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Fatty acid metabolism is altered in non-alcoholic steatohepatitis independent of obesity☆

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ABSTRACT

Background. Non-alcoholic steatohepatitis (NASH) is associated with changes in fatty acid (FA) metabolism. However, specific changes in metabolism and hepatic mRNA expression related to NASH independent of simple steatosis, obesity and diet are unknown.

Methods. Liver histology, serum and liver FA composition and estimated enzyme activities based on the FA ratios in cholesteryl esters and triglycerides were assessed in 92 obese participants of the Kuopio Obesity Surgery Study (KOBS) divided to those with normal liver, steatosis or NASH (30 men and 62 women, age 46.8 ± 9.5 years (mean ± SD), BMI 44.2 ± 6.2 kg/m^2). Plasma FA composition was also investigated in the Metabolic Syndrome in Men (METSIM) Study (n = 769), in which serum alanine aminotransferase (ALT) was used as a marker of liver disease.

Results. Obese individuals with NASH had higher activity of estimated activities of delta-6 desaturase (D6D, $p < 0.002$) and stearoyl-CoA desaturase 1 (SCD1, $p < 0.002$) and lower activity of delta-5 desaturase (D5D, $p < 0.002$) when compared to individuals with normal liver. Estimated activities of D5D, D6D and SCD1 correlated positively between liver and serum indicating that serum estimates reflected liver metabolism. Accordingly, NASH was associated with higher hepatic mRNA expression of corresponding genes *FADS1*, *FADS2* and

Abbreviations: NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; FA, fatty acid; VLCD, very low calorie diet; CE, cholesteryl esters; TG, triglycerides; PL, phospholipids; METSIM, Metabolic Syndrome in Men; ALT, alanine aminotransferase; KOBS, Kuopio Obesity Surgery Study; SCD1, stearoyl-CoA desaturase 1; D5D, delta-5 desaturase; D6D, delta-6 desaturase; DNL, de novo lipogenesis; SFA, saturated fatty acid.

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SCD. Finally, differences in FA metabolism that associated with NASH in obese individuals were also associated with high ALT in the METSIM Study.

Conclusions. We demonstrated alterations in FA metabolism and endogenous desaturase activities that associate with NASH, independent of obesity and diet. This suggests that changes in endogenous FA metabolism are related to NASH and that they may contribute to the progression of the disease.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) refers to a wide spectrum of liver damage that ranges from simple steatosis to liver cirrhosis affecting up to 30% of western population [1]. Non-alcoholic steatohepatitis (NASH) is characterized by inflammation and cytological ballooning in addition to steatosis [2,3]. Furthermore, patients with NASH have an increased risk of developing an end-stage liver disease and cardiovascular diseases [4,5]. Therefore, identifying the factors that contribute to the progression of NAFLD is important.

Liver fat accumulation is a result of imbalance between fatty acid (FA) uptake and disposal. Insulin resistance in adipose tissue leads to increased lipolysis and consequently to increase in the release of free FAs, which is the major source of triglycerides stored in the liver [6–8]. In animal studies, it has been suggested that individual free FAs promote oxidative stress and hepatic insulin resistance [9,10] and in humans free FAs have been associated with the severity of liver injury [11]. In fact, NASH has been associated with specific alterations in both hepatic and serum FA compositions when compared to normal controls and subjects with steatosis [12–16], suggesting a role of individual FAs in the development of NASH [17,18]. Recently, an association between circulating phospholipid profile and NASH was defined in obese individuals [19]. In addition to NASH-related changes in FA composition, the hepatic gene expression and liver FA composition differ between subjects with steatosis and NASH [16,20,21]. However, specific differences in serum, and especially in the liver, related to NASH independent of obesity and diet have not been investigated.

The aim of the present study was to investigate if FA metabolism is altered in individuals with NASH compared to those with normal liver and steatosis, independent of diet and obesity. To this aim we analyzed serum FA composition, liver FA composition and hepatic mRNA expression in morbidly obese individuals with histologically normal liver, simple steatosis or NASH after a standardized very low calorie diet (VLCD). We analyzed the relative amounts of FAs in cholesteryl esters (CE), triglycerides (TG) and phospholipids (PL) and calculated indices estimating activities of key enzymes regulating FA metabolism. Finally, the relevance of our results was studied in a large randomly selected population-based Metabolic Syndrome in Men Study (METSIM, n = 769), in which serum alanine aminotransferase (ALT) and previously published NASH score were used as markers of liver disease [22,23].

2. Methods

2.1. Kuopio Obesity Surgery Study (KOBS)

All patients undergoing obesity surgery in Kuopio University Hospital are recruited into an ongoing study investigating metabolic consequences of obesity surgery [24,25]. Present study contains cross-sectional baseline data from 92 subjects (30 men and 62 women, age 46.8 ± 9.5 years (mean ± standard deviation (SD)), body mass index (BMI) 44.2 ± 6.2 kg/m^2) (Table 1), who were accepted for Roux-en-Y gastric bypass operation. All patients with alcohol consumption of >2 doses per day or patients with previously diagnosed liver diseases not related to obesity were excluded from the study. All subjects were instructed to follow a preoperative VLCD for an average of 4 weeks. Patients consumed special products designed for VLCD and the daily energy intake was aimed to be 600–800 kcal. The study protocol has been approved by the Ethics Committee of the Northern Savo Hospital District (54/2005, 104/2008 and 27/2010), and was performed in accordance with the Helsinki Declaration. Written informed consent was obtained from the subjects.

2.2. Population-Based METSIM Study

In order to validate the findings in a randomly selected population sample, we analyzed data on 769 men from the cross-sectional METSIM study (Supplementary Table 1). The study protocol has been described earlier [26]. In short, all men aged 45–70 years and included in the population register of Kuopio, eastern Finland, were contacted with a letter. A total of 10,197 men participated in the METSIM study in 2005–2010. All patients with alcohol consumption of >2 doses per day were excluded from statistical analyses. A subsample of 769 men, aged from 47 to 75 years, who participated consecutively in a follow-up examination in 2010–2011, had a blood sample drawn. Subjects were divided based on the median of serum ALT value (24 U/l) to low ALT group (n = 386) and to the high ALT group (n = 383). The METSIM study was approved by the Ethics Committee of the University of Kuopio and Kuopio University Hospital (17/2004) and was conducted in accordance with the Helsinki Declaration. All participants gave a written informed consent.

2.3. Clinical Measurements and Laboratory Determinations

BMI was calculated as weight (in kilograms) divided by height (in meters) squared. Serum glucose concentration was measured by enzymatic hexokinase photometric assay (Konelab Systems Reagents; Thermo Fischer Scientific, Vantaa, Finland).