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Diet-induced obesity alters protein synthesis: tissue-specific effects in fasted versus fed mice

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Abstract

The influence of obesity on protein dynamics is not clearly understood. We have designed experiments to test the hypothesis that obesity impairs the stimulation of tissue-specific protein synthesis after nutrient ingestion. C57BL/6J mice were randomized into 2 groups: group 1 (control, n = 16) was fed a low-fat, high-carbohydrate diet, whereas group 2 (experimental, n = 16) was fed a high-fat, low-carbohydrate diet ad libitum for 9 weeks. On the experiment day, all mice were fasted for 6 hours and given an intraperitoneal injection of ^2H_2O. They were then randomized into 2 subgroups and either given a sham saline gavage or a liquid-meal challenge. Rates of protein synthesis were determined via the incorporation of [^{2}H]alanine (5 hours postchallenge) into total gastrocnemius muscle protein, total liver protein, and plasma albumin. High-fat feeding led to an increase in total body fat ($P < .001$) and epididymal fat pad weights ($P < .001$) and elevated fasting plasma glucose levels ($P < .01$). Diet-induced obesity (*a*) did not affect basal rates of skeletal muscle protein synthesis, but did impair the activation of skeletal muscle protein synthesis in response to nutrient ingestion ($P < .05$), and (*b*) slightly reduced basal rates of synthesis of total hepatic proteins and plasma albumin ($P = .10$), but did not affect the synthesis of either in response to the meal challenge. In conclusion, there are alterations in tissue-specific protein metabolism in the C57BL/6J mouse model of diet-induced obesity. This model may prove to be helpful in future studies that explore the mechanisms that account for altered protein dynamics in obesity.
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1. Introduction

The incidence of obesity and its accompanying morbidities has reached epidemic proportions, creating major health care challenges and costs [1,2]. The association of a variety of endocrine alterations and changes in the concentration of circulating hormones typically seen with obesity has led to the description of a metabolic syndrome, characterized by hyperinsulinemia, glucose intolerance, dyslipidemia, hypertension, and increased risk of diabetes and coronary heart disease. Although there is general agreement regarding the association between obesity and impaired regulation of carbohydrate and lipid metabolism, the influence of obesity on protein metabolism is somewhat controversial [3-6]. Some studies have found significant differences in protein metabolism in obese vs nonobese human subjects [6-8], whereas other studies have not [5,9,10].

One possible explanation for some of the apparent differences in protein metabolism may be found in the study of Jensen and Haymond [7]. Namely, they examined whether obesity was associated with abnormalities in leucine turnover in the postabsorptive state in age-matched premenopausal women. The obese women had increased whole-body proteolysis, as measured by leucine carbon flux, compared with nonobese women. In addition, differences in body fat distribution (ie, upper body vs lower body obesity) were associated with abnormalities in protein metabolism (upper body obesity impaired the antiproteolytic response to insulin when compared with lower body obesity and nonobese women) [7]. Thus, the location of the excess body fat plays an important role.

The observations reported above are consistent with the hypothesis that insulin's action as an anabolic hormone on suppressing protein breakdown and stimulating protein

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synthesis could be impaired in obesity. However, conflicting data have been obtained from studies that have used the insulin-clamp method in combination with isotope tracers. Insight into a possible explanation of the apparent discrepancies may be found in the work of DeFronzo and colleagues. For example, Luzi et al [8] demonstrated that although proteolysis is sensitive to regulation via insulin, the dose of insulin affects the conclusions that are drawn; for example, certain differences between obese vs control subjects were observed at a low dose of insulin but not at a high dose of insulin. Those studies suggest that the dose-response (ie, insulin-proteolysis) requires attention and that one may overcome certain defects depending on the experimental design. In addition, although glucose production and lipolysis can be suppressed ~100% when high doses of insulin are infused (eg, ~40 mU insulin per square meter per minute) [11], it appears that maximal suppression of proteolysis (which also occurs at an infusion rate of ~40 mU insulin per square meter per minute) only results in a ~25% reduction in endogenous leucine flux [12]. Consequently, it may be that certain discrepancies in the literature arise from (*a*) the narrow apparent range of insulin sensitivity of proteolysis, (*b*) the fact that high doses of insulin may mask subtle defects in insulin action, and (*c*) the possible heterogeneity within the obese population.

As with studies of proteolysis, the interpretation of studies regarding insulin-mediated stimulation of protein synthesis warrants caution because protein synthesis requires the presence of amino acid substrates. For example, Tessari et al [13] found that both hyperinsulinemia and hyperaminoacidemia were required to stimulate net leucine deposition into body protein in postabsorptive healthy subjects. Namely, hyperinsulinemia decreased endogenous leucine rate of appearance (Ra) (ie, proteolysis), whereas hyperaminoacidemia (alone or in combination with hyperinsulinemia) increased leucine Ra [13]. Chevalier et al [14] recognized this point and used an "insulin and amino acid clamp" to study protein turnover in obese vs lean women. They demonstrated that protein catabolism was equally suppressed in both obese and lean women. However, protein synthesis was less stimulated in the obese group; as well, the amino acid infusion rates required to maintain baseline levels were also lower [14].

In reviewing the literature on protein turnover in obesity, we found that a substantial number of investigators have relied on measurements of leucine flux [15] in either a basal state or during an insulin clamp ± amino acids. Presumably, the controversies regarding protein dynamics in obesity are not related to limitations in the method(s) because most studies that we have reviewed used the same tracer (ie, carbon-labeled leucine). Although measurement of leucine flux during a clamp provides unique insight because one can independently study physiological parameters (eg, test the effect[s] of insulin vs amino acids), an important and unaddressed question centers on whether there is (ab)normal protein synthesis after a meal in obese vs lean subjects. Therefore, we initiated a study to contrast protein synthesis in the fasted vs the fed state and to determine whether the response(s) to a mixed meal is impaired. Attention was directed toward measuring protein synthesis in skeletal muscle and liver (including plasma albumin) because the synthesis of these proteins is generally most responsive to nutritional status [16]. The use of labeled leucine is difficult under these conditions because the bolus of food will perturb the steady-state isotope labeling; therefore, rates of protein synthesis were determined using ^2H_2O, a newly developed method by our laboratory that is well suited for studying the response to a short-term perturbation (eg, feeding) [17].

2. Materials and methods

2.1. Supplies

Unless noted, chemicals and reagents were purchased from Sigma-Aldrich (St Louis, MO). The ^2H_2O was purchased from Cambridge Isotopes (Andover, MA). Gas chromatography and mass spectrometry supplies were purchased from Agilent Technologies (Wilmington, DE). Diets D12450B (70% carbohydrate, 20% protein, and 10% fat) and D12451 (35% carbohydrate, 20% protein, and 45% fat) were purchased from Research Diets (New Brunswick, NJ).

2.2. Biological

Male C57BL/6J mice (~14 g) were purchased from Jackson Laboratory (Bar Harbor, ME) and randomized into 2 groups (n = 16 per group). Group 1 (control) was fed a low-fat, high-carbohydrate (LF) diet, whereas group 2 (experimental) was fed a high-fat, low-carbohydrate (HF) diet ad libitum for 9 weeks. Mice were housed 4 per cage. On the experimental day, food was removed from all cages (t = 0 minute); at t = 180 minutes, all mice were given an intraperitoneal injection of ^{2}H-labeled saline (0.50 mL). At 90 minutes post-^2H_2O (t = 270 minutes), 8 mice from each diet group were given a saline gavage (0.75 mL, sham); the remaining 8 mice from each group were given a substrate gavage (0.75 mL of a liquid meal calculated to deliver 3.75 kcal and consisting of 19% fat, 53% carbohydrate, and 25% protein; prepared by mixing soybean oil, Nestle Carnation evaporated milk (Glendale, CA), Nestle Carnation sweetened condensed milk, potato starch, Beneprotein, and egg albumin). At 570 minutes (ie, 5 hours postgavage), mice were sedated using isoflurane, blood was collected via cardiac puncture and epididymal fat pads, liver and skeletal muscle (gastrocnemius) were dissected and quick-frozen in liquid nitrogen, and plasma was isolated and frozen. The rationale behind quantifying protein synthesis over 5 hours was based on a previous study in which we found that albumin synthesis is stimulated for several hours after a meal [17]. Rates of skeletal muscle protein synthesis were also measured in that experiment and found to yield a similar time-dependent response as plasma albumin (not shown). This study was approved by and conducted in compliance

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