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Original article

# Effect of 10% dietary protein intake on whole body protein kinetics in type 2 diabetic adults☆

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SUMMARY

*Background & aims:* Insulin resistance of protein metabolism occurs in obesity and type 2 diabetes (T2D). Hyperaminoacidemia during a simulated fed steady-state clamp compensates for this resistance. We tested whether decreasing protein intake affects the response to insulin with or without added amino acids, and if this response differs by sex.

*Methods:* Protein intake was reduced from usual (15%) to 10% of an isoenergetic diet energy for 11 days, in T2D obese men ($n = 8$) and women ($n = 10$). Whole-body leucine kinetics (1-$^{13}$C-leucine, surrogate for protein) were determined postabsorptive and during a hyperinsulinemic (~600 pmol/L), hyperglycemic (8 mmol/L), isoaminoacidemic, followed by hyperaminoacidemic clamp and compared to those of T2D men on a 17% protein diet.

*Results:* Initial negative nitrogen balance approached equilibrium by day 10 but remained lower than with the 17% protein diet. During the hyperinsulinemic, isoaminoacidemic clamp, total leucine flux was less, with both lower endogenous rates of appearance (catabolism) and nonoxidative rates of disposal (synthesis), resulting in net balance at zero. With hyperaminoacidemia, net balance increased to 0.39 ± 0.09 μmol/kgLBM·min in men, significantly less than in men on 17% protein (0.98 ± 0.09, $p < 0.01$). There were no sex differences in clamp responses with 10% protein.

*Conclusions:* After 11 days of 10% protein diet, there was a slight improvement in insulin sensitivity, but a blunted anabolic response to hyperaminoacidemia. Longer-term consequences of lesser anabolic efficiency at reduced protein intakes require study and may contribute to increased risk of sarcopenia in persons with T2D with aging.



## 1. Introduction

Insulin is an anabolic hormone necessary for the metabolism of all macronutrients. Resistance to its action is recognized in obesity and type 2 diabetes (T2D) for glucose, lipid and protein metabolism [1–3]. The protein metabolic abnormalities have received the least attention and consequently, this impedes evidence-based definition of specific dietary recommendations in treatment guidelines. Current usual protein intakes at a range of 15–20% of isoenergetic diets (1.0–1.2 g/kg BW·d) have been considered sufficient [4]. Of greatest concern is the relatively low level of evidence for the latter, especially during weight reduction and in relation to metabolic control of the diabetes. Sources of uncertainly about recommendations include extrapolations from studies performed in the postabsorptive state that showed no difference in protein breakdown with insulin therapy [5–7] or a low energy diet

*Abbreviations:* BCAA, branched-chain amino acids; BW, body weight; FFA, free fatty acids; FFM, fat free mass; HbA1c, glycated hemoglobin; EAA, essential amino acids; HyperAA, hyperaminoacidemic hyperinsulinemic clamp; IsoAA, iso-aminoacidemic hyperinsulinemic clamp; KIC, α-ketoisocaproic acid; LBM, lean body mass; MUHC, McGill University Health Centre; Ra, rate of appearance; Rd, rate of disappearance; REE, resting energy expenditure; RQ, respiratory quotient; TAA, total amino acids; TBS-T, tris-buffered saline containing 0.1% Tween 20; T2D, type 2 diabetes.

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[8], compared with non-diabetic controls [9–15]. Furthermore, suppression of whole body protein breakdown in response to hyperinsulinemia, which causes amino acid concentrations to decrease, was shown not to differ from non-diabetic controls [1,7,11,14,15]. However, protein breakdown was less suppressed in insulin-resistant obese than in non-obese subjects during a hyperinsulinemic, hyperaminoacidemic clamp which resulted in a lesser net balance during this simulated fed state [3]. When amino acids were maintained at postabsorptive concentrations to isolate insulin effects from those of decreased amino acids during a hyperinsulinemic, euglycemic clamp, protein anabolism was less in obesity [16] and even less with diabetes, but only in men [1]. Greater 24-h integrated fed-fasted protein breakdown and lower net balance in T2D versus obese controls have been reported with protein intakes of 0.85 g/kg BW·d [17]. These results suggested that protein needs may be increased in obesity and T2D to achieve net protein equilibrium. Since these results were 24-h integrated fed-fasted, it is unclear when the failure to maintain equilibrium occurred, i.e. in the fed and/or fasted state, nor which kinetic variables contributed to the negative net balance. Such impairment may partly explain the accelerated decline in muscle mass, strength and functional capacity in T2D with aging [18,19]. Since both insulin and amino acids are responsible for protein anabolism and insulin secretion decreases with duration of T2D [20], insufficient dietary protein may pose an additional challenge to the maintenance of lean body mass with advanced age.

Hence, it is unknown whether consuming protein at the lower limit of 10% of energy in the Dietary Reference Intakes for healthy persons is sufficient in those with insulin resistance of protein metabolism. We reported a normal anabolic response to hyperaminoacidemia at levels simulating a generous protein intake of 17% of energy [2], but we questioned whether protein intake at 10% of energy is sufficient to compensate for the insulin resistance of protein anabolism in T2D. Therefore, the present study was designed to test the impact of decreasing protein intake to 10% of an isoenergetic diet for 11 days, on protein metabolism, hormone and substrate concentrations, and insulin resistance. The results are compared with metabolically and anthropometrically matched subjects from our prior study using the same approach with protein contributing 17% of energy [2]. As sex differences were observed in a previous study [1], we included both men and women to test for a sex effect in the responses to the clamps after adaptation to the reduced protein diet.

## 2. Methods

### 2.1. Subjects and diet

Middle-aged women ($n$ = 10) and men ($n$ = 8) with T2D, overweight and obese, were recruited from in-hospital posters and referral. Screening and exclusion criteria are those described in [2]. Written consent was obtained as prescribed by the institutional ethics review board of the McGill University Health Centre (MUHC). Premenopausal women were studied during the follicular phase. Each volunteer was admitted for 8 days to the hospital Clinical Investigation Unit. Caffeine consumption and acetylsalicylic acid were stopped. Successful recruitment required acceptance of differing diabetes medications in 17 participants, that included metformin, 15; sulfonylurea, 11; thiazolidinedione, 3; dipeptidyl peptidase-4 inhibitor, 1; insulin, 1; doses were adjusted to maintain hyperglycemia. Fourteen participants were treated with statins and 11 with antihypertensive agents. All were weight stable. Medications were held on the experiment day.

Protein intake was reduced to 10% of energy for four days prior, then upon admission. The diet was isoenergetic (with the patients' habitual diets), protein-controlled and divided into five meals of equal energy and protein content, ingested from 8:00 to 20:00 h. It provided 60% of energy from carbohydrate, 30% from fat, and 10% from protein (0.71 ± 0.02 g/kg BW·d, 1.24 ± 0.04 g/kg FFM·d). The diet consisted mainly of a commercial formula (Ensure®, Abbott Laboratories, St. Laurent, QC, Canada) and orange juice, bran cereal, 2% fat milk, whole wheat bread, applesauce, lettuce, tomato, cucumber, olive oil and salad dressing. Indirect calorimetry (TrueOne® 2400 Canopy System, Parvo Medics, Sandy, UT) was used to estimate energy requirements from measured resting energy expenditure (REE), multiplied by a 1.5 activity factor and verified by 24-h food recall and daily weights. When glycosuria was present, it was quantified and an equivalent energy supplement was given (two-thirds glucose polymer [Polycose®; Abbott Laboratories] and one-third vegetable oil). Day 3–11 nitrogen (N) balance was calculated as in [8].

Participants were sedentary based on MONICA Optional Study of Physical Activity [21]and Beake [22] questionnaires. Physical activity was limited to short walks. World Health Organization 1995 criteria were used for waist and hip circumferences. Body composition was obtained by bioimpedance analysis (RJL-101A Systems, Detroit, MI) with equations for obese persons, for diet calculations (g protein/FFM). LBM was determined from dual energy x-ray absorptiometry (Lunar Prodigy Advance; GE Healthcare, Madison, WI) and used for normalizing clamp kinetic variables. Subjects began consuming the 59% carbohydrate, 24% fat, 17% protein diet at home for 7 days, then continued it during admission for 4 days as in [2]. Any pre-meal capillary glucose >15 mmol/L (Accuchek III; Boehringer Ingelheim, Mannheim, Germany) was treated with subcutaneous short-acting insulin.

### 2.2. Meal test protocol

On day 10, a meal test was conducted to assess the postprandial responses to a meal with 10% energy from protein. An antecubital catheter was inserted at 07h45 for blood sampling, and at 08h00, subjects consumed a 700 kcal breakfast with 148 g carbohydrate (77% of energy), 19 g protein (10%), and 11 g fat (13%) composed of Ensure®, bran cereal, applesauce, orange juice and 2% milk. Blood samples were collected at baseline and 30, 45, 60, 90, 120, and 150 min post meal for determination of serum insulin and plasma glucose and branched-chain amino acid (BCAA) concentrations to be used as a target concentration for the hyperaminoacidemic clamp.

### 2.3. Clamp experiment protocol (Fig. 1)

On day 12, catheters were inserted in an antecubital vein for infusions and a contralateral dorsal hand vein retrograde for arterialized blood sampling, using the heated box technique. After baseline fasting samples, an oral bolus of 0.1 mg/kg BW of $NaH^{13}CO_2$ (Cambridge Isotope Laboratory, Andover, MA) and a 0.5 mg/kg BW intravenous L-[1-$^{13}$C]-leucine bolus (Cambridge Isotope Laboratory, Andover, MA), was followed by a constant infusion rate of 0.008 mg/kg BW·min. After 2.5 h (time 0), a primed infusion of biosynthetic human insulin (1.2 mU/kg FFM·min) (Humulin R; Eli Lilly Canada Inc, Toronto, ON) was started and maintained for 5 h. Low $^{13}$C 20% glucose (Avebe b.a., Foxhol, Netherlands) and 10% amino acid solution (TrophAmine® 10% without electrolytes; B. Braun Medical, Irvine, CA) were infused at variable rates to maintain constant concentrations of glucose at 8 mmol/L and of total BCAA (a marker of total amino acids) at each individual's postabsorptive concentrations (IsoAA). Rates of infusion were based on plasma glucose and total BCAA concentrations measured at 5 min intervals by a rapid fluorometric assay (see 2.4). After 5 h, amino acid infusion was raised to achieve each