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Review

Effects of aging and insulin resistant states on protein anabolic responses in older adults

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ABSTRACT

Insulin is the principal postprandial anabolic hormone and resistance to its action could contribute to sarcopenia. We developed different types of hyperinsulinemic clamp protocols to measure simultaneously glucose and protein metabolism in insulin resistant states (older adults, obesity, diabetes, etc.). To define effects of healthy aging in response to insulin, we employed the hyperinsulinemic, euglycemic and isoaminoacidemic (HYPER-1) clamp. The net whole-body anabolic (protein balance) response to hyperinsulinemia was lower in the elderly vs young ($p = 0.007$) and was highly correlated with the clamp glucose rate of disposal ($r = 0.671$, $p < 0.001$), indicating insulin resistance of protein metabolism concurrent with that of glucose. Differences in insulin resistance due to aging were observed predominantly in men, with older ones exhibiting significant lower anabolism compared with young ones. As most of the anabolism occurs during feeding, we studied the fed-state metabolic responses with aging using the hyperinsulinemic, hyperglycemic and hyperaminoacidemic clamp, including muscle biopsies. Older women showed comparable whole-body protein anabolic responses and stimulation of mixed-muscle protein synthesis by feeding to the young. The responses of skeletal muscle insulin signaling through the Akt-mTORC1 pathway were also unaltered, and therefore consistent with muscle protein synthesis results. Given that type 2 diabetes infers insulin resistance of protein metabolism with aging, we studied 10 healthy, 8 obese, and 8 obese type 2 diabetic elderly women using the HYPER-1 clamp. When compared to the group of young lean women to define the effects of obesity and diabetes with aging, whole-body change in net protein balance with hyperinsulinemia was similarly blunted in obese and diabetic older women. However, only elderly obese women with diabetes had lower net balance than lean older women. We conclude that with usual aging, the blunted whole-body anabolic response in elderly subjects is mediated by the failure of insulin to stimulate protein synthesis to the same extent as in the young, especially in men. This blunted response can be overcome at the whole-body and muscle levels during an intravenous fed state supplying a generous amount of protein, in active healthy elderly women. Obese elderly women with and without type 2 diabetes have insulin resistance of protein anabolism at the whole-body level, but this resistance is worsened with diabetes when glucose metabolism is further impaired. More investigation is needed to determine the exact role of insulin in promoting anabolism with aging. The findings from our group are relevant for the field of sarcopenia research as they provide a rationale to offer low cost nutritional interventions for overcoming this detrimental condition associated with aging and diabetes.

1. Introduction

It is well recognised that insulin resistance of glucose metabolism, the primary mechanism underlying the development of type 2 diabetes mellitus, increases with aging (Ferrannini et al., 1996). Not surprisingly, the incidence of type 2 diabetes increases steeply after the age of 50 reaching its zenith between age 75–80 years, at which age about 25% of older adults meet diagnostic criteria of type 2 diabetes (Kirkman et al., 2012). Many factors contribute to increased insulin resistance with aging, including gain in adipose tissue, decreased physical activity, changes in hormonal milieu, inflammation, excess energy intake and several diseases and their treatments (Lee and Halter, 2017). Of interest is that when several of these factors were considered, age itself was found to play only a minor role in the insulin resistance of aging (Ferrannini et al., 1996). Although less well documented, insulin resistance of glucose also appears to extend to protein metabolism in reducing protein anabolism which could contribute to the loss of lean tissue, specifically of muscle mass, a condition called sarcopenia when

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strength is also affected (Cruz-Jentoft et al., 2010). The latter contributes to functional limitations and loss of autonomy of older adults (Cruz-Jentoft et al., 2010). The coexistence of obesity and sarcopenia imposes greater limitations on the physical performance of older adults compared with sarcopenia alone (Rolland et al., 2009) though recent analysis from the Framingham cohort showed independent but no synergistic effect (Dufour et al., 2013). Of note, a 6-year longitudinal study of body composition in older adults disclosed that individuals with type 2 diabetes, particularly women and individuals not under diabetes treatment, lost more muscle mass than non-diabetic individuals (Park et al., 2009).

Body proteins are in a continuous turnover, or flux, with new proteins being synthetized and others being degraded or broken down. Rates of protein flux, oxidation, synthesis and breakdown can be assessed with isotopically-labeled amino acids and are referred to as rates of protein kinetics. Lean body mass, including muscle tissue depends on the overall balance between rates of muscle protein synthesis and breakdown. If protein synthesis is greater than breakdown, anabolism (accretion) takes place, whereas the reverse would lead to catabolism (losses). The main daily regulators of anabolism are amino acids derived from feeding, circulating insulin and physical activity. Under the effects of feeding, protein anabolism ensues and losses occurred during overnight fasting are substituted to maintain body integrity with corresponding changes in rates of protein kinetics reflecting these events (Volpi et al., 1999). Situations of net catabolism include malnutrition, inflammatory conditions mediated through cytokines and inactivity, and especially bed rest (English and Paddon-Jones, 2010), the latter due to induction of insulin resistance (Dirks et al., 2016).

2. Brief review of the translation control of protein metabolism

The intracellular pathways regulating protein kinetics are now better understood though not fully characterized. Briefly, the stimulation of protein synthesis results from activation of the insulin signaling and amino acid sensing pathway serine-threonine kinase B and mammalian target of rapamycin complex 1 (Akt, mTORC1) through phosphorylation of key proteins. Binding of insulin causes its receptor to auto phosphorylate, leading to recruitment of the insulin receptor substrate 1 (IRS1), activation of PI3K, and phosphorylation-induced activation of Akt by PDK1. Akt interacts with mTORC1 containing Raptor and proline-rich Akt substrate of 40 kDa (PRAS-40). mTOR signaling is also regulated by intracellular AA levels, particularly leucine (Hara et al., 1998). Raptor serves to recruit substrate to mTOR whereas PRAS-40 inhibits mTORC1 activity by preventing substrate binding (Wang et al., 2007). Akt relieves the inhibition of PRAS-40. mTOR directly phosphorylates the eukaryotic initiation factor 4E-BP-1 (eIF4E-binding protein-1), which is an inhibitor of the eIF4E, to relieve translational suppression. eIF4E interacts with the mRNA 5′cap structure to facilitate the recruitment of ribosomes and promote translation. mTOR also directly phosphorylates ribosomal protein S6 kinase (p70S6K) which acts upon rpS6, enabling the last step of the assembly of the 48S preinitiation complex, its binding to the 60S subunit, and the start of elongation process.

The ubiquitin–proteasome pathway responsible for the bulk of myofibrillar protein degradation is partly regulated by the forkhead box O 3a (FoxO3a) transcription factor (De Bandt, 2016). Upon Akt phosphorylation by insulin signaling, the phosphorylation of FoxO3a prevents its entry in the nucleus and thus the induction of ubiquitin ligase expression, which are involved in proteolysis (Stitt et al., 2004), may be a potential mechanism explaining insulin's inhibition of protein breakdown.

3. The hyperinsulinemic clamp

Insulin being the principal postprandial anabolic hormone, its deficiency or resistance to its action would predispose to a lesser anabolism and therefore contribute to protein losses and sarcopenia over time. Consequently, protein anabolism, like glucose uptake, is dependent on optimal insulin action which is maximal during feeding. Assessing the insulin sensitivity of protein metabolism in concert with that of glucose requires maintenance of circulating amino acids during a hyperinsulinemic, euglycemic clamp, the gold standard to measure insulin resistance of glucose. During a clamp, many amino acids, notably the branched-chain amino acids (BCAA), decline markedly due to the suppression of protein degradation. This limited amino acid availability as substrates for synthesis therefore masks the true anabolic effect of insulin. To overcome this limitation and test for insulin resistant states (older adults, obesity, diabetes, etc.), for glucose as well as protein metabolism, we developed different types of hyperinsulinemic clamp experiments consisting of 3 h of postabsorptive state followed by 3.5 h of hyperinsulinemia with varying glucose and amino acid target levels and their corresponding infusion rates. Anabolism is defined as the change in net balance (protein synthesis - breakdown) occurring from the end of the postabsorptive to the end of the clamp states. Depending on the hypothesis under testing, we can evaluate the effects of insulin alone using the hyperinsulinemic, euglycemic and isoaminoacidemic clamp (glycemia maintained at 5.5 mmol/L and amino acids at postabsorptive level; HYPER-1) or those of hyperglycemia using the hyperinsulinemic, hyperglycemic and isoaminoacidemic clamp (glycemia at 8 mmol/L; HYPER-2) or in conjunction with hyperaminoacidemia thus mimicking a meal situation (aminoacidemia at 2–3-fold postabsorptive levels; HYPER-3). In the postabsorptive period and for the duration of the clamp, rates of glucose kinetics are assessed with primed continuous infusions of D-[3-^{3}H]glucose and protein kinetics with labeled amino acid infusions, i.e., dilution of [1-^{13}C]-leucine for whole-body and incorporation of L-[ring-$^{2}H_5$]phenylalanine for muscle protein synthesis, measured as fractional synthesis rate (FSR). Hyperinsulinemia is achieved with an insulin infusion of 40 mU/m^2/min. The target glycemia is maintained with infusion of 20% (w/v) potato starch-derived glucose (Avebe b.a., Foxhol, The Netherlands) while that of aminoacidemia with maintenance of individual plasma amino acids by an infusion of 10% TrophAmine™ (B. Braun Medical Inc., Irvine, CA). Stable aminoacidemia is made possible with a rapid BCAA enzymatic fluorometric assay with frequent feedback adjustment of the amino acid infusate (Chevalier et al., 2004).

Blood and expired air samples are collected at predetermined times and indirect calorimetry is performed prior to and before ending the insulin infusion. At similar time periods, glucose kinetics and substrate oxidation are calculated from the dilution of D-[3-^{3}H] glucose specific activity using the “hot GINF” method (Finegood et al., 1987). Leucine kinetics are calculated per the reciprocal model (Matthews et al., 1980). Depending on the study design, prior to and 2 h after start of the insulin, ~100 mg vastus lateralis muscle biopsies are obtained with a Bergstrom needle under sterile conditions and local anesthesia. Mixed-muscle FSR are determined as the rate of increase in protein-bound phenylalanine enrichment measured by gas chromatography–mass spectrometry over that of the intracellular free pool (Mackenzie et al., 2003). Immunoblotting of muscle samples are performed for signaling proteins (Adegoke et al., 2009).

4. Participants and other procedures

Young and older subjects were recruited through advertisements. Older adults were community-dwelling and independent in performing instrumental daily activities. All subjects were screened by medical history, physical examination and laboratory investigation. Screening included an oral glucose tolerance test (OGTT) for elderly participants. All participants were required to be non-smokers and over 65 years of age for older adults. Healthy subjects required a BMI between 18.5 and 27.0 kg/m^2, and obese subjects with and without type 2 diabetes, a BMI ≥ 30 kg/m^2. Exclusion criteria were unstable weight for the preceding 6 months, abnormal dietary habits, diabetes complications,