The reduction of muscle mass and increased protein catabolism in aging can determine the occurrence of metabolic alterations—such as hyperglycemia and reduced insulin sensitivity—in elderly subjects with diabetes mellitus. Therefore, the aim of the study was to evaluate the effect of nutritional supplementation with oral amino acid mixture (OAAM) in elderly subjects with type 2 diabetes. This approach was conducted in an attempt to antagonize muscle catabolism by means of increased endogenous protein synthesis and to improve glucose metabolism and insulin sensitivity. A randomized, open-label, crossover study was conducted in poorly controlled (glycosylated hemoglobin level [HbA1c] > 7%) elderly subjects (age range, 65 to 85 years) with type 2 diabetes. OAAM significantly reduced fasting and postprandial blood glucose and HbA1c, whereas all parameters remained substantially unchanged in the group treated with placebo. Fasting insulin levels and insulin resistance increased at baseline in all subjects with diabetes and decreased during OAAM supplementation. These results persisted also after crossover from OAAM to placebo. No changes in blood lipid levels, creatinine, homocysteine, and urinary albumin excretion rate were observed throughout the study, whereas a mild but significant increase of high-density lipoprotein cholesterol was found after OAAM supplementation. We suggest that increased amino acid availability for skeletal muscle function and strength could ameliorate metabolic control and insulin sensitivity in elderly patients with poorly controlled type 2 diabetes.

Skeletal muscle is the largest tissue in the body and contains 50% of the body’s proteins. Muscle is among the main targets of insulin action that promote protein anabolism; however, this occurs only in the presence of either normal or high systemic amino acid concentrations, thus regulating the rate of glucose uptake, storage, and utilization. In fact, 75% of the glucose in a carbohydrate meal is taken up in the postprandial state by muscle and stored mainly as glycogen. This tissue is therefore important for glucose metabolism in normal and pathologic clinical conditions (ie, obesity and diabetes mellitus) and represents an attractive therapeutic target for the prevention of peripheral insulin resistance and type 2 diabetes.1,2

The reduction of muscle mass and function in the elderly could potentially result in impaired glucose utilization and storage because of impaired insulin sensitivity, a condition frequently observed in such patients. It is well recognized that a reduction in insulin activity is often present in critically ill elderly patients, as well as in elderly patients with impaired glucose tolerance and overt diabetes. Dietary amino acid and protein requirements could be significantly increased in elderly patients with diabetes in an effort to antagonize muscle catabolism and to stimulate net muscle protein synthesis.3–7

Anabolic conditions that are able to enhance endogenous protein synthesis and adenosine triphosphate production by cells could potentially induce beneficial effects in restoring muscle integrity and metabolic functions and, consequently, enhance insulin activity and sensitivity of muscle. This situation could be useful in elderly patients with poorly controlled diabetes and could be achieved by amino acid supplementation; amino acids have been shown to decrease insulin resistance8 and reduce glycosylated hemoglobin (HbA1c).9

The present investigation was undertaken to evaluate whether increased nutritional support with an oral amino acid mixture (OAAM) composed of essential amino acids could improve glycemic control and insulin sensitivity in elderly patients with poorly controlled type 2 diabetes.

SUBJECTS AND METHODS

Subjects and study protocol: We conducted a randomized, open-label, crossover study of OAAM ver-
sus placebo in 34 consecutive elderly subjects with type 2 diabetes. All subjects gave their written informed consent to participate in the study. The age range of the subjects was 65 to 83 years, and the length of time since diabetes diagnosis ranged from 5 to 15 years. Body weight (expressed as body mass index; range, 18 to 23) was within normal limits. All patients had diabetes that was poorly controlled, as defined by metabolic control, with HbA1c >7% (range, 7.2% to 10.5%). In all, 25 patients were treated with oral hypoglycemic agents (9 with metformin alone, 8 with the combination of metformin with glimepiride, 5 with the combination of repaglinide with metformin, 3 with glimepiride), and 9 patients were treated with recombinant human insulin.

Exclusion criteria for enrollment were as follows: severe diabetic neuropathy and retinopathy, overt diabetic nephropathy (urinary albumin excretion rate >200 μg/min), diabetic ketoacidosis, renal or hepatic failure, coronary and peripheral macroangiopathy, arterial hypertensive disease, and other systemic diseases.

OAAM (449 kcal/day) and placebo were ingested as snacks at 10:00 AM and 6:00 PM, maintaining a total daily caloric intake of 1,600 ± 370 kcal (55% carbohydrates, 30% lipids, 15% proteins). Breakfast, lunch, and dinner were scheduled at 7:00 AM, 12 noon, and 7:30 PM. The OAAM preparation (Big One; Professional Dietetics SRL, Milan, Italy) contained 8 g/day of amino acids, fractionated in the following manner: 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-tyrosine, 0.06 g; L-tryptophan, 0.04 g. Total lipids were 0.43 g, and carbohydrates were 8.15 g.

The randomized study consisted of 5 phases: (1) a run-in period and baseline examination 2 weeks before OAAM or placebo administration; (2) randomization into a 16-week treatment period with OAAM or placebo; (3) a washout period of 2 weeks (from week 16 to week 18); (4) crossover of the OAAM group to placebo and the placebo group to OAAM; and (5) a second period of 16 weeks of treatment either on OAAM or on placebo. Of the 34 subjects, 18 were strictly assigned to group A (initial randomization to OAAM) and 16 were assigned to group B (initial randomization to placebo). The crossover of group A to placebo and of group B to OAAM was scheduled after week 18 (end of the 2-week washout period). OAAM and placebo treatments were given in addition to conventional antidiabetic therapy.

The following measures were obtained at baseline (2 weeks before the study and at the start) and after 4, 8, 12, 16, 18, 22, 26, 30, and 34 weeks: body mass index, arterial blood pressure, fasting blood glucose, postprandial (1 hour and 2 hours) blood glucose, HbA1c, total and high-density lipoprotein (HDL) cholesterol and triglycerides (TGs), fasting serum insulin, homocysteine, urinary albumin excretion rate (expressed as micrograms per minute in a 24-hour collection specimen), serum creatinine, and the degree of insulin resistance. Insulin resistance was estimated using the homeostasis model assessment for insulin resistance (HOMAIR): fasting insulin (μU/mL) × fasting blood glucose (mmol/L)/22.5.10 The normal HOMAIR value measured in our control population of 350 healthy elderly subjects (age range, 67 to 81 years) was <2.4.

Laboratory procedures: Blood glucose (fasting and postprandial), creatinine, total and HDL cholesterol, and TGs were determined by using a fully automated method following the single manufacturer’s protocols (Dasit-Ise Autoanalyzer; Dasit, Bareggio, Italy). HbA1c and serum homocysteine were measured by high-performance liquid chromatography (Bio-Rad Diagnostics Group, Hercules, CA). Fasting serum insulin was determined by fluoroimmunoassay (Delfia-insulin; PerkinElmer Life Sciences Inc., Boston, MA). Urinary albumin excretion rate was measured by fully automated computerized immunonephelometry (Dade Behring, Milan, Italy).11 Data were expressed as mean ± SD; 2-way analysis of variance with the Fisher protected least-significant test and unpaired Student t test were used for statistical analysis. A p value <0.05 by the 2-tailed test was considered significant.

RESULTS

Neither body weight nor arterial blood pressure levels varied in group A or group B during the study. The results of the fasting and postprandial blood glucose levels are represented in Figures 1 through 3. A significant reduction in fasting blood glucose was demonstrated in group A as soon as 8 weeks after initiation of nutritional support with OAAM. The reduction was more pronounced at 16 weeks (Figure 1). The effect was maintained during the washout period and after crossover from OAAM to placebo (from week 18 to week 34). Conversely, fasting blood glucose was unchanged in group B from baseline through 18 weeks; however, a significant decrease of fasting blood glucose was found when these patients were assigned to treatment with OAAM (after the crossover at week 18).

Similarly to what was found regarding fasting blood glucose, a significant but more consistent and early decrease of postprandial blood glucose (at 1 and 2 hours) was observed in group A (Figures 2 and 3); this decrease was consistent throughout crossover from OAAM to placebo (from week 18 to week 34). Conversely, postprandial blood glucose remained unchanged in group B during treatment with placebo (from baseline to week 16), and only a borderline significant reduction of postprandial blood glucose was observed at week 16. However, a significant and persistent decrease of postprandial blood glucose was found when these patients were treated with OAAM (ie, after the crossover of week 18). Hence, in both groups of elderly subjects with diabetes, the decrease of fasting and postprandial blood glucose was strongly associated with OAAM supplementation, paralleling the period of treatment with OAAM and persisting in group A after crossover to placebo.
Figure 4 shows the mean variations of HbA\textsubscript{1c} in groups A and B throughout the 34-week follow-up period. A highly significant reduction of HbA\textsubscript{1c} levels was demonstrated by 8 weeks in subjects initially randomized to OAAM supplementation (group A), and these findings were maintained in these subjects after the crossover from OAAM to placebo. HbA\textsubscript{1c} remained unchanged in subjects initially randomized to placebo (group B), but it was significantly reduced from week 26 to week 34, after crossover from placebo to OAAM supplementation.

Figures 5 and 6 report serum insulin and HOMA\textsubscript{IR} variations in groups A and B during the study. Fasting serum insulin levels in groups A and B were significantly higher than the insulin levels of the control population of 350 healthy elderly subjects with no diabetes (10.6 ± 2.2 μU/mL, \(p < 0.001\)). A significant reduction in insulin concentration was observed after 8 weeks in subjects with diabetes initially randomized for OAAM supplementation (group A), and these results were consistent throughout OAAM treatment and after crossover to placebo. Similar to what was observed in group A, serum insulin levels were significantly reduced after crossover from placebo to OAAM supplementation (from week 26 to week 34) in group B.

HOMA\textsubscript{IR} exhibited a similar pattern of decrease as did serum insulin levels. In effect, a significant de-
crease of HOMA IR was demonstrated as early as 8 weeks after randomization in subjects treated with OAAM supplementation (group A), and this pattern was maintained up to the end of the follow-up period and after crossover from OAAM to placebo. HOMA IR remained unchanged in subjects initially randomized to placebo (group B) and then was significantly reduced from week 22 to week 34 after crossover from placebo to OAAM supplementation.

Table 1 summarizes the other biochemical variables evaluated in groups A and B throughout the study period. No changes in serum creatinine, homocysteine, or urinary albumin excretion rate were demonstrated during placebo and OAAM supplementation. Total cholesterol and TG levels remained unchanged, whereas HDL-cholesterol levels were slightly but significantly increased after OAAM supplementation in both groups.

**DISCUSSION**

Nutritional supplementation with OAAM significantly improved metabolic control and insulin sensitivity in elderly subjects with poorly controlled type 2 diabetes. These data suggest a beneficial effect of OAAM supplementation in the regulation of glucose metabolism and of insulin activity in elderly patients with type 2 diabetes.

Our data clearly demonstrate that short-term (16 weeks) OAAM supplementation decreases fasting and postprandial hyperglycemia and reduces HbA1c levels.
within 8 weeks of treatment. These metabolic effects were maintained during the crossover with placebo, even after the end of OAAM support.

Several hypotheses can be proposed to explain the mechanisms involved in this phenomenon. It is well known that free amino acid plasma concentrations are increased in long-term diabetes. This fact can be dependent on increased protein turnover and catabolism, on increased gluconeogenesis, or on decreased synthesis of structural proteins. OAAM or parenteral amino acid supplementation may scavenge glucose, thereby decreasing hyperglycemia and glycemic excursions and sparing the protein amino acids. OAAM may also positively affect protein anabolism by lowering amino acids in plasma and by stimulating net muscle protein synthesis and glucose storage by insulin-sensitive tissues. This process is particularly evident in elderly subjects when OAAM supplementation was introduced along with several other strategies to counteract skeletal muscle loss and sarcopenia.

The capability of OAAM to increase protein anabolism and muscle tissue synthesis is of definite importance in restoring blood glucose levels and in improving insulin sensitivity. Amino acids also upregulate insulin receptor synthesis and its autophosphorylation in animals and in individuals with type 2 diabetes. Insulin activity, in addition to insulin secretion, could therefore be potentially enhanced during amino acid supplementation in subjects with diabetes, thus contributing to normalizing blood glucose levels. This fact is demonstrated in our study. Fasting hyperinsulinemia was significantly decreased during OAAM, and insulin sensitivity, which was evaluated by HOMA-IR.
MAIR, was also improved after the end of OAAM supplementation and after crossover to placebo. The amelioration of insulin sensitivity and the reduction of fasting hyperinsulinemia can be considered important metabolic consequences of oral amino acid support in these elderly subjects with diabetes. These effects could be dependent on recovery of insulin action on the muscle target or could be due to an insulin-dependent increase of skeletal muscle anabolism and mass.

On the other hand, it has been well demonstrated that oral supplementation with arginine, glycine, and cysteine improves insulin sensitivity and muscle glucose metabolism; it has also been shown that leucine enhances protein synthesis in the skeletal muscle through an insulin-dependent mechanism.17–21 All of these mechanisms could contribute to enhance the insulin-dependent action of glucose removal from circulation and thereby enhance blood glucose reduction in postabsorptive conditions. The improvement of insulin sensitivity on skeletal muscle could permit increasing protein synthesis and glucose uptake and utilization within this tissue. In effect, adequate insulin availability is necessary for the maintenance of protein synthesis and of normal glucose metabolism in skeletal muscle. The final metabolic effect may be represented by increased consumption and use of glucose by muscle itself, thereby decreasing the circulating glucose. These effects appear to be demonstrated in these elderly subjects during short-term supplementation with OAAM.

The well-known substrate competition between insulin-mediated glucose disposal and amino acids could contribute to hyperglycemia in elderly subjects with type 2 diabetes, when protein catabolism and muscle degradation (conditions frequently associated with aging) enhance the availability of circulating amino acids. OAAM could powerfully antagonize muscle catabolism, therefore reducing the amount of free amino acids in the circulation and increasing the insulin-mediated glucose disposal in peripheral tissues. On the other hand, OAAM supplementation used in this study did not induce a consistent load of amino acids.22,23 MET oxidation and homocysteine levels, a marker frequently associated with cardiovascular disorders in diabetes and other diseases.24 Nevertheless, it is very important to observe that the content of leucine in the composition of our mixture did not significantly influence homocysteine levels, and homocysteine remained unchanged throughout the period of supplementation with OAAM and after the crossover to placebo.

Based on the results of this pilot study and previous double-blind pilot clinical trials, we hypothesize that oral amino acids may be promising antidiabetic agents that may be useful as a nutritional supplement in the treatment of elderly patients with type 2 diabetes. In order to avoid the negative interaction between amino acids and carbohydrates when ingested together, the recommended timing of administration for OAAM is the preprandial state. The absence of side effects during the study suggests a potentially useful long-term role for OAAM supplementation in this population.

### TABLE 1

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<th>Homocysteine (µmol/l)</th>
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HDL = high-density lipoprotein; UAER = urinary albumin excretion rate. *p < 0.001 vs baseline.
Nevertheless, well-planned, long-term clinical trials should be conducted to confirm these results.