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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.

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

with the policies of the Case Western Reserve University Institutional Animal Care and Use Committee.

2.3. Analytical

Food intake and body weights were measured weekly. Caloric intake was calculated by multiplying the gram of food consumed by the caloric density of each diet (ie, 3.8 kcal per gram low-fat diet vs 4.7 kcal per gram high-fat diet).

Body composition was determined via dissection of epididymal fat pads and ^{2}H-dilution. Briefly, the ^{2}H-labeling of body water was determined as described by McCabe et al [18]; the ^{2}H-dilution yields a direct measure of water mass, from which one can estimate total lean mass and fat mass.

The concentration of plasma glucose was determined in the fasted mice (ie, those given the sham saline gavage) using stable isotope dilution. A known volume of plasma (5 μL) was spiked with a known quantity of [6,6-$^2H_2$] glucose (5 μL of a 1-mg/mL solution); samples were then deproteinized via the addition of 10 vol of methanol (100 μL). The supernatant was evaporated to dryness, reacted to form the "oxime-trimethysilyl" derivative, and analyzed by gas chromatography–mass spectrometry under electron impact ionization; the concentration of glucose was determined from the ratio of *m*/*z* 319 to 321 [19].

The fractional rates of protein synthesis in liver, plasma albumin, and skeletal muscle were determined from the incorporation of [^{2}H]alanine using a precursor-product relationship. Briefly, samples were homogenized in trichloroacetic acid (TCA; 0.1 g of tissue in 1000 μL of 10% TCA, wt/vol) and centrifuged for 10 minutes at 4000 rpm. The protein pellet was washed twice with 5% TCA and then hydrolyzed for 20 hours in 1 mL of 6 N HCl at 100°C. To determine rates of plasma albumin synthesis, ~200 μL of plasma was treated with 1 mL of 10% TCA. The protein pellet was washed twice with 5% TCA; albumin was then extracted from the pellet into 100% ethanol [17]. After the evaporation of ethanol, samples were hydrolyzed in 1 mL of 6 N HCl at 100°C.

An aliquot of a hydrolyzed protein sample was dried by vacuum centrifuge for 30 to 60 minutes. The samples were then reacted to form the "methyl-8" derivative of alanine, made by mixing acetonitrile, methanol, and methyl-8 reagent (Pierce, Rockford, IL; 1:2:3, vol:vol:vol) and heating the sample at 75°C for 30 minutes [17]. The sample was transferred to a gas chromatography–mass spectrometry vial and analyzed using an Agilent 5973N-MSD equipped with an Agilent 6890 GC system. A DB17-MS capillary column (30 m × 0.25 mm × 0.25 μm) was used in all assays. The initial temperature program was set at 90°C and held for 5 minutes, increased by 5°C per minute to 130°C, and increased by 40°C per minute to 240°C and held for 5 minutes, with a helium flow of 1 mL/min. Alanine elutes at ~12 minutes. The mass spectrometer was operated in the electron impact mode. Selective ion monitoring of *m*/*z* 99 and 100 (total ^{2}H-labeling of alanine) was performed using a dwell time of 10 milliseconds per ion.

2.4. Calculations

2.4.1. Protein synthetic rate

The rate of protein synthesis was calculated using the equation:

$$^2\text{H}-\text{labeling protein}-\text{derived alanine (percentage)} / [^2\text{H}-\text{labeling body water (percentage)} \times 3.7 \times \text{time(hour)}],$$

where the factor 3.7 represents an incomplete exchange of ^{2}H between body water and alanine, that is, 3.7 of the 4 carbon-bound hydrogens of alanine exchange with water [17,20] This equation assumes that the ^{2}H-labeling in body water

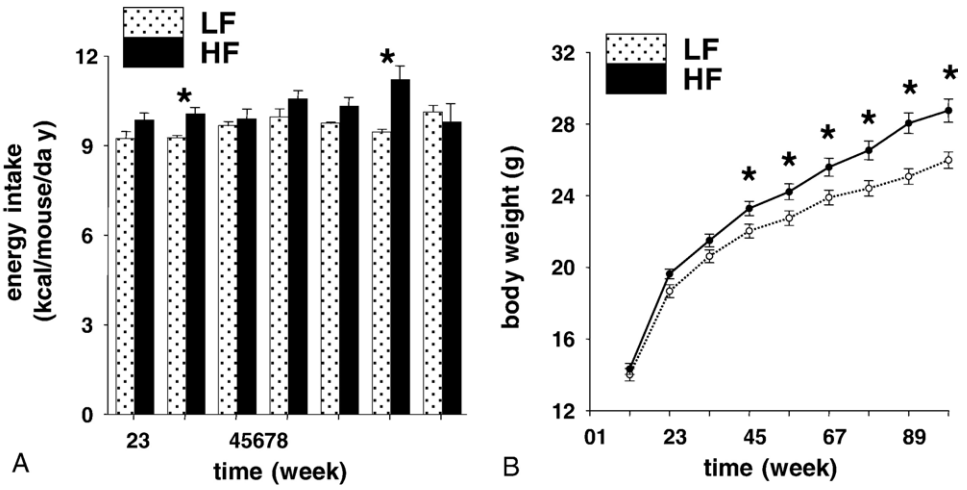

Fig. 1. Energy intake and growth. Male C57BL/6J mice were allowed ad libitum access to either an LF diet or an HF diet. Energy intake (A) was determined by weighing the food that remained on each cage at the end of every week and dividing by 4 (ie, the number of mice per cage). The body weight (B) of individual mice was determined at the end of each week. The data are expressed as mean ± SEM (n = 16 per diet group per time point). *$P < .05$.

equilibrates with free alanine more rapidly than alanine is incorporated into newly made protein and that protein synthesis is linear over the study [21].

The percentage of change of protein synthesis in various samples was determined by comparing the individual mice in an experimental group (eg, liquid meal gavage, fed group) against the mean of the respective control group (eg, sham saline gavage, fasted group) using the equation:

$$[(\text{fed}_{\text{mouse } x} - \text{fasted}_{\text{mean}})/\text{fasted}_{\text{mean}}] \times 100,$$

where "$\text{fed}_{\text{mouse } x}$" refers to a single mouse in the fed group. The mean ± SEM was calculated.

2.4.2. Statistics

The sample size required for this study was estimated by performing power calculations (5% level of significance with 80% power) using data acquired in experiments with a similar design in which we measured protein synthesis in basal and stimulated states. Unless noted, data are expressed as mean ± SEM. Comparisons of various parameters (eg, body mass, percentage of fat, blood glucose concentration) between low-fat–fed vs high-fat–fed mice were made using 1-tailed *t* tests assuming equal variance, and the effects of nutritional state (fed vs fasted) and obesity (low-fat vs high-fat diet) on protein synthesis were determined using a 2-way analysis of variance (SPSS version 14.0; SPSS, Chicago, IL).

3. Results

Fig. 1 demonstrates that regardless of the dietary composition, total caloric intake was similar between the mice in each group when expressed as kilocalories consumed per mouse per day (Fig. 1A). For example, significant differences were only observed over 2 of the experimental weeks. In addition, the cumulative caloric intake was similar when data are expressed as kilocalories consumed per mouse over the course of the study: 503 ± 6 vs 518 ± 16 total kcal per mouse, LF vs HF diet, respectively (P = .134). Consumption of the HF diet, as compared with the LF diet, promoted a greater increase in body mass (Fig. 1B), which was related to an increase in total body fat and epididymal fat: ~65% and ~92% increases, respectively. That is, total body fat accounted for 28.1% ± 1.7% vs 17.3% ± 1.9% of body weight (n = 16 each, P < .001) and epididymal fat reached 1.00 ± 0.08 g vs 0.52 ± 0.02 g (n = 16 each, P < .001) in HF- vs LF-fed mice, respectively. Total lean body mass was similar in the 2 groups: 20.4 ± 0.8 g vs 21.4 ± 0.7 g in HF- vs LF-fed mice, respectively (n = 16 each, P = .151).

Mice on the HF diet developed elevated fasting plasma glucose: 8.85 ± 0.37 mmol/L vs 7.05 ± 0.19 mmol/L in mice on the LF diet (n = 5-7 each, P < .01). Although we were not able to measure plasma insulin concentrations in this study, we have measured fasting plasma insulin in mice fed the respective diets for 1, 4, and 12 weeks [22]. In that study, we found no difference after 1 week of dietary intervention (ie, ~60 pmol/L in each diet group), whereas fasting plasma insulin was doubled (P < .01) in HF- vs LF-fed mice after 4 and 12 weeks of intervention, that is, ~113 vs ~66 pmol/L and 140 vs 75 pmol/L, respectively. These observations support the development of a model of diet-induced obesity with impaired fasting glucose.

Fig. 2 demonstrates the fractional rates of protein synthesis in total muscle and liver proteins and plasma

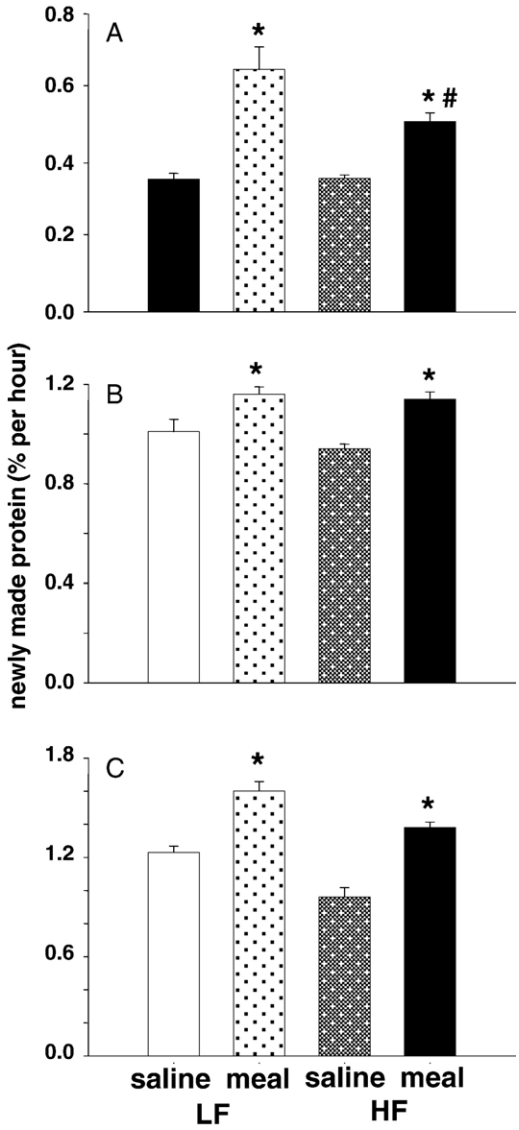

Fig. 2. Effect of diet-induced obesity on protein synthesis. Mice were maintained on either an LF or an HF diet for 9 weeks. Protein synthesis was quantified after the injection of ^2H_2O via the incorporation of ^2H-labeled alanine; mice were studied during either the fasted (saline gavage) or the fed (liquid meal) state. Muscle, liver, and plasma albumin syntheses were quantified (A, B, and C, respectively). The data are expressed as the percentage of newly made protein per hour; mean ± SEM (n = 6-8, muscle and liver data; n = 3-4, albumin data). *P < .05 fed vs fasted, #P < .05 fed vs fed.

albumin. The relative differences in basal values between muscle and liver and the relative agreement between liver and plasma albumin are in accord with the literature [23]. Furthermore, consistent with other reports, one would expect a stimulation of protein synthesis in fed (liquid meal) vs fasted (sham saline) mice, as we observed [17]. These data demonstrate that diet-induced obesity leads to an impaired activation of skeletal muscle protein synthesis (Fig. 2A, an increase of 95% ± 18% in LF-fed mice vs 43% ± 7% in HF-fed mice, respectively). Although there is no apparent effect on the stimulation of total liver protein synthesis or plasma albumin (Fig. 2B, an increase of 16% ± 3% in LF-fed mice vs 21% ± 3% in HF-fed mice and Fig. 2C, an increase of 38% ± 3% in LF-fed mice vs 44% ± 4% in HF-fed mice, respectively), there is a slight reduction in basal hepatic protein synthesis and albumin synthesis.

4. Discussion

Because obesity is typically associated with insulin resistance and because insulin regulates protein dynamics, it is not unreasonable to suspect that obesity would alter protein synthesis. However, the literature regarding the relationship between obesity and protein metabolism is somewhat controversial. The primary aim of our study was to quantify rates of protein synthesis in obesity. Although we considered various metabolic models, we decided to use the high-fat–fed C57BL/6J mouse vs a genetic model (eg, the *ob*/*ob* mouse) because one can examine the time course for the induction of obesity and related diseases (eg, type 2 diabetes mellitus) and/or the reversal of obesity via changes in the macronutrient composition of the diet [24,25]. In addition, because this strain is often used as the background onto which genetic modulations are introduced, it is possible to address questions regarding interactions between diet and genetics. We also considered the experimental conditions; for example, should studies be performed in a basal and/or a stimulated state? We chose to contrast fasting vs feeding (as compared with an insulin-glucose clamp) to simulate a more reasonable physiological scenario(s). Because we used an oral gavage to deliver the liquid meal, special attention was given to minimize the stress. For example, mice were briefly sedated before the gavage was given; and the controls (fasted groups) were given a sham gavage of saline.

It is of interest to note that in the seminal studies of Surwit and colleagues [25] on diet-induced obesity in C57BL/6J mice, the data are often expressed as a "feed efficiency" (ie, gram of fat gained per kilocalorie consumed). Over the course of our studies [26], we became intrigued by the fact that when C57BL/6J mice are given ad libitum access to either an HF or an LF diet, animals typically consume a similar amount of calories; yet the phenotypes are widely different. As expected, in the current study, we observed a significant change in body composition in mice that were fed the HF vs the LF diet; there was approximately a doubling of total body fat and epididymal fat (there was no difference in total lean mass). We were surprised by the fact that the total caloric intake was only slightly different over the course of the study (Fig. 1A, HF-fed mice consumed ~15 kcal more than LF-fed mice over the course of the study). Although one would not require a large caloric imbalance to explain the fact that the final difference in body mass was ~2.5 g in HF- vs LF-fed mice, other factors could influence the development of obesity including (*a*) the patterns of food intake (eg, small frequent meals vs larger boluses of food), (*b*) the partitioning of nutrients after a meal, and/or (*c*) altered energy expenditure (eg, a difference in activity) [25]. For purposes of the current study, the physiological/biochemical mechanisms that account for the development of obesity are not important; nevertheless, these observations suggest the merit of future studies in C57BL/6J mice to obtain new knowledge regarding the pathogenesis of diet-induced obesity. Namely, the development of obesity in C57BL/6J mice is clearly not characterized by overt hyperphagia but more likely by some subtle factor(s) [25]. A careful dissection of the etiology of diet-induced obesity in this model may improve our understanding regarding fundamental causes of excess weight gain.

In the current study, attention was directed toward quantifying the synthesis of total protein in skeletal muscle and liver (including plasma albumin) because these end products are sensitive to nutritional status [27] and because these sites constitute major depots for protein after a meal [16]. For example, one can estimate that ~5% of the increase in whole-body protein synthesis during feeding is related to an increase in albumin synthesis [28] and that a substantial fraction of the remainder is related to an increase in the synthesis of skeletal muscle protein [16]. Consistent with the literature, we observed a stimulation of protein synthesis in fed vs fasted mice regardless of the pool that was studied (Fig. 2); for example, a 2-fold increase in muscle protein synthesis after a meal was reported by others [29].

Our data demonstrate that diet-induced obesity leads to an impaired stimulation of skeletal muscle protein synthesis (Fig. 2A, an increase of ~95% in LF-fed mice vs ~43% in HF-fed mice, respectively). Although this observation is intriguing, we cannot state whether this is a direct and/or an indirect effect, that is, whether there is an impaired activation of translation initiation and/or whether there is an altered digestion/absorption of the meal. Furthermore, it has been speculated that if skeletal muscle protein degradation is likely to be constant from day to day under normal physiologic conditions, then the activation of protein synthesis in response to feeding must elicit efficient resynthesis of muscle proteins to prevent net protein breakdown and maintain skeletal muscle mass [30]. Considering the latter, if basal rates of skeletal muscle protein synthesis are similar in obese mice when compared with normal mice, and obese mice synthesize less skeletal muscle protein in response to nutrient ingestion (as shown), there must be an adaptive mechanism(s) for protein sparing in this

model; otherwise, net protein breakdown would occur. The last point is somewhat supported by the fact that we did not observe major differences in lean body mass.

Hepatic insulin resistance has been reported in rodent models after high-fat feeding. For example, Park et al [31] demonstrated that high-fat feeding to C57BL/6J mice resulted in insulin resistance in liver (and skeletal muscle and adipose tissue) after only 3 weeks of dietary intervention. In addition, Samuel et al [32] found impaired hepatic insulin action after 3 days of high-fat feeding in rats. Because albumin synthesis is sensitive to insulin [33], one might predict a substantial alteration in liver/albumin synthesis in HF-fed animals. However, we did not observe any apparent defects in the activation of total liver protein synthesis or plasma albumin in the fed state (Fig. 2B and C, respectively). We suspect that the apparent "normal response" of hepatic protein synthesis may be related to the nature of our experimental design vs that used in insulin-clamp studies. For example, in our study, a fairly large bolus of food was given (equal to ~35% of the daily intake), which could be sufficient to overcome more subtle defects that can be detected using the insulin clamp; in addition, nutrients (eg, amino acids) can stimulate translation independent of insulin. We believe that this should not be construed as a weakness in our data because one could argue that our design is somewhat more physiological than that which is achieved using the insulin clamp. More importantly, the apparent discrepancies between our data and those in the literature suggest the need for additional studies in this area and the need to determine the contribution of direct and/or indirect factors, for example, a primary impairment in insulin action vs a primary alteration in the response of the β-cell to secrete insulin and/or a primary defect in digestion/absorption. Lastly, our data suggest a tendency for reduced synthesis of total hepatic proteins and plasma albumin in the basal (fasting) state. These observations may be related to a modest increase in fat deposition in the liver (not shown) and suggest the importance of studies that more closely examine whether the magnitude and/or the duration of fatty liver impacts the synthesis/secretion of hepatic protein(s).

To this point, we have considered the nutritional-physiological model and the meaning of the biological data; however, another important area concerns the methodology that we used to quantify protein synthesis. For example, labeled water was used in a few early studies of protein dynamics [34-36]; we have recently revisited the use of ^2H_2O [17,20,37] and $H_2^{18}O$ (unpublished observations). The ^2H_2O method is based on establishing a precursor-product relationship; that is, after the administration of ^2H_2O, one determines the rate of protein synthesis by measuring the incorporation of ^2H-labeled alanine into a protein(s) of interest. In our studies, we have assumed that the precursor labeling is that of body water and that the product labeling is that of protein-bound alanine divided by 3.7, and that there is rapid equilibration between hydrogen in body water and free alanine. Our previous experiments and data in the literature validate these assumptions. First, studies of enzyme reaction mechanisms demonstrate that it is not possible to exchange all 4 of the carbon-bound hydrogens of alanine [38,39]; our findings and the recent study by Belloto et al [40] agree with those reports. Second, tracer studies using various isotopes have demonstrated that alanine flux is rapid [41-43]; for example, the pool turnovers over several times per hour in humans. Again, our experiments in humans [20] and rodents [17] are consistent with those data and with the fact that alanine is a central metabolic intermediate. Last, other recent reports have examined the use of ^2H_2O to measure protein synthesis in vivo [40,44].Those investigators have also concluded that measuring the incorporation of ^2H-labeled alanine into proteins provides a reliable measure of protein synthesis, even when comparing the ^2H_2O method against the steady-state leucine infusion [40].

Although there is a slight discrepancy in the literature regarding the total number of exchangeable hydrogens in free alanine [17,20,40,44] (eg, Busch and colleagues [44] found that the labeling of alanine is 4 times that of water), one could argue that the critical factors are (*a*) the stability of this number over the time course of a study and (*b*) whether perturbations affect the value. As demonstrated by our group [17,20] and by Belloto and colleagues [40], it appears that the ^2H-labeling of alanine is remarkably stable (albeit at ~3.7 times that of body water) and that the labeling of plasma alanine reflects that in different tissues. We believe that the use of ^2H_2O offers an advantage over the use of labeled leucine, especially in studies where the experimental design involves non–steady-state metabolism. For example, a major concern in tracer studies is the stability of the precursor labeling, especially over a prolonged period and/or during conditions where substrate flux may suddenly change (eg, after a meal). Thus, the use of labeled leucine infusion would have been somewhat problematic in our studies because animals would have required catheters to maintain a constant tracer infusion and the bolus of food would have affected the precursor labeling [45]. Perhaps the "flooding dose" would have been reasonable because the expansion of the amino acid pool via the flood of tracer might have minimized any perturbation that occurred via digestion/absorption of dietary protein. However, a potential concern regarding the flooding dose centers on the duration of the study; that is, ^2H_2O permits experiments over a broad window, therein allowing one to capture and integrate more metabolic activity vs the flooding dose that is typically used over ~60 minutes (and in some cases, only ~10 minutes). We previously demonstrated that in short-term studies (eg, those lasting several hours), it is possible to simply administer an intraperitoneal priming bolus of ^2H_2O and therein maintain a steady state of labeling [17]. For example, because the $t_{1/2}$ of ^2H in body water in mice (fed the respective diets described herein) is ~2.5 days [26], if the initial target enrichment is 2.5% ^2H-labeling, then after 5 hours, one expects to find a ~6% decrease in the ^2H-labeling of body water, that is, from ~2.50% to ~2.35%. A

recent study by our group used ^2H_2O to measure protein synthesis in the heart in rats fed various diets, including some with a high salt regimen [46], which is known to increase water turnover. In that study, we were able to obtain serial measurements of water labeling over ~6 hours. Although we could detect a slight difference in the ^2H-labeling of samples obtained at 60 vs 300 minutes postinjection (~5% decrease), we could not detect differences between the groups, implying a highly stable precursor labeling within and between groups.

In summary, diet-induced obesity in C57BL/6J mice appears to alter normal protein dynamics. This model may prove useful in future studies because one can readily induce/reverse disease via dietary manipulation. Furthermore, because tissue biopsies can be obtained, one should be able to identify molecular mechanisms that explain various findings. Although our data differ in regards to some results that have been obtained using insulin clamps, it is important to note that our studies used a design that yields an integrative response, whereas "clamp studies" allow one to focus on a specific component of the physiological response; these 2 approaches complement each other. Finally, because ^2H_2O is suitable for use in humans, it is possible to translate observations between clinical and basic science; of course, studies of albumin synthesis are more practical because an examination of muscle protein synthesis requires tissue biopsies.

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