Institute for Clinical and Experimental Medicine

Monitoring of hepatic fat metabolism using magnetic resonance methods

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Study protocol

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(translation of original Study protocol in Czech dated April 2016)

Introduction

The epidemic of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis is becoming a major challenge currently faced by health systems worldwide. Importantly, the first stage of the disease is simple steatosis caused by excessive fat accumulation in the liver (in the absence of significant alcohol consumption). Hepatic fat comes from three major sources – non-esterified fatty acids (NEFA) released from adipose tissue, dietary fat, and hepatic *de novo* lipogenesis (DNL). However, hepatic triglycerides (TG) are subsequently metabolized or, more importantly, quickly secreted from the liver in very-low-density lipoproteins (VLDL), which explains why TG should not accumulate in the liver. Steatosis develops when the delicate balance between these processes is disrupted and hepatic TG production outweighs TG export to other tissues.

In healthy non-steatotic subjects, the daily turnover of hepatic TG might even exceed hepatic fat content (HFC). It is estimated that up to 20% of dietary fat is delivered to the liver both as TG of chylomicron remnants and spillover fatty acids released from dietary TG by lipoprotein lipase in the circulation and not captured by extrahepatic tissues. Therefore, a sufficient amount of dietary fat provided to healthy non-steatotic subjects should induce changes in HFC detectable using ^{1}H magnetic resonance spectroscopy (^{1}H-MRS) even within hours. However, liver fat accumulation may be strongly affected by the coadministration of other nutrients, especially carbohydrates, which induce regulatory responses diverting metabolism to carbohydrate oxidation and fat storage and, possibly, also affecting HFC.

The current epidemic of NAFLD seems to be tightly linked to excessive fructose consumption. However, it has not been clarified yet whether fructose is just a marker of increased caloric intake leading to insulin resistance and ectopic fat accumulation or the culprit directly involved in fat accumulation in the liver. It has been documented that metabolism of fructose, contrary to that of glucose, can indeed induce metabolic changes possibly promoting hepatic fat accumulation but up to now there is no direct proof that fructose consumption induces immediate fat accumulation in the liver.

In this study we therefore aim to determine, using ^{1}H-MRS, the immediate effect of high-fat load on HFC and whether such an effect can be modified by fructose and/or glucose coadministration. The fat load (150 g, whipping cream) will be administered subjects after

baseline measurement of HFC. Because of relatively short-term impact of simple sugars on regulatory pathways, the glucose and fructose will be administered to subjects in three doses in 2-hour intervals.

Objective(s)

The objectives of the present study are as follows:

- To determine whether high-fat load (150 g of fat) induces an increase in hepatic fat content (HFC) that is detectable by magnetic resonance spectroscopy (MRS) 3 and 6 hours after administration
- To determine whether changes in HFC induced by high-fat load can be affected by coadministration with glucose at three doses of 50 g throughout the experiment
- To determine whether changes in HFC induced by high-fat load can be affected by coadministration with fructose at three doses of 50 g throughout the experiment

Design

The present study is a randomised, controlled, crossover design in which 2 groups of 10 subjects – 10 nonobese healthy males with normal hepatic fat content (HFC) as determined by proton magnetic resonance spectroscopy (^{1}H-MRS) and 10 nonobese nondianbetic males with hepatosteatosis (HFC more than 5%) will complete total six examinations lasting approximately 8 hours. The examinations will differ in the dietary load and will be carried out in a randomised order and will consist of three measurements of hepatic fat in (HFC) – before and 3 and 6 hours after defined dietary load.

Methods

Screening

The subjects will firstly undergo a screening visit to the laboratory in which written informed consent will be obtained. Further to this, anthropometric measures (height, weight, hip and waist circumferences) and blood sample will be taken for biochemical parameters that are used as inclusion/exclusion criteria (cholesterol, glucose, AST, ALT, HbA1c). Moreover, oral glucose tolerance test will be carried out to exclude diagnosis of diabetes mellitus. If the subjects meet the inclusion criteria, they will be invited to examination in which their hepatic fat is measured using ^{1}H-MRS. Based on the results of this examination (Examination

A), they will be included into group with normal HFC (less than 5% of fat in the liver) or into group with steatosis (more than 5% of fat in the liver).

Experiments/examinations

All subjects included into the study will comlete six examinations. They will be asked not to change their everyday habits on the day before the study and consume their last meal no later than 10 PM. They will come to the MR department at 7:30/8:15, sign the informed content for MR examination and pass the first MR examination. After that the cannula for blood draws will be inserted into the antecubital vein and the first blood sample taken; the volunteers will then receive the first „meal“ (whipping cream and/or fruit tea with 50 g of glucose or 50 g of fructose). The time when they will finish the „meal“ will be time 0 of the examination. The dietary interventions will be:

- High-fat load: 150 g of fat – 470 ml of whipping cream
  High-fat load + glucose: 470 ml of whipping cream and fruit tea with 50 g of glucose administered to volunteers at the same time as cream (time 0) and then 2 and 4 hours later
- High-fat load + fructose: 470 ml of whipping cream and fruit tea with 50 g of fructose administered to volunteers at the same time as cream (time 0) and then 2 and 4 hours later
- Fasting: no food administered during experiment
- Glucose: fruit tea with 50 g of glucose administered to volunteers at time 0 and then 2 and 4 hours later
- Fructose: fruit tea with 50 g of fructose administered to volunteers at time 0 and then 2 and 4 hours later

During each examination volunteers will not consume any other food and will be allowed to drink only water or fruit tea (without sugar).

Nine more blood samples will be obtained at times 0.5, 1, 2, 2.5, 3, 4, 4.5, 5, and 6 hours, two other MR measurements will be carried out at time 3 and 6 hours (immediately after blood draws).

The Sampling protocol that will be used in each experiment is presented (Figure 1).

During the day of examination the person conducting the study with volunteer will fill in the questionnaire on physical activity, previous day's food and possible illnesses or used

medication. Moreover, during the day of examination, the body fat content and body composition will be determined by bioelectrical impedance analysis (BODYSTAT® 1500).

Figure 1. Sampling protocol of the study

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Protocol

Examination number: Date:

Name:

Phone number: Bodystat (#):

Height (cm): Weight (kg):

Waist (cm): Hip (cm):

	Time	Time planned	Time real	Comments	Vacutainers
MR examination					
Blood draw 0					9 ml violet (EDTA) 2 ml grey (EDTA, NaN3)
Cream / sugar	0 h				
Blood draw 1	0h 30				9 ml violet (EDTA)
Blood draw 2	1h				9 ml violet (EDTA)
Blood draw 3	2h				9 ml violet (EDTA)
Sugar	2h				
Blood draw 4	2h 30				9 ml violet (EDTA)
Blood draw 5	3h				9 ml violet (EDTA)
MR examination	3h				
Blood draw 6	4h				9 ml violet (EDTA)
Sugar	4h				
Blood draw 7	4h 30				9 ml violet (EDTA)
Blood draw 8	5h				9 ml violet (EDTA)
Blood draw 9	6h				9 ml violet (EDTA)
MR examination	6h				

Labeling of tubes:

subject number (01-20), experiment (A-F, see below), blood draw number (0-9), aliquot label (I-V), date

Experiments:

B fasting
C cream + glucose
D cream
E cream + fructose
F glucose
G fructose

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Methods

HFC will be measured by *in vivo* ^{1}H MRS. Examinations will be performed on a 3T whole body scanner (3T Trio Siemens, Germany). Standard PRESS (Point Resolved Spectroscopy sequence) single voxel spectroscopy will be used [echo time (TE) 30 ms and repetition time (TR) 4500 ms] to measure HFC. Voxel size of our volume of interest (VOI) will be set to have 40x30x25 mm and the VOI position in V/VIII liver segments will be carefully checked during all consecutive examinations. One image acquisition during each breathhold will be obtained with the measurement repeated three times. Relaxation times for saturation corrections will be measured using the same sequence (PRESS) with echoes: 30, 50, 68, 135, 180 and 270 ms. Spectra will be evaluated using LCModel version 6.2 (http://s-provencher.com/lcmodel.shtml). Three lipid signals of aliphatic protons in the range 0.0-3.5 ppm (-CH_2 1.2-1.4 ppm; -CH_3 0.8-0.9 ppm; -CH_2CH_2CH=CH 1.9-2.1 ppm) and water and olefinic protons signals (4.7 and 5.3 ppm) will be fitted and the percentage of HFC will be calculated according to Longo et al. (*J Magn Res Imaging 1995; 5: 281-5*).

Biochemistry

Blood will be collected into 8ml vacutainers with EDTA and immediately placed on the ice. The centrifugation will be carried out for 15 min at 4°C, 3000 g, and plasma divided into 5 aliquots (min. 0.5 ml) that will be stored at -80°C. One aliquot will be used for determination of TG, glucose, and NEFA (Lipid laboratory CEM IKEM), two other aliquots will be used for determination of insulin (IRMA, Beckman) and glucagon (RIA, Millipore), and two will serve as a reserve for repetition or other analyses.