Age Attenuates Leucine Oxidation after Eccentric Exercise

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Abstract

Aging may alter protein metabolism during periods of metabolic and physiologic challenge. The purpose of this study was to assess the effects of age on whole-body amino acid turnover in response to eccentric exercise and hyperglycemia-induced hyperinsulinemia. 16 healthy men were divided into young (N = 8) and older (N = 8) groups. Protein metabolism was assessed using a [1-^{13}C]-leucine isotopic tracer approach. Measures were obtained under fasted basal conditions and during 3-h hyperglycemic clamps that were performed without (control) and 48 h after eccentric exercise. Exercise reduced leucine oxidation in the younger men ($P < 0.05$), but not in older men. Insulin sensitivity was inversely correlated with leucine oxidation ($P < 0.05$), and was lower in older men ($P < 0.05$). Healthy aging is associated with an impaired capacity to adjust protein oxidation in response to eccentric exercise. The decreased efficiency of protein utilization in older men may contribute to impaired maintenance, growth, and repair of body tissues with advancing age.

Keywords

age; amino acid metabolism; insulin resistance; physical activity

Introduction

It is well established that aging is associated with multiple physiological changes including loss of muscle mass, decline in organ function, hormonal dysregulation, and metabolic disturbances. Despite the maintenance of what is considered to be healthy weight, body composition, and a normal response to a glucose tolerance test, there is still a decrease in insulin sensitivity with age [12]. While the precise role of insulin resistance in protein metabolism remains unclear, increased insulin resistance has been shown to lead to a reduced capacity of insulin to suppress whole body protein degradation [38], and in men with type 2 diabetes, insulin fails to increase protein synthesis [31]. This is of particular interest in the context of aging, considering maintenance and repair of tissues is important for continued healthy and independent living.

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Resistance exercise is known to have a potent anabolic effect in skeletal muscle [32] and is one of the most effective countermeasures for age-related sarcopenia – or loss of muscle mass. We have previously shown that resistance exercise reverses age-related losses in strength, even in very old adults [10]. However, aging is also marked by a diminished capacity to respond or adapt to resistance exercise [4, 34, 37]. It has been suggested that aging leads to an impaired anabolic response to amino acid ingestion, as well as an impaired insulin-stimulated ability to suppress protein breakdown [29]. Following an acute bout of eccentric exercise, older individuals excrete more 3-methylhistidine in urine compared to young controls [11], and experience substantially more ultra-structural muscle damage [24]. Eccentric exercise has also been shown to cause acute transient whole-body insulin resistance in healthy young subjects [17] likely due to a pro-inflammatory response [16, 17]. Additionally, the combination of eccentric exercise and hyperglycemia seem to have a compound effect on insulin resistance. Miles et al. have reported that after eccentric exercise, a high carbohydrate diet can induce insulin resistance in healthy young adults [28],

We have previously shown that combined eccentric exercise and hyperglycemia-induced hyper-insulinemia are associated with alterations in carbohydrate and lipid metabolism [21] and decreased pancreatic -cell function [22], all of which are indicative of deleterious changes in metabolic flexibility with advancing age. Herein, we report the effect of aging on whole-body protein synthesis, breakdown, and oxidation following the physiological challenges of an acute bout of eccentric exercise and hyperglycemia-induced hyperinsulinemia. Protein turnover was measured using a constant $[1\text{-}^{13}C]$ leucine infusion, which is a widely used method and is considered the "gold standard" of whole body protein turnover measurement [8]. We hypothesized that in older men, age-related declines in insulin sensitivity, compounded by the metabolic stress arising from eccentric exercise would attenuate whole-body protein metabolism when compared to the response of young healthy individuals. This approach was designed to provide insight into potential mechanisms of age-related changes in protein metabolism that may contribute to a decline in health.

Methods

Subjects

16 healthy sedentary men volunteered to participate in this investigation (○ Table 1). 8 of the participants were young (22 ± 2 years; BMI 23 ± 5 kg/m ^{2}) and eight were older (66 ± 6 years; BMI 25 ± 3 kg/m ^{2}). All of the men were sedentary as assessed by a physical activity questionnaire, with similar activity levels between the groups. Further, none of the subjects were engaged in any regular exercise regimen for at least 6 months prior to the study. The men had normal glucose tolerance as assessed by a 75 g oral glucose tolerance test [3]. Body composition was assessed by underwater weighing (UWW) as previously reported [22]. By design, the groups were comprised of young and older men who were closely matched for body weight and BMI. Both groups had similar fat-free mass, but the older men had higher ($P < 0.05$) fat mass. Exclusion criteria included acute or chronic diseases, obesity, medications that would affect metabolism, and a family history of type 2 diabetes. This investigation was carried out in accordance with the principles of the Declaration of Helsinki as well as Title 45, US Code of Federal Regulations, Part 46, Protection of Human Subjects, and is in accordance with the ethical standards in sport and exercise science research [13]. The research protocol was approved by our Institutional Review Board, and written informed consent was obtained from all subjects in accordance with Institutional Guidelines for the protection of human subjects.

Study design

A summary of the study design is presented in ⊙ Fig. 1. Subjects participated in 2 separate trials that were no less than 1 week apart. For each trial, the men were housed in the General Clinical Research Center for 3 nights and 2 days, in order to control their activity and nutrient consumption. Subjects consumed a similar eucaloric diet consisting of 60 % carbohydrate, 25 % fat, and 15 % protein. On day 1 of each trial, subjects remained inactive (control), or performed a single bout of eccentric exercise. The control trial was always performed first so as to avoid any of the residual metabolic consequences of eccentric exercise, which may last several days to weeks [30]. The ^{13}C-leucine tracer infusion and hyperglycemic clamp were performed 48-h after eccentric exercise. The 48-h time point was chosen based on previous findings indicating that peak muscle soreness, functional impairment [9], and transient insulin resistance [1, 2, 7] occurs approximately two days after eccentric exercise.

Exercise

For the eccentric exercise trial, subjects performed unilateral leg extension and chest press exercises using Universal weight machines (Universal Gym Equipment, Cedar Rapids, IA) as previously described [22]. Starting at approximately 0700h on day 1, subjects completed 10 sets of 10 repetitions of each exercise with the resistance initially set at 100 % of their concentric 3-repetition maximum, which had been determined no less than 2 weeks prior to the control trial. Subjects received the weight for each repetition at full extension of their arms or legs, and were instructed to lower the weight through their full range of motion in a slow and steady manner over the span of ~3 s in order to maximize the eccentric component of each repetition.

Isotopic tracer procedure and hyperglycemic clamp

Baseline blood samples were obtained from subjects for the measurement of fasting plasma glucose and insulin levels, and background ^{13}C-leucine abundance. The 5-h ^{13}C-leucine infusion (see below) started at approximately 0700h on day 3. A primed 20 % dextrose infusion (Harvard Apparatus, South Natick, MA) was used to maintain plasma glucose concentration at 10 mM as previously described [22].

An intravenous $[1-^{13}C]$-leucine infusion was used to assess plasma leucine flux (from protein breakdown), leucine oxidation, and non-oxidative leucine disposal (leucine uptake for protein synthesis) rates. At time 0, priming doses of $NaH^{13}CO_3$ (2.35 $\mu mol \cdot kg^{-1}$) and $[1-^{13}C]$-leucine (7.56 $\mu mol \cdot kg^{-1}$) were administered followed by a 5-h continuous infusion of $[1-^{13}C]$-leucine (0.126 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) using a calibrated syringe pump (Harvard Apparatus, South Natick, MA).

Blood samples were obtained, centrifuged and the plasma stored at − 70 °C until analyzed for ^{13}C- KIC enrichment using gas chromatography mass spectrometry (GC-MS; Agilent 6890N gas chromatograph and 5973N mass selective detector; Agilent, Palo Alto, CA) [35, 42]. Briefly, plasma KIC was isolated, the trimethylsilyl derivative was formed [36] and ^{13}C enrichment was quantified using GC-electron impact ionization-MS and selected ion monitoring (m/z 232 and 233). The GC-MS instrument response was calibrated using gravimetric standards of known isotope enrichment.

Exhaled breath samples were collected into a balloon (Airship Assembly, US Alcohol Testing of America, Rancho Cucamonga, CA), transferred to a 20-mL evacuated tube (Venoject, Terumo, Elkton, MD), and analyzed for $^{13}CO_2/^{12}CO_2$ enrichment (m/z 45 and 44) using a dual inlet-gas isotope ratio mass spectrometer (Thermo-Finnigan Delta + XL, Bremen, Germany) [43]. Resting carbon dioxide production rate was measured by indirect

calorimetry using a ventilated hood system before the start of the infusion and hourly throughout the study. Breath $^{13}CO_2$ enrichment values were used in conjunction with the CO_2 production (ml/min) rates to calculate the leucine oxidation rate [25].

Calculation of leucine kinetics

Leucine kinetics were calculated as previously described by Matthews et al. [25] using plasma ^{13}C- KIC enrichment at isotopic plateau to reflect the intracellular leucine pool [26]. The 20 % dextrose infusion alters background ^{13}C abundance and can confound the ^{13}C-leucine oxidation measures [39]. To correct for this, breath $^{13}CO_2$ enrichments were adjusted using a previously established correction factor [0.00039 mole percent excess (MPE) $\times$ the subject's mean glucose disposal rate in mg glucose $\cdot$ kgFFM^{-1} $\cdot$ min^{-1} for a given clamp]. The 0.00039 MPE value was estimated from breath $^{13}CO_2$ enrichment and mean glucose disposal rate data reported by Toth et al. [39].

Statistics

Statistical analysis was carried out using StatView Version 5.0.1 (SAS Institute, Cary, NC). Primary dependent variables were analyzed by repeated-measures ANOVA with the main effects of group (young vs. older), trial (control vs. eccentric exercise), and condition (basal vs. clamp) in combination with Bonferroni post hoc analysis. Within a given age group, comparisons between trials and between conditions were performed using repeated-measures ANOVA, with follow-up analyses by Fisher's PLSD post hoc test. Pearson product-moment correlations were used to explore univariate relationships between selected outcomes. Statistical significance was set at $P < 0.05$. All values are expressed as mean $\pm$ SE.

Results

Eccentric resistance exercise

The young men had greater lower body strength as evidenced by their ability to lower significantly more weight during the leg exercises (○ Table 1, $P < 0.05$). Based on chest press performance, upper body strength was not different between the 2 groups. As previously reported [22], creatine kinase was significantly elevated above baseline for both the young and older men 48 h after exercise, and upper and lower body muscle soreness was significantly elevated 24 and 36 h after exercise. There were no age-related differences in creatine kinase or muscle soreness.

Hyperglycemic infusion

Eccentric exercise did not change the amount of glucose required to maintain hyperglycemia in either the young (10.2 ± 0.8 vs. 9.6 ± 0.5 ml $\cdot$ kg FFM^{-1} $\cdot$ min^{-1}, control vs. eccentric exercise, respectively) or older (6.4 ± 0.6 vs. 6.2 ± 0.6 ml $\cdot$ kg FFM^{-1} $\cdot$ min^{-1}, control vs. eccentric exercise, respectively) groups. However, the glucose infusion rate that was required to maintain hyperglycemia was significantly lower ($P < 0.05$) in the older men during both trials, indicating impaired insulin sensitivity. Basal plasma glucose and glucose levels during the hyperglycemic clamp were not different between the 2 groups. In response to the hyperglycemic clamp, insulin concentrations increased ($P < 0.05$) after the control [238 ± 28 vs. 266 ± 49 pM (10–180 min), for young vs. old, respectively], and eccentric exercise [254 ± 37 vs. 245 ± 38 pM (10–180 min) for young vs. old, respectively] conditions. Insulin concentrations were also not different between the groups during basal or clamp conditions. However, as previously reported, eccentric exercise caused a compensatory increase in glucose-stimulated insulin secretion from pancreatic -cells in the younger group, whereas the older group could not mount a similar response [22].

Leucine kinetics

Basal nonoxidative leucine disposal, a measure of protein synthesis, and basal leucine flux, which is representative of protein breakdown were not different between the groups during the control or the eccentric exercise trial, and there was no age-related effect after eccentric exercise (○ Table 2). However, synthesis and breakdown declined ($P < 0.05$) during the hyperglycemic clamp in both groups during the control, and eccentric exercise trial. Compared to control, eccentric exercise did not alter sythesis or breakdown in either group.

In young men, hyperglycemia alone suppressed leucine oxidation ($P < 0.05$). Eccentric exercise further decreased leucine oxidation ($P < 0.05$), which declined further during hyperglycemia after exercise ($P < 0.05$) (○ Fig. 2). In the young men, a significant main effect of trial ($P < 0.05$) and condition ($P < 0.05$) in the absence of an interaction indicates that the effects of hyperglycemia and eccentric exercise were independent and additive. In contrast to the younger group (age-by-condition interaction; $P < 0.05$), the older men did not suppress leucine oxidation in response to hyperglycemia during the control trial, or the eccentric exercise trial (○ Fig. 2). Furthermore, there was a trend for higher leucine oxidation ($P < 0.1$) in the older compared to the young group during hyperglycemia. When data from the 2 groups were combined, correlation analyses revealed an inverse relationship between clamp glucose infusion rate and clamp leucine oxidation after eccentric exercise ($r = -0.53$, $P < 0.05$; ○ Fig. 3a). In addition, body fat (%) was positively correlated with leucine oxidation during the eccentric exercise clamp ($r = 0.51$, $P < 0.05$; ○ Fig. 3b).

Discussion

In this study, we show for the first time that healthy, young men suppress leucine oxidation during hyperglycemia alone, after eccentric exercise alone, and show an even greater suppression (additive) when hyperglycemia and eccentric exercise are combined. In contrast, healthy, normal-weight, older men with normal glucose tolerance failed to suppress leucine oxidation during the separate and combined challenges of hyperglycemia and eccentric exercise.

It was anticipated that impaired insulin sensitivity between the older men, as determined during the hyperglycemic clamp, would translate into alterations in protein synthesis and breakdown. However, age did not effect whole body changes in protein synthesis or breakdown, indicating insulin-stimulated amino acid uptake was not altered with age. In support of this observation, Katsanos et al. [15] found no effect of insulin sensitivity on skeletal muscle amino acid uptake.

In young men, the hyperglycemia-driven decrease in leucine oxidation coincides with previously reported decreases in tumor necrosis factor- (TNF-) secretion from mononuclear cells [18], a potent inducer of insulin resistance [14]. Alternatively, the failure to decrease leucine oxidation in the older group is consistent with an absence of TNF- suppression [18], and an impaired ability to clear glucose [20] during hyperglycemia. The addition of eccentric exercise further exacerbated age-related differences in the oxidation of leucine during hyperglycemia. Eccentric exercise causes transient insulin resistance that is not just localized to muscle, but induces whole-body insulin resistance [1, 17], that is likely due to a global pro-inflammatory response [7, 23, 28]. The young men in our study suppressed leucine oxidation after eccentric exercise, indicating either compensation or recovery from a transient exercise-induced inflammatory response. However, the older men did not decrease leucine oxidation, which coincides with impaired pancreatic -cell function [22], and decreased carbohydrate oxidation [21], both indicators of inflammatory perturbations. This disparity indicates a decline in the efficient use of amino acids, which could account for age-related decreases in tissue function and repair.

The underlying cause of age-related changes in protein metabolism is likely driven by the "normal" accumulation of adipose tissue, particularly abdominal fat, with age [5, 19]. Increased adiposity is linked to a myriad of metabolic abnormalities, including insulin resistance (reviewed in [27]). Our data support this relationship considering that the higher body fat among our older men was significantly correlated with the insulin sensitivity and leucine oxidation during hyperglycemia after eccentric exercise. It may be speculated that with advanced age, the increased adiposity leads to a higher prevailing level of chronic low-grade inflammation, which has been shown in muscle [6]. This subsequently shifts metabolism towards continued use of amino acids for fuel, rather than protein synthesis in response to stressors like eccentric exercise and hyperglycemia.

Our data show a definite impact of age on leucine metabolism, but the contribution of discrete organ systems or tissues underlying these changes require further investigation, given the use of whole-body protein measurements. For example, clear disconnects between whole body protein turnover and muscle turnover have been demonstrated previously [33, 40, 41]. Thus, alterations in leucine metabolism in muscle or other metabolically active tissues may not be reflected in whole-body measurements. Other limitations include the measurement of only leucine kinetics, which may not be reflective of all amino acids.

In conclusion, we found leucine oxidation was not changed in response to physiologic challenges in older men, while it was suppressed in young men. This implies a lower efficiency of amino acid utilization in older men, which may contribute to impaired maintenance and repair of body tissues. These data extend our knowledge about metabolic alterations that accompany healthy aging, especially alterations in amino acid oxidation that are only revealed under conditions of physiological stress.

Acknowledgments

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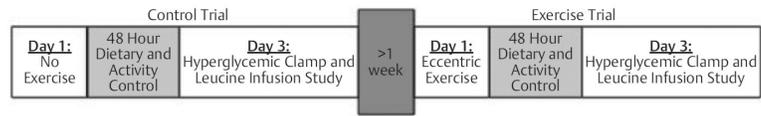
Fig. 1.
Study timeline. Trials were completed no less than 1 week apart, with the control trial preceding the eccentric exercise trial.
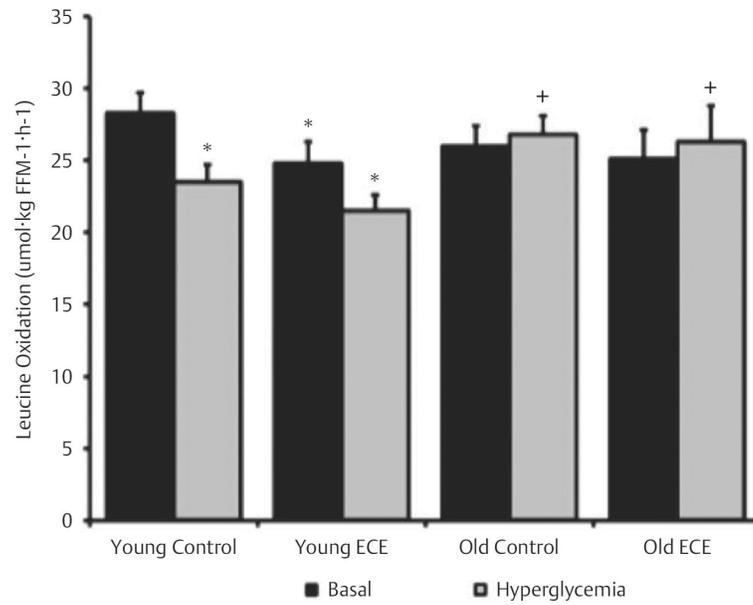
Fig. 2.
Comparisons of leucine oxidation (μmol $\cdot$ kg FFM^{-1} $\cdot$ h^{-1}) between young and old, before and during a hyperglycemic clamp, and for control eccentric resistance exercise. *Decrease ($p < 0.05$) from control basal condition. + an age-related difference ($p < 0.05$) in response to hyperglycemia.
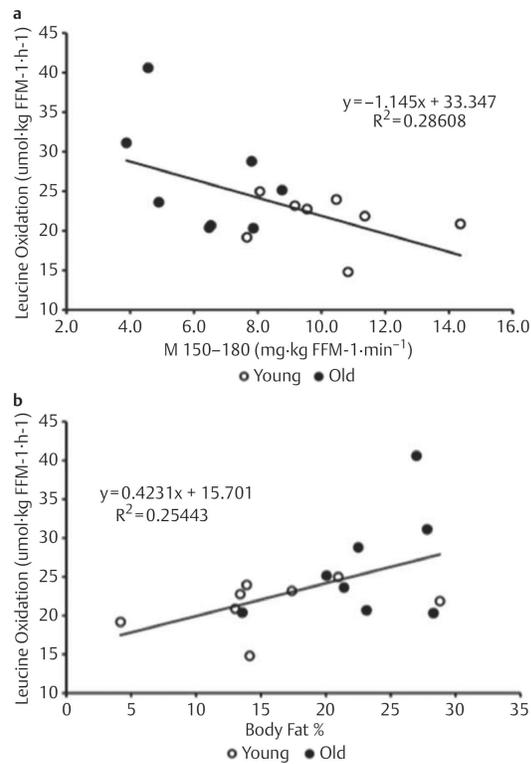
Fig. 3.
a Correlation between clamp leucine oxidation (μmol · kg FFM^{-1} · h^{-1}) after eccentric exercise and insulin sensitivity as represented by M 150–180 (mg · kg FFM^{-1} · min^{-1}) ($r = -0.53$, $P < 0.05$). **b** Correlation between clamp leucine oxidation (μmol · kg FFM^{-1} · h^{-1}) after eccentric exercise and percentage body fat ($r = 0.51$, $P < 0.05$).

Table 1

Subject characteristics.

	Young (n = 8)	Old (n = 8)
age (yr)	22 ± 1	66 ± 2
height (cm)	181.0 ± 1.0	172.1 ± 2.1[*]
weight (kg)	76.1 ± 5.5	75.7 ± 4.2
BMI (kg/m^2)	23.4 ± 1.9	25.5 ± 1.2
UWW %Fat	15.7 ± 2.5	23.0 ± 1.7[*]
FFM (kg)	63.9 ± 3.0	58.5 ± 2.9
fat mass (kg)	12.2 ± 3.0	17.2 ± 1.7[*]
3RM R Leg (kg)	33.6 ± 2.3	24.1 ± 2.7[*]
3RM L Leg (kg)	32.3 ± 2.3	24.1 ± 2.3[*]
3RM chest (kg)	50.0 ± 7.7	35.9 ± 4.5
FPG (mg/dl)	91 ± 1	94 ± 2
FPI (pM)	52 ± 2	57 ± 4

Values are means ± SE. BMI, body mass index; UWW, body composition as determined by hydrostatic weighing; FFM, fat free mass; FPG, fasting plasma glucose; FPI, fasting plasma insulin.

[*] Significantly different between young and old, $p < 0.05$

Table 2

Leucine whole body breakdown and synthesis kinetics (μmol $\cdot$ kg FFM^{-1} $\cdot$ h^{-1}) of young and old subjects before and during hyperglycemia, and before and after eccentric exercise.

		BREAKDOWN		SYNTHESIS	
		Basal	**Hyperglycemia**	**Basal**	**Hyperglycemia**
control	*Young*	152.2 ± 1.8	126.8 ± 1.0[*]	124.0 ± 1.4	103.3 ± 1.2[*]
	Old	144.5 ± 1.7	123.4 ± 0.9[*]	118.4 ± 1.5	96.6 ± 0.7[*]
eccentric exercise	*Young*	146.9 ± 2.1	123.9 ± 2.0[*]	122.2 ± 1.9	102.4 ± 1.9[*]
	Old	141.6 ± 1.0	126.9 ± 1.1[*]	116.5 ± 1.2	100.5 ± 1.1[*]

Values are mean ± SE.

[*] Significant difference ($p < 0.05$) from basal conditions