

# Effects of oral amino acid supplementation on myocardial function in patients with type 2 diabetes mellitus

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**Background** Diabetes mellitus is associated with an increased rate of cardiac amino acid catabolism that could interfere with cardiac function.

**Methods** We assessed the effects of an oral amino acids mixture (AAM) on myocardial function in patients with type 2 diabetes mellitus (DM2). We studied 65 consecutive patients with DM2 who had normal resting left ventricular ejection fraction (LVEF) and did not have obstructive coronary artery disease (CAD). After baseline evaluations, patients were randomized to receive, in a single-blinded fashion, AAM (12 grams/day) or placebo for 12 weeks, after which, treatment was crossed over for another similar period. At baseline and at the end of each treatment, 2-dimensional echocardiography at rest and during isometric exercise (handgrip) was performed, as were biochemical assays. Twenty adults, matched for age, sex, and body mass index served as control subjects.

**Results** At baseline and during AAM or placebo treatment, resting left ventricular dimensions and LVEF in patients with DM2 did not differ from those of control subjects. In patients with DM2, at baseline and during placebo treatment, peak handgrip LVEF decreased significantly in comparison with the resting value ( $63\% \pm 9\%$  vs  $56\% \pm 9\%$ ,  $P < .001$ ; and  $62\% \pm 6\%$  vs  $55\% \pm 8\%$ ,  $P < .001$ ). During AAM treatment, peak handgrip LVEF did not differ from resting value ( $66\% \pm 11\%$  vs  $64\% \pm 9\%$ ,  $P =$  not significant). Thus, exercise LVEF was higher during AAM treatment than both baseline and placebo treatment ( $66\% \pm 11\%$  vs  $56\% \pm 9\%$  and vs  $55\% \pm 8\%$ ,  $P < .001$ ). In contrast to placebo treatment, after the AAM supply, a decreased glycosylated hemoglobin level was observed ( $7.0\% \pm 1.3\%$  vs  $7.6\% \pm 1.8\%$ ,  $P < .05$ ).

**Conclusions** Myocardial dysfunction is easily inducible with isometric exercise in patients with DM2 who have normal resting LV function and do not have CAD. An increased amino acid supply prevents this phenomenon and improves metabolic control. (*Am Heart J* 2004;147:1106–12.)

Cardiovascular complications contribute significantly to morbidity and mortality in the diabetic population.<sup>1</sup> In addition, a specific cardiomyopathy has been described<sup>2</sup>: this diabetic cardiomyopathy leads to an increased incidence of congestive heart failure in the absence of coronary artery disease (CAD). The mechanisms underlying this condition have not been completely clarified. Small vessel disease, endothelial dysfunction, abnormalities in  $\beta$ -adrenergic pathway,

changes in contractile proteins, and alteration in intermediary metabolism have been proposed as factors contributing to myocardial failure.<sup>3–7</sup>

Investigations into the effects of diabetes mellitus on myocardial macronutrient selection have focused on glucose and fatty acids as substrates of interest. However, it has been shown that hyperglycemia is associated with a significant arterial-venous difference of branched-chain amino acids across myocardium.<sup>8,9</sup> These data strengthen the concept that diabetes mellitus is associated with an increased or poorly regulated rate of amino acid cardiac catabolism.<sup>10</sup> Moreover, in patients who have fasted, are untrained, and have type 2 diabetes mellitus (DM2), in the absence of nutritional intake, the amino acids needed to produce muscle proteins at an increased rate after exercise are largely derived from protein breakdown.<sup>11,12</sup> Thus, the aim of this study was to determine whether an increased intake of amino acids could exert a beneficial

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**Table I.** Main demographic and baseline characteristics of the study population

|  | Controls | Type 2 diabetic patients |
|--|----------|--------------------------|
| Sex (M/F)  | 16/4     | 46/19                    |
| Age (y)  | 64 ± 5   | 67 ± 9                   |
| Duration of the disease (y)                            | –        | 9 ± 6                    |
| Total cholesterol (mg/dL)                              | 220 ± 37 | 222 ± 37                 |
| HDL-cholesterol (mg/dL)                                | 56 ± 9   | 54 ± 14                  |
| LDL-cholesterol (mg/dL)                                | 140 ± 34 | 139 ± 33                 |
| Triglycerides (mg/dL)                                  | 121 ± 57 | 150 ± 82                 |
| Hypertension (%)                                       | 60       | 81                       |
| Hypoglycaemic treatment (diet/oral agents/insulin) (%) | –        | 28/66/6                  |
| Additional treatments (%)                              |          |                          |
| Hypolipidemic drugs                                    | –        | 4                        |
| ACE inhibitors   | 30       | 58                       |
| Calcium-channel blockers                               | 12       | 16                       |
| β-Blockers   | 1        | 2                        |
| Diuretics  | 16       | 10                       |
| Antiplatelet agents                                    | 21       | 45                       |
| Current smoking (%)                                    | 4        | 5                        |
| Alcohol intake (%)                                     | 40       | 37                       |

effect on myocardial function, both in resting state and during exercise, in patients with DM2.

## Methods

### Study patients

We studied 65 consecutive patients with DM2 who had normal resting left ventricular (LV) function (evaluated as LV ejection fraction [LVEF]) and did not have obstructive CAD. All patients were enrolled through outside general physicians and not by our institution's Diabetic Clinic nor by the Echocardiography Laboratory, so as not to bias or favor some subgroups of patients with DM2. This decision was made to study patients with DM2 as they are encountered in real world practice. The main demographic and baseline characteristics of the study population are summarized in Table I. All patients were nonsmokers. The subjects were active and living on their own with no limitation in ambulation, but were untrained. No patient had significant coronary vessel obstruction (≥50%) by using selective coronary angiography. Exclusion criteria were: (1) diabetic proliferative retinopathy, (2) clinical evidence of autonomic neuropathy, (3) overt diabetic nephropathy (creatinine level >1.4 mg/dL), and (4) poorly controlled arterial hypertension (>160/90 mm Hg during therapy).

In each subject, the habitual daily amount of calories and the qualitative composition of the diet, verified by a dietician, was 1600 ± 370 Kcal/day, containing 55% of total caloric intake in carbohydrates, 30% in lipids, and 15% in proteins. The diet was verified to exclude differences in dietetic regimens. During placebo or amino acid (AA) treatment, each subject followed his or her habitual daily diet.

**Table II.** Composition of the amino acid mixture

|                    | Per 100 g | Grams per day | Biosynthesis |
|--------------------|-----------|---------------|--------------|
| KJ                 | 1666      | 450           | –            |
| Protein (N × 6,25) | 0.67      | 0.18          | –            |
| Carbohydrates      | 45.29     | 12.21         | –            |
| Lipids             | 2.38      | 0.64          | –            |
| L-leucine          | 13.889    | 3.8           | essential    |
| L-lysine           | 7.222     | 2             | essential    |
| L-isoleucine       | 6.944     | 1.9           | essential    |
| L-valine           | 6.944     | 1.9           | essential    |
| L-threonine        | 3.889     | 1.1           | essential    |
| L-cystine          | 1.667     | 0.4           | nonessential |
| L-istidine         | 1.667     | 0.4           | essential    |
| L-phenylalanine    | 1.111     | 0.3           | essential    |
| L-methionine       | 0.556     | 0.2           | essential    |
| L-tyrosine         | 0.333     | 0.1           | nonessential |
| L-tryptophane      | 0.222     | 0.1           | essential    |

The study protocol was approved by the local ethical committee of the school of medicine, and written informed consent was obtained from all participants after the nature of the procedure was explained.

### Study design

This study was a randomized, single-blind, crossover study of AAs versus placebo. Patients were blinded to the study medication. The label appearances were identical, but the flavor was different. The flavor was designed to have as much as possible of an orange taste, but certainly it did not have the same taste as a non-AA. However, patients were not informed about the taste of AA or placebo. The detailed composition of the AA is summarized in Table II. This formulation contains all indispensable and 2 nonessential AAs (tyrosine and cystine), in a complex ratio that was planned to match with metabolic requirements in conditions of elevated demand.<sup>13,14</sup> Seventy-five percent of human nitrogen needs are covered by only 5 AAs<sup>13</sup>: leucine, isoleucine, valine, threonine, and lysine.

The study consisted of 4 phases: (1) baseline examination with anthropometrical, metabolic, and cardiac evaluations, and then randomization to either AA or placebo; (2) 12-week maintenance period either receiving AA or placebo, followed by anthropometrical, metabolic, and cardiac evaluations; (3) 1-week placebo washout phase, and (4) 12-week maintenance period either receiving AA or placebo, again followed by anthropometrical, metabolic, and cardiac evaluations. During both treatments, each patient performed home blood glucose monitoring to avoid any possible serious metabolic alteration. Concomitant medications were kept identical. Both placebo and AA were ingested as snacks with tap water at 10:00 AM, 4:00 PM, and 10:00 PM. Individual dietary intake was accurately assessed throughout the study to prove adequate adherence to diet and AA supplementation. Height and weight were measured with a standardized protocol. Body mass index (BMI; weight/height<sup>2</sup>) was used as an estimate of overall adiposity. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in the sitting position,

with a standard mercury sphygmomanometer and cuff of appropriate size; the average of 3 measurements 5 minutes apart was recorded. Hypertension was defined as the current use of antihypertensive medication.

Twenty healthy subjects matched for age, sex, and BMI served as control subjects. All control subjects were enrolled through the same general physicians as the patients with DM2. Control subjects were not randomized to receive AA or placebo, but were examined only once to derive limits for values of dimensions and function of the left ventricle at rest and during isometric exercise. A high number of control subject had hypertension (Table I), as were the diabetic patients, so we matched them for levels of arterial pressure values. With the exception of hypertension, the control group had, on average, low-density lipoprotein cholesterol values  $>130$  mg/dL, to match values in the diabetic group, but were otherwise healthy. The control subjects were all nonsmokers, without family history of CAD, symptoms suggestive of myocardial ischemia, or electrocardiographic signs or history of myocardial infarction.

On a separate occasion, in 10 patients with DM2 and in 10 control subjects, baseline concentrations of each AA present in the mixture and their plasma level excursion after oral intake of 12 g were determined. Blood samples were obtained at 0, 20, 40, 60, 90, and 120 minutes after the AA load. AAs were determined with a standard high-performance liquid chromatography method.<sup>15</sup>

### Echocardiographic analysis

Echocardiographic examinations were performed by cardiologists with no knowledge of patient identity and experimental condition using Hewlett-Packard Sonos 5500 echocardiographic equipment. Patients were instructed not to give any information to the cardiologists about the therapy they received (study therapy or other medications routinely assumed). Measurements of LV volumes and LVEF were done by echocardiographers who did not perform the studies. Examinations were furthermore coded and read by 2 independent observers, who were blinded to patient identity, order of the study (rest vs isometric exercise), or intervention (placebo or AA). Echocardiographic analysis was performed with the digitized cine loop method (Pre Vue III System, Nova MicroSonics). LV volumes were calculated with an ellipsoid biplane area-length method.<sup>16</sup> LVEF was derived as end-diastolic volume minus end-systolic volume divided by the end-diastolic volume. LV endocardial echocardiograms in the apical 4- and 2-chamber views, for a minimum of 2 to 4 cardiac cycles, were digitized at end-diastole (R wave peak) and end-systole (time of smallest cavity area) by 2 independent observers. A discrepancy of more than 10 mL for LV volume required that the echocardiographic tracing be analyzed by a third observer. Agreement was achieved by consensus. Changes in LVEF were considered significant when they were outside the 95% confidence limits of interstudy variability.

### Isometric exercise

We choose isometric instead of isotonic exercise because it has the same capacity to provoke LV dysfunction in patients with DM2 who had normal resting LV function.<sup>5</sup> More-

over, isotonic exercise (treadmill or bicycle) has several technical problems because of the body movements, high respiratory rate, and high heart rate, all of which contribute substantially to reduce the image quality of the left ventricle and so introduce some errors in measurements of LV dimensions and function. During isometric exercise, the patient lies in the left lateral recumbent position (as at rest), and differences in the quality of resting and exercise images are absent. During handgrip, arterial pressure was measured every 30 seconds with an oscillometric method (Nippon Colin Co). LV function was assessed with 2-dimensional echocardiography in apical 4- and 2-chamber views. The difference in LVEF at peak handgrip between placebo or AA treatment was chosen as the primary marker of the effectiveness of the AA because of the known negative effects of DM2 on myocardial function in a condition of acute overload induced with exercise. Maximal voluntary contraction was determined by means of a dynamometer. Maximal voluntary contraction of the right hand was measured before the first echocardiographic study. Forty percent of this effort was then used to induce isometric exercise during the echocardiographic study in all situations (baseline, placebo, or AA mixture). Moreover, during handgrip, the contraction effort of the right hand was continuously monitored with a dynamometer, and the patient was encouraged to maintain the pre-imposed value for 3 minutes. Patients were instructed to avoid performing the Valsalva manoeuvre during handgrip. Heart rate and electrocardiographic tracings were continuously monitored.

### Statistical analysis

Results are expressed as the mean value plus or minus SD for normally distributed variables (ejection fraction, end-diastolic and end-systolic volume indices, age, blood pressure, heart rate, and blood chemical variables). Comparison were made with the paired (resting vs peak handgrip values, placebo vs AA mixture) or unpaired (control subjects vs patients with DM2) Student *t* test as appropriate. Multiple comparisons were performed with a 2-way repeated measurement analysis of variance, followed by the Fisher protected least significant difference test. The primary end point, differences for LVEF at peak handgrip during placebo or AA treatment, was prospectively defined. We determined the number of patients needed to treat to detect a difference  $\geq 6$  units in LVEF, with a type I error (2-sided *P* value) of .01 and a type II error (beta) of 0.1 (power of 0.9). Using data from a previous study (SD of LVEF equal to 11) in which LVEF at peak handgrip in patients with DM2 decreased to  $>5$  units compared with the values at rest, we calculated that it would be sufficient to treat 49 patients, in a crossover design (single group study with 2 measurements, placebo vs AA), to detect a significant difference in LVEF at peak handgrip. Assuming that no more than 30% of the patients would be lost to follow-up or withdraw from the study, 64 patients would be enough to test our initial hypothesis. For all statistical analysis, we

**Table III.** Baseline amino acid concentrations and maximal percentage increase in blood plasma after 12 grams of oral intake in 10 controls and 10 diabetic patients

|                 | Controls                        |                                 | DM2                             |                                 |
|-----------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
|                 | Baseline concentration (μmol/L) | Maximal percentage increase (%) | Baseline concentration (μmol/L) | Maximal percentage increase (%) |
| L-leucine       | 127 ± 25                        | 451 ± 159                       | 147 ± 15*                       | 385 ± 69                        |
| L-lysine        | 169 ± 26                        | 217 ± 66                        | 190 ± 28                        | 178 ± 31                        |
| L-isoleucine    | 48 ± 7                          | 454 ± 148                       | 64 ± 12†                        | 407 ± 274                       |
| L-valine        | 228 ± 28                        | 237 ± 68                        | 289 ± 40†                       | 189 ± 17*                       |
| L-threonine     | 126 ± 39                        | 154 ± 37                        | 138 ± 35                        | 146 ± 27                        |
| L-cystine       | 13 ± 16                         | 111 ± 34                        | 62 ± 10*                        | 109 ± 9                         |
| L-istidine      | 79 ± 14                         | 138 ± 31                        | 80 ± 21                         | 110 ± 22*                       |
| L-phenylalanine | 45 ± 6                          | 136 ± 20                        | 49 ± 9                          | 135 ± 23                        |
| L-methionine    | 23 ± 4                          | 140 ± 41                        | 26 ± 3                          | 136 ± 18                        |
| L-tyrosine      | 50 ± 10                         | 113 ± 23                        | 65 ± 6                          | 102 ± 10                        |
| L-tryptophane   | NA                              | NA                              | NA                              | NA                              |

\**P* < .05, patients with diabetes versus controls.

†*P* < .01, patients with diabetes versus controls.

**Table IV.** Metabolic variables

|                                 | Controls   | DM2        |            |            |
|---------------------------------|------------|------------|------------|------------|
|                                 |            | Baseline   | Placebo    | Amino acid |
| BMI (kg/m <sup>2</sup> )        | 26.2 ± 3.2 | 27.7 ± 3.7 | 27.6 ± 3.6 | 27.6 ± 3.7 |
| Fasting plasma glucose (mg/dL)  | 93 ± 8     | 155 ± 44*  | 157 ± 46   | 153 ± 34   |
| Fasting plasma insulin (μU/mL)  | 17 ± 15    | 16 ± 9     | 15 ± 8     | 15 ± 7     |
| HbA <sub>1c</sub> (%)           | 4.2 ± 0.7  | 7.7 ± 1.9* | 7.6 ± 1.8* | 7.0 ± 1.3† |
| Albumin excretion rate (mg/day) | 24 ± 52    | 33 ± 77    | 35 ± 80    | 34 ± 87    |

\**P* < .001, controls versus patients with diabetes.

†*P* < .05, patients with diabetes: amino acid versus placebo/baseline.

used the Statistical Package for Social Sciences software package version 10.1 for Windows (SPSS, Chicago, Ill). A *P* value ≤.05 with the 2-tailed test was considered to be statistically significant.

## Results

### Metabolic parameters

Metabolic parameters are shown in Tables III and IV. The study medication was generally well tolerated. The only symptom reported in a few patients receiving the AA (1.5%) was dyspepsia. They all concluded the study. The evaluation of plasma AA concentrations, assessed in 10 patients with DM2 and 10 control subjects, showed plasma baseline concentrations of branched chain AAs and cystine significantly higher in patients with DM2 than in control subjects. However, a higher maximal percentage increase from baseline concentration in control subjects was observed for valine and istidine in comparison with patients with

DM2. Glycated hemoglobin levels decreased significantly during AA treatment in comparison with placebo treatment or baseline (*P* < .05). Both plasma glucose and insulin levels slightly declined, but the difference was not statistically significant. No significant changes occurred in metabolic parameters during placebo.

### Resting LV function

Measurements of LV end-diastolic volume index (LVEDVI; 66 ± 9 mL/m<sup>2</sup>; range, 54–74 mL/m<sup>2</sup>) and LVEF (64% ± 9%; range, 58%–72%) in the control group were used to determine the mean and 95% confidence limits of normal values. Resting values of LV dimensions and function are listed in Table V. At the baseline examination, in patients with DM2, LVEDVI (67 ± 14 mL/m<sup>2</sup> vs 66 ± 9 mL/m<sup>2</sup>, *P* = not significant) and LVEF (63% ± 9% vs 64% ± 9%, *P* = not significant) did not differ from those in control subjects. Four patients had an enlarged left ventricle, as indi-

**Table V.** Resting and handgrip variables

|                             | DM2      |           |           |            |
|-----------------------------|----------|-----------|-----------|------------|
|                             | Controls | Baseline  | Placebo   | Amino acid |
| <b>Resting</b>              |          |           |           |            |
| HR (beats/min)              | 70 ± 9   | 72 ± 14   | 74 ± 10   | 72 ± 10    |
| SBP (mm Hg)                 | 132 ± 12 | 155 ± 14* | 153 ± 16* | 152 ± 15*  |
| DBP (mm Hg)                 | 76 ± 9   | 88 ± 11†  | 89 ± 9†   | 86 ± 10†   |
| LVEDVI (mL/m <sup>2</sup> ) | 66 ± 9   | 67 ± 14   | 68 ± 12   | 66 ± 14    |
| LVESVI (mL/m <sup>2</sup> ) | 24 ± 10  | 25 ± 9    | 26 ± 9    | 24 ± 8     |
| LVEF (%)                    | 64 ± 9   | 63 ± 9    | 62 ± 6    | 64 ± 9     |
| <b>Handgrip</b>             |          |           |           |            |
| HR (beats/min)              | 89 ± 16  | 90 ± 10   | 90 ± 9    | 94 ± 11    |
| SBP (mm Hg)                 | 156 ± 30 | 198 ± 20* | 200 ± 20* | 194 ± 23*  |
| DBP (mm Hg)                 | 90 ± 20  | 110 ± 16* | 111 ± 12* | 107 ± 14*  |
| LVEDVI (mL/m <sup>2</sup> ) | 70 ± 9   | 72 ± 12   | 72 ± 14   | 71 ± 12    |
| LVESVI (mL/m <sup>2</sup> ) | 14 ± 6   | 32 ± 10*  | 32 ± 9*   | 24 ± 8†‡   |
| LVEF (%)                    | 78 ± 9   | 56 ± 9*   | 55 ± 8*   | 66 ± 11†‡  |

HR, Heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVEDVI, left ventricular end-diastolic volume-index; LVESVI, left ventricular end-systolic volume-index; LVEF, left ventricular ejection fraction.

\* $P < .001$  controls versus patients with diabetes.

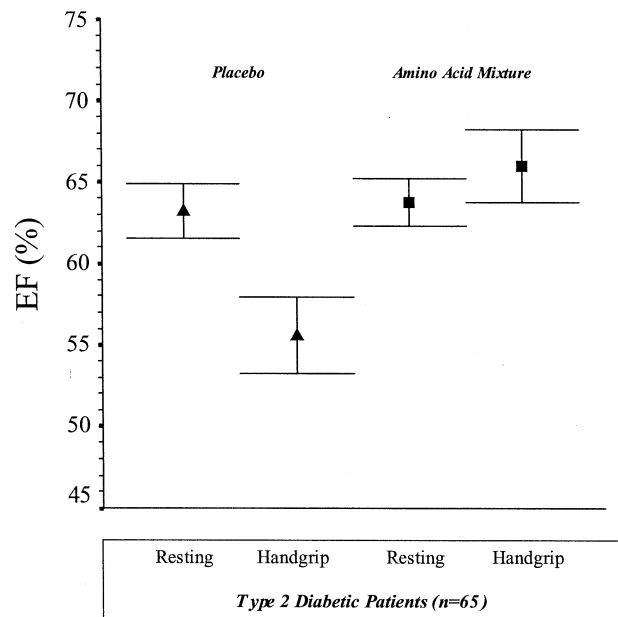
† $P < .005$  controls versus patients with diabetes.

‡ $P < .001$  patients with diabetes: amino acid versus placebo.

cated by an LVEDVI greater than the upper normal limit. All patients had a normal LVEF. Moreover, no differences were detected in resting LVEDVI and LVEF during AA treatment in comparison with baseline evaluation or placebo treatment. Heart rate did not differ between control subjects and patients with DM2 independently of experimental conditions (baseline, placebo, AA), whereas SBP ( $155 \pm 14$  mm Hg vs  $132 \pm 12$  mm Hg,  $P < .001$ ) and DBP ( $88 \pm 11$  mm Hg vs  $76 \pm 9$  mm Hg,  $P = .002$ ) were higher in patients with DM2.

### LV response to handgrip

In patients with DM2, during the experimental conditions (baseline, placebo, AA), heart rate increased at peak exercise without significant differences compared with that of control subjects, whereas at peak handgrip SBP and DBP were higher. LVEF response to handgrip in the control group was characterized by a  $>5$ -unit increase in all but 2 subjects, who showed no changes. In this group, the mean LVEF peak value was significantly higher than the mean resting value ( $78\% \pm 9\%$  vs  $64\% \pm 9\%$ ,  $P < .005$ ). At baseline, 50 of the patients with DM2 (77%) had a  $>5$ -unit decline in LVEF, and the remaining 15 patients (23%) had a normal response to handgrip (5 patients had a significant increase in LVEF, whereas 10 patients showed no change in LVEF). At peak handgrip, LVEF was significantly lower than the resting value ( $56\% \pm 9\%$  vs  $63\% \pm 6\%$ ,  $P < .001$ ) (Table V).

**Figure 1**

LVEF (%) for patients with DM2 ( $n = 65$ ) at rest (*resting*) and at peak handgrip (*handgrip*), during placebo (*black triangles*) or during AA mixture (*black squares*).

During placebo treatment, peak stress LVEF decreased in comparison with resting values in a similar manner as during the baseline evaluation (Figure 1). In patients with DM2 after AA administration, peak stress LVEF ( $66\% \pm 11\%$  vs  $64\% \pm 9\%$ ,  $P =$  not significant; Figure 1) did not differ from resting values. In 42 of 50 patients with abnormal LV response to handgrip at baseline condition (84%), LVEF did not decline from resting values (in 38 patients, LVEF increased by  $>0.05$  units, and in 4 patients LVEF did not change). As a consequence, during AA treatment, patients had significantly higher peak stress LVEF than values obtained during placebo treatment ( $66\% \pm 11\%$  vs  $55\% \pm 8\%$ ,  $P < .001$ ).

### Discussion

This study was performed to assess the effects of oral AAs on myocardial function in patients with DM2, a population at risk for heart failure.<sup>2</sup> The study group consisted of patients with DM2 who had normal resting LV function and did not have obstructive CAD. In these patients, a LV dysfunction induced with isometric exercise could represent an early phase of diabetic cardiomyopathy.<sup>17,18</sup> Previous papers have shown that intravenous infusion of an AA, such as glutamine/gluta-

mate and aspartate, improves cardiac hemodynamic performance in patients with CAD.<sup>19,20</sup> This beneficial effect takes place at rather low plasma concentrations.<sup>21</sup> Our results show that the baseline circulating levels of the branched chain AAs were significantly higher in patients with DM2. Some studies reported disturbances of protein metabolism in diabetes mellitus,<sup>10,22</sup> but this has been excluded by other studies.<sup>23</sup> In our study, the blunted plasma maximal increase in some AAs after oral ingestion could also suggest a decreased absorption of these substrates.<sup>24</sup> The adequacy of this dosage is supported by the plasma AA increase after their ingestion: the observed peak plasma levels of each AA present in the mixture are similar to those previously shown to decrease proteolysis and increase protein synthesis.<sup>25-27</sup> Moreover, a chronic nutritional approach is considered not only more physiological, but even superior to the aggressive approach in preventing protein loss.<sup>28</sup> We thus hypothesize that, in patients with DM2, this supplementation might have positive effect on myocardial contractility.

The results of this study show that myocardial dysfunction induced with isometric exercise is prevented or attenuated during treatment with increased AA supply. During placebo treatment, peak stress LV pump function decreases and is significantly less than during AA treatment. Resting parameters of LV function did not change significantly.

Although speculative, a hypothesis can be put forward to explain our findings. Both diabetes mellitus and chronic heart failure are characterized by nitrogen metabolism alterations, the former because of the presence of insulin resistance, which can alter protein anabolism both in skeletal and heart muscle.<sup>29,30</sup>

Although we cannot exclude a priori possible interactions between the AAs and the other medications, the important role of glutamate in cardiac metabolism could be inferred from our findings. Most of the AAs in the mixture, such as phenylalanine, tyrosine, lysine, and leucine, feed into the Krebs cycle and may become glutamate through alpha-ketoglutaric acid, and histidine could also contribute to glutamate via alpha-ketoglutaric acid, emphasizing the crucial cardioprotective role of this AA by improving aerobic substrate metabolism and adenosine triphosphate production via a direct metabolic effect on the heart.<sup>31</sup> Thus, in addition to arginine's crucial role in cardiovascular function,<sup>32</sup> the other AAs may also contribute to improve heart function in patients with DM2. Moreover, our data support the findings of McNulty and colleagues,<sup>33</sup> who showed that although plasma levels of glucose and insulin independently regulate the proportional contribution of exogenous glucose to myocardial glycolytic and Krebs cycle flux in vivo in a dose-dependent manner, nonglucose substrates continue to supply  $\geq 40\%$  of myocardial Krebs cycle flux.

The increased availability of branched chain AAs may also explain the amelioration of myocardial dysfunction associated with exercise; they are the principal AAs taken up by the heart, and their net uptake is increased by increasing their concentrations.<sup>10,34</sup>

At variance with some findings,<sup>35,36</sup> but in agreement with other findings,<sup>37</sup> we found that chronic oral administration of an AA improved the metabolic control assessed with glycated hemoglobin level. This finding was initially not taken into account for significant changes and thus was not prospectively hypothesized. We are fully aware that a better metabolic control may contribute to obtain an improvement in myocardial function. Moderate diabetes mellitus may alter the cardiac myosin isoform profile in a way comparable with that which occurs in a patient with more severe DM2<sup>38</sup>; thus, the improved metabolic control could positively influence the myocardial contractile function by reversing the low adenosine triphosphatase isoform, V<sub>3</sub>, to the high adenosine triphosphatase cardiac isoform, V<sub>1</sub>.

### Study limitations

In this study, we cannot entirely exclude that patients could have been able to figure out what they were actually taking, because of the very stringent flavor of AAs. The possible bias was largely minimized by using an AA mixture with as much as possible of a orange taste, like the placebo.

This study was designed and powered as an echocardiographic study of LV function. It should be considered a conceptual study, which provides insight into the mechanisms of early myocardial dysfunction in patients DM2 and the possibility to attenuate a metabolic disorder potentially responsible for progressive myocardial deterioration. Further trials involving larger numbers of patients will be needed to confirm our findings and to determine whether this approach may have real clinical benefit in the long term.

In conclusion, myocardial dysfunction is easily inducible with isometric exercise in patients with DM2 who have normal resting LV function and do not have CAD. An increased AA supply, in addition to conventional therapy, significantly attenuated this phenomenon. Although the precise underlying pathophysiological mechanism(s) is (are) not completely known, these data may eventually be important in designing optimal dietetic approach for patients with DM2 to prevent progressive myocardial dysfunction.

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