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Original Article

Effects of a whey protein supplementation on intrahepatocellular lipids in obese female patients

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SUMMARY

Background & aims: High protein diets have been shown to improve hepatic steatosis in rodent models and in high-fat fed humans. We therefore evaluated the effects of a protein supplementation on intrahepatocellular lipids (IHCL), and fasting plasma triglycerides in obese non diabetic women.
Methods: Eleven obese women received a 60 g/day whey protein supplement (WPS) for 4-weeks, while otherwise nourished on a spontaneous diet, IHCL concentrations, visceral body fat, total liver volume (MR), fasting total-triglyceride and cholesterol concentrations, glucose tolerance (standard 75 g OGTT), insulin sensitivity (HOMA IS index), creatinine clearance, blood pressure and body composition (bio-impedance analysis) were assessed before and after 4-week WPS.
Results: IHCL were positively correlated with visceral fat and total liver volume at inclusion. WPS decreased significantly IHCL by 20.8 ± 7.7%, fasting total TG by 15 ± 6.9%, and total cholesterol by 7.3 ± 2.7%. WPS slightly increased fat free mass from 54.8 ± 2.2 kg to 56.7 ± 2.5 kg, $p = 0.005$). Visceral fat, total liver volume, glucose tolerance, creatinine clearance and insulin sensitivity were not changed.
Conclusions: WPS improves hepatic steatosis and plasma lipid profiles in obese non diabetic patients, without adverse effects on glucose tolerance or creatinine clearance.
Trial Number: NCT00870077, ClinicalTrials.gov

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterized by an elevated intrahepatocellular lipid (IHCL) concentration. Incidence of NAFLD is frequently increased in obese patients, and is considered as the hepatic component of the metabolic syndrome. It is tightly associated with the metabolic complications of obesity, i.e. insulin resistance, impaired glucose tolerance, and dyslipidemia.[1,2]

Several reports suggest that a high protein intake may improve NAFLD. In high-fat fed rats, increasing the proportion of protein in the diet reduced hepatic steatosis and dyslipidemia.[3,4] In healthy human male subjects in whom IHCL concentrations had been nearly doubled by a 4-day hypercaloric, high-fat feeding, increasing the dietary protein intake reduced significantly IHCL concentration.[5] These observations suggest that a high protein intake may exert beneficial effects in NAFLD patients. We therefore hypothesized that increasing the dietary protein intake in the same range as that which reduced IHCL in high-fat fed subjects[5] would also reduce IHCL concentrations in obese patients. To evaluate this

Abbreviations: IHCL, intrahepatocellular lipids; WPS, whey protein supplementation; MR, Magnetic Resonance; ^{1}H-MRS, ^{1}H- Magnetic Resonance Spectroscopy; NAFLD, non-alcoolic fatty liver disease; OGTT, oral glucose tolerance test; HOMA, homeostasis assessment model; HOMA IS, HOMA of insulin sensitivity; NEFA, non esterified fatty acids; BOHB, beta-hydroxybutyrate.

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hypothesis, we assessed the effects of a 4-week supplementation with 60 g/day whey protein (Whey Protein Supplement : WPS) in obese non diabetic female patients.

2. Research design and method

2.1. Participants

11 obese female patients, aged 38 ± 2 years, were recruited at the obesity clinics of the Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne, Switzerland. They had a mean body weight of 99.7 ± 5.3 kg, a mean height of 1.63 ± 0.02 m, and a mean BMI of 37.6 ± 1.8 kg/m^2. None had liver or renal disease, nor was on antidiabetic or antilipemic agents. They were sedentary (less than two sessions of physical activity per week). All reported a daily alcohol intake less than 20 g. The experimental protocol was approved by the Ethical Committee of Lausanne University School of Medicine and was registered at ClinicalTrial.gov (Trial Number: NCT00870077, ClinicalTrials.gov). Subjects gave their written informed consent before participating in the study.

2.2. Study design

After inclusion, subjects reported in the morning after an overnight fast to the Cardiomet Clinical Investigation Center (Cardiomet CIC) of the Lausanne University Hospital. Their body weight and blood pressure were measured and their body composition was assessed by bioelectrical impedance analysis. Thereafter, they underwent a standard 75 g oral glucose tolerance test (OGTT) with measurement of plasma glucose and insulin at time 0 and 120 min. Fasting plasma triglycerides, total cholesterol, HDL cholesterol, urea, creatinine, ASAT, ALAT and urinary urea and creatinine concentrations were assessed using a bench-top clinical chemistry analyzer (RX Monza, Randox Laboratories Ltd, Crumlin, UK). Plasma insulin and glucagon were measured by radio-immuno assays using kits from Millipore, St Charles, Missouri, USA). A 24 h urine collection was obtained for determination of urea and creatinine excretion. Total nitrogen excretion was calculated assuming that urea accounted for 85% of total urinary nitrogen and that extra-renal nitrogen losses were 2 g/day. Total energy expenditure and net substrate oxidation rates were measured during 45 min before and over the 120 min after oral glucose ingestion by indirect calorimetry (Deltatrack II, Datex Instruments,Heslinki, Finland)

On the following day, intrahepatocellular lipids (IHCL), visceral fat volume, and total liver volume were measured by clinical Magnetic Resonance (MR) methods at the Department of Clinical Research of University Bern.

IHCL content was determined by ^{1}H MR spectroscopy (MRS) on a clinical 3 T MR system (TIM Trio, Siemens Medical, Germany) using the whole body coil for excitation. A volume of interest (2.5 × 2.5 × 3 cm^3) was localized in the liver using the body array surface coils for signal detection and a double echo localization sequence combined with Siemens' 2D "prospective acquisition correction".

(PACE) scheme,[6] based on a 2D gradient echo image to monitor the position of the diaphragm for triggering in expiration (MRS echo time TE 30 ms, TR according to the breathing cycle between 2.5 and 6 s, 4000 Hz spectral width, 2048 data points). MRS was preceded by fast spin echo MRI (HASTE [Half Fourier Acquisition Single Shot Turbo Spin Echo], echo time 89 ms, repetition time 1030 ms, flip angle 150°, nominal resolution 1.7 × 1.3 × 5 mm^3) in three planes using the same PACE triggering to visualize the liver and to reliably reproduce the placement of the ROI in follow-up examinations. The ROI was placed evading large vessels and proximity to extrahepatic fat. The magnetic field distribution over the ROI was optimized in breath-hold using the manufacturers automated gradient shim routine. For choice of proper flip angle, a B_1 mapping scan was recorded in expiration prior to MRS. MR spectra were recorded with water presaturation to determine the lipid and metabolite spectra (32 acquisitions, 60 Hz suppression bandwidth, center frequency at 3 ppm) and without water suppression to acquire the water signal as internal standard (16 scans, center frequency at 4.7 ppm). Automatic fitting of the MR spectra was performed with the home-written software FiTAID allowing for the use of Voigt lines and implementation of prior knowledge restraints.[7] The lipid spectrum was modeled using 9 Voigt lines to describe all spectral components and initial model optimizations based on an average spectrum from several subjects, further 5 lines were used to cover the metabolites and residual water. Absolute quantification was performed in analogy to Bortolotti et al[5] and was based on the peak areas of the methylene protons that are not neighbors of an allylic or carboxylic carbon, basic assumptions on lipid composition, the water peak area from non-water-suppressed scans, an assumed liver water content and relaxation corrections based on literature values.[8] Results were expressed as volume percentage of lipid.

Volumes of the liver and visceral adipose tissue (VAT) were determined using T_1-weighted images of the abdomen, recorded in breath-hold (multi-spin-echo technique, echo train length 7, echo spacing 7.6 ms, repetition time 452 ms, echo time 38 ms, flip angle 130°, 30 axial slices in 6 slabs covering the pelvis at the lower end and the diaphragm at the upper end, slice thickness of 10 mm, gap between slices 10 mm, 5 slices per breath-hold sequence, acquisition matrix 256 × 147 with a resolution of 2 mm/pixel, body coil was used for excitation and signal acquisition). Volumetry was performed using a semi-automatic implementation of the point counting method, which represents a sparse sampling scheme whereby an operator accepts or rejects points from a regular grid that covers the targeted anatomic structure in a random orientation.[9] Visceral fat was counted on images between pelvis and the upper end of the diaphragm.

After these initial measurements, WPS was provided as bags containing 20 g of commercialized whey protein (WheyProtein94®, Sponser, Wollerau, Switzerland), with instructions to consume the content of one bag diluted into 300 ml water 30 min before breakfast, lunch and dinner. Total WPS supplementation amounted to 60 g/day Their food and drink intake was otherwise left *ad libitum*. The study was performed as an open label, unblinded, uncontrolled study.

1, 2 and 3 weeks after the beginning of WPS, volunteers returned to the cardiomet CIC and fasting blood sample was obtained for the measurement of plasma triglycerides, total cholesterol, HDL cholesterol, urea, creatinine, ASAT, ALAT and glycemia. 24-h urine collections were also obtained to measure urea and creatinine excretion. Compliance to WPS was assessed by collecting the empty supplementation bags.

After 4-weeks WPS, all measurements performed at inclusion, OGTT and MR determination of IHCL, visceral fat volume, and liver volume were repeated.

2.3. Analytic procedures

After collection, blood and urine samples were sent to the Central Laboratory of CHUV for measurements of fasting plasma total- triglycerides, total cholesterol, HDL cholesterol, urea, creatinine, ASAT, ALAT and 24 h urea and creatinine excretion. For the other blood parameters, blood was centrifuged at 4 °C for 10 min, at 3600 rpm, and plasma were stored at −20 °C/−80 °C until further analysis. Glucose concentrations were measured by the glucose oxidase method with a Beckman Glucose Analyzer II (Beckmann Glucose Analyzer II, Beckmann Instruments, Fullerton, CA). Plasma