Nutritional Supplements with Oral Amino Acid Mixtures Increases Whole-Body Lean Mass and Insulin Sensitivity in Elderly Subjects with Sarcopenia

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Decreases in whole-body lean mass can cause sarcopenia, a disease frequently found in the elderly. This condition is frequently associated with frailty and disability in aging as well as the onset and progression of several geriatric syndromes. Sarcopenia therefore must be managed with multidimensional approaches that include physical training, nutritional support, and metabolic and anabolic treatment. The purpose of our study was to assess the effect of an orally administered special mixture of amino acids (AAs) in elderly subjects with reduced lean body mass and sarcopenia. A randomized, open-label, crossover study was conducted in 41 elderly subjects (age range: 66–84 years) with sarcopenia, assigned to 2 distinct treatments (AAs and placebo). All subjects had normal body weight (body mass index within 19–23). The AA treatment consisted of 70.6 kcal/day (1 kcal = 4.2 kJ) of 8 g of essential AA snacks, given at 10:00 AM and 5:00 PM. Lean mass was measured with dual-energy x-ray absorptiometry in leg, arm, and trunk tissues. Significant increases in whole-body lean mass in all areas were seen after 6 months and more consistently after 18 months of oral nutritional supplementation with AAs. Fasting blood glucose, serum insulin, and homeostatic model assessment of insulin resistance (an index of insulin resistance) significantly decreased during AA treatment. Furthermore, a significant reduction in serum tumor necrosis factor–α (TNF-α) and a significant increase in both insulin-like growth factor–1 (IGF-1) serum concentrations and in the IGF-1/TNF-α ratio were also found. No significant adverse effects were observed during AA treatment. These preliminary data indicate that nutritional supplements with the oral AA mixture significantly increased whole-body lean mass in elderly subjects with sarcopenia. The improvement in the amount of whole-body lean mass could be linked to increased insulin sensitivity and anabolic conditions related to IGF-1 availability. **(Am J Cardiol 2008;101[suppl]:69E–77E)**

Skeletal muscle is the largest single amount of tissue in the body and contains >50% of the body's proteins. Muscle tissue also is among the main targets of insulin action that actively promotes protein anabolism, which occurs in the presence of normal or high systemic amino acid (AA) concentrations.

Sarcopenia, defined as a reduction in lean mass and muscle strength, is considered a frequent hallmark of the aging process and is present in elderly patients with various clinical syndromes. Sarcopenia is viewed as the consequence of multiple medical, behavioral, and environmental factors that are common in older people.[1–5]

Several possible mechanisms leading to sarcopenia have been identified, including loss of α-motor neurons in the spinal column, impairment of endogenous growth hormone and insulin-like growth factor–1 (IGF-1) secretions, deficiency of androgen and estrogen production, inadequate protein intake, upregulation of catabolic cytokines, and reduced physical activity.[5,6] It is generally accepted that age-associated changes in body composition may depend on lower levels of anabolic hormones, neuromuscular alterations, and on the decline in muscle protein turnover.[7–9]

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These impairments in the quantity and quality of contractile protein contribute to physical disability and frailty, loss of independent function, risk of falling and fractures, and increased healthcare costs.[1,10,11] Therefore, the reduction in muscle mass and prolonged inactivity in elderly subjects could decrease insulin sensitivity and consequently impair glucose uptake, storage, and use in peripheral tissues and particularly in muscle.

Within this context, dietary AA and protein requirements could be significantly increased in elderly patients in an effort to antagonize muscle catabolism and to stimulate protein synthesis.[12–14] The recovery of anabolic conditions that enhance endogenous protein synthesis and adenosine triphosphate (ATP) production by cells could potentially induce beneficial effects to restore muscle integrity and metabolic functions and, consequently, enhance insulin activity and sensitivity. This condition could be beneficial in elderly patients with sarcopenia and could be easily achieved with AA supplementation.[15–19]

In an attempt to verify this hypothesis, this study was undertaken to evaluate whether increased nutritional support with an oral AA special mixture, composed of essential AAs, could improve lean mass (muscle mass) and insulin sensitivity in elderly outpatients with sarcopenia.

Subjects and Methods

Subjects and experimental design: A randomized, open-label, crossover study of AAs versus placebo was designed and conducted in 41 consecutive elderly outpatients with overt sarcopenia and reduced whole-body lean mass. The diagnosis of sarcopenia was confirmed by clinical evaluations (physical and anthropometric) and by instrumental validation with dual energy x-ray absorptiometry (DEXA) (DPX-L Lunar; Radiation Corporation, Madison, WI) that demonstrated reduced lean mass in leg, arm, and trunk tissues. All subjects gave their informed consent.

The age of the subjects with sarcopenia ranged from 66–84 years; body weight (expressed as body mass index) ranged from 19–23. The mean and standard deviation of total body fat mass of subjects with sarcopenia, measured by bioelectrical impedance analysis, was 26.5% ± 3.6 %.

All subjects had good glycometabolic control and were free of diabetes mellitus and metabolic and cardiovascular disorders. Nevertheless, all elderly subjects with sarcopenia had moderate insulin resistance and hyperinsulinemia. A group of age-matched healthy elderly subjects without sarcopenia was analyzed in order to compare laboratory features and DEXA values.

The oral AA mixture (Big One; Professional Dietetics, Milan, Italy), administered as a nutritional support, and isocaloric placebo were ingested as snacks at 10:00 AM and 5:00 PM, maintaining a total daily caloric intake of 2,000 ± 280 kcal (55% carbohydrates, 30% lipids, 15% proteins; 1 kcal = 4.2 kJ). Breakfast, lunch, and dinner were normally scheduled at 8:00 AM, 1:00 PM, and 8:00 PM. The AA preparation (70.6 kcal/day) contained 8 g/day of AAs, divided as follows: L-leucine, 2.5 g; L-lysine, 1.3 g; L-isoleucine, 1.25 g; L-valine, 1.25 g; L-threonine, 0.7 g; L-cysteine, 0.3 g; L-histidine, 0.3 g; L-phenylalanine, 0.2 g; L-methionine, 0.1 g; L-thyrosine, 0.06 g; and L-tryptophan, 0.04 g.

This randomized protocol study was divided into different phases: (1) a run-in period of 30 days and the baseline evaluation, performed before administration of either AAs or placebo; (2) randomization in 2 different groups of subjects for 4 months of treatment with AAs (group A) or placebo (group B); (3) a washout period of 15 days for both groups; (4) crossover of the AA group to placebo and of the placebo group to AAs; (5) a second period of 4 months of treatment with AAs or placebo; and (6) a final treatment period of 8 months with AAs for both groups (maintenance treatment period with AAs). At the beginning of the randomization, 19 patients were assigned after baseline to group A and 22 patients to group B.

The following parameters were examined at baseline and after 4, 6, 8, and 16 months: body mass index, arterial blood pressure, fasting blood glucose, fasting serum insulin, IGF-1, tumor necrosis factor–α (TNF-α), and IGF-1/TNF-α ratio. The degree of insulin resistance was estimated by homeostatic model assessment of insulin resistance (HOMA-IR) using the following equation: fasting insulin (microunits per milliliter) × fasting blood glucose (calculated as millimoles per liter)/22.5. The normal HOMA-IR value determined in our control population of 480 healthy elderly subjects (age range, 65–83 years) was <2.3.

Laboratory and statistical procedures: Blood samples were collected by venipuncture and centrifuged (3,000 rpm for 5 minutes) at 5°C in a refrigerated centrifuge (Heraeus, Hanau, Germany). Blood glucose was determined by using a fully automated method (DASIT-ISE Autoanalyzer; DASIT-ISE, Bareggio, Italy). Fasting serum insulin was determined by fluoroimmunoassay (Delfia-insulin; Perkin Elmer Life Sciences Inc., Boston, MA).

IGF-1 and TNF-α were extracted and measured in 500 μL of supernatants of cultured peripheral blood mononuclear cells (PBMC) of elderly subjects with and without sarcopenia. Hence, IGF-1 and TNF-α were determined as a secretion product of circulating PBMC and analyzed by specific highly sensitive enzyme-linked immunosorbent assay (Quantikine human; R&D Systems Inc., Minneapolis, MN). The intra- and interassay precisions were, respectively, <5% and <8%. The sensitivity of the methods were, respectively, <0.1 ng/L and <0.5 pg/mL. PBMC were separated by Ficoll-Hypaque density centrifugation and concentrated at measured density of 7.75×10^6 cells/mL in complete RPMI (Roswell Park Memorial Institute) medium. PBMC were incubated for 20 hours at 37°C in a 5% CO_2 incubator (Heraeus BB6220; Heraeus); IGF-1 was elevated after 20 hours of exposure to growth hormone (Sigma Chimica, Milan, Italy) concentrated at 2 μg/mL. The com-

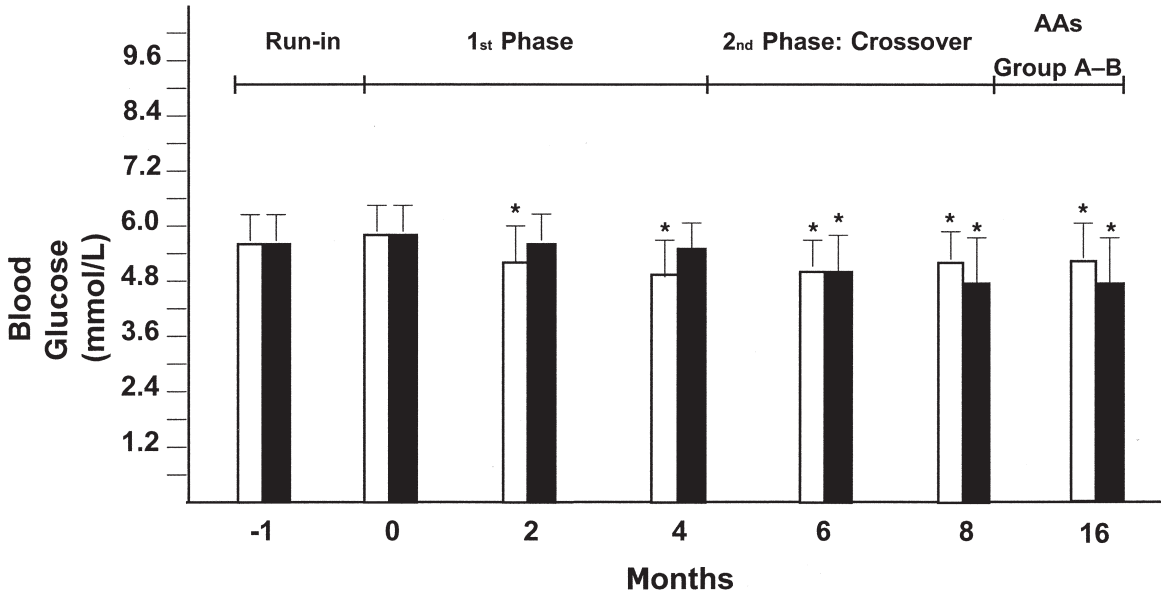

Figure 1. Mean ± SD values of fasting blood glucose in elderly subjects with sarcopenia in group A (*open bars*) and group B (*solid bars*) during supplementation with amino acids (AAs). *p <0.05 vs baseline.

plete PBMC extraction and incubation procedure has been described in detail in previous studies.[20–23]

Data were expressed as mean ± SD. The crossover treatment trial was analyzed as described by Doehner and associates.[24] Repeated measures analysis of variance and the paired Student *t*-test were used for statistical analysis where appropriate. A p value <0.05 was considered statistically significant.

Results

A mild but significant increase in body weight was demonstrated at the end of the study (after 16 months) in both groups of elderly subjects with sarcopenia. In effect, the mean values of body mass index significantly increased in Group A from 20.9 ± 1.2 to 22.3 ± 1.7 (p <0.05), and in Group B from 20.6 ± 1.4 to 22.5 ± 1.9 (p <0.01); whereas the total body fat mass remained unchanged throughout the study (from 26.5% ± 3.6% to 26.9 ± 3.9 %). No changes in arterial blood pressure levels or heart rate were found (data not shown).

Figure 1 shows the mean variations in fasting blood glucose levels during the study. Blood glucose concentrations at baseline were within normal limits (<6.0 mmol/L), even if the mean blood glucose value of matched healthy elderly subjects was <5.2 mmol/L. Nevertheless, a mild but significant reduction in blood glucose levels was found in both groups after just 2 months of treatment with AAs and also at the end of the study.

Figure 2 shows the mean changes in fasting serum insulin levels during the study. Insulin levels at baseline were significantly increased in both groups of elderly subjects with sarcopenia compared with matched healthy controls (14.7 ± 3.2 μU/mL vs 9.3 ± 2.1 μU/mL; p<0.001). A significant reduction in fasting insulin levels was demonstrated in group A after 2 months of treatment with AAs. The reduction was more pronounced from 4 months to 16 months of treatment. Insulin remained unchanged in group B before AA treatment (AAs started after the 4-month placebo trial), whereas a significant decrease in insulin was found when these patients were assigned to AA treatment (from the crossover at 4 months to the 16 months' trial with AAs).

Figure 3 displays the insulin resistance pattern evaluated by the HOMA-IR analysis during the study. HOMA-IR levels at baseline significantly increased in both groups of elderly subjects with sarcopenia compared with matched healthy controls (4.8 ± 0.6 ratio vs 1.8 ± 0.2 ratio; p <0.001). Similar to that found for fasting insulin, a significant decrease in HOMA-IR was demonstrated in group A after 2 months' treatment with AAs. This decrease became more pronounced as the study continued and was also observed in group B when these subjects were recruited for AA treatment (from the crossover at 4 months to the end of the study at 16 months).

Hence, in both groups of elderly subjects with sarcopenia, decreases in blood glucose, insulin, and the insulin-resistance index (HOMA-IR) were associated with AA supplements, persisting also in group A after the crossover to placebo.

Figures 4 and 5 report TNF-α and IGF-1 concentrations, evaluated in the supernatants of PBMC of elderly subjects with sarcopenia (groups A and B) during the study. TNF-α and IGF-1 concentrations were respectively significantly increased and reduced in both groups of elderly subjects

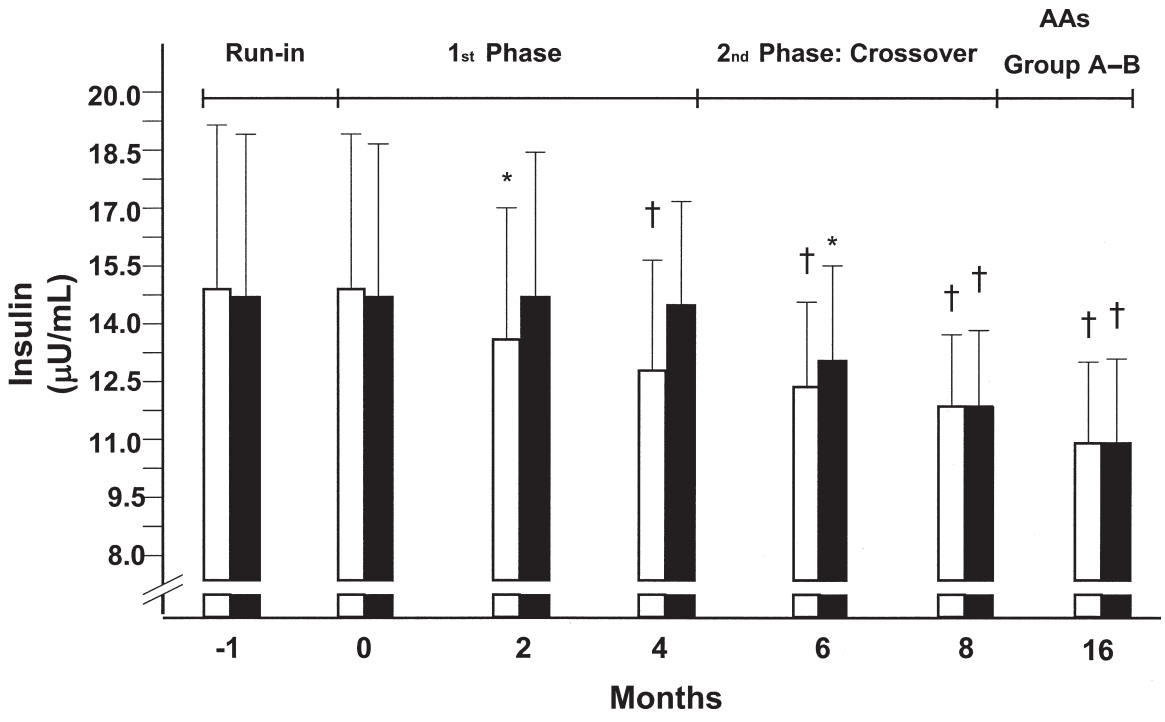

Figure 2. Mean ± SD values of fasting serum insulin in elderly subjects with sarcopenia in group A (*open bars*) and group B (*solid bars*) during supplementation with amino acids (AAs). *p <0.01 vs baseline; †p <0.001 vs baseline.

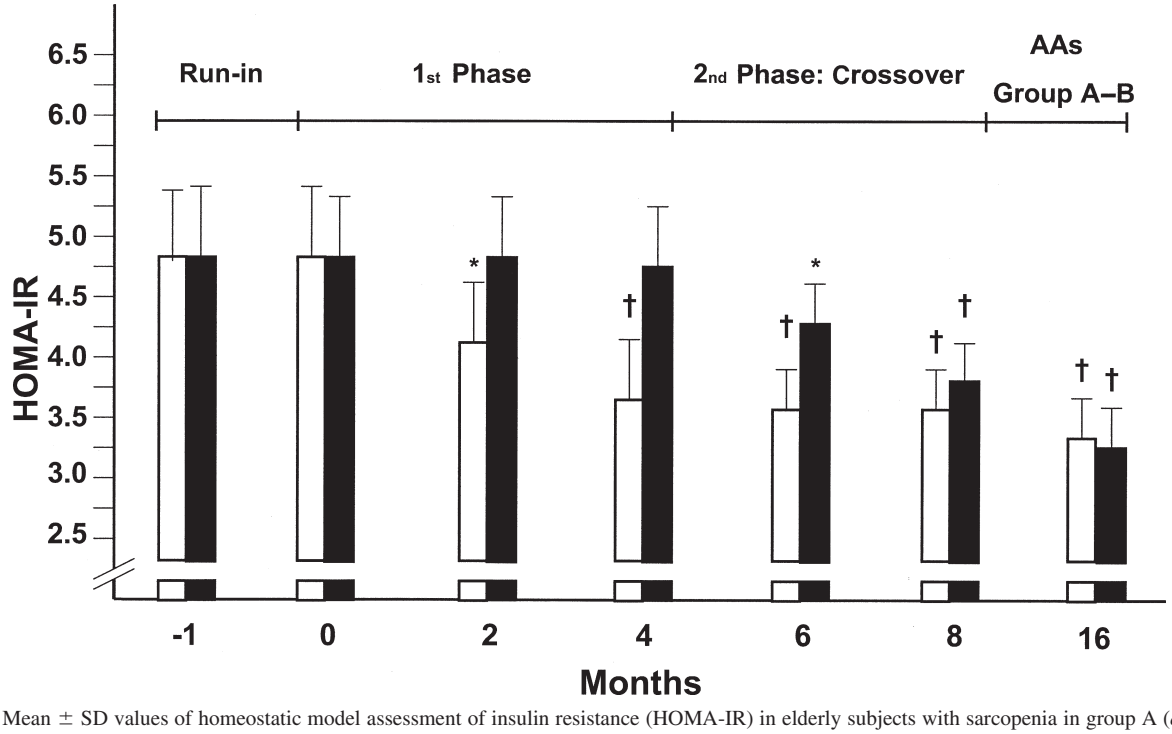

Figure 3. Mean ± SD values of homeostatic model assessment of insulin resistance (HOMA-IR) in elderly subjects with sarcopenia in group A (*open bars*) and group B (*solid bars*) during supplementation with amino acids (AAs). *p <0.01 vs baseline; †p <0.001 vs baseline.

with sarcopenia compared with matched healthy controls (160 ± 33 pg/mL and 14 ± 2 ng/L, p <0.001, vs 86 ± 18 pg/mL and 19 ± 3 ng/L, p <0.001, respectively). A significant reduction in TNF-α levels was found after 2 months in elderly subjects with sarcopenia initially randomized to AA supplements (group A), and these results were reinforced during the treatment and also after the crossover to placebo. In parallel with the decrease in TNF-α, a significant increase in IGF-1 was found during AA supplementation in group A, and this increase was more consistent after 8 and 16 months,

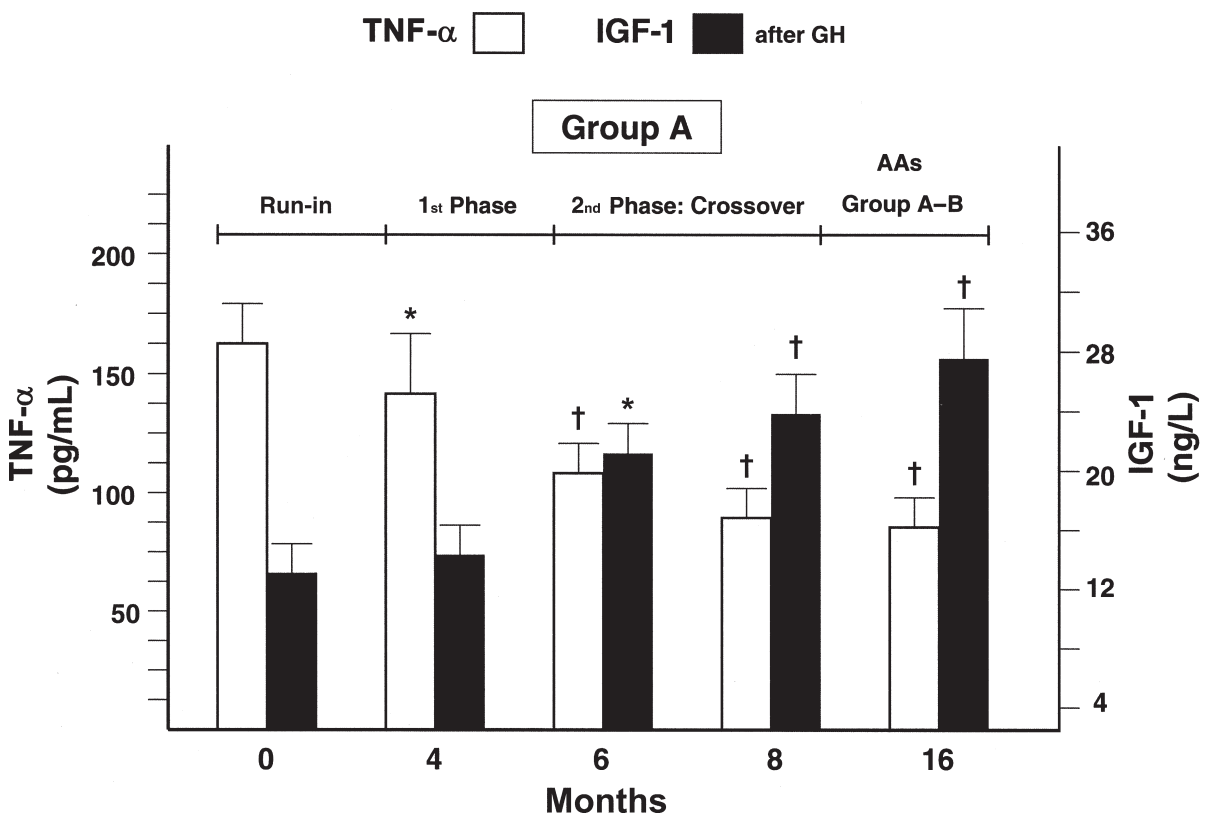

Figure 4. Mean ± SD values of tumor necrosis factor–α (TNF-α) and insulin-like growth factor–1 (IGF-1) in elderly subjects with sarcopenia in group A during supplementation with amino acids (AAs). GH = growth hormone. *p <0.01 vs baseline; †p <0.001 vs baseline.

also persisting during crossover to placebo. Similar to that observed in group A, TNF-α levels were significantly reduced and IGF-1 concentrations significantly increased after crossover from placebo to AA supplementation (from month 8 to month 16) in group B. TNF-α and IGF-1 of group B exhibited a similar pattern of changes compared with group A.

Figure 6 shows changes in TNF-α and IGF-1 patterns represented as the IGF/TNF ratio index. It is clear that both groups of elderly subjects with sarcopenia demonstrated an increase in the IGF/TNF ratio after taking AA supplements (at 2 months in group A, 8 months in group B) and that this increase persisted until the end of the study. Furthermore, the increase of IGF/TNF ratio persisted after the crossover to placebo in group A.

Finally, Figure 7 displays changes in whole-body lean mass evaluated by DEXA in elderly subjects with sarcopenia. Whole-body lean mass values (expressed in kilograms) significantly decreased in the sarcopenic groups (A and B) compared with matched nonsarcopenic controls. This reduction was significant in all regions evaluated by DEXA: legs ($p < 0.001$), arms ($p < 0.001$), and trunk tissue ($p < 0.01$) (data not shown). A significant increase in whole-body lean mass was found in both groups of subjects with sarcopenia after 8 and 16 months of AA supplements, reaching the normal values found in age-matched nonsarcopenic healthy controls at the end of the study.

Interestingly, AA supplements did not influence renal function (data not shown).

Discussion

Sarcopenia, the pathologic decrease in whole-body lean mass in the elderly, is characterized by increased frailty, polypathology, and disability related to hypocynetic conditions. This deleterious condition of aging carries high economic and social costs. Therefore, the correction of sarcopenia can be considered a primary outcome of geriatric rehabilitative procedures.

Our clinical investigation clearly demonstrated that long-term nutritional supplementation with a special mixture of oral AAs significantly increased lean body mass in elderly subjects with sarcopenia until they normalized fat-free body mass within 16 months of treatment. This observation could be very important in the clinical management of sarcopenia, and indicates the positive approach of nutritional support in the correction of one of the most important conditions associated with geriatric syndromes.

It is well known that advanced adult age is associated with changes in body composition, such as increased total fat mass and decreased bone and muscle mass. Severe loss of muscle mass, or sarcopenia, is characterized by a decrease in the total number of muscle fibers, reduced thigh

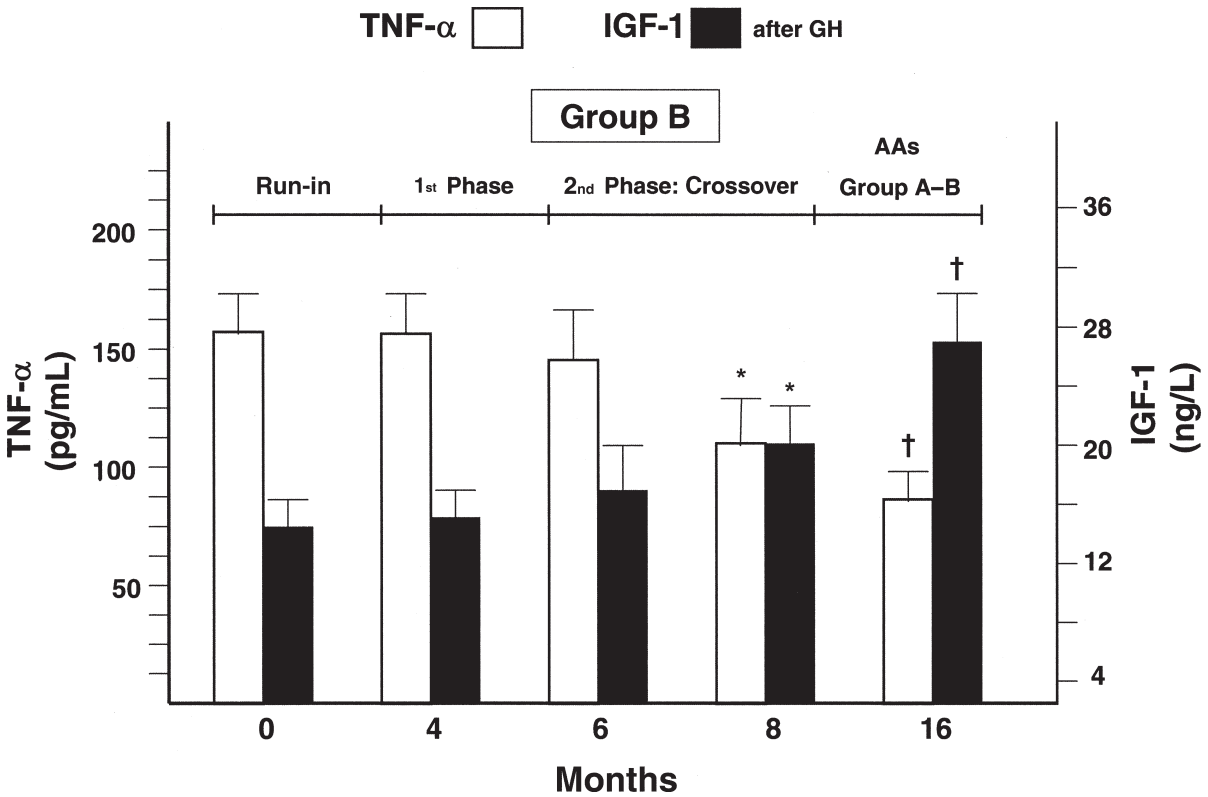

Figure 5. Mean ± SD values of tumor necrosis factor–α (TNF-α) and insulin-like growth factor–1 (IGF-1) in elderly subjects with sarcopenia in group B during supplementation with amino acids (AAs). GH = growth hormone. *p <0.01 vs baseline; †p <0.001 vs baseline.

cross-sectional areas, and decreased muscle density associated with increased intramuscular deposition of fat. Loss of skeletal muscle mass may be a common pathway by which multiple diseases contribute to disability. In effect, decreased muscle mass is associated with an increased risk for morbidity, mortality, and disability in old age, but the mechanisms by which this occurs are not fully understood.

Geriatricians are very interested in the study of sarcopenia because of its detrimental effect on physical function and functional ability. A number of physiologic functions that depend on muscle tissue have a critical effect on human metabolism: Muscles are an important reserve of body proteins and energy that can be used in extreme conditions of stress or malnutrition. AAs can be mobilized during acute infections and used as building blocks for antibodies; hormones are produced and catabolized within muscle tissue. Thus, it should not be surprising that reduced muscle mass has a negative impact on metabolic adaptation and immunologic responses to disease, reducing the body's ability to resist environmental challenges and so inducing frailty, disability, and further loss of muscle mass.[1,10,11]

Current views consider sarcopenia to be the consequence of multiple medical, behavioral, and environmental factors typically present in the elderly.[1–5] Certain conditions involve the nervous system, exercise and training, hormonal pathways (eg, growth hormone–IGF-1 and testosterone decrease), nutrition, muscle protein turnover, proinflammatory conditions, and the load of reactive oxygen species within the muscle mitochondria.[3,6,8,9,25–28]

Regarding the age-associated decline in muscle mass, we can hypothesize a reduction of whole-body lean mass loss by nutritional implementation with essential AAs. Indeed, AAs can antagonize muscle catabolism, improving global protein synthesis in skeletal and cardiac muscle.[12,29–31]

Anabolic conditions that enhance endogenous protein synthesis and ATP production could potentially benefit the restoration of muscle integrity and functions and, consequently, improve insulin sensitivity in muscles. This situation could be very useful in elderly patients with sarcopenia and could potentially be achieved through use of AA supplements.[15–19,31,32] In effect, experimental observations demonstrate that AAs decrease insulin resistance[33,34] and reduce glycosylated hemoglobin concentrations.[35]

Our data indicate that long-term nutritional supplementation with AAs causes protein anabolism in elderly subjects with sarcopenia. This condition improves muscle protein synthesis, whole-body lean mass, and, consequently, insulin sensitivity, with beneficial effects on metabolic and physical status. Our data demonstrated that use of AA supplements in elderly subjects with sarcopenia resulted in several important beneficial changes, including an increase in muscle anabolism, as demonstrated by cytokine and growth factor pathways. In effect, we found a reduction in catabolic cytokines (TNF-α) and increased anabolic growth factors

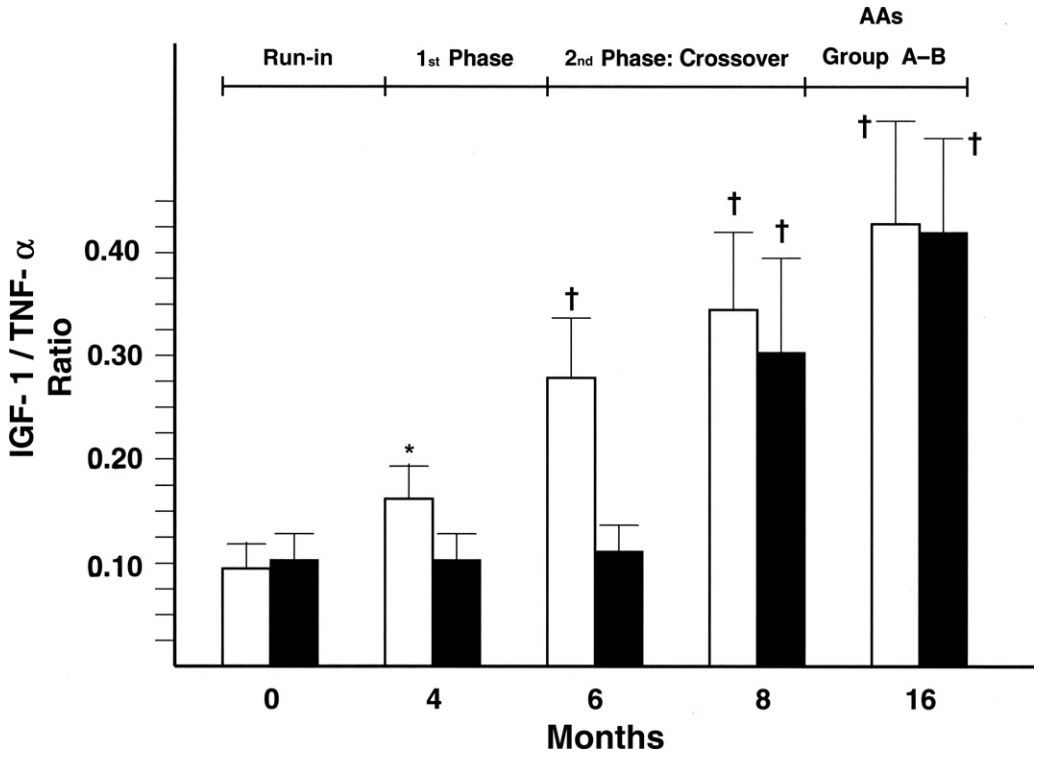

Figure 6. Mean ± SD values of insulin-like growth factor–1/ tumor necrosis factor–α (IGF-1/TNF-α) ratio in elderly subjects with sarcopenia in group A (*open bars*) and group B (*solid bars*) during supplementation with amino acids (AAs). *p <0.01 vs baseline; †p <0.001 vs baseline.

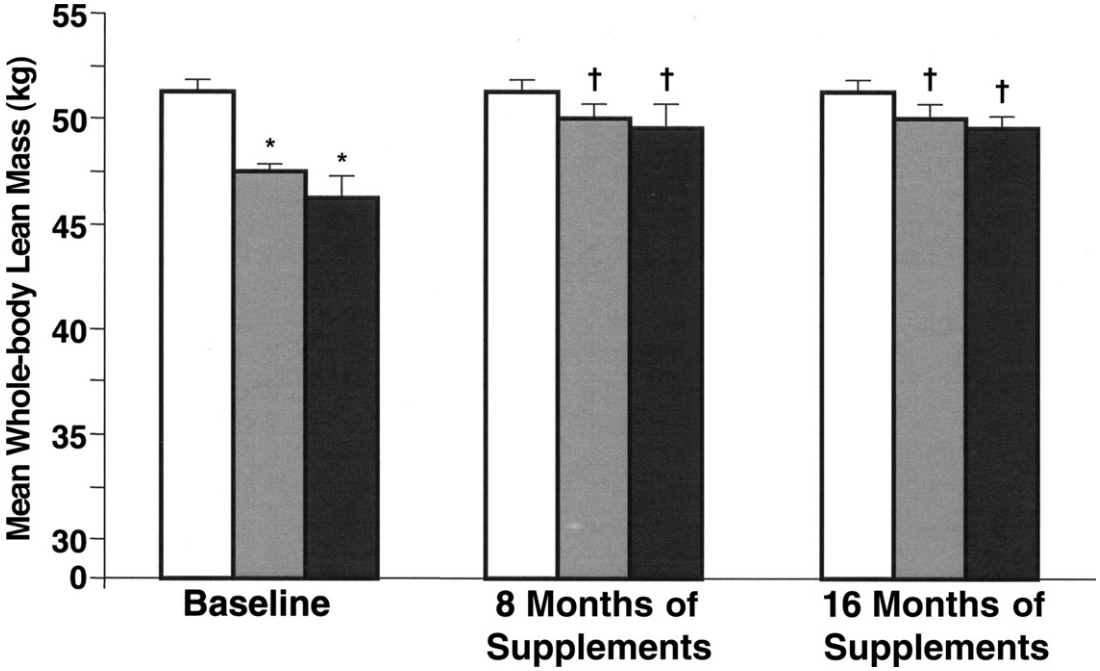

Figure 7. Mean values of whole-body lean mass evaluated by dual x-ray absorptiometry (DEXA) in elderly subjects with sarcopenia in group A (*light gray bars*) and group B (*dark gray bars*) during supplementation with amino acids (AAs) compared with nonsarcopenic matched controls (*white bars*). *p <0.01 vs nonsarcopenic subjects; †p <0.01 vs baseline.

(IGF-1), with a consequent increase in the IGF-1/TNF-α ratio, thus demonstrating a transition from catabolic to anabolic conditions. Therefore, use of nutritional AA supplements in elderly subjects with sarcopenia causes an anabolic shift, suggesting that AA treatment could be extended to most geriatric syndromes in which this disorder is present. These data are confirmed by the evident increase in the whole-body lean mass after 8–18 months of treatment.

Furthermore, the increase in lean body mass is presumably the cause of improved insulin sensitivity observed in elderly patients with sarcopenia, as demonstrated by reduced fasting insulin levels, HOMA-IR, and fasting blood glucose concentrations. AAs can potentially decrease hepatic blood glucose flow by increasing insulin sensitivity in skeletal muscle, obtaining a double effect, ie, restoring muscle mass and activity and inducing anabolism and increased glucose uptake. Therefore, we have demonstrated that sarcopenia and insulin resistance are strongly associated in elderly people and that essential AAs can correct both alterations by means of increasing mitochondrial activity and ATP production by cells.

Conclusion

Our study shows that nutritional supplementation with oral AA mixtures increases whole-body lean mass in elderly subjects with sarcopenia. More studies need to be performed, both along these lines and also to develop new perspectives about the nutritional approach to sarcopenia.

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The authors who contributed to this article have disclosed the following industry relationships:

Sebastiano B. Solerte, MD, PhD, has no financial arrangement or affiliation with a corporate organization or a manufacturer of a product discussed in this article.

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