

Effect of Long-term Dietary Protein Intake on Glucose Metabolism in Man

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Introduction

As long-term effects of dietary protein on insulin secretory capacity and glucose metabolism are unknown our study focussed on this aspect. 0.8 g kg⁻¹ day⁻¹ dietary protein is the recommended daily allowance calculated by the World Health Organization for healthy adults (1). However, most people in the industrialized countries consume more dietary protein than recommended by the WHO.

We set out to answer the following questions: 1) what are the mechanisms by which protein intake affects hepatic glucose metabolism in man, and 2) does long-term high protein diet modify pancreatic insulin secretory capacity? Using stable isotopes of the glucose molecule, but avoiding complicated isotopomer distributions (2) we estimated liver glucose metabolism by enrichment of liver glycogen with ingested ¹³C-labelled glucose over a period of one week.

Probands and Methods

Subjects with constant protein intake of 1.87 ± 0.26 g kg⁻¹ day⁻¹ (1.25 - 2.41), named high protein (HP) group, and with 0.74 ± 0.08 (0.57 - 0.80), normal protein (NP) group, were matched (n=9) according to sex, age, and calorie intake (Table 1). They underwent an intravenous glucose tolerance test (0.3 g kg⁻¹ 50% glucose injected as bolus, glucose and insulin plasma concentrations followed for 10 min) and a euglycemic hyperinsulinemic clamp with infusion of [6,6-²H]-glucose combined with calorimetry (Deltatrac calorimeter).

To estimate net gluconeogenesis the usual diet was enriched by U-[¹³C]-glucose for one week, and breath and plasma were sampled on a daily basis (3). During five days subjects ingested 2.4 g U-[¹³C]-glucose per day added to their main meals. Three days before and during this time respiratory gas exchanges and breath ¹³CO₂ were measured every morning in the postabsorptive state under resting conditions after an overnight fast. A plasma sample was taken at the same time for the determination of plasma ¹³C-enrichment.

Calculations

Since indirect calorimetry estimates net substrate oxidation rates, glucose oxidation determined in the postabsorptive state is exclusively accounted for by oxidation of endogenous glycogen. Net carbohydrate oxidation corresponds to oxidation by peripheral tissues of glucose issued from glycogen hydrolysis. Assuming that breath CO₂ has an enrichment in ¹³C identical with that of glycogen, Gay et al. (3) have proposed the following equation: ¹³C glycogen enrichment = breath ¹³CO₂ * VCO₂ / VCO₂(CHO) * 0.8, where VCO₂(CHO) is the fraction of total VCO₂ used for oxidation of carbohydrate and 0.8 is the recovery factor for ¹³CO₂ in breath. Fractional gluconeogenesis (expressed in %) was calculated as ¹³C-glycogen enrichment minus plasma U-[¹³C]-glucose divided by ¹³C-glycogen enrichment.

Results

Diet

Protein intake in both groups was assessed by seven-day food records. Carbohydrate intake was significantly lower in the HP group. Energy production calculated from indirect calorimetry correlated significantly with the estimates obtained from the food questionnaire (R² = 0.83, p < 0.0001).

Glucose stimulated insulin release

Acute insulin response to glucose (mean insulin above basal from 2-10 min after glucose injection) correlated to protein intake by a second grade function (Figure 1). Protein consumption of all probands had a range of 0.6 to 2.2 g kg⁻¹ and day.

Glucose turnover

At a low plasma insulin level (circa 40 pM) hepatic glucose output was 12% higher in the HP group (12.2 ± 0.51 for HP and 10.9 ± 0.54 μmol kg⁻¹ min⁻¹ for the NP group, p < 0.01, Figure 2). During the hyperinsulinemic clamp HGO was equally suppressed in both dietary groups. Overall glucose disposal at 100 pM was slightly, but significantly lower in the HP group (4.7 ± 2.6 HP vs. 4.3 ± 2.2 mg kg⁻¹ min⁻¹ NP, p < 0.05). Plasma glucagon in the presence of low insulin levels was elevated in the HP group, suppression of glucagon secretion by higher insulin concentrations was not different (Figure 3).

	High Protein	Low Protein	p
Number	9,00	9,00	
Age	30.2±5.1	29.7±6.3	n.s.
BMI (kg/m ²)	23.4±2.2	22.7±2.0	n.s.
Sex (f/m)	7/6	7/6	

Table 1

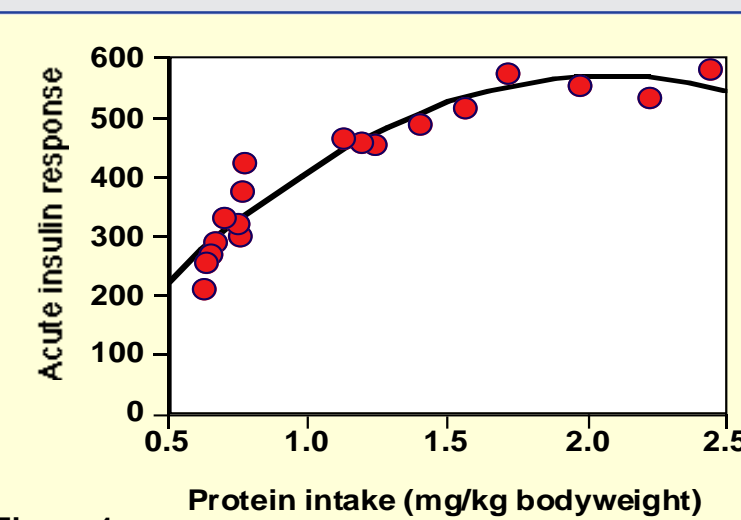


Figure 1

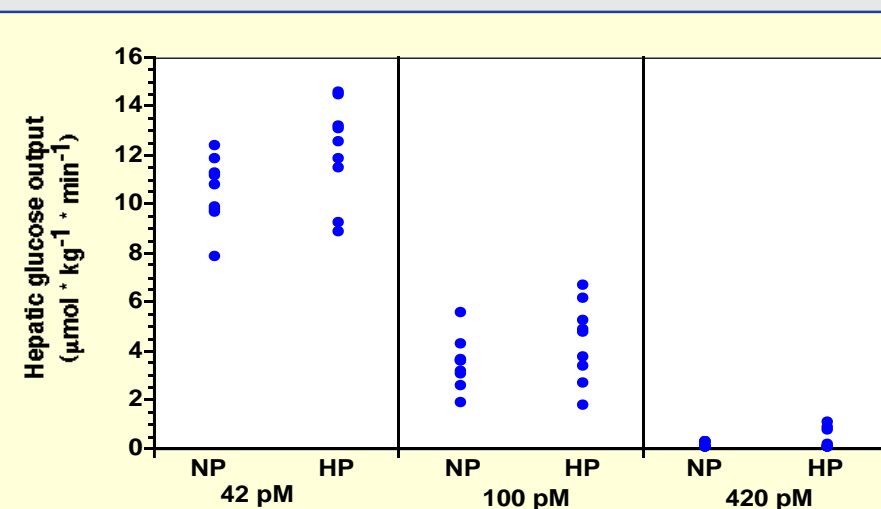


Figure 2

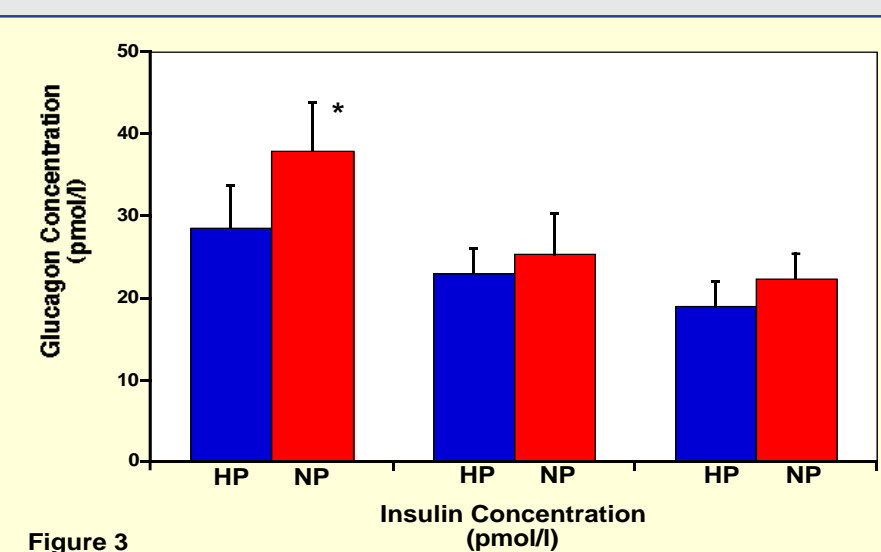


Figure 3

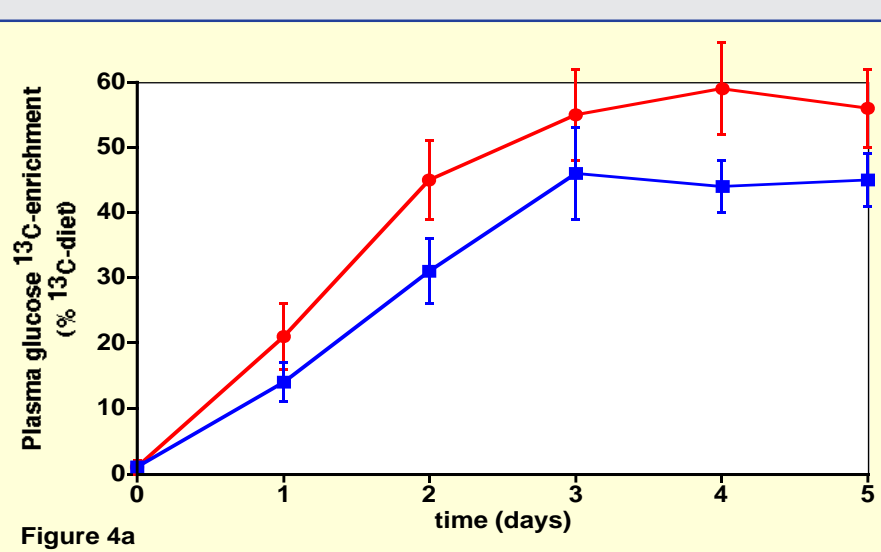


Figure 4a

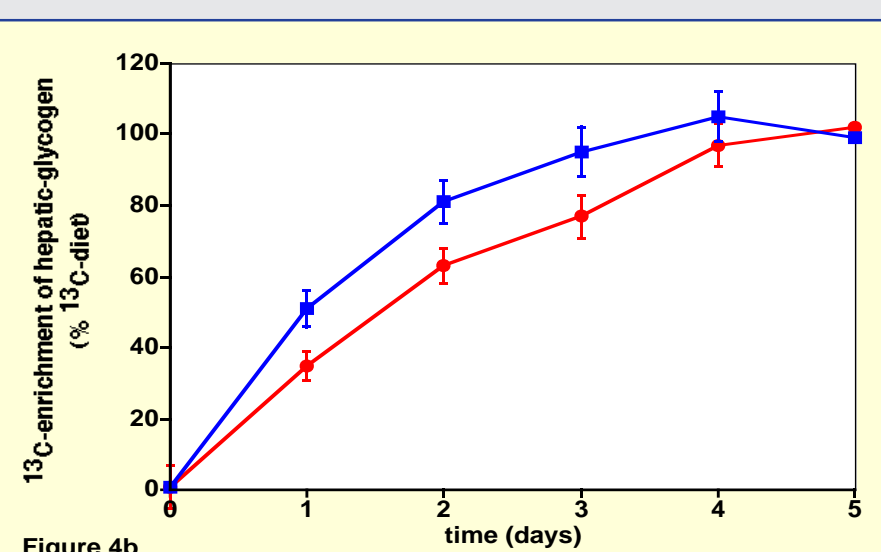


Figure 4b

Hepatic glycogen accumulation

Plasma ¹³C-glucose enrichment increased rapidly when the ¹³C-enriched diet was started and reached a plateau after four days (Figure 4a). At the plateau plasma ¹³C-glucose enrichment represented about 50% of the ¹³C-carbohydrate enrichment of the diet for both groups. Resting, post-absorptive breath ¹³CO₂ also increased progressively when the ¹³C-enriched diet was started reaching a plateau after five days (Figure 4b). The ¹³C-enrichment of hepatic glycogen, i.e. of glucose oxidized at rest, assumed to be essentially of hepatic origin, was 104 ± 8 % of the ¹³C-enrichment of dietary carbohydrates.

In subjects from the NP group plasma U-[¹³C]-enrichment represented 51.5 ± 2.9% of the enrichment of hepatic glycogen as calculated from ¹³C-enrichment of breath samples. This corresponded to a fractional gluconeogenesis of 48.7 ± 6.2%. By contrast, net gluconeogenesis in the HP group was 68.7 ± 3.5% (p < 0.02). The fraction of glucose produced by gluconeogenesis calculated from the one-week ²H-enrichment was 36.8 ± 3.4% in the NP group and 42.1 ± 4.9% in the HP group (p < 0.05).

The time required to reach 50% of hepatic glycogen was an estimated 19.2 ± 3.8 h in the NP group and 16.2 ± 3.1 h in the HP group (p < 0.02); i.e. glycogen turnover was increased in the HP group.

Discussion and Conclusion

The secretion of insulin into the blood stream is elevated by the consumption of dietary protein during a meal. Dietary protein is associated with stimulation of growth hormone and insulin-like growth factor (4,5), and it is known that in states of increased metabolic demand, nutrient availability, and growth hormone production - as for instance pregnancy - an expansion of islet tissue is found (6). This implies, that a high protein diet may increase the capacity for insulin secretion by expansion of islet tissue. The ¹³C-glucose turnover in plasma and air showed that dietary protein modified glucose metabolism over a period of several weeks. Substrates delivered by subsequent protein-rich meals reduced overall glucose disposal in both low-insulin and high-insulin states. Based on our calorimetric data glucose oxidation was reduced by high protein intake. In addition, hepatic glucose production was increased in close association with plasma glucagon which was still stimulated hours after the last protein rich meal. Therefore, individuals with high protein diet and activated gluconeogenesis and glycogenolysis showed to have an increased hepatic glucose production, however a reduced overall glucose disposal with the result of rising fasting blood glucose.

We conclude, that a high protein diet is accompanied by increased stimulation of glucagon and insulin within the endocrine pancreas. This is associated with high glycogen turnover and gluconeogenesis.

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