not translate into higher liver fat scores. It has been supported that levels of physical

activity, the nutrient intake, the initial liver fat and the body mass are some factors that determine the reduction of the IHTG content by the physical activity. Even though in this work the co-twin groups had significant discordance in the LTPA there was no statistical significant difference on liver fat score. It is presumed that the explanation to this result is the absence of abnormal liver fat scores, the lack of substantial BMI deference nutrient and total energy intake between the active and inactive co-twins. It is worth mentioning that once VAT is associated positively with hepatic steatosis it is rather important to encourage the chronic adherence in physical activity in order to minimize a potential future increase in hepatic fat and subsequent possible metabolic lesions.

Moreover, additionally to the correlation of liver fat with the visceral fat other cardio-metabolic risk factors have been found to be associated with these ectopic fat areas. Several studies have supported the notion that fat distribution is stronger correlator with risk factors of cardio-metabolic abnormalities than anthropometrically-determined obesity. Both visceral fat and liver fat are considered the main determinants of this association even though it is not yet fully elucidated which variable plays the major role.

Therefore, we performed a cross-sectional analysis to examine the association of liver fat with variables of abdominal fat, anthropometrics, blood lipids, glucose homeostasis and physical activity in a sample consisted from healthy young adults in middle thirties who had no overt health issues. The findings depicted a series of significant moderate positive correlations between liver fat scores and the anthropometric measurements such as WC and BMI as well as with variables of abdominal adipose tissue like VAT and SAT and total fat percentage. Also, there existed an association between liver fat and the level of 30min insulin of the OGTT. Interestingly, the association between liver fat and VAT remained significant moderate and positive after statistical adjustment of SAT, WC and BMI. The positive correlations of these observational studies are not translated as causal relationships between HS and the cardio-metabolic factors but they confirm the link between the ectopic fat areas with the local and systemic functions. Based on these results and previous perspective studies, it can carefully be suggested that even apparently healthy young adults should be careful with their nutritional and activity habits in order to minimize the future risk of cardio-metabolic abnormalities.

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