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Lower Muscle Protein Synthesis in Humans with Obesity Concurrent with Lower Expression of Muscle IGF-1 Splice Variants

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Abstract

Objective: We tested the hypothesis that expression of IGF-1 protein and mRNA splice variants is lower in skeletal muscle of humans with obesity that have lower mixed-muscle protein fractional synthesis rate (MMP-FSR) when compared to humans without obesity (i.e, controls).

Methods: We studied 9 subjects with obesity (OB, BMI = 35 ± 3 kg/m^2, MMP-FSR = 0.06 ± 0.02 %/h) and 9 subjects without obesity (W-OB, BMI = 24 ± 3 kg/m^2, MMP-FSR = 0.08 ± 0.02 %/h) (for both BMI and MMP-FSR p<0.05). MMP-FSR and mitochondrial protein FSR (MITOP-FSR) were measured following an overnight fast.

Results: Along with lower MMP-FSR, OB subjects displayed lower MITOP-FSR (p=0.03) compared to W-OB subjects. Expression of IGF-1 (p = 0.04) and IGF-1R (p<0.01) proteins were lower in muscle of OB subjects. OB subjects had lower (p<0.05) mRNA expression of the *IGF1* variants Eb and Ec. We show that lower protein synthesis in muscle of humans with obesity occurs concurrently with lower expression of muscle IGF-1 and IGF-1R proteins, as well as lower mRNA expressions of the *IGF1* splice variants.

Conclusions: Our findings indicate that lower protein synthesis observed in muscle of humans with obesity may result from diminished muscle *IGF1* gene expression.

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Keywords

protein metabolism; obesity; muscle IGF-1; IGF-1 splice variants; mitochondrial protein synthesis

INTRODUCTION

Obesity is a growing health concern worldwide. Recent estimates indicate that more than 40% of adults in the United States alone are obese (1). In addition to its profound health implications in increasing risk for Type 2 diabetes, heart disease, and several types of cancer, obesity has negative effects on overall skeletal muscle physiology, such as reducing muscle quality (2) and disrupting the cellular proteome (3). Previous studies from our laboratory (4–6), as well as others, have found lower protein synthesis in skeletal muscle of humans with obesity (4, 5, 7–10). However, the physiological mechanisms underlying lower protein synthesis in skeletal muscle of humans with obesity remain unknown.

Available evidence shows that lower protein synthesis in skeletal muscle of humans with obesity is observed in the presence of lower concentrations of the plasma insulin-like growth factor 1 (IGF-1) (5). IGF-1 is essential for proper maintenance of skeletal muscle protein metabolism and function (11, 12) and it is a potent stimulus for protein synthesis (11, 13). It can exert its effects on skeletal muscle as both an endocrine (i.e., circulating) and autocrine/paracrine (i.e., within muscle) factor. Although both circulating and muscle IGF-1 may stimulate protein synthesis in muscle, muscle IGF-1 may be a more potent stimulus for protein synthesis due to its proximity to muscle cells. In this regard, it is critical to understand potential impairments in *IGF1* gene expression intrinsic to muscle as they may explain lower protein synthesis observed in muscle of humans with obesity.

Maintaining adequate levels of skeletal muscle IGF-1, and thus IGF-1 signal within muscle, occurs through continuous transcription and translation of the *IGF1* gene in muscle. Following transcription, alternative splicing of the *IGF1* gene involves a process that yields three different variants of the gene, which in humans are *IGF1* Ea, IGF-1 Eb, and IGF-1 Ec (14). Each variant encodes for a pro-IGF-1 protein differing from the rest by a short peptide sequence of amino acids located at the carboxylic end of the newly formed protein. These short sequences, known as E peptides, are named according to the *IGF1* mRNA variant from which they are translated (i.e., Ea, Eb, or Ec). After translation of the mRNA *IGF1* variant (i.e., *IGF1* + E peptide), the E peptide is cleaved and mature IGF-1 is generated (14, 15). Although most studies have focused on the physiological implications of mature IGF-1 for skeletal muscle protein synthesis (16, 17), current evidence shows that the pro-IGF-1 isoforms, as well as individual E peptides have important roles in muscle protein metabolism. For instance, Barton et al. (18) demonstrated that IGF-1 Ea and Eb variants exert permissive-like effects on inducing muscle hypertrophy in mouse skeletal muscle. More specifically, the degree of muscle hypertrophy was greater in the presence of IGF-1 Ea and IGF-1 Eb than in the presence of mature IGF-1 protein alone. On the other hand, Fornaro et al. (19)reported that the mouse IGF-1 Eb isoform (Ec in humans) was not capable of inducing proliferation or differentiation in human myoblast cell lines. The role that the

expressions of the different IGF-1 E variants play in regulating growth in skeletal muscle of humans remain unknown.

We hypothesized that lower protein synthesis in skeletal muscle of humans with obesity occurs concurrently with lower gene expression of IGF-1 in skeletal muscle. Hence, we determined IGF-1 protein expression and mRNA expression of *IGF1* variants in muscle of humans with obesity that, when compared to humans without obesity, are characterized by lower protein synthesis in skeletal muscle.

METHODS

Experimental Design

We studied humans with obesity (OB) that when compared to a group of humans without obesity (W-OB) had a lower rate of synthesis of mixed-muscle protein (i.e., overall muscle protein). Lower IGF-1 gene expression in skeletal muscle of humans with obesity characterized by lower protein synthesis in muscle can implicate a causal role of this anabolic factor in sustaining lower protein synthesis in muscle of humans with obesity.

Participants and Screening Procedures

All procedures involved in this study were approved by the Institutional Review Board at the Mayo Clinic prior to any participant's enrollment in the study. Before attending their first in-person visit, volunteers were screened over the phone to ensure they met initial criteria for inclusion in the study. Interested participants were excluded if they reported liver, renal or heart disease, history of gastro-intestinal surgery, participation in a weight loss regimen, use of any nutritional supplements, being vegan or vegetarian, and if they were engaged in regular physical activity more than two days a week. Moreover, all subjects included in the study were determined to be physically inactive, based on a series of questions that included the type of physical activity, the number of times/days per week performed, the duration per session, the level of effort/intensity, and the number of months/years the subject has been engaging in the physical activity. Therefore, subjects included in the study were physically inactive based on current consensus classifying individuals that do not meeting current physical activity recommendations as physically inactive (20, 21)

Participants arrived at the Clinical Studies Infusion Unit at Mayo Clinic in Scottsdale, Arizona, between 7:00 AM and 8:00 AM, for further screening, and following an overnight fast of at least 10 hours. After providing them with details about the study procedures, participants read and signed a written consent form. Then, participants underwent screening, which included medical history, physical examination, electrocardiogram, and standard blood and urine tests. Participants underwent an oral glucose tolerance test (OGTT), which was administered to estimate insulin sensitivity, and by calculating the Matsuda Insulin Sensitivity Index (22). Calculation of the Mastuda ISI is based on the responses of plasma glucose and insulin concentrations during the OGTT, with higher ISI values indicating greater whole-body insulin sensitivity (22).

The body composition was measured using bioelectrical impedance analysis (BIA) (BIA 310e, Biodynamics Corp., Shoreline, Washington). Maximal oxygen uptake was evaluated

using an incremental cycle ergometer test (20 W/min) to volitional exhaustion and by continuously monitoring expired gases using a metabolic cart (MedGraphics Metabolic Cart, Saint Paul, Minnesota).

Stable Isotope Infusions and Muscle Biopsy Procedures

Participants were instructed to not change their diet and to abstain from exercise and alcohol consumption for a minimum of three days before their study visit. Procedures were carried out at the Clinical Studies Infusion Unit at Mayo Clinic and started at ~7:30 AM after subjects verbally confirmed they had completed a 10-h overnight fast and complied with the dietary and exercise instructions. One catheter was placed into an antecubital arm vein for infusion and another in a retrograde fashion into a dorsal hand vein for collection of blood samples, performed using the heated-hand technique. D10-leucine (L-[2,3,3,4,5,5,5,6,6,6-$^{2}H_{10}$]leucine) was continuously infused for 300 min at a rate of 0.15 μmol/kg fat-free mass (FFM)/min (priming dose, 6.4 μmol/kg FFM) to determine the synthesis rates of muscle proteins. Muscle biopsy samples were collected from the vastus lateralis muscle at 120 and 300 min after the start of the d10-leucine infusion. Tissue for isolating muscle mitochondria was processed immediately. The remaining muscle tissue was stored in liquid nitrogen after the removal of any visible fat and connective tissue and blotting the sample dry. Blood samples were collected for determinations of d9-leucine enrichment and various biochemical parameters, including plasma IGF-1, glucose, insulin, and total, essential, and branched-chain amino acids.

Stable isotope enrichment determination

Intravenous infusion of d10-leucine results endogenously in the irreversible formation of d9-leucine (23), which constitutes the stable isotope amino acid tracer we used here to measure synthesis rates of muscle proteins, and similar to what we have previously reported (24). Measurements of d9-leucine enrichment in blood and in mixed-muscle and mitochondrial proteins were performed following standard procedures in our laboratory (24). In summary, proteins were precipitated in 1 mL of blood mixed with 15% sulfosalicylic acid (SSA), while ~15 mg of tissue from each muscle biopsy was homogenized in SSA to precipitate muscle proteins for the measurement of mixed-muscle protein synthesis. Precipitated proteins in muscle samples were hydrolyzed using 6 N HCl. Amino acids from the blood SSA-supernatant and those from the muscle HCl hydrolysate were purified by passing them through cation-exchange columns. D9-leucine enrichment was measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS) (24).

The enrichment of muscle mitochondrial proteins with d9-leucine was determined in muscle mitochondrial protein isolates. Mitochondria in the muscle biopsy were isolated from ~80 mg of fresh tissue using differential centrifugation, and according to procedures we have described previously (25). Specifically, after removing blood, fat and visible connective tissues, the muscle was first minced with scissors in ice-cold solution containing (mM), 100 KCl, 40 Tris-HCl, 10 Tris-Base, 5 MgCl2, 1 EDTA, and 1 ATP (pH 7.5), followed by homogenization of the tissue in a glass-to-glass Potter-Elvehjem homogenizer. The homogenate was then centrifuged (800 x g), and the collected supernatant was further centrifuged at 14,000 x g. The collected pellet containing mitochondria was resuspended

in a medium containing (mM) 100 KCl, 40 Tris-HCl, 10 Tris-Base, 5 MgCl2, 1 EDTA, 0.2 mM ATP (pH 7.5), and centrifuged at 7000 x g. This mitochondrial pellet was then resuspended in the same medium and centrifugated at 4000 x g. A final mitochondrial pellet was retrieved and resuspended in mannitol-sucrose buffer (mM) (220 Mannitol, 70 Sucrose, 10 Tris-HCl, 1 EGTA (pH 7.4), and was used for the determination of mitochondrial protein synthesis. Similarly, to the procedures used for mixed-muscle proteins, mitochondrial protein isolates were hydrolyzed with 6 N HCl and processed for d9-leucine enrichment by LC-MS/MS. Protein synthesis in mixed-muscle and mitochondrial proteins was determined as the fractional synthesis rate (FSR) of protein using the precursor-product approach, and following procedures we have previously reported (24).

Immunosorbent assays

For the determination of muscle IGF-1 protein expression, muscle biopsy samples weighing ~25 mg were homogenized in RIPA buffer (Abcam, cat. no. ab288006) using 20 μL of buffer per 1 mg of tissue. Before homogenization, 10 μL per 20 mg of tissue of a protease inhibitor cocktail (Sigma-Aldrich, cat. no. P8340) were added to the RIPA buffer. After homogenizing the muscle, the samples were allowed to rest for about 20 min on ice and then centrifuged for 20 min at 4 °C. The supernatant was collected and transferred to a new tube, and protein concentration in these samples was measured using the DC protein assay kit (Bio-Rad, cat. no. 5000112). Expressions of IGF-1 and IGF-1 receptor (IGF-1R) proteins in the supernatant were measured using commercially available ELISA assays (Abcam, cat. no. ab108873 and ab275102, respectively). We evaluated total IGF-1 protein content rather than free IGF-1 protein content in muscle because the mRNA expression of *IGF-1*, which is a main end-point of our investigation, is biologically related directly to the total rather than free IGF-1 in muscle. Therefore, and because IGF-1 found in muscle is bound to carrier proteins, we employed a pretreatment step of the samples before assaying for IGF-1 protein in order to disassociate IGF-1 from its carrier proteins within the samples, and by following the manufacturer's instructions. The content of measured proteins in the muscle was normalized to the total muscle protein content. Circulating plasma IGF-1 concentrations were measured using an ELISA procedure (ALPCO Diagnostics, cat. no. 22-IGFHU-E01) following the manufacturer's instructions.

Quantification of *IGF1* mRNA expression

Approximately 30 mg of skeletal muscle was homogenized in QIAzol lysis reagent (QIAGEN, cat. no. 79306) and total RNA was extracted using a commercially available total RNA extraction kit (QIAGEN, cat. no. 217084). Reverse transcription was carried out using a thermal cycler (ProFlex™ 3 × 32-well PCR System, Thermo Fisher Scientific Inc., cat. no. 4484073) to synthesize complementary DNA using SuperScript IV VILO master mix (Thermo Fisher Scientific Inc., cat. no. 11766500), which included a step to eliminate genomic DNA using ezDNase enzyme (Thermorfisher, cat. no. 11766051). The mRNA expression of the following *IGF1* variants was quantified in muscle using predesigned TaqMan assays (Thermo Fisher Scientific Inc., Waltham, MA): IGF-1 Ea (Hs01547657_m1), IGF-1 Eb (Hs01555481_m1), and IGF-1 Ec (Hs03986524_m1). Overall muscle IGF-1 mRNA expression was measured using the assay Hs01547656_m1. Real-time PCR measurements were performed in the QuantStudio™ 6 Flex Real-Time PCR System

(Thermo Fisher Scientific Inc., cat. no. 4485691) using TaqMan™ Fast Advanced Master Mix (Thermo Fisher Scientific Inc., cat. no. 4444556). Measured mRNA values for the target genes were normalized to 18S rRNA (assay: Hs99999901_s1). Table 1 provides details for each TaqMan assay used and according to reporting standards established in the field (26, 27). The results were analyzed using the comparative method, and differences between groups were determined by comparing the $2^{-\Delta\Delta Ct}$ values (28).

Other biochemical measurements

The plasma concentrations of total, essential, and branched-chain amino acids were measured using high-performance liquid chromatography (29). Plasma glucose concentrations were determined using an automated glucose analyzer (YSI2300, Yellow Springs, Ohio).

Statistical analysis

Data normality was determined using the Levene's test and supplemented by graphical information. Independent *t*-tests were used to compare differences between humans with obesity and humans without obesity. Cohen's *d* was calculated to determine effect size for each comparison. Pearson's product-moment correlation coefficient (*r*) was calculated to measure the strength of association between variables of interest. $2^{-\Delta\Delta Ct}$ values displayed a nonnormal distribution and were thus log transformed prior to statistical analyses. Alpha level was set at $p \leq 0.05$ and all data are presented as mean ± SD. All statistical analyses were carried out using R Studio.

RESULTS

The descriptive characteristics of the participants are presented in Table 2. The group of humans without obesity displayed greater insulin sensitive than the group of humans with obesity based on their calculated Matsuda ISI value. By design, the OB group had significantly lower mixed-muscle protein FSR than the W-OB group ($p = 0.042$, $d = 1.10$). The group with obesity also had significantly lower mitochondrial protein FSR ($p = 0.026$, $d = 1.22$) (Figure 1). Figure 2 shows the plasma d_9-leucine enrichment in the two groups over the course of the infusion study. There were no significant ($p > 0.05$) main effects for plasma d9-leucine enrichment for either group or time for the plasma d9-leucine enrichment.

Muscle IGF-1 protein expression was significantly lower in the OB group compared with the W-OB group ($d = 1.15$, Figure 3A). Additionally, muscle IGF-1 receptor protein expression was significantly lower in the OB group than in the W-OB group ($d = 1.88$, Figure 3B). There were no significant differences in the mRNA expressions of overall IGF-1 ($d = 0.74$, Figure 4A) or IGF-1 Ea ($d = 0.76$, Figure 4B) between the groups. However, subjects with obesity showed significantly lower mRNA expression of the IGF-1 variants Eb ($d = 1.39$, Figure 4C) and Ec ($d = 1.17$, Figure 4D) when compared to the subjects without obesity.

As presented in Table 3, mitochondrial protein FSR was strongly correlated with the mRNA expressions of overall IGF-1 as well as all IGF-1 isoforms within the W-OB ($p < 0.05$), but not within the OB ($p > 0.05$) group. We also carried out the same correlations within males and females regardless of their BMI and observed no significant correlations between

mitochondrial protein FSR and expression of any of the IGF-1 variants within the male cohort. Within the female subjects, most correlations did not reach statistical significance, but approached it (Overall IGF-1: $r = 0.53$, $p = 0.065$; IGF-1 Ea: $r = 0.55$, $p = 0.078$; IGF-1 Eb: $r = 0.54$, $p = 0.088$; IGF-1 Ec: $r = 0.18$, $p = 0.044$). However, considering that the study was not designed to address sex differences and the small sample size, we are limited to draw any conclusions from these findings. No significant correlations ($p > 0.05$) were found between mixed-muscle protein FSR and mRNA expressions of either overall IGF-1 or its IGF-1 variants within either the W-OB or the OB groups. Protein synthesis in muscle (either mixed muscle or mitochondrial) did not correlate with measured IGF-1 protein content in muscle, although the correlation between mixed-muscle protein FSR and IGF-1 protein in muscle approached statistical significance ($p = 0.06$) in the W-OB group Graphical depiction of correlations of various parameters of interest, along with Pearson's correlation and statistical significant values are included in the Supplemental file 1.

Discussion

The main goal of this study was to determine whether low protein synthesis observed in skeletal muscle of humans with obesity occurs concurrently with lower gene expression of muscle-specific IGF1 and its mRNA variants. We found lower muscle IGF-1 protein expression and mRNA expressions of the muscle IGF-1 variants Eb and Ec in skeletal muscle of subjects with obesity. In addition, the expression of the IGF-1 receptor was lower in the group of subjects with obesity than in the group of subjects without obesity.

Although currently several lines of evidence show lower protein synthesis in skeletal muscle of humans with obesity (4, 5, 7–10), the underlying mechanisms sustaining this response in muscle of humans with obesity remain elusive. Our novel findings show that lower skeletal muscle protein synthesis observed in obesity may result from lower IGF-1 gene expression in the muscle of these individuals. Although muscle protein synthesis was not measured, Sullivan et al. (30) reported also lower IGF-1 gene expression in muscle of humans with obesity, in line with our findings in the present study. In our study, mixed-muscle protein synthesis tended to significantly ($P = 0.06$) and directly correlate with the content of IGF-1 protein in muscle within the W-OB, but not within the OB ($P = 0.13$), group. Previous research shows that overexpression of IGF-1 in the skeletal muscle of mice induces muscle growth (31), suggesting that IGF-1 produced locally in the skeletal muscle stimulates muscle protein anabolism. The apparent positive correlation we observed between muscle IGF-1 protein and mixed-muscle protein synthesis in our healthy subjects without obesity is in line with evidence from Fryburg et al (17), who demonstrated that increased delivery of IGF-1 in muscle via arterial infusion of IGF-1 stimulates directly protein synthesis in muscle. The absence of significant corresponding correlation in humans without obesity suggests that IGF-1 signaling may be disrupted in the muscle of humans with obesity due to lower IGF-1R content we observed in the muscle of these individuals.

Although all pro-IGF-1 variants (i.e., IGF-1 + E peptide sequence) ultimately yield mature IGF-1 in muscle, each variant exerts unique effects on skeletal muscle anabolism. To illustrate, Barton et al. (18) demonstrated that viral-mediated delivery of IGF-1 Ea and Eb (equivalent to the Ec isoform in humans) in muscles of mice induced hypertrophy and

strength gains. In another study from the same research group (32), the authors investigated the effects of IGF-1 Ea and Eb variants on muscle size and reported significant muscle hypertrophy induced by each variant. Interestingly, this occurred in the absence of changes in mature IGF-1. Additionally, Ascenzi et al. (33) demonstrated that overexpression of only the IGF-Ea variant was able of inducing muscle hypertrophy in adult mice. However, in the latter report, overexpression of both variants protected against age-associated loss of muscle mass. Altogether, these lines of evidence demonstrate that *IGF1* gene variants play critical role in muscle anabolism. In our study, mRNA expressions of overall IGF-1 and IGF-1 Ea were not different between the two subject groups. However, mRNA expressions of IGF-1 variants Eb and Ec were lower in the subjects with obesity. It is reasonable to speculate that, among the *IGF1* mRNA variants within muscle, expressions of the Eb and Ec IGF-1 mRNA variants may have unique role in regulating protein synthesis in human muscle. Studies using cell culture models to investigate the potential biological roles of the Eb peptide of the *IGF1* on non-skeletal muscle cells have demonstrated that, despite IGF-1R inactivation, the Eb peptide is still able to induce proliferation of human bronchial epithelial cells (34). The Eb variant has also been shown to induce proliferation and migration in human cell lines (i.e., HeLa and U2-OS cells) (35). In terms of skeletal muscle, the Ec variant is linked to stretch-induced hypertrophy (36) and its expression has been shown to increase after injury, and immediately before satellite cell activation. Such findings however are not consistent, as another study found no effects of IGF-1 Ec on cell proliferation or differentiation in a human myoblast cell line (19). Thus, the exact mechanisms through which *IGF-1* splice variants may regulate skeletal muscle protein metabolism remain currently unknown.

We found that, and similar to what we have previously reported (4, 5), lower synthesis rate of mixed-muscle protein in humans with obesity includes lower synthesis rate of proteins within the mitochondrial protein pool. The present study provides novel evidence linking the *in vivo* regulation of human skeletal muscle mitochondrial protein synthesis to gene expression of *IGF-1* variants in muscle. Specifically, muscle mitochondrial protein synthesis was highly correlated with the gene expression of *IGF1* variants in the subjects without obesity. Previous studies have demonstrated the importance of IGF-1 for mitochondrial biogenesis and cell survival in non-muscle cells (31, 32). In muscle cells, Guan et al. (33) reported that IGF-1 signaling increases the expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and stimulates mitochondrial biogenesis. In our study, we observed strong positive correlations between mitochondrial protein synthesis and mRNA *IGF-1* expression and its splice variances in our healthy humans without obesity, suggesting a direct link between mRNA IGF-1 gene expression and mitochondrial protein synthesis in muscle (Table 3). Lack of a corresponding correlations in our subjects with obesity may relate to the existence of variable degree of impairments in *IGF1* gene expression and its splice variants in the muscle of humans with obesity. Notably, the expression of muscle *IGF-1* mRNA, but not protein, correlated with the mitochondrial protein synthesis, which points attention to the role of pro-IGF-1 as opposed to the mature IGF-1 protein in playing a role in mitochondrial protein synthesis. Thus, our findings provide new direction into our efforts to understand regulation of muscle mitochondrial protein synthesis by muscle IGF-1, and pave the way for future research exploring the role of *IGF1* gene variants in the regulation of mitochondrial protein synthesis in humans with

obesity. In regards to the latter, it is reasonable to speculate that either the pro-IGF-1 variants or the individual E peptides of IGF-1 (or both) have role in regulating mitochondrial protein synthesis in muscle.

IGF-1 enhances protein synthesis in skeletal muscle by binding to its receptor embedded in the muscle fiber membrane. Both *in vitro* (37) and *in vivo* (32) studies have demonstrated that a functional IGF-1R is required for the IGF-1 Ea and Eb isoforms to exert their physiological effects on skeletal muscle. It has also been shown that deletion of both IGF-1 and insulin receptors in skeletal muscle causes growth impairment and severe muscle atrophy in mice (38). In the current study we found that not only the IGF-1 protein but also the IGF-1R protein was markedly reduced in our group of humans with obesity when compared to that of humans without obesity. It is noted that it was not a main aim of this investigation to evaluate IGF-1 receptor signaling in muscle, but rather *IGF-1* mRNA and link its expression to the IGF-1 protein content in muscle. For that reason, we measured total muscle IGF-1 protein, rather than free IGF-1 protein that interacts directly with the IGF-1 receptor. Therefore, the direct relationship between IGF-1 protein and its receptor cannot be described based on our data. However, our secondary finding of a decreased IGF-1R protein content in the muscle of humans with obesity suggests that the IGF-1 signaling component of the IGF-1 biology in muscle appears to be impaired in humans with obesity.

We recognize that despite studying individuals with maximal oxygen uptake values comparable between groups, we cannot exclude the possibility that subjects with obesity had lower overall fitness and/or physical activity levels. Therefore, and given that it was not experimentally feasible to characterize the everyday physical activity levels of our subjects in a definitive manner, our study is possibly limited in its ability to attribute the observed findings on obesity, per se (as opposed to physical activity levels). However, the majority of individuals with obesity are characterized by low physical activity levels (39) and, therefore, these findings are still applicable to the general population of humans with obesity. Our study is limited in its capacity to describe signaling of IGF-1 in skeletal muscle. Because our goal was to link mRNA IGF-1 expression to IGF-1 protein expression, we measured total IGF-1 protein in muscle, which encompasses IGF-1 protein that is found both bound and unbound to its carrier proteins. Therefore, considering that it is the free IGF-1 that binds to its receptor and initiates intracellular signaling, we are unable to describe whether the free IGF-1 protein concentration in muscle differs between groups, and which can have implications for IGF-1 signaling in muscle.

In conclusion, this study demonstrates that lower protein synthesis in skeletal muscle of humans with obesity occurs concurrently with lower muscle gene expression of *IGF1* and muscle IGF-1R content, and that these responses alone or in combination may explain lower rate of protein synthesis in muscle of humans with obesity. Lower mRNA expression of the IGF-1 variants Eb and Ec in muscle of humans with obesity likely contribute to lower expression of mature IGF-1 protein in these individuals. Moreover, and as opposed to mature IGF-1 protein, lower expression of the muscle IGF-1 Eb and Ec mRNA splice variants may have a unique role in sustaining lower synthesis of mitochondrial proteins in muscle of humans with obesity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data sharing statement:

any data and information related to this study will be available for sharing upon formal request made to the corresponding author.

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Study Importance Questions

- What is already known about this subject?
  - Prior research has demonstrated that protein synthesis is lower in skeletal muscle of humans with obesity; however, the underlying biological mechanisms that sustain this response remain unknown.
  - IGF-1 is a growth factor that regulates protein synthesis in skeletal muscle; reduced gene expression of muscle *IGF1* splice variants may reduce IGF-1 protein in muscle and thus sustain lower protein synthesis observed in skeletal muscle of humans with obesity.
- What are the new findings in your manuscript?
  - IGF-1 mRNA and protein expressions are reduced in muscle of humans with obesity, and along with reduced IGF-1 receptor protein content in the muscle of these individuals; these responses can explain, at least in part, lower protein synthesis observed in muscle of humans with obesity.
  - The mRNA expression of overall *IGF1* and its splice variants were highly correlated with mitochondrial protein synthesis in skeletal muscle of healthy humans without obesity, but not in humans with obesity.
- How might your results change the direction of research or the focus of clinical practice?
  - The results of this study pave the way for future research addressing the causal role of reduced expression of *IGF-1* splice variants as well as that of IGF-1 receptor in muscle of humans with obesity as potential culprits sustaining lower protein turnover in muscle of these individuals.
  - This study provides a foundation for future research on the implications of the expression of muscle *IGF-1* splice variants as key molecular modulators of protein turnover specifically within the mitochondrial protein pool in skeletal muscle.

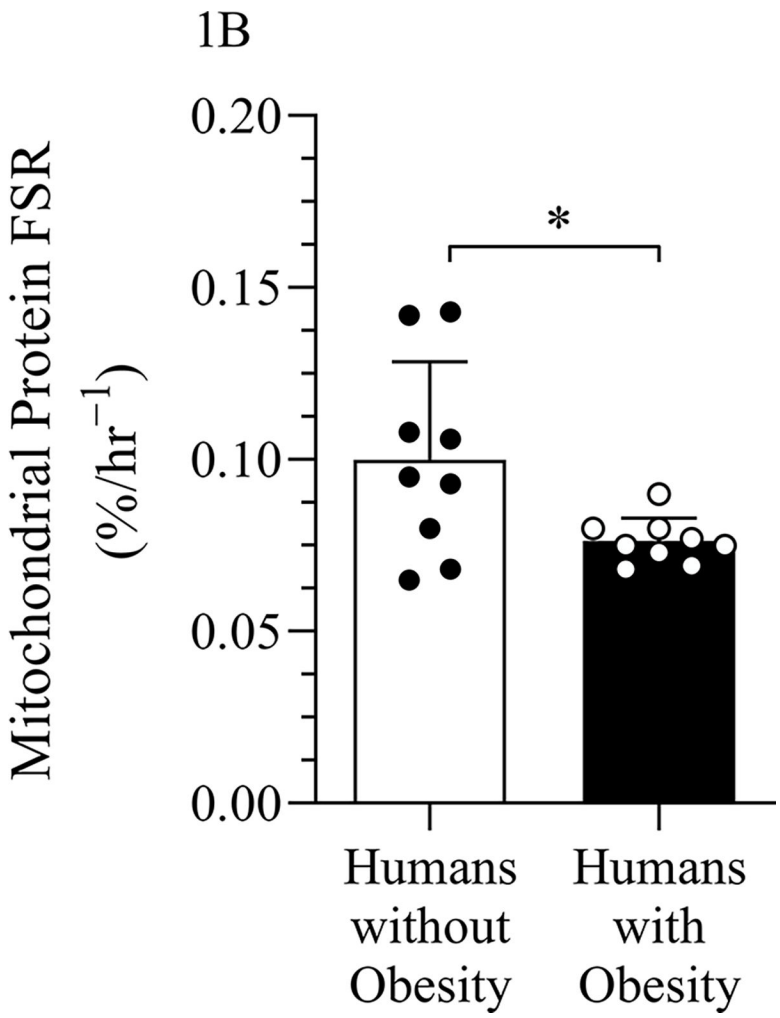

Figure 1.
Mitochondrial protein fractional synthesis rate (FSR) in humans with obesity and humans without obesity. *Significant group difference at $p \leq 0.05$.

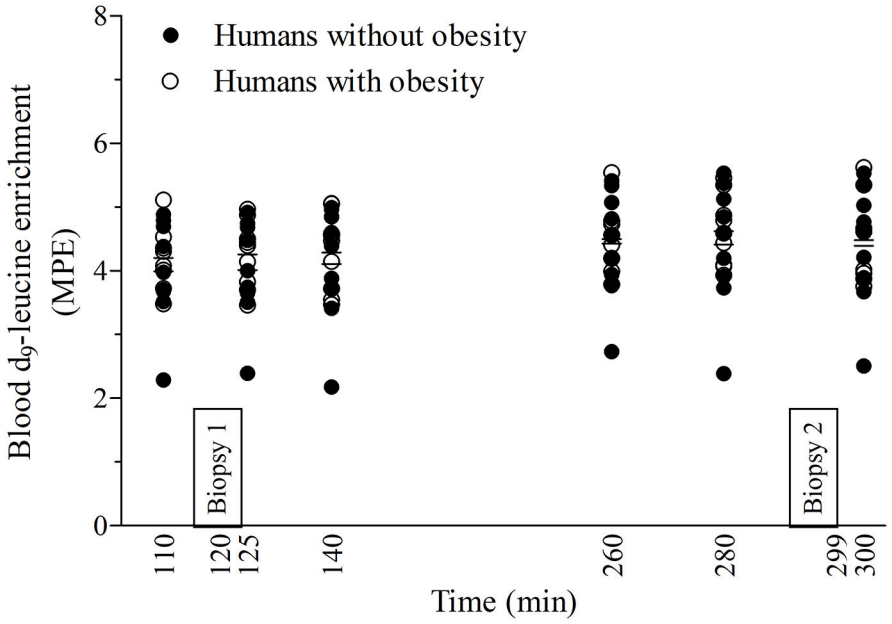

Figure 2.

Plasma d9-leucine enrichment in humans with obesity and humans without obesity is shown along the time points corresponding to the collection of muscle biopsy samples during the infusion study (MPE: molar percent excess).

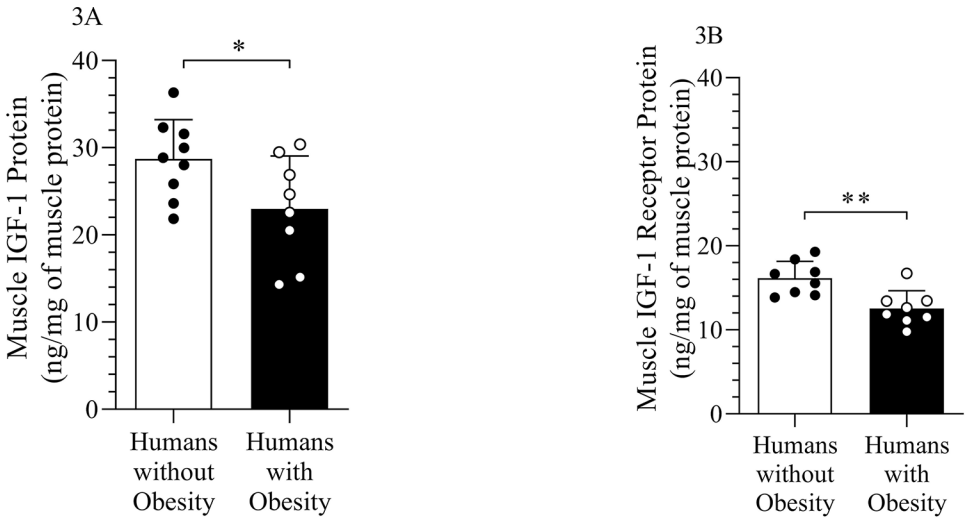

Figure 3.

A: IGF-1 protein expression, B: IGF-1 receptor expression in the skeletal muscle of humans with obesity and humans without obesity. *Significant group difference at $p \leq 0.05$, **Significant group difference at $p \leq 0.01$.

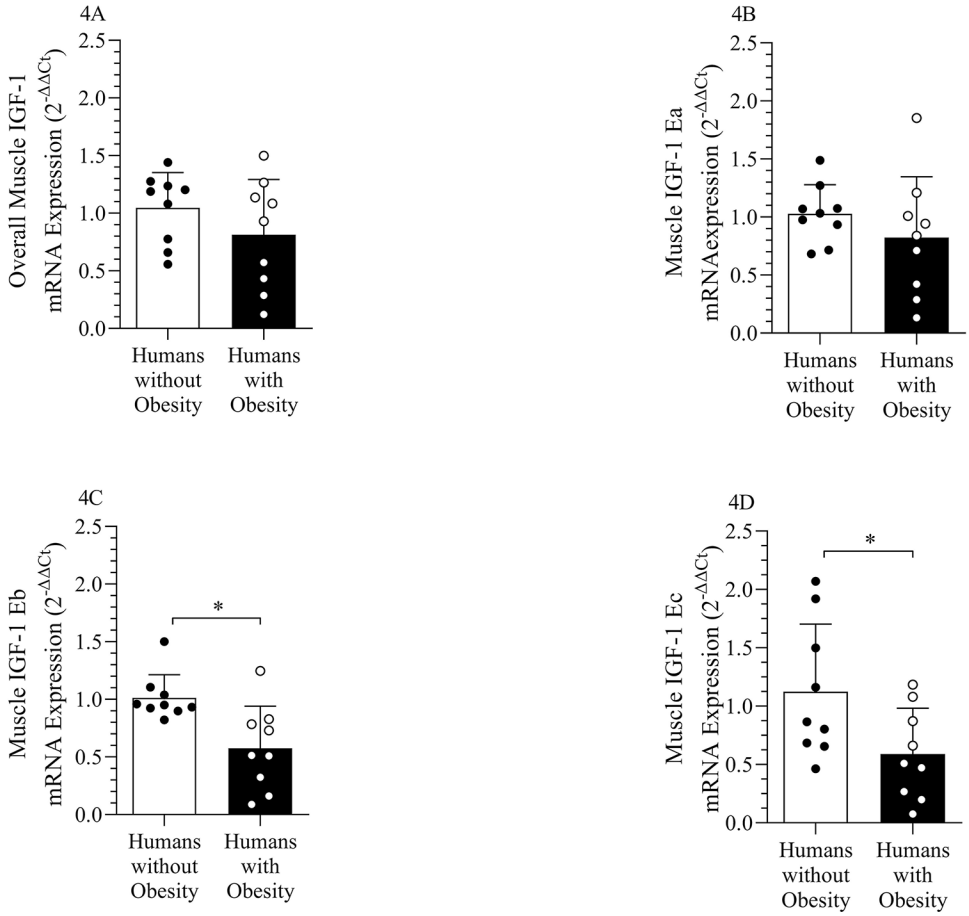

Figure 4.

A: mRNA expression of overall *IGF1*and its variants (Ea, Eb, and Ec) in the skeletal muscle of humans with obesity and humans without obesity. *Significant group difference at $p \leq 0.05$.

Table 1.

Amplicon context sequences used for the quantification of genes of interest using RT-qPCR

Gene symbol	TaqMan assay ID	Amplicon context sequence	GenBank accession number	Exon boundary
IGF1	Hs01547656_m1	CTCTGCGGGGCTGAGCTGGTGGATGCTCTTCAGTTCGTGTGTGGAGACAGGGGCTTTTATTTCAACAAGC CCACAGGGTATGGCTCCAGCAGTCGGAGGGCGCCTCAGACAGGCATCGTGGATGAGTGCTGCTTCCG	NM_000618	2 - 3
IGF1 Ea	Hs01547657_m1	GCCTGCCAAGTCAGCTCGCTCTGTCCGTGCCCAGCGCCACACCGACATGCCCAAGACCCAGAAGGAAGTA CATTTGAAGAACGCAAGTAGAGGGAGTGCAGGAAACAAGAACTACAGGATGTAGGAA	NM_000618	3 - 4
IGF1 Eb	Hs01555481_m1	CTGCCAAGTCAGCTCGCTCTGTCCGTGCCCAGCGCCACACCGACATGCCCAAGACCCAGAAGTATCAGCC CCCATCTACCAACAAGAACACGAAGTCTCAGAGAAGGAAAGGAAGTACATTTGAAGAAC	NM_001111283	3 - 4
IGF1 Ec	Hs03986524_m1	ACACCGACATGCCCAAGACCCAGAAGTATCAGCCCCCATCTACCAACAAGAACACGAAGTCTCAGAGAAG GAAAGGAAGTACATTTGAAGAACGCAAGTAGAGGGAGTGCAGGAAACAAGAACTACAGGATGTAGGAAGA CCC	NM_001111283.2	4 - 5
18S	Hs99999901_s1	CAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAA TTACCCACTCCCGACCCGGGGAGGTAGTGACGAAAAATAACAATACAGGACTCTTTCGAGGCCCTGTAAT TGGAATGAGTCCACTTTAAATCCTTTAACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGG TAATTCCAGCTCCAATAGCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCTTGGGAGCG GGCGGGCGGTCCGCCGCGAGGCGAGCCACCGCCCGTCCCCGCCCCTTGCCTCTCGGCGCCCCCTCGATGC TCTTAGCTGAGTGTCCCGCGGGGCC	X03205.1	1

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Table 2.

Study participant descriptive characteristics

	Humans without obesity (*n* = 9)	Humans with obesity (*n* = 9)	p-value
Sex (males/females)	3/6	4/5	-
Age (years)	33 ± 7	33 ± 12	0.962
Height (m)	1.72 ± 0.09	1.69 ± 0.09	0.641
Weight (kg)	69.7 ± 12.1	100.9 ± 15.4	< 0.001
BMI (kg/m^2)	23.5 ± 2.7	35.0 ± 3.2	< 0.001
Waist circumference (cm)	82.31 ± 5.80	105.44 ± 10.15	< 0.001
Hip circumference (cm)	101.69 ± 7.03	119.75 ± 11.57	0.002
Waist-to-hip ratio (cm)	0.81 ± 0.06	0.88 ± 0.07	0.044
Fat mass (kg)	17.52 ± 5.64	35.40 ± 10.57	< 0.001
Lean body mass (kg)	52.22 ± 11.74	65.59 ± 10.85	0.015
Body fat percentage (%)	25.44 ± 7.94	34.88 ± 7.42	0.019
Plasma glucose (mg/dL)	84.3 ± 5.42	87.95 ± 6.67	0.221
Plasma insulin (μIU/mL)	4.18 ± 1.10	8.91 ± 9.54	0.006
Plasma IGF-1 (ng/mL)	193.97 ± 43.27	199.68 ± 89.67	0.857
Plasma total amino acids (μmol/L)	1475.67 ± 266.65	1757.00 ± 504.88	0.159
Plasma EAA (μmol/L)	622.67 ± 100.58	734.11 ± 186.66	0.134
Plasma BCAA (μmol/L)	338.22 ± 50.85	416.89 ± 89.55	0.036
Matsuda ISI	9.45± 2.82	4.64 ± 4.34	0.013
HOMA-IR	0.88 ± 0.28	1.92 ± 0.93	0.005
VO_{2max} (ml/kg FFM/min)	27.42 ± 6.64	22.50 ± 6.54	0.133
MMP-FSR ($\%/hr^{-1}$)	0.083 ± 0.016	0.065 ± 0.019	0.042

BMI = body mass index, IGF-1 = insulin-like growth factor 1, EAA = essential amino acids, BCAA = branched-chain amino acids, MMP-FSR = mixed-muscle protein fractional synthesis rate, VO_{2max} = maximal oxygen consumption, HOMA-IR = homeostatic model assessment for insulin resistance, Matsuda ISI = Matsuda Insulin Sensitivity Index.

Table 3.

Pearson's product moment correlation coefficient (r) between *IGF1* gene expression and protein synthesis in skeletal muscle

	IGF1 **Protein**	**Overall *IGF1*** **mRNA**	***IGF1*****Ea** **mRNA**	***IGF1*****Eb** **mRNA**	***IGF1*****Ec** **mRNA**
Humans without obesity					
MMP-FSR	$r = 0.66$	$r = -0.55$	$r = -0.46$	$r = -0.27$	$r = -0.32$
	$p = 0.06$	$p = 0.13$	$p = 0.21$	$p = 0.48$	$p = 0.40$
MITOP-FSR	$r = -0.16$	$r = 0.86$**	$r = 0.94$**	$r = 0.76$*	$r = 0.80$**
	$p = 0.68$	$p < 0.01$	$p < 0.01$	$p = 0.02$	$p < 0.01$
Humans with obesity					
MMP-FSR	$r = -0.58$	$r = -0.31$	$r = -0.30$	$r = -0.29$	$r = -0.05$
	$p = 0.13$	$p = 0.41$	$p = 0.44$	$p = 0.45$	$p = 0.89$
MITOP-FSR	$r = 0.49$	$r = -0.08$	$r = -0.23$	$r = -0.13$	$r = -0.62$
	$p = 0.21$	$p = 0.83$	$p = 0.54$	$p = 0.73$	$p = 0.08$

MMP-FSR = mixed-muscle protein fractional synthesis rate, MITOP-FSR = mitochondrial protein fractional synthesis rate.