Improvement of Blood Glucose Control and Insulin Sensitivity During a Long-Term (60 Weeks) Randomized Study with Amino Acid Dietary Supplements in Elderly Subjects with Type 2 Diabetes Mellitus

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A decrease in lean muscular mass causes sarcopenia, a disease frequently found in the elderly population. The reduction of muscle mass may be responsible for reduced insulin sensitivity and decreased glucose uptake, thus increasing the risk for hyperglycemia and insulin-resistance syndrome in elderly subjects with type 2 diabetes mellitus. We therefore wanted to determine the effect of a special mixture of oral amino acids (AAs) on elderly subjects with type 2 diabetes. A randomized, open-label, crossover study was conducted in 34 subjects with diabetes (age range, 65–85 years) assigned to 2 distinct treatments (AAs and placebo). In spite of treatment with oral hypoglycemic drugs or insulin, all subjects were in poor metabolic control (glycated hemoglobin [HbA_{1c}] >7%). The subjects studied had normal body weight (ie, body mass index within 19–23). AAs consisted of 70.6 kcal/day (1 kcal = 4.2 kJ) of 8 g of AA snacks, given at 10.00 AM and 5.00 PM. Fasting and postprandial (1 hour and 2 hours) 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 of HbA_{1c} levels was found throughout the study. No significant adverse effects were observed during the active treatment. We suggest that nutritional supplementation with a special mixture of oral AAs is safe and significantly improves metabolic control and insulin sensitivity in poorly controlled elderly subjects with type 2 diabetes. This effect was consistent during the long-term observation period of 60 weeks and was also present after the crossover from AAs to placebo. **(Am J Cardiol 2008; 101[suppl]:82E–88E)**

Skeletal muscle is the main target of glucose use and insulin activity that causes protein anabolism in adequate systemic amino acid (AA) concentrations. Hence, glucose is taken from nutrients by the muscles and stored by insulin activity as glycogen. Therefore, this tissue may be important for glucose metabolism and could be an original target to treat metabolic disorders, eg, insulin resistance, reduced glucose tolerance, and type 2 diabetes mellitus.[1,2] Reduced muscle mass (defined also as sarcopenia) is very frequent in old age.[3–8] Furthermore, this condition could be associated with the reduced activity of anabolic hormones and with muscle protein turnover disorders.[9–11]

Sarcopenia and physical inactivity in the elderly could potentially induce hyperglycemia and insulin resistance and both conditions can lead to poorly controlled type 2 diabetes.

We can therefore hypothesize that dietary AA supplements in elderly patients with diabetes could antagonize muscle catabolism and glucose derangement.[12–14] Moreover, AAs have been shown to increase insulin sensitivity[15] and to decrease glycated hemoglobin (HbA_{1c}) levels.[16]

In a preliminary study, we found that nutritional supplements with a special mixture of oral AAs improved glycemic control and insulin sensitivity in subjects with poorly controlled type 2 diabetes.[17] In an attempt to confirm the long-term effects of AAs on metabolic control of subjects

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with diabetes, this investigation was designed with a long-term follow-up period of 60 weeks. We studied elderly subjects with type 2 diabetes associated with insulin resistance and poor metabolic control.

Materials and Methods

We recruited 34 consecutive elderly outpatients with type 2 diabetes and assigned them to a randomized, open-label, crossover study with AAs versus placebo. All subjects gave their informed consent.

The age of subjects ranged from 65–83 years; body weight (expressed as body mass index) was within normal limits for the age group studied (range, 19–23). All subjects were in poor glycometabolic control, with HbA_{1c} >7% at the beginning of the study (range, 7.2%–10.5%) despite treatment with oral hypoglycemic drugs or recombinant human insulin. Exclusion criteria for enrollment were as follows: severe diabetic microangiopathy and neuropathy, diabetic ketoacidosis, renal or hepatic failure, coronary and peripheral macroangiopathy, and arterial hypertensive disease.

The oral AAs, produced as nutritional support (Big One; Professional Dietetics, Milan, Italy), and the 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 respectively. The AAs 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 2 weeks and the baseline evaluation, performed before administration of AAs or placebo; (2) randomization into 2 different groups of subjects for 16 weeks of AA treatment (group A) or placebo (group B); (3) a washout period of 2 weeks for both groups; (4) crossover of the AA group to placebo and of the placebo group to AAs (week 18); (5) a second period of 16 weeks of AA treatment or placebo; and (6) a final treatment period of 26 weeks with AAs for both groups (maintenance treatment period with AAs). At the beginning of the randomization, after baseline, 18 patients were assigned to group A (initial randomization to AAs) and 16 patients to group B (initial randomization to placebo).

AAs and placebo were given in association with conventional antidiabetic treatment.

The following parameters were examined at baseline and after 2, 4, 8, 16, 18, 22, 26, 30, 34, and 60 weeks: body mass index, arterial blood pressure, fasting blood glucose, postprandial blood glucose (1 hour and 2 hours), HbA_{1c}, fasting serum insulin, and the homeostatic model assessment of insulin resistance (HOMA-IR). HOMA-IR values were calculated as follows: fasting insulin (μU/mL) × 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.

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). HbA_{1c} was measured by high-performance liquid chromatography (HbA1c-HPLC; Bio-Rad Diagnostics Group, Hercules, CA).

Data were expressed as mean ± SD. The crossover treatment trial was analyzed as described by Doehner and colleagues.[18] Repeated measures ANOVA and the paired Student's *t*-test were used for statistical analysis where appropriated. A p-value <0.05 was considered statistically significant.

Results

Body weight, arterial blood pressure, and renal function remained unchanged during the study (data not shown). The data concerning fasting and postprandial blood glucose levels are reported in Figures 1–3. A significant decrease of fasting blood glucose was found in group A as early as 8 weeks after the start of nutritional AA supplements. The decrease in fasting blood glucose levels was more significant at 16 weeks (Figure 1). This effect was maintained during the washout period and the crossover from AAs to placebo (from week 18 to week 34) and was more pronounced after restarting the AA treatment from week 34 to week 60. On the other hand, fasting blood glucose remained unchanged in group B from baseline to week 18, whereas significantly reduced fasting blood glucose was found when these patients were assigned to AA treatment (after the crossover at week 18 to week 60).

Similar to the fasting blood glucose levels, a significant but more consistent decrease in postprandial blood glucose (at 1 and 2 hours) was found in group A (Figures 2 and 3). This decrease was present throughout the crossover from AAs to placebo (from week 18 to week 34) and was more pronounced after restarting the AA treatment from week 34 to week 60. On the contrary, postprandial blood glucose remained unchanged in group B during placebo treatment. However, a significant and persistent decrease in postprandial blood glucose was found when these patients were treated with AAs (from week 18 to week 60). Therefore, AA supplements were strongly associated with the decrease in fasting and postprandial blood glucose levels in both groups of elderly subjects with diabetes.

Figure 4 shows the mean changes in HbA_{1c} levels in groups A and B throughout the 60-week follow-up protocol study. A significant decrease in HbA_{1c} levels was found by 8 weeks in subjects with diabetes who were initially randomized to AAs (group A), and these changes

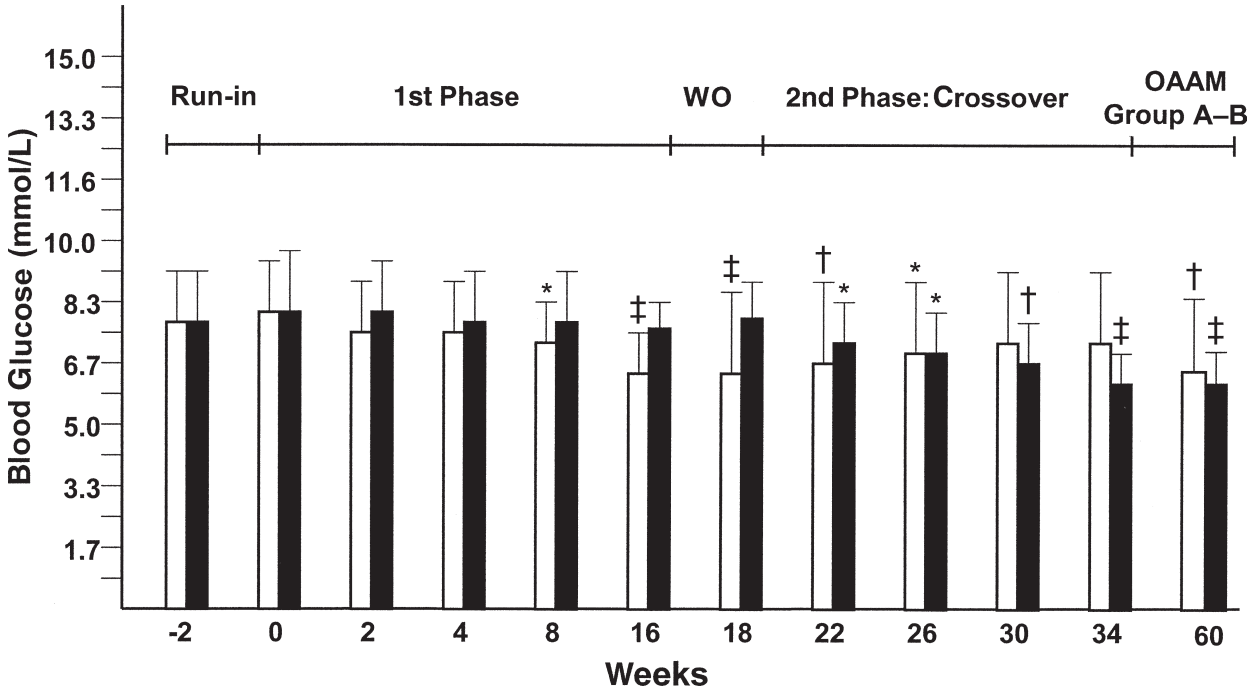

Figure 1. Mean (±SD) fasting blood glucose values at 7:00 AM in group A (*open bars*) and group B (*solid bars*) during supplementation with amino acids and placebo. OAAM = Oral Amino Acid Maintenance; WO = washout. *p <0.05 vs baseline; †p <0.01 vs baseline; ‡p <0.001 vs baseline.

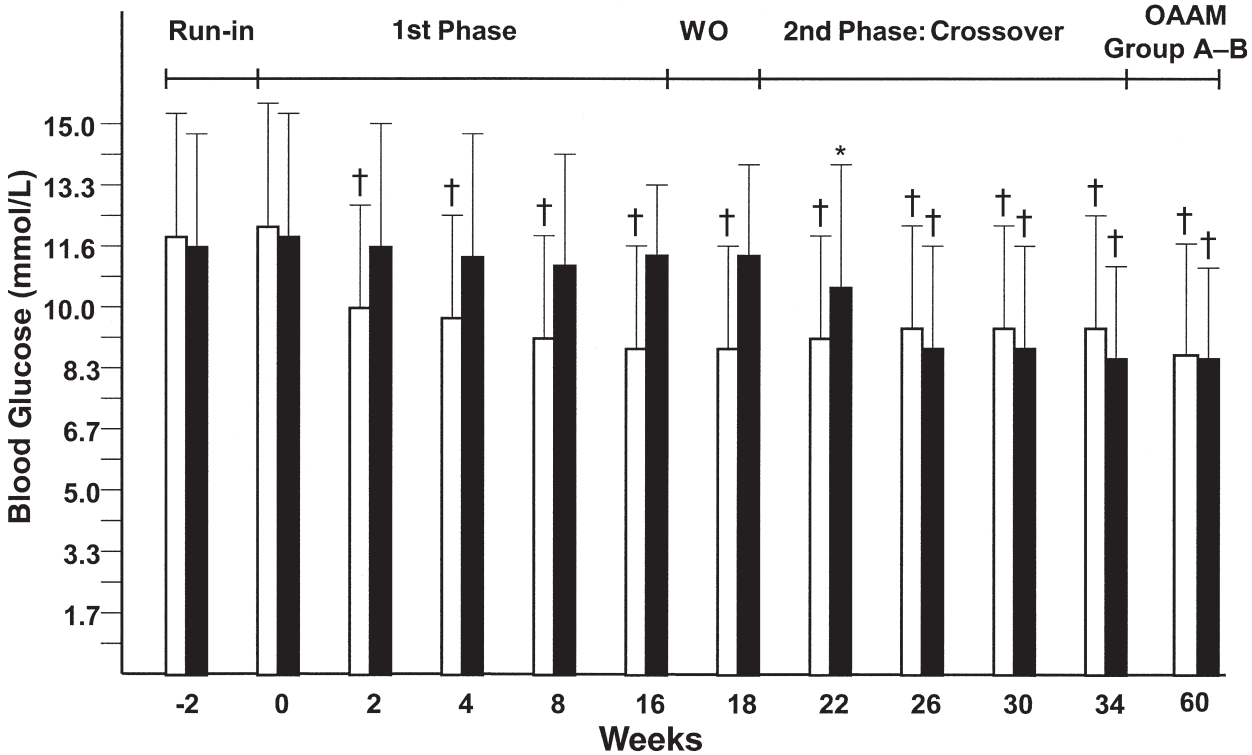

Figure 2. Mean (±SD) 1-hour postprandial blood glucose values in group A (*open bars*) and group B (*solid bars*) during supplementation with amino acids and placebo. OAAM = Oral Amino Acid Maintenance; WO = washout. *p <0.01 vs baseline; †p <0.001 vs baseline.

were maintained after the crossover from AAs to placebo and remained more pronounced after restarting the AA treatment (from week 34 to week 60). On the contrary, HbA_{1c} remained unchanged in subjects with diabetes who were initially randomized to placebo (group B), while HbA_{1c} was significantly reduced, from week 26 to week 60, after crossover from placebo to AA supplements.

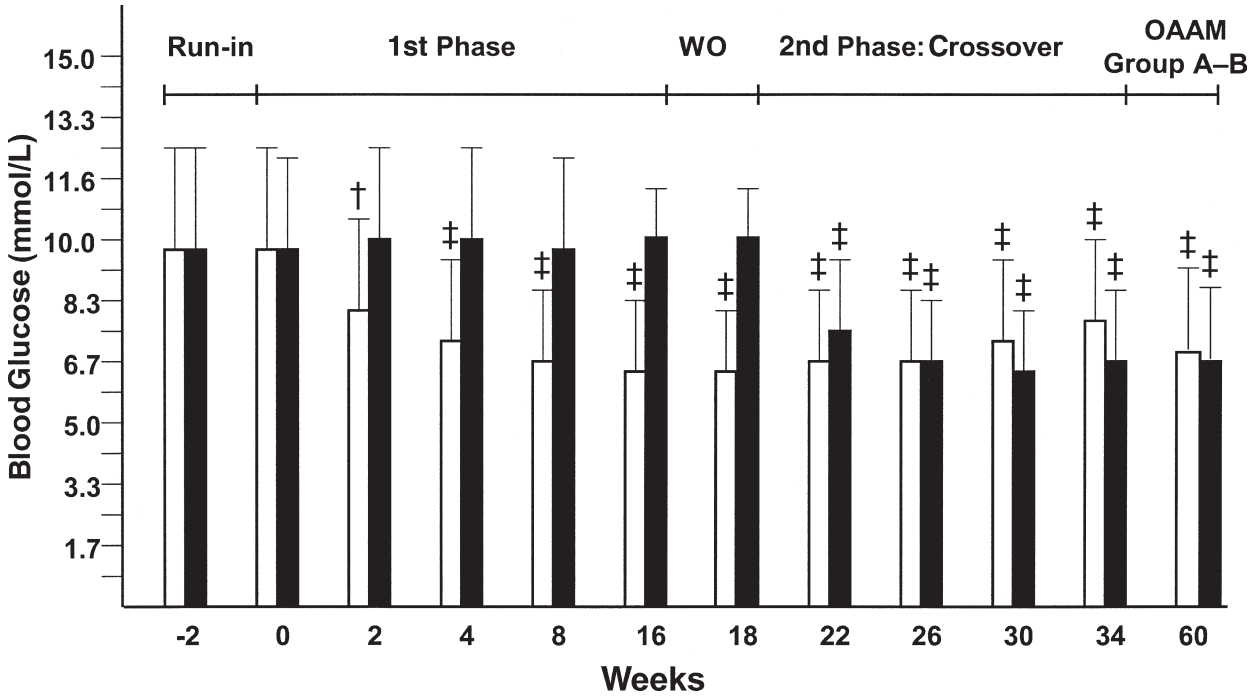

Figure 3. Mean (±SD) 2-hour postprandial blood glucose values in group A (*open bars*) and group B (*solid bars*) during supplementation with amino acids and placebo. OAAM = Oral Amino Acid Maintenance; WO = washout. *p <0.05 vs baseline; †p <0.01 vs baseline; ‡p <0.001 vs baseline.

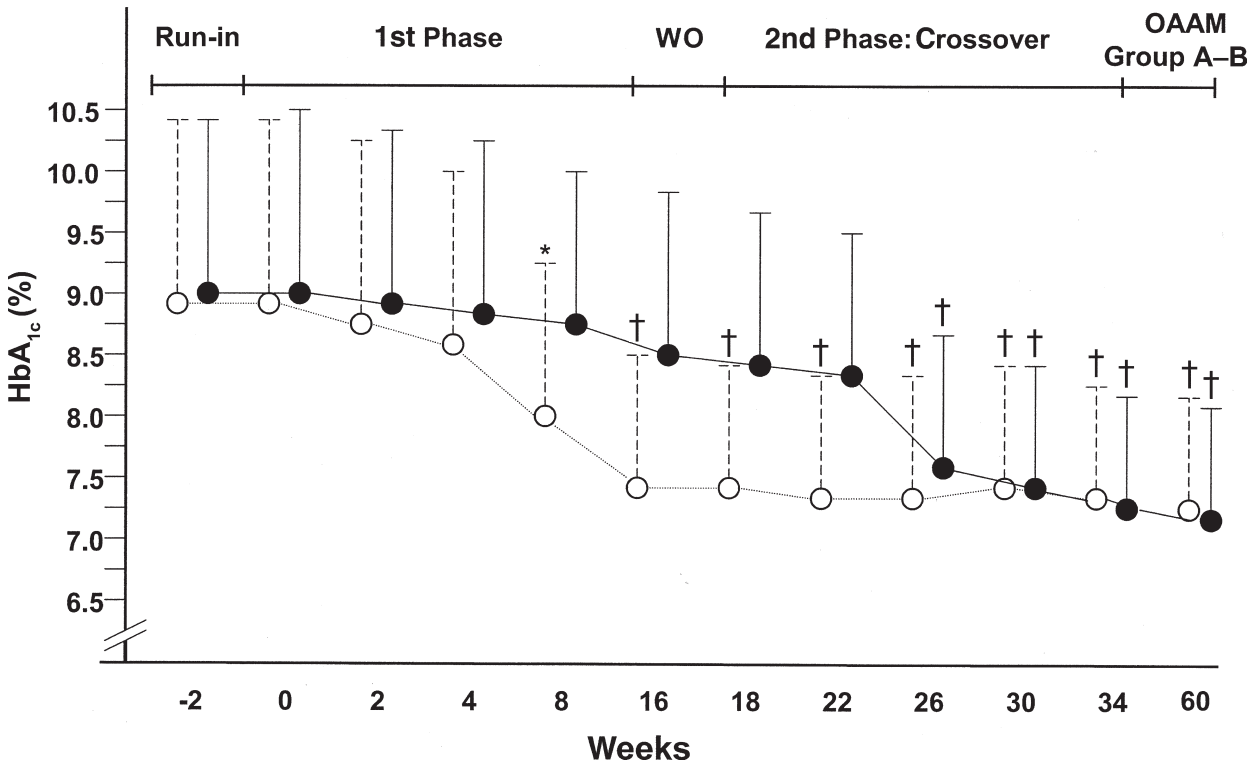

Figure 4. Mean (±SD) glycated hemoglobin (HbA_{1c}) values in group A (*open circles*) and group B (*solid circles*) during supplementation with amino acids and placebo. OAAM = Oral Amino Acid Maintenance; WO = washout. *p <0.01 vs baseline; †p <0.001 vs baseline.

Finally, Figures 5 and 6 show fasting serum insulin and HOMA-IR changes 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 age-matched subjects with no diabetes (10.6 ± 2.2 μU/mL; p <0.001). A significant decrease in insulin levels was demonstrated after 8 weeks in subjects initially randomized to AA supplements (group A),

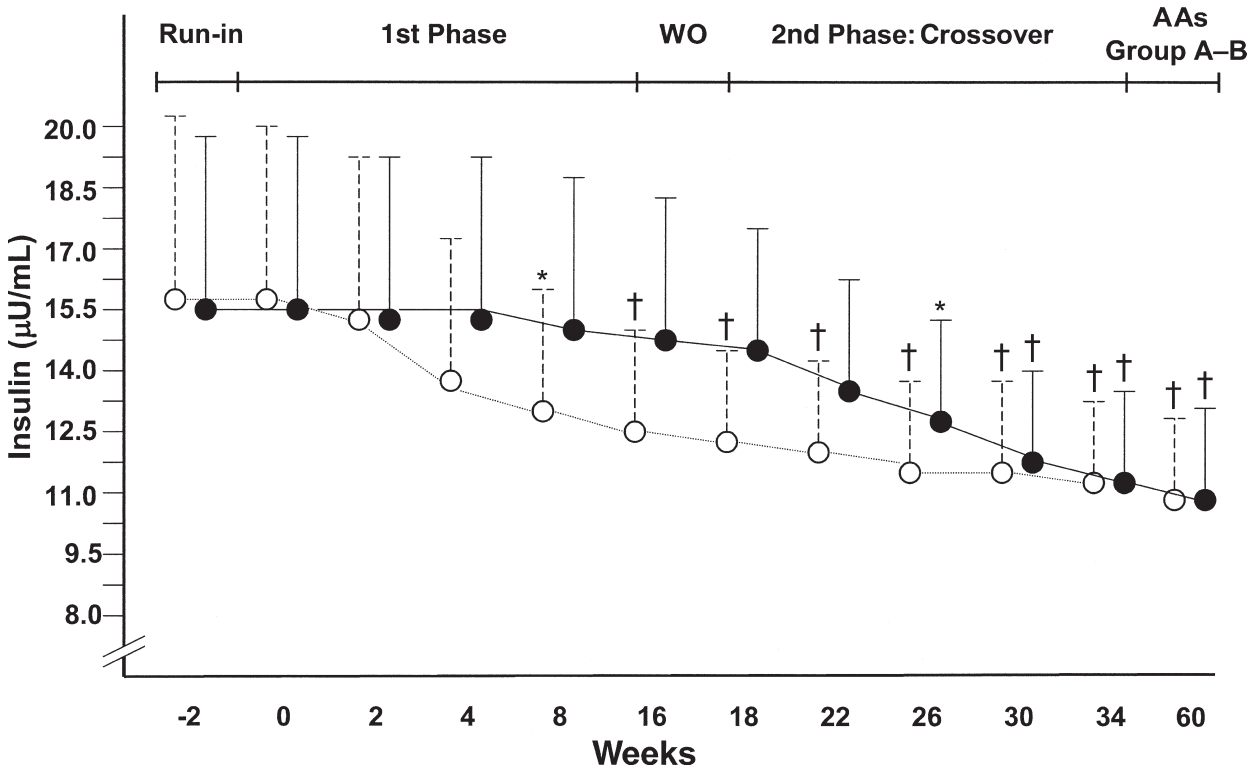

Figure 5. Mean (±SD) fasting serum insulin values in group A (*open circles*) and group B (*solid circles*) during supplementation with amino acids and placebo. OAAM = Oral Amino Acid Maintenance; WO = washout. *p <0.01 vs baseline; †p <0.001 vs baseline.

and these results were consistent during AA treatment and after the crossover to placebo. Similar to group A, serum insulin levels significantly decreased after crossover from placebo to AA supplements (from week 26 to week 60).

HOMA-IR (Figure 6) decreased in a pattern similar as did fasting serum insulin levels. HOMA-IR significantly decreased in group A subjects 8 weeks after AA supplements and this pattern was maintained throughout the study and after the crossover from AAs to placebo. On the other hand, HOMA-IR remained unchanged in subjects with diabetes initially randomized to placebo (group B) and then significantly decreased from week 22 to week 60 after crossover from placebo to AA supplements. No significant adverse effects were observed during the active treatment.

Discussion

Our study clearly demonstrated that, given in conjunction with conventional antidiabetic therapy, use of AA supplements in elderly subjects with poorly controlled type 2 diabetes is safe and significantly improves metabolic control and insulin sensitivity. Moreover, these changes were observed during a 60-week follow-up study, suggesting that AA supplements are an important long-term therapeutic strategy for regulating glucose metabolism in elderly patients with type 2 diabetes. In addition, our investigation demonstrated that both short-term (ie, fasting and postprandial blood glucose levels) and long-term (ie, HbA_{1c}) metabolic parameters improved during AA treatment, and these effects were maintained during the washout period and the period of crossover to placebo.

The mechanism by which AAs improve glucose homeostasis may have several different aspects. AAs can induce protein anabolism by lowering AA levels in plasma[19] and by increasing muscle protein synthesis and glucose storage in insulin-sensitive tissues.[20–22] This ability of AAs to increase protein anabolism and muscle tissue synthesis may play an important role in improving blood glucose control and insulin sensitivity. Furthermore, AAs upregulate insulin-receptor synthesis and its autophosphorylation in experimental and clinical type 2 diabetes.[23,24] Insulin activity, in addition to insulin secretion, could therefore be enhanced during AA supplementation in subjects with diabetes, thus contributing to normalized blood glucose levels. In effect, our investigation demonstrated that fasting hyperinsulinemia significantly decreased with the use of AA supplements, and insulin sensitivity (measured by HOMA-IR) improved after finishing AA supplements and after the crossover to placebo. The improvement in insulin sensitivity and the reduced fasting hyperinsulinemia could be considered important metabolic consequences of oral AA support in our group of elderly subjects with type 2 diabetes. These effects could be dependent on either recovery insulin activity on the muscle target or an insulin-dependent increase of skeletal muscle anabolism and mass. On the other

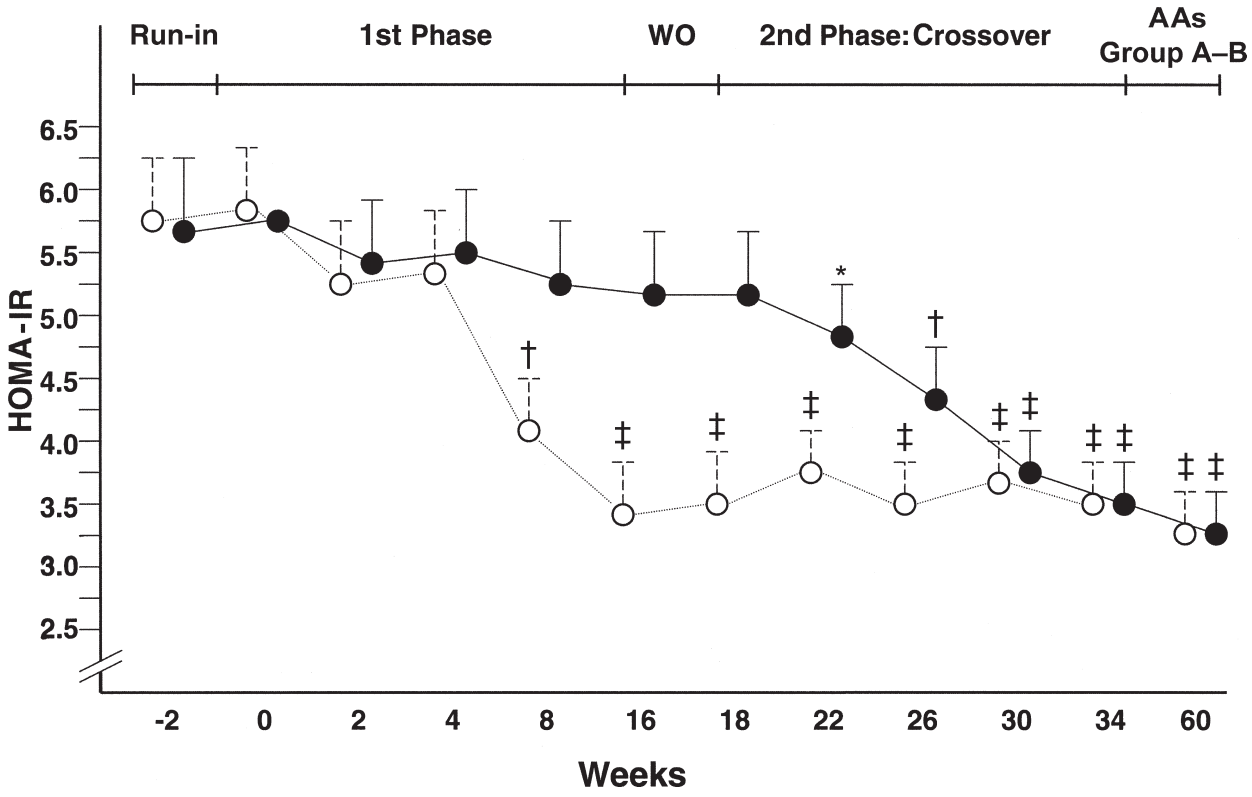

Figure 6. Mean (±SD) homeostatic model assessment of insulin resistance (HOMA-IR) values in group A (*open circles*) and group B (*solid circles*) during supplementation with amino acids and placebo. WO = washout. *p <0.05 vs baseline; †p <0.01 vs baseline; ‡p <0.001 vs baseline.

hand, it has been demonstrated that oral supplements with arginine, glycine, and cysteine improve insulin sensitivity and glucose metabolism in the muscle, whereas leucine enhances protein synthesis in skeletal muscles through an insulin-dependent mechanism.[25–28] All of these mechanisms could contribute to the removal of blood glucose in postabsorptive conditions by increasing glucose uptake and use in skeletal muscle.[29–31] The final metabolic effect may be related to increased glucose consumption by muscles, thereby decreasing the pool of circulating glucose. These effects were demonstrated in elderly subjects with type 2 diabetes during short-term supplementation with AAs, and progressively improved throughout the long-term 60-week observation period.

Conclusion

Our data show that AA supplements positively influence metabolic control in elderly subjects with type 2 diabetes.

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