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The influence of sex on the protein anabolic response to insulin

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Abstract

We hypothesize that sex influences whole-body protein anabolism in the postabsorptive state and in response to hyperinsulinemia. Kinetics of 3-^{3}H-glucose and ^{13}C-leucine were studied in 16 men and 15 women after energy- and protein-controlled diets, before and during a hyperinsulinemic, euglycemic, isoaminoacidemic clamp. In the postabsorptive state, women had 20% higher rates of leucine Ra (protein breakdown) and nonoxidative Rd (synthesis) adjusted for fat-free mass than men but net leucine balance was as negative. In response to hyperinsulinemia, leucine oxidation rates increased only in women and the change in net leucine balance was less than in men. Net leucine balance during the clamp correlated with rates of glucose disposal. Thus, women showed greater protein turnover rates when adjusted for fat free mass in the postabsorptive state, and lesser insulin sensitivity of protein anabolism and net protein accretion. A relationship exists between the protein anabolic response to insulin and the insulin sensitivity of glucose metabolism.

1. Introduction

Many metabolic differences between men and women have been identified. Some are related to their differences in body composition, but others remain even when these are taken into account. Whether the latter applies to regulation of whole-body protein turnover and fuel homeostasis is not clear. Using the single-dose ^{15}N-glycine method, neither protein breakdown nor synthesis were different by sex in young adults when expressed per fat free mass (FFM) [1]. Using the ^{14}C-leucine kinetic method, Volpi et al [2] also found that postabsorptive protein breakdown and synthesis also expressed per FFM were comparable between sexes in young subjects matched for body mass index (BMI). However, leucine oxidation was significantly less in women. Using the ^{13}C-leucine method, Boirie et al [3] reported that postabsorptive flux, breakdown, oxidation, and synthesis, again per FFM, were higher in young men than in women. In that study, breakdown was no longer greater in men at 2 levels of clamped hyperinsulinemia with circulating amino acid concentrations held constant by exogenous infusion [3]. Differing protocols (including prior diet control), turnover methodologies, and statistical analyses may explain these differing results. In particular, analyses presenting variables divided by, rather than adjusted for, FFM have been questioned [4].

We used the hyperinsulinemic, euglycemic, isoaminoacidemic clamp technique [5] and ^{13}C-leucine as tracer to study protein metabolism in postabsorptive and hyperinsulinemic states and demonstrated increased whole-body protein synthesis with high insulin and plasma amino acids maintained at postabsorptive levels in lean young men [6]. We also compared obese to lean women and found less increase in synthesis and net protein accretion (synthesis minus breakdown) in the obese subjects [7]. Furthermore, net protein accretion correlated negatively with all indices of adiposity. These results suggested that in obese women, insulin resistance of protein metabolism occurs concurrently with that of glucose. In both of these studies, we isolated the effect of insulin on protein turnover from that of amino acids by maintaining their postabsorptive concentrations constant. This approach contrasts with previous studies in which increases in plasma amino acids due to higher infusion rates contributed to the stimulation of protein synthesis [8] and those in which decreases in plasma amino acids in the absence of infusion failed to show altered insulin effects in obesity [8-10]. It became apparent that insulin stimulation of protein synthesis and intergroup differences could not be assessed in the face of the hypoaminoacidemia induced by

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hyperinsulinemia [11]. Indeed, the activity of initiation factor of messenger RNA translation, eIF2B is reduced with decreased plasma amino acid concentrations [12].

Previous studies have reported protein turnover data in pooled groups of men and women [8,13,14] or have assessed one sex only [5,9,15]. This study explored whether there is a sex effect in both postabsorptive and insulin-stimulated protein turnover resulting in one or more of synthesis, breakdown, oxidation, and net protein accretion being affected.

2. Materials and methods

2.1. Subjects and diet

Healthy men (n = 16) and women (n = 15) matched for BMI, age, and glucose tolerance were recruited and screened by medical history, physical examination, and laboratory investigation, as previously detailed [6]. They were admitted to the McGill University Health Centre-Royal Victoria Hospital Clinical Investigation Unit after giving written informed consent. This protocol was approved by the Human Ethics Review Committee of the hospital. Women with regular menstrual cycles were studied during the follicular phase. Data from 10 of the men and 13 of the women form part of different subject groups in our other studies that do not address sex effects [6,7].

During the 7 days before the clamp experiment, subjects received an individualized formula-based isoenergetic diet, according to resting metabolic rate measured by indirect calorimetry (Deltatrac, Sensor Medics, Yorba Linda, Calif), multiplied by a physical activity factor of 1.6 to 1.7. The diet provided 15% of energy as protein. Details of the diet and dietary protocol have been recently described [6]. Nitrogen balance studies were conducted during the last 3 days of the adaptation diet, as previously described [16]. Body composition was assessed by bioelectrical impedance analysis using the RJL-101A Systems (Detroit, Mich) instrument [17] and using equations validated for lean [18] and overweight [19] men and women.

2.2. Hyperinsulinemic, euglycemic, isoaminoacidemic clamp protocol

The hyperinsulinemic clamp experiment was performed according to the detailed procedure recently published [6], with target plasma glucose levels at 5.5 mmol/L and maintenance of individual subjects' postabsorptive plasma branched-chain amino acid (BCAA) concentrations. All subjects were studied after an overnight fast. Briefly, at 8:00 AM, subjects had catheters inserted in an antecubital vein for infusions and in a contralateral hand vein (retrogradely) for blood sampling. The hand was placed in a heating box at 65°C to 70°C to arterialize the venous blood [20]. Glucose turnover was studied using a primed (22 μCi [814 kBq]) and continuous infusion (0.22 μCi/min [8.14 kBq/min]) of [3-^{3}H]-glucose, maintained for the duration of the clamp. At the same time, a primed (0.5 mg/kg), constant infusion of [1-^{13}C]-leucine was started at 0.008 mg/kg per minute to study leucine kinetics [21] after an oral bolus of 0.1 mg/kg of $NaH^{13}CO_3$. A primed infusion of biosynthetic regular human insulin (Humulin R, Eli Lilly Canada Inc, Toronto, ON) was started 180 minutes later and maintained at a rate of 40 mU/m^2 per minute for at least 210 minutes. At 184 minutes, sterile 20% (wt/vol) potato starch–derived glucose (Avebe b.a., Foxhol, The Netherlands) in water with added [3-^{3}H]-glucose (the "hot GINF" method [22]) was infused at variable rates adjusted to reach and maintain euglycemia based on measurements of glucose every 5 minutes. Baseline concentrations of plasma individual amino acids (AAs) were maintained with an infusion of a 10% AA solution (10% TrophAmine without electrolytes, B. Braun Medical Inc, Irvine, Calif) by feedback adjustments of infusion rates based on plasma BCAA concentrations, measured every 5 minutes.

Blood samples were collected for glucose, BCAA, insulin, and tritiated glucose-specific activity analyses every 10 minutes for 40 minutes before the insulin infusion, then every 30 minutes until the last 40 minutes, at which time they were again drawn at 10-minute intervals. Indirect calorimetry was performed for 20 minutes before and during the last 30 minutes of the insulin infusion [23] for total carbon dioxide measurement for determination of leucine oxidation and for nonprotein respiratory quotient calculations using the known nitrogen excretion rates of the previous day to determine substrate oxidation. Glucose turnover was calculated as specified in Refs [22,24] and substrate oxidation as in Ref [23]. Expired air samples were collected into a breath collection balloon, transferred to 10-mL Vacutainer tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) and stored at room temperature. Leucine kinetics were calculated according to the stochastic model of Matthews et al [21] using plasma α-ketoisocaproic acid (α-KIC) as an index of the precursor pool enrichment (reciprocal model).

2.3. Assays

Plasma glucose was measured by the glucose oxidase method (GM7 Micro-Stat, Analox Instruments USA, Lunenberg, Mass). Assays for immunoreactive insulin and glucagon, and glucose-specific activity were the same as in Refs [22,25]. Plasma total BCAA concentrations were measured by a rapid enzymatic, fluorometric assay [6]. Individual plasma AAs were determined by ion-exchange high-performance liquid chromatography with postcolumn ninhydrin detection [26] using a Beckman High-Performance Liquid Chromatography System (Beckman Coulter Inc, Fullerton, Calif). Plasma free fatty acid (FFA) concentrations 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, Calif) after derivatization with *N*-methyl-*N*-(*tert*-

Table 1
Subject characteristics

	Men	Women	*P*
n	16	15	
Age (y)	30 ± 2	30 ± 3	–
Height (cm)	178 ± 2	160 ± 2	<.001
Weight (kg)	81 ± 5	62 ± 3	.004
BMI (kg/m^2)	25.4 ± 1.4	24.3 ± 1.4	–
FFM (kg)	62.3 ± 1	42.4 ± 1	<.001
Body fat (%)	20.8 ± 2.6	30.2 ± 2.5	.014
Waist circumference (cm)	89.6 ± 3.9	76.4 ± 3.5	.018
Waist-hip ratio	0.90 ± 0.02	0.77 ± 0.02	<.001
Energy intake (MJ/d)	12.06 ± 0.32	8.95 ± 0.21	<.001
(adjusted for FFM)	(10.29 ± 0.29)	(10.83 ± 0.31)	–
Protein intake (g/d)	109 ± 3	78 ± 3	<.001
(adjusted for FFM)	(89 ± 3)	(98 ± 3)	–
OGTT (mmol/L)	5.5 ± 0.4	5.8 ± 0.4	–

Values are means ± SEM. Groups were compared by unpaired *t* tests. OGTT indicates plasma glucose 2 hours after a 75-g oral glucose tolerance test; –, not significant.

butyldimethylsilyl) trifluoroacetamide (Regis Technologies Inc, Morton Grove, Ill) to yield a TBDMS derivative of HIC. Expired air was analyzed for $^{13}CO_2$ enrichment by isotope ratio mass spectrometry on a Micromass 903D (Vacuum Generators, Winsforce, UK).

2.4. Validation studies of background enrichment of expired $^{13}CO_2$ and plasma ^{13}C-KIC, and ^{13}C bicarbonate recovery

We have previously found a 10.1% ± 1.6% dilution in the background enrichment of expired $^{13}CO_2$ in lean young men, mainly due to infusion of the potato starch–derived glucose with a natural low ^{13}C content [6]. The same effect was later tested in 4 lean young women to test a possible sex difference, and their percentage of dilution was not significantly different from that of men. Thus, leucine oxidation rates for both lean men and women groups were corrected using the same dilution factor. No dilution effect in plasma ^{13}C-KIC was found. The recovery of ^{13}C from the bicarbonate pool was also assessed in the postabsorptive state, and during the hyperinsulinemic clamp in 12 subjects, and recovery factors of 0.671 in the postabsorptive state and 0.799 during the clamp period were used for the calculation of leucine oxidation rates.

2.5. Statistical analyses

Results are presented as means ± SEM. Subject characteristics were compared by unpaired *t* test. The sex effect was assessed by analysis of covariance, at both baseline and clamp periods, with FFM as covariate when it was found to have a significant predictive value on the dependent variable, from prior regression analyses [4]. Repeated-measures analysis of variance (ANOVA) was used to assess the effect of the clamp intervention (response to insulin) and the sex effect on the response to the clamp. Thus, for those variables adjusted for FFM, data are presented as means adjusted for FFM. Pearson coefficient was used for all correlations, and when they required controlling for other variables, partial correlation was used. Significance level was defined as $P < .05$. The analyses were performed with SPSS 11.0 for Windows (SPSS Inc, Chicago, Ill).

3. Results

Body composition and characteristics of the subjects are shown in Table 1. The women were significantly shorter and weighed less than the men, had lower FFM and waist-hip ratio, but had higher body fat as percentage of weight. Total energy intake and protein intake per day were lower in women, but not when adjusted for FFM. The diet was well tolerated, weight was maintained during the study, and nitrogen balance was at equilibrium or slightly positive (data not shown).

Baseline plasma glucose was not different and was clamped at 5.5 mmol/L in all subjects. The difference in glucose infusion rates between women and men did not reach statistical significance when adjusted for FFM (340 ± 51 vs 522 ± 48 mg/min, $P = .056$). There was no sex effect on baseline glucose rates of appearance (Ra) and rates of disappearance (Rd) when adjusted for FFM (data not shown). Glucose Ra was suppressed to zero in both groups during clamp. Plasma insulin, glucagon, and the glucagon-insulin ratio did not differ between sexes either at baseline or during the clamp (Table 2). Insulin increased, glucagon decreased, and their ratio decreased with the clamp to comparable values.

Data for gas exchange and nonprotein substrate use are shown in Table 3, in which all means presented are adjusted for FFM. V_{O_2} and V_{CO_2} (mL/min) were significantly higher in men at baseline and during the clamp. The small but significant increase in V_{O_2} during the clamp was seen only in men, whereas V_{CO_2} increased in both groups. Nonprotein respiratory quotient (RQ) did not differ between sexes at baseline; it increased in all subjects during the

Table 2
Plasma hormones in men and women at baseline and during the hyperinsulinemic clamp

		Men (n = 16)	Women (n = 15)	Clamp effect	Sex effect
Insulin (pmol/L)	Baseline	65 ± 4	79 ± 7		
	Clamp	587 ± 23	637 ± 30	<.001	–
Glucagon (pmol/L)	Baseline	19 ± 1	21 ± 1		
	Clamp	14 ± 1	16 ± 1	<.001	–
Glucagon/insulin (pmol/L)	Baseline	0.29 ± 0.03	0.27± 0.03		
	Clamp	0.02 ± 0.00	0.02 ± 0.00	<.001	–

Values are means ± SEM. The effect of the clamp intervention was assessed by repeated-measures ANOVA, with sex as the main factor. No significant clamp by sex interaction was found. – indicates not significant.

Table 3
Nonprotein substrate use in men and women at baseline and during the hyperinsulinemic clamp

		Men (n = 16)	Women (n= 15)	Clamp effect	Sex effect	Clamp × sex
V_{O_2} (mL/min)	Baseline	260 ± 8	198 ± 4			
	Clamp	267 ± 8*	197 ± 5	–	<.001	.018
V_{CO_2} (mL/min)	Baseline	211 ± 7	158 ± 4			
	Clamp	237 ± 7	180 ± 4	<.001	<.001	–
npRQ	Baseline	0.82 ± 0.01	0.79 ± 0.01			
	Clamp	0.92 ± 0.01*	0.94 ± 0.01*	<.001	–	<.001
REE (MJ/d)	Baseline	6.26 ± 0.21	6.92 ± 0.22	<.001	–	–
	Clamp	6.53 ± 0.20	7.13 ± 0.21			
Glucose Rd (mg/min)	Baseline	133 ± 8	128 ± 8			
	Clamp	505 ± 47	352 ± 50	<.001	–	–
Oxidative glucose Rd (mg/min)	Baseline	63 ± 15	78 ± 16			
	Clamp	155 ± 13	169 ± 14	<.001	–	–
Nonoxidative glucose Rd (mg/min)	Baseline	70 ± 17	50 ± 18			
	Clamp	350 ± 45	183 ± 48	<.001	–	–
Lipid oxidation (mg/min)	Baseline	57 ± 7	61 ± 8			
	Clamp	21 ± 6	25 ± 6	<.001	–	–
Serum FFAs (μmol/L)	Baseline	462 ± 28	735 ± 51**			
	Clamp (90 min)	140 ± 12*	99 ± 11*,**			
	Clamp (plateau)	117 ± 8***	99 ± 11*	<.001	.005	<.001

Values are mean ± SEM. Means adjusted for FFM are presented except for V_{O_2}, V_{CO_2}, and npRQ. The effect of the clamp intervention was assessed by repeated-measures ANOVA, with sex as the main factor, without FFM as covariate. npRQ indicates nonprotein RQ; Rd, rate of disposal; –, not significant.

* $P < .05$ by paired t test vs baseline.

** $P < .05$ by unpaired t test vs men.

*** $P < .05$ by paired t test vs 90 minutes.

clamp, but more so in women, as indicated by the significant clamp × sex interaction. There was no sex effect on resting energy expenditure (REE) at baseline and during hyperinsulinemia. Resting energy expenditure increased in all subjects during the clamp. There were no sex differences for baseline and clamp glucose Rd, oxidative or nonoxidative glucose Rd. Furthermore, there was no sex effect on their significant response to hyperinsulinemia. Lipid oxidation did not differ between sexes at baseline and during the clamp and decreased similarly in both groups. Free fatty acids were higher in women at baseline, were suppressed more than in men at 90 minutes, but did not differ at plateau.

Postabsorptive plasma leucine, total BCAA, indispensable, dispensable, and total AA were higher in men, and because they were "clamped" at this level, also during hyperinsulinemia (Table 4). Plasma indispensable AA increased only in women, whereas plasma dispensable AA decreased only in men. Overall, the total AA concentrations did not change significantly from baseline during the clamp and were thus maintained. However, there was a clamp × sex interaction because of a decrease in plasma AA concentrations in men. Plasma AA concentrations were maintained with rates of AA infusion that did not differ between women and men when adjusted for FFM (37 ± 3 vs 43 ± 3 mg/min).

Leucine kinetic data are presented in Table 5 as means in μmol/min, adjusted for FFM. Total leucine Ra was lower in men at baseline, but increased in both groups during the clamp to values that no longer differed. Baseline and clamp

Table 4
Plasma AA concentrations in men and women at baseline and during the hyperinsulinemic clamp

		Men (n = 16)	Women (n = 15)	Clamp effect	Sex effect	Clamp × sex
Plasma leucine	Baseline	137.1 ± 4.8	110.8 ± 2.5			
	Clamp	148.2 ± 4.4	125.7 ± 4.0	<.001	<.001	–
Plasma BCAA	Baseline	422 ± 14	348 ± 8			
	Clamp	421 ± 12	362 ± 10	–	<.001	–
Plasma indispensable AA	Baseline	943 ± 24	793 ± 22			
	Clamp	952 ± 18	867 ± 25*	.001	<.001	.005
Plasma dispensable AA	Baseline	1568 ± 35	1386 ± 43			
	Clamp	1434 ± 16*	1370 ± 47	<.001	.017	.004
Plasma total AA	Baseline	2511 ± 55	2179 ± 49			
	Clamp	2386 ± 31*	2237 ± 58	–	.001	.003

Values are means ± SEM in μmol/L. The effect of the clamp intervention was assessed by repeated-measures ANOVA, with sex as the main factor. – indicates not significant.

* $P < .05$ vs baseline by paired t test.

oxidation rates did not differ significantly between sexes and increased significantly only in women. Endogenous leucine Ra (protein breakdown) was higher in women at baseline and during the clamp, and decreased by the same amount during the clamp. No sex differences were found in the leucine infusion rates. Baseline nonoxidative leucine Rd (protein synthesis) was significantly higher in women and increased during the clamp to values that no longer differed by sex; net endogenous leucine balance (from subtracting breakdown from synthesis) was as negative in men as in women at baseline, but increased more in men during the clamp to levels that indicated net protein accretion in men but equilibrium in women.

There was a significant correlation between the steady-state clamp rates of AA and glucose infusions, with $r = 0.640$ and $P < .001$, indicating a relationship between the sensitivity to insulin of glucose and that of AA metabolism. Furthermore, clamp net endogenous leucine balance (net protein accretion) correlated with clamp nonoxidative glucose Rd (partial $r = 0.569$, $P = .001$) and with clamp glucose Rd (partial $r = 0.615$, $P < .001$) after controlled for FFM, further indicating a relationship between rates of these components of glucose and AA metabolism. The change in net endogenous leucine balance in response to hyperinsulinemia correlated with the change in V_{O_2} ($r = 0.435$, $P = .014$), in REE ($r = 0.427$, $P = .017$), and negatively with that of RQ ($r = -0.552$, $P = .001$).

Table 5
Leucine kinetics in men and women at baseline and during the hyperinsulinemic clamp

Leucine kinetics adjusted for FFM (μmol/min)	Men (n = 16)	Women (n = 15)	Clamp effect	Sex effect	Clamp × sex
Total Ra					
Baseline	120 ± 6	145 ± 6*			
Clamp	145 ± 7	161 ± 7	<.001	–	–
Oxidation					
Baseline	28 ± 2	28 ± 2			
Clamp	29 ± 3	39 ± 3	<.001	–	.053
Endogenous Ra					
Baseline	120 ± 6	145 ± 6*			
Clamp	98 ± 7	121 ± 7	<.001	.046	–
Infusion rate					
Clamp	46 ± 3	40 ± 3		–	
Nonoxidative Rd					
Baseline	93 ± 5	117 ± 5*			
Clamp	116 ± 7	122 ± 7	<.001	–	–
Net balance					
Baseline	−28 ± 2	−28 ± 2			
Clamp	17 ± 3	1 ± 4*	<.001	.049	.012

Means ± SEM are adjusted for FFM. The effect of the clamp intervention was assessed by repeated-measures ANOVA without FFM as covariate. Total Ra indicates total leucine flux, including exogenous leucine infusion during the clamp period; endogenous Ra, index of protein breakdown; nonoxidative Rd, index of protein synthesis; net balance, synthesis − breakdown; –, not significant.

* $P < .05$ vs men at the same period, by ANCOVA with FFM as covariate.

4. Discussion

The main findings of our study are that women show greater protein turnover rates in the postabsorptive state and less insulin sensitivity of protein (anabolism) compared with men. Our approach to the application of the ^{13}C-leucine technique has been to rigorously control nutrient intakes and maintain low intensity of physical activity [27] for a whole week, then to define first the postabsorptive state, then in the same study, the responses to high physiological insulin levels while maintaining circulating levels of both glucose and AAs in the postabsorptive range [8,9]. This enables the definition of the insulin effect without being confounded by the substantial, physiologically relevant changes in circulating AA of other study protocols, whether downward (without AA infusion) or upward (with higher infusion rates of AA). The latter exerts independent effects upon protein turnover [28].

Comparing sex differences when studying whole-body protein metabolism requires the use of a reference compartment of body composition. Because body weight and composition differ between men and women, we chose to normalize the metabolic data for the lean tissue compartment, using a regression-based approach [4]. This method accounts for the nonzero intercept of the relationship between the dependent variable and FFM. The only way of definitively quantifying the roles and responses of the key target tissues of insulin's effects on protein metabolism is to study the individual tissues, a more invasive approach [27,29]. The definition of the whole-body responses is the best noninvasive way available to serve as the basis for justification for deploying the more complex protocols.

As summarized in the Introduction, differing results have been obtained in studies of the influence of sex on protein turnover [1-3]. These are due to subject selection, varying nutrition before study (not controlled and often not mentioned), different methods (no AA infusion, or infusion producing hyperaminoacidemia that intentionally or not, mimics the postprandial state), and normalization of data by presenting them as ratios with different denominators. Our subjects did show sex differences in the postabsorptive state. Whole-body leucine turnover was higher in women than men and may be the consequence of different lean tissue distribution. Women are characterized by less skeletal muscle mass and proportionately greater visceral mass, the latter known to turn over 2 to 3 times more rapidly [30]. Higher rates of protein turnover have been shown in insulin resistant states such as obesity [7] and type 2 diabetes [31], but to our knowledge not in women compared with men. We also found that the proportion of leucine oxidation to total flux not adjusted for FFM was significantly lower, and that of protein synthesis to total flux significantly higher in women than in men, by 6% ($P < .001$). Short et al [27] also reported a 9% lower ratio of leucine oxidation to flux in women than in men. These results are consistent with those of Volpi et al [2] showing lower postabsorptive leucine

oxidation rates in women, albeit normalized by FFM as a ratio. If oxidation rates were normalized by FFM as a ratio in the present study, they would also be lower in women than in men (0.44 ± 0.02 vs 0.58 ± 0.02, $P < .001$).

With hyperinsulinemia, an increase in leucine oxidation was found only in women. In contrast, net protein accretion was less than in men. These responses indicate that from the same infusion rates of AA, women showed less insulin sensitivity of protein anabolism than men. This lesser sensitivity of protein to insulin may be related to the relatively higher adiposity of women, as we have found with aging (unpublished data) and in obesity [7]. In fact, when net protein accretion adjusted for FFM was also controlled for percentage of body fat, it was no longer different between sexes. Of interest were the significant correlations between the change in net protein accretion with hyperinsulinemia and those in REE and VO_2, the latter increasing only in men, which would be consistent with the greater energy expenditure associated with a greater protein accretion [32]. Sex effects on the insulin sensitivity of protein synthesis remain to be assessed in the postprandial state and in response to hyperaminoacidemia alone, and to the postprandial state in which both hyperglycemic and hyperaminoacidemic responses occur.

Protein anabolism was correlated with markers of insulin sensitivity of glucose, suggesting that the lesser insulin sensitivity in women paralleled that of glucose. Other studies, but not all [33], have shown greater insulin resistance of glucose metabolism in girls than boys after adjusting for confounding variables of body composition and physical activity levels [34]. Greater prevalence of type 2 diabetes in girls than boys [33], and in adult women than men at any given BMI [35,36], has also been reported. This has led to a sex insulin hypothesis, proposing that an intrinsic insulin resistance in women could confer metabolic advantages in reproduction [37].

The present study cannot provide a mechanistic explanation for the sex differences in protein metabolism found. It is clear however that the differences in body composition, namely, a lesser FFM and higher percentage of body fat, contributed most importantly to the lesser insulin sensitivity of protein in women. But sex hormones and other humoral mediators of insulin responsiveness need to be considered [38,39]. Sex differences have been reported in the response to exercise, showing a lower oxidation of AAs in women than in men [40]; in addition, nitrogen balance was less negative in female endurance athletes when dietary protein intake was reduced [41]. Other factors are fiber-type distributions [42] and intramyocellular fat differences between sexes. The parallel between insulin sensitivity of protein and glucose will inform the design of the tissue-specific studies required to understand the cellular mechanisms involved.

Our results have implications for the understanding of insulin resistance in all situations in which it occurs, including obesity, the metabolic syndrome, perhaps some aged persons, and type 2 diabetes mellitus. In each group, where applicable, the recognition of sex differences becomes important not only for carbohydrate and lipid metabolism, but for protein as well. We therefore suggest that differences between men and women mandate studies that are sex-specific in relation to strategies for nutritional intervention, especially in refining recommendations for protein requirements in the previously mentioned conditions.

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