

# Effects of Alpha-hydroxy-isocaproic acid upon Body Composition in a Type I Diabetic Patient with Muscle Atrophy – A Case Study

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Research involving dietary supplement interventions for sarcopenia and osteopenia in type 1 diabetes patients is scarce. Here we present a case study of a type 1 diabetic patient that was treated with supplemental alpha-hydroxy-isocaproic acid ( $\alpha$ -HICA $\dagger$ ) for 120 days. Several measures of body composition by dual x-ray absorptiometry, blood markers, and maximum voluntary contraction parameters were assessed at baseline and after 120 days. The patient's baseline weight was 73.2 kg, which increased to 75.2 kg by the 120-day assessment. Salient mass distribution changes included increases of trunk fat mass (+0.4 kg), trunk fat free mass (+0.2 kg), total trunk mass (+0.2 kg), and a decrease of 8 percent in trunk fat mass contribution. Handgrip strength increased by 58.84 N, whereas isometric force in the leg press decreased by 347.15 N. Amelioration of BMD Z-scores from -0.7 to 0.5 and T-scores from -1.0 to -0.9 were noted. Importantly, full hematologic measures and weekly nutritional counselling assessments revealed no signs of adverse effects with  $\alpha$ -HICA supplementation. Due to the imperative of maintaining FFM, strength and bone mass in these patients, additional research is necessary to confirm these promising results and to clarify whether leucine and/or one of its derivatives might be clinically useful.

## INTRODUCTION

Diabetes mellitus (DM) is a global pandemic of chronic hyperglycemia characterized by diminished

insulin secretion and/or low sensitivity in target cells, and an increase in hepatic glucose production [1]. Worldwide, the prevalence of DM is currently estimated at 415 million, or 8.3 percent of the global population [2]. The

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$\dagger$ Abbreviations:  $\alpha$ -HICA, alpha-hydroxy-isocaproic acid; DM, diabetes mellitus; UPP, ubiquitin proteasome pathway; SMM, skeletal muscle mass; DXA, dual X ray absorptiometry; BMC, bone mineral content; BMD, bone mineral density; FM, fat mass; FFM, fat free mass (lean + BMC); TM, total mass; B, baseline; AbsC, absolute change; L, left; R, right; kcal, kilocalorie; TDEE, Total daily energy expenditure; pg, picogram; ng, nanogram; mg, milligram; g, gram; kg, kilogram; N, Newton; MVC, maximal voluntary isometric contraction; kHz, kilohertz; LDL, low-density lipoprotein; cm<sup>2</sup>, centimeter square; HDL, high-density lipoprotein; dL, deciliter; mL, milliliter;  $\mu$ L, microliter; fL, femtoliter; IU, international unit; U, units; mmol, millimole; DLD, daily living disability; IGF-1, insulin-like growth factor; IL-6, interleukin-6; STAT3, signal transducer and activator of transcription 3; CRP, C-reactive protein; GH, growth hormone.

Keywords: alfa-hica, diabetes, body composition, strength, leucine, intervention

Author Contributions: FJT made all laboratory evaluations with the exception of bloodwork, wrote the manuscript, CPM and CNM assisted on measurements and development of the manuscript. CPM took the role of corresponding author. SLH and RK reviewed, assisted, and assessed data.

current trajectory predicts this burden will increase to 642 million people with the disease by 2040 [3]. Type 1 diabetes represents 5 to 10 percent of all DM cases, a figure that is progressively rising so that it too is regarded as an emerging epidemic in several countries [4]. This form of the disease is typically expressed during adolescence, but also occurs in older adults [5]. One of the severe consequences of DM is myopathy, associated with loss of both skeletal muscle mass and physical capacity [6]. Due to the important anticatabolic action of insulin in the ubiquitin proteasome pathway (UPP), both types of diabetes influence skeletal muscle mass (SMM), but differently, with type I resulting in greater SMM loss [7]. Interest in DM-associated skeletal muscle mass loss is high for two major reasons: 1) SMM is the major reservoir of post-prandial glucose uptake targeted by insulin and 2) loss of SMM leads to a dramatic reduction in global protein, which might impair mobility and response to critical illness [6,8].

Leucine metabolites are thought to decrease muscle protein breakdown by inhibiting the UPP [9]. Alpha-hydroxy-isocaproic acid ( $\alpha$ -HICA) is an end product of leucine metabolism in human tissues [10] with natural occurrence in several foods [11]. It has been regarded as an anti-catabolic substance in both *in vitro* and animal research [12,13]. *In vitro* research suggests that a possible mechanism for this effect is the inhibition of metalloprotein enzymes [13]. However, human  $\alpha$ -HICA research is scarce, with only one double-blind human study performed involving 15 young Finnish male soccer players [14]. In this work, the researchers reported that administration of 500 mg of  $\alpha$ -HICA, 3 times a day, over 4 weeks, led to a significant increase in body weight and whole body lean mass, while fat mass (FM) remained constant. Theoretically, several mechanisms could explain how both leucine or its downstream metabolites might be beneficial to individuals bearing DM [7,9]. Without additional confirmatory research, no definitive statement can be made. Fortunately, the safety of leucine and its derivatives is well established, even in elderly individuals, which permits further research with this compound in diabetic patients [15]. Within this context, we present a case study of a patient with type I diabetes, in which,  $\alpha$ -HICA presented favorable changes in fat free skeletal mass.

## CASE PRESENTATION

A 59-year old, non-smoking man diagnosed with type 1 diabetes for 30 years by his primary care physician, presented to our lab. The subject is sedentary with minimal physical activity due to serious mobility limitations which precluded any type of training or exercise on his daily routine. Medical records showed loss of body

weight and presumably muscle mass for over one year. The patient had a long history of successive hypoglycemic crises, one associated with cardiac arrest. Fifteen years before, the patient suffered a grand mal seizure that caused permanent damage to the right hip, eventually requiring a complete hip replacement. The primary care physician believed that the combination of impaired ambulation and type 1 diabetes was the primary cause for the patient's more recent insidious muscle loss, especially in the lower limbs.

The patient used slow action insulin (Lantus®-Generis Farmacêutica SA, Amadora, Portugal)  $\approx$ 25 IU upon awakening depending on glycemic values and fast acting insulin (Humalog®-Lilly Portugal, Lisboa, Portugal)  $\approx$ 3 IU one hour after meals, also depending on glycemic values. The patient had a flash glucose monitoring system which was alternated between the right and the left arm. Additional medications included pregabalin 100 mg twice a day (Lyrica® Pfizer Ltda, Freiburg, Germany) for peripheral pain and the antiplatelet drug clopidogrel (KRKA, Cuxhaven, Germany) 75 mg before sleep to prevent arterial thrombosis.

## MEASUREMENTS

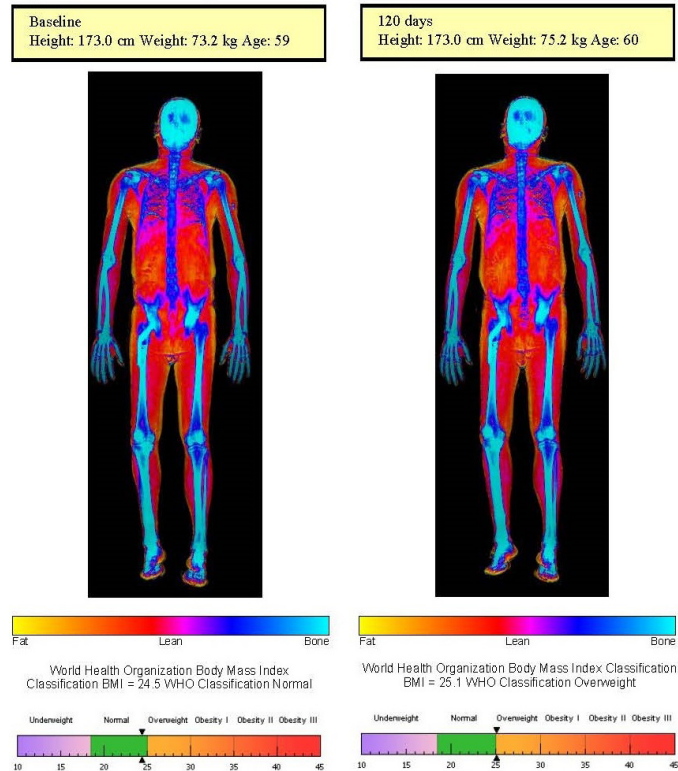
The subject was evaluated at baseline and after 120 days of supplementation with  $\alpha$ -HICA (from April to August 2017). Evaluations included blood analysis, body composition, and strength measures. All body composition measurements were performed after a 12 h fast. A meal replacement bar (Matrix Bar, Olimp Labs, Pustynia, Poland) was provided prior to the strength tests, comprised of 258 kcal (energy) = 26.2 g (protein) + 20.5 g (carbohydrates) + 8 g (fat). During the whole intervention the subject received guidance from his doctor and a registered dietitian. Subject was instructed not to change his physical activity during the intervention, maintaining only his professional daily activity as an administrative assistant.

### Anthropometry

Height was measured to the nearest 0.1 cm with a stadiometer (Seca, Hamburg, Germany), using standardized procedures [16]. Body mass was assessed to the nearest 0.1 kg using a weight scale (Seca, Hamburg, Germany).

### Dual X-ray Absorptiometry (DXA)

The patient underwent a whole-body DXA scan on a Hologic Explorer-W, fan-beam densitometer (Hologic, Waltham, Massachusetts, USA) according to the manufacturer guidelines [17]. The DXA scan included whole body measurements of bone mineral content (BMC), bone mineral density (BMD), absolute and relative FM,



**Figure 1.** DXA Baseline vs. 120 days.

and fat free mass (FFM). The equipment measures the attenuation of X-rays pulsed between 70 and 140 kV synchronously with the line frequency for each pixel of the scanned image. A step phantom with six fields of acrylic and aluminum of varying thickness and known absorptive properties was scanned to serve as an external standard calibrator for the analysis of different tissue components. The same technician positioned the patient, performed the scan, and executed the analyses (software QDR for Windows version 12.4, Hologic, Waltham, Massachusetts, USA) according to the operator's manual using the standard analysis protocol. The coefficients of variation in our laboratory, based on 10 young active adults (five males and five females), is 1.6% for BMC, 1.7% for FM, and 0.8% for FFM [18].

### Strength

Before each assessment the participant was familiarized with the specific strength test. Maximal isometric forearm strength was determined using a hydraulic hand dynamometer model 5030J1 (Jamar, Sammons Preston, Inc, Bolingbrook, IL, USA) with visual feedback [19]. The dynamometer was adjusted to the subject's dominant hand with each trial lasting approximately 5 seconds. The best of three maximal trials was recorded to the nearest 2 kg (19.61 N). The same adjustment of the dynamometer

was used for all tests. The evaluation of the maximal lower strength was made performing three rapid maximal voluntary isometric contractions (MVC's).

The evaluation of the maximal knee extension strength was performed on a custom-made horizontal leg press device (Model 4090E; HBP Exclusive Line) instrumented with an aluminum platform equipped with four load cells (Shear Beam Load Cell - Flintec BK2). During the test, the patient was positioned with the hip and knee joints at angles of 100° and 110°, respectively. He was then instructed to produce the maximal force as quickly as possible and sustain the effort for 3 seconds. The force signal was A/D converted (MP100 – Biopac Systems Inc., 16 bits) with a sample rate of 1 KHz. Acq-Knowledge software (Biopac Systems Inc.) was used to analyze the highest value across the three MVC's to the nearest 2.0 N/mV. All strength measures were performed in a fed state (a meal replacement bar was provided to the patient 30 minutes prior to testing).

### Diet

The patient followed a diet designed by a registered dietitian from a previous hospital admission. The diet comprised 2200 kcal (138 g protein  $\approx$ 25% TDEE, 303 g carbohydrates  $\approx$ 55% TDEE, and 49 g fat  $\approx$ 20% TDEE) and its composition was assessed from baseline

Table 1. Body composition characteristics: Baseline vs. 120 days.

DXA	FM-B (g)	FM-120 (g)	AbsC (g)	FFM-B (g)	FFM-120 (g)	AbsC (g)	TM-B (g)	TM-120 (g)	AbsC (g)	Fat-B (%)	Fat-120 (%)	AbsC (%)
L Arm	820	858	+38	3023	3003	-20	3843	3861	+18	21.3	22.2	+0.9
R Arm	691	729	+38	3626	3247	-379	4318	3977	-341	16.0	18.3	+2.3
Trunk	10577	11005	+428	27380	29639	+2259	37957	40644	+2687	27.9	27.1	-0.8
L Leg	2037	2020	-17	8699	8295	-404	10736	10315	-421	19.0	19.6	+0.6
R Leg	1989	2086	+97	8473	8258	-215	10462	10344	-118	19.0	20.2	+1.2
Subtotal	16114	16699	+585	51201	52442	+1241	67316	69140	+1824	23.9	24.2	+0.3
Head	1062	1065	+2	4185	4153	-32	5247	5218	-29	20.2	20.4	+0.2
Total	17176	17764	+588	55386	56595	+1209	72562	74359	+1797	23.7	23.9	+0.2

Abbreviations: DXA-Dual X ray absorptiometry, B-Baseline, AbsC-Absolute change, FM-Fat mass, FFM-Lean + BMC, TM-Total mass, % Fat- Percent fat contribution, L-left, R-right

every 30 days through three-day food records [20] (three non-consecutive days, one being a weekend day) using certified software (Food Processor, Esha Research, Inc., Salem, Oregon, USA). Weekly nutrition counseling was provided by a registered dietitian to enhance compliance with the hospital diet.

### Supplementation Protocol

The supplement consisted of 500 mg  $\alpha$ -HICA per tablet (HICA, Onsalesit, SA, Funchal, Portugal). The patient was instructed to take one tablet (500 mg) each day at breakfast, lunch, and dinner, with a liquid beverage, as indicated by Mero *et al.* [14]. The daily dosage was provided in individual bags (three tablets per bag). Compliance was assessed when the patient returned empty bags to the dietitian during the weekly nutrition counselling session.

### Blood Analysis

Blood samples were taken under physician order at a local hospital and processed onsite in a certified laboratory. Venous blood was withdrawn from the antecubital vein after a 12 h fast and analyzed with automated equipment according to standard procedures.

### Results

The patient achieved 98.4% compliance with the supplementation protocol during the 120-day treatment period. At baseline, the patient's weight was 73.2 kg, which increased to 75.2 kg by the 120-day assessment (Figure 1). Table 1 depicts regional body composition changes comparing baseline and 120 days. During that same period, the absolute FM, FFM, and total mass increased by 588, 1209, and 1797 g, respectively, along with a 0.2 percent increase in fat. Salient mass distribution changes included increases in trunk FM of +428 g, trunk FFM of +2259 g, total trunk mass of +2687 g, with a decrease of 0.8 percent in trunk fat contribution.

Absolute change in total bone area decreased by 67.78 cm<sup>2</sup>, whereas absolute change in BMC and BMD increased by 1.38 g and 0.036 g/cm<sup>2</sup>, respectively. Thoracic spine area decreased 34.08 cm<sup>2</sup>, while lumbar spine area increased 19.76 cm<sup>2</sup>. Right leg BMC decreased 24.39 g, while the BMC increased 21.21 g and 18.86 g in the pelvis and lumbar spine, respectively. BMD decreased 0.520 g/cm<sup>2</sup> and 0.117 g/cm<sup>2</sup> but increased in all other areas.

Redistribution of BMC in all areas led to a slight amelioration of BMD Z-scores from -0.7 to -0.5, with T-scores following this trend changing from -1.0 to -0.9.

Table 2 lists the change in T- and Z-scores from baseline to 120 days. Handgrip strength increased by 58.84 N, whereas isometric force in the leg press decreased by

**Table 2. BMD T and Z scores: Baseline vs. 120 days.**

Score	Baseline	120 days	Critical Values
T-score (% age matched)	-1.0	-0.9	> -1 Normal
Z-score (standard deviations)	-0.7	-0.5	> 2

Note: Age matched (31,31), Expected value for age (31)

**Table 3. Strength measures: Baseline vs. 120 days.**

Test	Baseline	120 days
Handgrip (N)*	480.53	539.37
Leg press (N)*	2954.63	2607.48

\*Best out of 3 attempts

**Table 4. Blood composition changes baseline vs 120 days.**

Marker	Baseline	120 days	Change
Hemoglobin (g/dL)	14.6	14.7	0.1 ↑
Erythrocytes x (10 <sup>6</sup> /μL)	4.83	4.86	0.03 ↑
Hematocrit (%)	44.8	45.0	0.2 ↑
Mean corpuscular volume (fL)	92.8	92.5	0.3 ↓
Red cell distribution width (%)	12.4	12.2	0.2 ↓
Platelet count x (10 <sup>3</sup> /μL)	298	301	3 ↑
Ferritin (ng/mL)	80.9	79.0	1.9 ↓
Transferrin (mg/dL)	241.0	233.4	7.6 ↓
Serum iron (μg/dL)	78	86	8 ↑
Glucose (mg/dL)	217	252	35 ↑
Glycated hemoglobin (%)	9.6	9.3	0.3 ↓
Median glucose (mg/dL)	229	220	9 ↓
Insulin (μU/mL)	10.9	11.2	0.3 ↑
Total cholesterol (mg/dL)	193	192	1 ↓
LDL cholesterol (mg/dL)	103	100	3 ↓
HDL cholesterol (mg/dL)	62	64	2 ↑
Triglycerides (mg/dL)	141	139	2 ↓
Albumin (g/dL)	3.7	3.7	=
C reactive protein (mg/dL)	0.182	0.123	0.059 ↓
Uricemia (mg/dL)	6.4	7.0	0.6 ↑
Creatinine (mg/dL)	1.62	1.52	0.1 ↓
Aspartate aminotransferase (U/L)	21	26	5 ↑
Alanine aminotransferase (U/L)	28	31	3 ↑
Gamma-glutamyl transpeptidase (U/L)	37	47	10 ↑
Alkaline phosphatase (U/L)	92	87	5 ↓
Sodium (mmol/L)	142	142	=
Potassium (mmol/L)	5.3	4.9	0.4 ↓
Chlorine (mmol/L)	106.0	105.0	1 ↓
Free testosterone (pg/mL)	12.28	9.39	2.89 ↓



**Table 5. Estimated dietary intake from baseline to 120 days.**

	Energy (kcal)	Protein (g)	Carbohydrates (g)	Fat (g)	Protein (g/kg body weight)
<b>Baseline</b>	2200	138	303	49	1.9
<b>120 days</b>	2540	146	318	76	1.9

347.15 N (Table 3). Blood markers changes from baseline to 120 days are presented in Table 4. No salient changes in blood markers were noted. Estimated dietary intake from baseline to 120 days is presented above (Table 5). A slight increase in energy was noted (+340 kcal) mainly due to an increase in fat intake (+27 g).

## DISCUSSION

### Body Composition

Relevant changes associated with  $\alpha$ -HICA administration, in both body weight and FFM, have been previously reported in the literature in 15 healthy young soccer players [14]. However, these changes were of small magnitude (+0.3 kg for both body weight and absolute FFM) in the  $\alpha$ -HICA group. The significant increase in FFM was attributed to a variation in the lower extremities (0.4 kg). Our patient increased body weight by 2 kg and total FFM by 1.2 kg, which is almost 4-fold the previous reported values. In our patient, the main contributor to absolute FFM increase was the trunk area (+2.2 kg), whereas in the previous study lower extremities accounted for the significant differences. In our patient, the trunk also contributed to a major increase in FM (+0.4 kg), which was 0.2 percent higher than baseline. Differences between our patient and the previously reported study [14] can be attributed to the fact that our patient was older and had a diagnosed metabolic disease, but also to the duration of our study (120 days vs 4 weeks). The incremented FFM in lower extremities in the previous study may be attributed to participation in soccer with primary use of the lower body as a muscular stimulus. Since our subject was sedentary and substantially limited in the lower body from previous hip replacement surgery, it is plausible that the upper body was more stimulated than the lower body, thus leading to greater muscle mass preservation. Although changes in FM were detected, they were not clinically relevant given the duration of the study.

### Bone Mineral Density

Bone mineral density is an expression of BMC/area ( $\text{g}/\text{cm}^2$ ) in which values are typically expressed in relative values: T-scores (standard deviations) and Z-scores (% age matched) [21]. In our patient, only trivial changes in BMD were detected per area, however, changes in Z-score and T-score were noted. Our patient had an initial

T-score of osteopenia (-1.0) and after 120 days this value was ameliorated to normal BMD (0.9) [21]. The Z-score also followed this trend but did not reach the reference value expected for age (>2) [22]. Notwithstanding, clinical relevance cannot be established from these changes regarding both Z-score and T-score, due to the short duration of the study. Gains in BMD are important for bone strength; however, they need to be sustained over time. The follow-up schedule after initiation of a new therapy is usually superior to 6 months, typically 1 year [23]. Although the interaction between FFM increase and BMC is well supported from both animal [24] and human studies [25], longer trials are required to establish clinical relevance between  $\alpha$ -HICA supplementation and bone health. Nevertheless, plausible mechanisms will be further discussed in the mechanisms section.

### Strength

Grip-strength is a practical and informative measure of muscle strength in middle-aged and elderly individuals due to the relationship between grip strength and favorable prognosis in health-related events [19,26]. Key health-related prognostic factors are functional limitation, functional decline, daily living disability (DLD), and mortality [19]. Muscle weakness is expected in type 1 diabetics due to FFM loss and neuropathy [27]. Our patient increased grip strength by 58.84 N in his dominant side (right-handed), which is quite substantial when compared to the baseline value and healthy population of the same age [28]. Due to the importance of strength in DLD, comorbidities, and mortality we find these results extremely encouraging, certainly warranting further investigation. Our subject's lower body strength, measured through maximal voluntary isometric contractions, declined -347.15 N, which was expected due to mobility limitations and deficient lower body stimulation. Given the circumstances, whether a strength increase would be noted in the absence of mobility limitations could not be established.

### Blood Markers

No salient changes in blood markers were observed after 120 days of  $\alpha$ -HICA supplementation, except for a slight decrease in free testosterone and glycated hemoglobin. A drop in free testosterone (>20%) was observed in our patient (albeit within the laboratory reference

range), however this did not seem to negatively impact FFM gains. Adult men with type 1 diabetes have a tendency to hypogonadism, however do not present different total testosterone levels or impaired pituitary-gonadal axis, only slightly non-statistically significant lower free testosterone levels, when comparing with healthy control subjects [43]. This is further confirmed in larger studies, with type 1 diabetes patients presenting total testosterone, free testosterone, calculated free testosterone, and bioavailable testosterone values in the middle of the normal range [43]. Since fluctuations within a normal physiological range do not influence muscle protein synthesis [44,45], we conclude that this decrease has small clinical relevance in which concerns body composition or strength. Albeit a slight decrease in glycated hemoglobin (-0.3%) was detected with supplementation, it seems unlikely that this might have significantly influenced strength and body composition. In fact, some rodent model studies have suggested that leucine and its downstream metabolites might improve insulin sensitivity [43], however studies in humans also suggest the direct supply of amino acids as one of the main pathways involved [55]. Conversely, research in younger subjects with type 1 diabetes mellitus has suggested that leucine supplementation might lead to hyperglycemia with higher exogenous insulin administration being required for glycemic control [37]. Since glycated hemoglobin is influenced by small dietary changes (particularly in the last 30 days prior to assessment) [56], seasonal variations [57], and other factors [58], we cannot directly attribute body composition or strength outcomes to this blood marker. Altogether,  $\alpha$ -HICA did not adversely influence blood markers. This finding is consistent with recent studies of leucine and leucine-derivatives ( $\alpha$ -HICA included) administered safely to elderly populations [15,29,30]. Administration of  $\alpha$ -HICA for 120 days was well tolerated and produced no adverse effects upon our patient. Our findings confirm the marked safety profile noted in the literature [15,29,30]; hence,  $\alpha$ -HICA may be used in this population with confidence.

### Diet

A small energy increase was noted from baseline to day 120. According to food records, this increase was mainly due to an increased fat intake (+27 g), while protein and carbohydrate displayed only minor changes. There is no compelling evidence to support fat intake alone regarding these body composition and strength outcomes, however while ingesting sufficient protein, an energy increase might have contributed to some of the results reported herein. Notwithstanding, some research with leucine derivatives, shows improvements in body composition and strength with no significant increase in energy or macronutrients, both in healthy and mus-

cle-wasting conditions [31]. Therefore, the relevance of this 340 kcal increase while supplementing with  $\alpha$ -HICA, requires further investigation.

### Plausible Mechanisms

In “brittle” type 1 diabetes patients with rapidly changing insulin and glucose levels, protein degradation is enhanced, leading to a highly catabolic state reflected by accelerated muscle mass loss [6,32]. Such enhanced protein degradation contrasts with augmented splanchnic protein synthesis [33], likely attributable to increased availability of amino acids from skeletal muscle protein breakdown [7]. Both protein synthesis and degradation require high amounts of energy [33], and in the presence of chronically high circulating levels of glucagon and enhanced hepatic gluconeogenesis [34], a negative energy balance is easily attained, followed by weight loss. Insulinopenic animal models show an increase in UPP activity [35], phenotypically resembling type I diabetics. Therefore, it is reasonable to suspect that inhibiting UPP may lead to higher FFM retention. Both leucine and its downstream metabolites have shown the ability to inhibit muscle protein degradation [9]. In fact, recent research indicates that one leucine derivative ( $\beta$ -hydroxy- $\beta$ -methylbutyrate) might slow protein degradation through an alternative pathway to insulin [36]. One possible mechanism for the protective effect of  $\alpha$ -HICA upon muscle mass is through insulin-like growth factors (IGF-1), since leucine metabolites have displayed the capacity to enhance IGF-1 expression in skeletal muscle [9], although the evidence is equivocal [37]. Patients with type 1 diabetes may also experience reduced expression of IGF-1 [38], which attenuates anabolism, since this factor is involved in the regulation of skeletal muscle mass [39]. Another possible mechanism for  $\alpha$ -HICA-related muscle mass preservation is cortisol modulation, given the frequent elevation of this hormone in type 1 diabetics. Leucine metabolites have displayed promising effects in both reducing [40] and modulating cortisol [41]. This catabolic hormone is linked with increased protein degradation in animal models of type 1 diabetes [42].

Another plausible mechanism is the modulation of inflammatory cytokines by both leucine and downstream metabolites. Leucine has displayed, in tumor-bearing rats, the ability to modulate interleukin-6 (IL-6) levels, leading to protein preservation [46], while other leucine derivatives have exerted promising effects in modulating other inflammatory markers [47]. Coincidentally, IL-6 levels are upregulated in several type 1 diabetic subjects [42] and are associated with muscle wasting, presumably due to enhanced myostatin expression [48]. Rodent models suggest that elevated levels of IL-6 might induce muscle atrophy from both downregulation of growth factor-mediated intracellular signaling [49] and through

IL-6 ligands such as STAT3, since phosphorylation of this signal transducer is elevated in the presence of high levels of IL-6 [50]. Cachexic conditions observed in DM are complicated by the fact that chronic elevation of inflammatory markers such as C-reactive protein (CRP) and IL-6 are not only influenced by skeletal muscle [49], but also by the level of physical activity [51]. Acute phase response elevations in CRP and IL-6 follow a downward trend after exercise in healthy individuals [52], whereas in DM, CRP and cytokine levels remain largely unchanged [53]. In wasting conditions, the pattern of immunomodulation of inflammatory mediators and regeneration of muscle tissue differs from acute phase responses to chronic responses [54]. Subsequently, some researchers have focused solely on immunomodulation to attenuate inflammation in DM [51]. Evidence from clinical trials using interleukin antagonists reinforces the need to focus on methods of controlling inflammatory pathways outside of physical activity, such as dietary, nutraceutical or pharmaceutical interventions, as part of an overall immunomodulatory strategy in managing DM [51].

The changes observed in BMD were intriguing and worthy of further attention. A direct mechanism to explain the influence of  $\alpha$ -HICA on BMD is presently unknown. Some amino acids have been linked with bone health but not with leucine or its derivatives [59]. In fact, research with leucine metabolites shows no effect in bone mass of healthy young individuals [60]. Studies using leucine derivatives in populations with type 1 diabetes are insufficient in the literature. One possible mechanism that might explain amelioration of bone health by  $\alpha$ -HICA involves IGF-1. With aging and certain disease states, bone remodeling and formation tends to be seriously impaired due to low production of growth factors like growth hormone (GH) and IGF-1 [25]. Since GH and IGF-1 are necessary for osteoblast differentiation and function, decreased systemic and local skeletal production of IGF-1 as well as increased levels of growth factor binding proteins might down-regulate bone modeling in older individuals which is not adequate to maintain BMD [61]. Given that both aging and type 1 diabetes reduce IGF-1 expression, it is possible that  $\alpha$ -HICA might present favorable effects through the expression of IGF-1. A positive protein balance could also enhance skeleton health [62]. It has been observed that essential amino acids might improve bone matrix collagen protein [63]. In this regard, some amino acids like leucine or its downstream metabolites might present actions through their anabolic properties [64]. It is estimated that one-third of bone volume is 50 percent protein, which is involved in the synthesis of type I collagen through post-translational modifications of several amino acids [65]. Another factor that might influence bone health and strength is vitamin D. Albeit not being assessed in this case-study, a recent

cross-sectional study suggests that in the Portuguese population the median 25(OH)D is 35.9 nmol/L [66]. This value is deemed insufficient and inadequate according to the Endocrine Society and the Institute of Medicine, respectively [67]. Hence, it is unlikely that a vitamin D increase might have improved BMD. In which concerns strength, improvements in elderly populations have been reported with serum values ranging between 24.7 nmol/L and 74.9 nmol/L [68], therefore it is not clear whether a seasonal increase in vitamin D would have influenced strength results.

Given that, in some studies, leucine exerted a greater capacity to stimulate muscle protein synthesis and elicited a similar capacity to modulate muscle protein breakdown [69], it is unclear whether the effect of  $\alpha$ -HICA would be superior to leucine or to any other downstream leucine metabolite. In fact, some research indicates that a leucine rich diet might be beneficial in reducing protein degradation in adolescents with type 1 diabetes [37]. Further research should seek to clarify whether supplementing  $\alpha$ -HICA or leucine itself produces any benefit in skeletal muscle mass preservation in patients with type 1 diabetes. To further explore this issue, double-blind, placebo-controlled randomized trials are necessary, since this study is obviously a limited one case clinical study.

## LIMITATIONS

Due to the long duration of this case study (120 days), intermediate measures for body composition, strength and blood markers should have been performed for additional control, perhaps every 30 days. To establish clinical relevance for BMD gains, longer duration studies are mandatory. This study pertains to a single patient clinical case study; therefore, any conclusions should be drawn with extreme caution recognizing its inherent limitations.

## CONCLUSION

To our knowledge, this is the first reported case in which a type 1 diabetic patient with documented loss in muscle mass has been studied before and after supplementation with a leucine metabolite. Importantly, in this patient a full panel of blood biomarkers and weekly visits for nutritional counselling revealed no signal of an adverse effect. Salient increases in FFM, especially in the trunk area, hand grip strength, and BMD were noted. Due to the imperative of maintaining FFM and strength in these patients, additional research is necessary to clarify whether leucine and/or one of its derivatives might be clinically useful.

**Acknowledgments:** The authors would like to show



their appreciation to Vanessa Marques for all her support and assistance during all evaluations. Gratitude is also extended to Prof. Maria João Valamatos for all her kind support during strength evaluation.

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