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Original Article

Effects of dietary protein on post-prandial lipid metabolism in healthy humans

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SUMMARY

Background & aims: Protein supplementation reduces intrahepatic lipids in high fat fed subjects, but the mechanisms are not known. We therefore assessed the effects of dietary proteins on post-prandial lipid metabolism in healthy humans.
Methods: Seven healthy young males were studied on 3 occasions: after ingestion of a low (0.5 g/kg) or a high (1.5 g/kg) protein meal and a high protein meal administered after 4 days on a high (1.5 g/kg) protein diet. Net substrate oxidation, exogenous fat oxidation, glucose and glycerol kinetics, hormones and substrates concentration were monitored at baseline and over 6 h post-prandial.
Results: The high protein meal decreased post-prandial NEFA, glycerol and beta-hydroxybutyrate concentrations. After 4 days on a high protein diet post-prandial *chylomicron-triglyceride* concentrations were in addition increased ($p < 0.05$).
Conclusions: Proteins added to a meal do not increase post-prandial total or exogenous lipid oxidation. After 4 days on a high protein diet, proteins even enhanced post-prandial *chylomicron-triglyceride* concentrations, suggesting an impaired chylomicron clearance.

1. Introduction

The metabolic syndrome, characterized by visceral obesity, altered glucose homeostasis, dyslipidemia, and high blood pressure, is highly prevalent in industrialized countries, and represents a major public health burden.[1] Insulin resistance is tightly associated with this syndrome,[2] and is thought to result of impaired mitochondrial function, impaired lipid oxidation, and lipotoxicity. Ectopic lipid deposition in the liver, leading to non-alcoholic fatty liver disease, is frequently encountered in association with the metabolic syndrome[3] and is closely linked to insulin resistance.[4]

Both dietary factors[5] and a low physical activity[6] are involved in the development of the metabolic syndrome. Among dietary factor, a high sugar intake[7] and a high saturated fat intake[8] can lead, in animal models and in humans, to the development of several features of the metabolic syndrome, including increased intrahepatic lipids. The effects of dietary protein are less well known. While amino-acid infusion can clearly impair hepatic and extrahepatic insulin's actions[9,10], several observations suggest that a high protein diet may have beneficial effects on the metabolic syndrome.[11] Co-ingestion of protein with carbohydrate tends to decrease post-prandial glycemia in healthy subjects and in insulin-resistant patients.[12] A high protein diet has also been shown to improve glucose homeostasis in patients with type 2 diabetes mellitus[13] Furthermore, a hypocaloric high protein diet has also been shown to enhance weight loss and improve glucose tolerance in obese patients.[14] Finally, a high protein diet reduced intrahepatic fat concentrations in carbohydrate fed rats,[15] in obese Zucker rats,[16] and in healthy human subjects receiving a high fat diet for 4 days.[17]

Given the role of lipotoxicity in the development of insulin resistance, the apparent beneficial effects of a high protein diet on glucose homeostasis and on intrahepatic lipids suggest that protein may improve insulin's action by promoting lipid oxidation. Several mechanisms may be postulated for protein-induced stimulation of hepatic and whole body lipid metabolism, including: a) increased

Abbreviations: GC-MS, gas chromatography-mass spectrometry; CD, control diet; GRa, glucose rate of apperance; GRd, glucose rate of disapperance; GlyRa, glycerol rate of appearance; GlyRd, glycerol rate of disappearance; HPD, high protein diet; HPM, high protein meal; LPM, low protein meal; NEFA, non esterified fatty acid; TG, triglyceride; VLDL, very-low density lipoprotein.

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whole body and hepatic energy expenditure,[18,19] which may stimulate lipid oxidation through an activation of AMP-dependant protein kinase (AMPK)[20]; b) protein-induced increased bile acid concentrations[17], which may stimulate lipid oxidation pathways through activation of the oxysterol receptor LXR.[21] To evaluate this hypothesis, we compared the metabolic effects of 2 test meals with a fixed carbohydrate and fat content but either a standard or a high protein content. To further evaluate possible long term effects of protein, the metabolic effects of the high protein meal were also tested after 4 days on a high protein diet. Whole body net lipid oxidation was measured with indirect calorimetry, while whole body lipolysis (predominantly adipose) was indirectly assessed by measuring glycerol turnover with deuterated glycerol. In addition ^{13}C-triolein incorporated in the test meals was used to calculate exogenous fat oxidation.

2. Materials and methods

2.1. Subjects

Seven healthy male volunteers were recruited amongst student at the University of Lausanne. They had a mean age of 21 ± 0.5 years, mean weight of 74 ± 3 kg, mean BMI of 23.2 ± 0.8 kg/m^2 and mean fat mass of 13 ± 0.8% evaluated from the skinfold-thickness measurements following the tables of Durnin and Womersley.[22] All were in good physical health based on medical history and a standard physical examination, were not on any medication at the time of the study, had a usual alcohol consumption <20 g/day and were non-smokers. Every participant provided an informed, written consent.

2.2. Experimental protocol

Every subject took part to 3 tests, separated by at least 3 weeks. During the 4 days preceding each test, they consumed a controlled diet, with all food items prepared and provided by the investigators to be consumed at home. Subjects were carefully instructed to prepare and consume all the food provided according to specific instructions, and to refrain from consuming any other food or beverage during this period. On two occasions, subjects consumed a weight-maintenance diet (control diet: CD) containing 140% basal energy requirements (calculated using the equation of Harris–Benedict multiplied by a coefficient of Physical Activity Level of 1.4). On the third occasion, subjects consumed the same control diet supplemented with 1.5 g/kg/day protein (high protein diet: HPD) (Table 1). The protein supplementation was provided as dairy products (skimmed milk powder, cottage cheese, and joghurt). After 4 days on one or the other of these two controlled diets, subjects underwent a metabolic test aimed at assessing substrate oxidation and glucose homeostasis in basal conditions and after ingestion of a test meal. One test meal was low in protein (low protein meal, LPM) contained 55% carbohydrate, 35% fat labelled with 1% ^{13}C-triolein (Cambridge Isotope Laboratories, Andover, MA, USA), and 10% protein as cottage cheese. The other test meal had the same carbohydrate and fat, and labelled triolein content but was enriched with dairy proteins (high protein meal, HPM). Detailed composition of these two test meals is given in Table 2.

Each subject was studied on three occasions, i.e. after 4 days controlled diet and with a control test meal (CD-LPM), after 4 days controlled diet with a high protein test meal (CD-HPM), and after 4 days high protein diet with a high protein test meal (HPD-HPM), according to a randomized sequence.

For each metabolic test, subjects came at the Cardiomet clinical investigation unit of the Centre Hospitalier Universitaire Vaudois at ca 7 am after an overnight fast. At their arrival, subjects were asked to void and were transferred to a bed where they remained quiet in a semi-recumbent position for the next 8 h. An indwelling teflon® catheter was inserted into a vein of one forearm, two continuous infusions were infused throughout the experiment, one of labelled 6,6 $^2H_2$-glucose (bolus: 2 mg/kg; continuous infusion: 40 μg/kg/min) and the other of labelled $^2H_5$-glycerol (bolus: 1 μmol/kg, continuous infusion 0.1 μmol/kg/min) (Cambridge Isotope Laboratories, Andover, MA, USA). A second indwelling catheter was inserted into a vein of the other forearm for periodic blood sampling. This arm was maintained in a thermostabilzed box heated at 50 °C to achieve partial arterialization of venous blood. Respiratory gas exchanges were continuously monitored by means of an open flow, continuous indirect calorimeter with a hood system (Deltatrak II, Datex Instruments, Helsinki, Finland). After a 105 min period allowed for tracer equilibration, and during which basal measurements were performed, the hood was removed for 30 min, the subject was asked to void again before ingesting one of the two test meals in 20 min. Measurements were thereafter pursued for another 360 min. Blood samples were collected every 30 min throughout the test for the measurement of plasma glucose (Beckmann Glucose Analyzer II, Beckmann Instruments, Fullerton, CA), urea (Urea Analyzer, Beckmann Instruments Fullerton, CA, USA) plasma insulin (RIA kit from Millipore, Billerica, MO, USA), plasma glucagon (RIA kit from Millipore, Billerica, MO, USA), plasma non esterified fatty acids (NEFA kit from Wako Chemical GmbH, Neuss, Germany), plasma Beta-hydroxybutyrate (BOHB) concentrations (kit from Boehringer Mannheim, Mannheim, Germany) and total plasma triglyceride (TG) concentrations (kit from Biomérieux, Marcy l'Etoile, France). For each blood collection time, lipoprotein subfractions were separated by ultracentrifugation and TG concentrations were measured in chylomicrons and VLDL subfractions. Plasma 6,6 $^2H_2$-glucose and $^2H_5$-glycerol were measured by GC–MS as previously described.[23,24] Breath collections were obtained at 30 min intervals throughout the test and were stored in 10 ml vacutainers until analyzed. Breath $^{13}CO_2$ isotopic abundance

Table 1
Energy repartition of the 4-day standardized diets, control diet (CD) and high protein diet (HPD).

	CD	HPD
Total Energy (kcal)	2585 ± 32	3000 ± 32
Total Macronutrients		
Carbohydrates (kcal)	1370 ± 22	1409 ± 14
Lipids (kcal)	810 ± 12	768 ± 8
Protein (kcal)	405 ± 5	830 ± 11

All values are expressed as mean ± SEM.

Table 2
Energy repartition and composition of the low protein meal (LPM), high protein meal (HPM).

	LPM	HPM
Total Energy (kcal)	772 ± 19	921 ± 24
Total Macronutrients		
Carbohydrates (kcal)	424 ± 11	424 ± 11
Lipids (kcal)	270 ± 6	270 ± 6
Protein (kcal)	77 ± 3	226 ± 7
Food items		
White bread (g)	70 ± 0.0	70 ± 0.0
Orange juice (g)	252 ± 4.4	265 ± 3.7
Sirup (g)	61 ± 4.6	23 ± 3.5
Olive Oil (g)	15 ± 0.2	15 ± 0.2
Butter (g)	13 ± 0.7	
Buttermilk (g)		149 ± 1.0
Serac (g)		109 ± 6.7
Skimmed milk (g)		51 ± 4

All values are expressed as mean ± SEM.