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Effect of obesity and type 2 diabetes on protein anabolic response to insulin in elderly women

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ABSTRACT

Obesity and type 2 diabetes have been shown to alter the insulin sensitivity of glucose and protein metabolism in middle-aged women. We aimed to determine whether these findings translate to the elderly who are at increased risk of muscle loss. We assessed whole-body protein (1-^{13}C-leucine) and glucose (3-^{3}H-glucose) kinetics in 10 healthy (age: 71.6 ± 1.8 years; BMI: 23.2 ± 0.8 kg/m^2), 8 obese (age: 72.9 ± 1.3; BMI: 33.1 ± 1.0) and 8 obese well-controlled type 2 diabetic (age: 69.8 ± 1.6; BMI: 34.4 ± 1.5) elderly women in the postabsorptive state and during a hyperinsulinemic, euglycemic, isoaminoacidemic clamp. All subjects followed an isoenergetic, protein-controlled diet for 6 days preceding the clamp. The net protein anabolic response to hyperinsulinemia was similarly blunted in obese (0.08 ± 0.06) and obese type 2 diabetic women (0.06 ± 0.04) compared to healthy women (0.24 ± 0.05 μmol·kg fat free mass^{-1}·min^{-1}; ANOVA $p = 0.018$). In contrast, the insulin-mediated glucose disposal (healthy: 9.72 ± 0.67) was decreased with obesity (6.96 ± 0.86) and further with diabetes (5.23 ± 0.27 mg·kg fat free mass^{-1}·min^{-1}; ANOVA $p < 0.001$). Endogenous glucose production was not completely suppressed during the clamp only in diabetic women. Thus, the glucose infusion rate was the lowest in this group. Obese elderly women with and without type 2 diabetes have a similar degree of insulin resistance of protein anabolism, despite worse glucose metabolism in type 2 diabetes. Similar to previous findings in middle-aged women, obesity exerted a blunting effect on protein anabolism, which may contribute to the development of sarcopenic obesity. Our results suggest that the presence of type 2 diabetes at an advancing age does not further aggravate this effect.

1. Introduction

The increase in the proportion of elderly individuals is the most significant demographic change in diabetes prevalence across the globe (Wild et al., 2004). As the aging of the population meets the escalating obesity prevalence, more individuals are entering their senior years with excess adiposity, and in turn, with type 2 diabetes. Furthermore, older individuals with type 2 diabetes are at increased risk of sarcopenia—the excessive loss of muscle mass with age (Park et al., 2009). Sarcopenia manifests when a negative muscle protein balance (synthesis < breakdown) ensues over time and contributes to the functional decline and loss of independence common to old age (Janssen et al., 2002). The coexistence of sarcopenia and obesity has been shown to pose greater physical hindrances than sarcopenia alone (Rolland et al., 2009), which raises the possibility that type 2 diabetes may impose additional deterioration. Studying protein metabolism at the whole-body level in obese type 2 diabetic elderly persons is a critical first step to understanding the potential anabolic defects that may accelerate their rate of muscle loss.

Protein anabolism, like glucose uptake, is dependent on optimal insulin action. Assessing the insulin sensitivity of protein metabolism in concert with that of glucose requires maintenance of circulating amino acids during a hyperinsulinemic, euglycemic clamp (Chevalier et al., 2004). In healthy young adults, protein breakdown is suppressed and synthesis stimulated in response to hyperinsulinemia, achieving positive net protein balance (Chevalier et al., 2004). This anabolic response defines the insulin sensitivity of protein metabolism (Chevalier et al., 2006). We previously showed a diminished protein anabolic response to insulin with aging, attributable in part to greater body fat proportion (Chevalier et al., 2006). In middle-aged adults, obesity blunts the protein anabolic response to insulin, and type 2 diabetes heightens

Abbreviations: ANOVA, analysis of variance; BCAAs, branched-chain amino acids; BIA, bioelectrical impedance analysis; BMI, body mass index; FFAs, free fatty acids; FFM, fat free mass; HOMA-IR, homeostatic model assessment of insulin resistance; HPLC, high-performance liquid chromatography; OGTT, oral glucose tolerance test; R_a, rate of appearance; R_d, rate of disappearance; REE, resting energy expenditure; SEM, standard error of the mean.

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this impairment, in men (Pereira et al., 2008). As the suppression of protein breakdown was not resistant to insulin (at high postprandial levels) in these obese and type 2 diabetic subjects, the blunted response was mediated by a lesser stimulation of protein synthesis. In contrast, type 2 diabetes did not impart further impairments than those conveyed by obesity alone, in middle-aged women (Pereira et al., 2008). However, given the epidemiological observation that type 2 diabetes accelerates the rate of thigh muscle area decline in elderly women (Park et al., 2009), we hypothesized that those with type 2 diabetes would have worsened protein anabolism than age- and body composition-matched controls. Accordingly, the present study aimed to explore how obesity and type 2 diabetes alter insulin's effect on whole-body protein and glucose metabolism in elderly women.

2. Materials and methods

2.1. Study design

The study was carried over seven consecutive days for each of the healthy, obese, and obese type 2 diabetic elderly women who participated. After six days of dietary control, subjects participated in a metabolic protocol to assess whole-body glucose and protein kinetics in the postabsorptive state and during a hyperinsulinemic, euglycemic, isoaminoacidemic clamp.

2.2. Subjects, diet and body composition

Ten healthy, 8 obese, and 8 obese type 2 diabetic elderly women were recruited by advertisements in local newspapers and magazines oriented for older adults. All subjects were screened by medical history, physical examination and laboratory investigation as previously detailed (Pereira et al., 2008). This included an oral glucose tolerance test (OGTT) for the healthy and obese women. Notably, 75% of the diabetic women were taking hypoglycemic agents at screening; one woman was taking insulin and metformin, another was taking pioglitazone and gliclazide, and four women were taking metformin alone. All participants were required to be non-smokers and between 65 and 85 years of age. Healthy subjects required a BMI between 18.5 and 27.0 kg/m^2, and obese subjects with and without type 2 diabetes, a BMI ≥ 30 kg/m^2. Exclusion criteria included unstable weight for the preceding 6 months, abnormal dietary habits, diabetes complications, other active medical conditions, and use of medications (other than for diabetes treatment) that may affect metabolism. The type 2 diabetic subjects stopped their oral antihyperglycemic agents or took lower doses before starting the study with the aim of bringing fasting glucose levels to approximately 8.0 mmol/L. Ethical approval was obtained from the McGill University Health Centre Research Ethics Board, and all subjects provided written informed consent.

All subjects consumed an isoenergetic, protein-controlled formula-based diet for days 1–6 of the study. They spent days 1–3 at home and were admitted to the hospital Clinical Investigation Unit on day 4. Physical activity was restricted to walks in and around the hospital. Resting metabolic rate was measured by indirect calorimetry (Deltatrac®, Sensor Medics, Yorba Linda, CA) and was multiplied by a physical activity factor of 1.4–1.7 to determine energy requirements. Details of the dietary protocol have been previously described (Chevalier et al., 2004). Twenty-four hour urine was collected daily upon admission for the determination of nitrogen balance, and to measure urinary glucose concentrations in the type 2 diabetic subjects. Energy lost as glycosuria was added to their diet as 50% glucose polymer (Polycose: Ross, Abbott Laboratories, Columbus, OH; 3.8 kcal/g) and 50% canola oil (9.1 kcal/g). The main goal of the diet was to maintain body weight and zero nitrogen balance. Women with type 2 diabetes measured their capillary blood glucose concentration before each meal. When concentrations were >15 mmol/L or deemed high by the study physician, antihyperglycemic agents were adjusted accordingly.

Fat free mass was determined by bioelectrical impedance analysis (BIA) (RJL-101A Systems, Detroit, MI) using the Roubenoff equation for elderly women (Roubenoff et al., 1997). Skeletal muscle mass was also calculated according to the women-specific BIA equation of Janssen et al. (2000) in order to quantify absolute (% of body weight) and relative (per height-squared) skeletal muscle indices. Circumference measurements were taken at sites according to WHO procedures (World Health Organization, 1995).

2.3. Protein and glucose kinetic studies

On day 7 of the study, after an overnight fast, protein and glucose kinetics were assessed using labeled isotope methodology in the postabsorptive state and then during a hyperinsulinemic, euglycemic, isoaminoacidemic clamp. Primed (with 1.4 times the amount with hyperglycemia), continuous infusions of D-[3-^{3}H]glucose and ^{13}C-leucine were started 180 min prior to insulin and continued for the duration of the study (390 min). The hyperinsulinemic clamp experiment was performed according to the detailed procedure published (Chevalier et al., 2004) with the target of maintaining plasma glucose at 5.5 mmol/L and plasma branched-chain amino acids (BCAAs) at each subject's postabsorptive concentration during an insulin infusion of 40 mU/m^2·min (Humulin R®, Eli Lilly Canada Inc., Toronto, ON, Canada) for 210 min. Euglycemia was maintained with infusion of 20% (w/v) potato starch-derived glucose (Avebe b.a., Foxhol, The Netherlands) and isoaminoacidemia with infusion of 10% TrophAmine® (without electrolytes, B. Braun Medical Inc., Irvine, CA) by feedback adjustments of infusion rates based on plasma glucose and total BCAA concentrations measured every 5 min. Blood and expired air samples were collected every 10 min for 40 min prior to the insulin infusion, then every 30 min until the last 40 min, at which time they were again taken at 10 min intervals. Indirect calorimetry was performed for 20 min prior to and during the last 30 min of the insulin infusion. Glucose kinetics and substrate oxidation were calculated from the dilution of D-[3-^{3}H] glucose specific activity using the "hot GINF" method (Finegood et al., 1987). Leucine kinetics were calculated according to the reciprocal model of Matthews et al. (1980), from the dilution of plasma α-[^{13}C] keto-isocaproic acid (α-KIC) enrichment using the equation $Q = i\,[(E_i / E_{KIC}) - 1]$, where Q is leucine flux, i is [1-^{13}C]-leucine infusion rate, E_i is the enrichment of ^{13}C-leucine and E_{KIC} is the plasma α-KIC enrichment. Endogenous leucine rate of appearance (Leu R_a, an index of protein breakdown) was calculated as Leu $R_a = Q - I$, where I is the infusion of amino acids; non-oxidative leucine disposal (Non-ox R_d, an index of protein synthesis) was calculated as Non-ox R_d = Q − leucine oxidation. Leucine oxidation was obtained from $F^{13}CO_2/E_{KIC}$, where $F^{13}CO_2 = (VCO_2 \cdot {}^{13}CO_2$ enrichment$)/{}^{13}CO_2$ recovery factor based on results from our laboratory (Chevalier et al., 2006).

2.4. Assays

Plasma glucose was measured by the glucose oxidase method (GM7 Micro-Stat, Analox Instruments USA, Lunenberg, MA). Assays for immunoreactive insulin and glucagon, and glucose specific activity were performed as in Finegood et al. (1987) and Sigal et al. (1994). Plasma total BCAA concentrations were measured by a rapid enzymatic, fluorometric assay (Chevalier et al., 2004). Individual plasma amino acids were determined by reverse-phase high-performance liquid chromatography (HPLC) after pre-column derivatization with O-phthalaldehyde using a System Gold® HPLC System (Beckman Coulter Inc., Fullerton, CA). Serum free fatty acids (FFAs) were determined using the NEFAC test kit (Wako Chemicals USA, Inc., Richmond, VA). The [^{13}C] enrichment of plasma α-KIC was analyzed by gas chromatography–mass spectrometry (Hewlett-Packard GCMS 5988A, Palo Alto, CA) after derivatization with a quinoxalinol tert-butyldimethylsilyl ether derivative (Regis Technologies Inc., Morton Grove, IL). Expired air was analyzed for $^{13}CO_2$ enrichment by isotope ratio mass spectrometry on a Micromass 903D (Vacuum Generators, Winsford, United Kingdom).