Metabolic and endothelial effects of trimetazidine on forearm skeletal muscle in patients with type 2 diabetes and ischemic cardiomyopathy

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INSULIN RESISTANCE CHARACTERIZES type 2 diabetes. The impairment in insulin action in type 2 diabetes is multifaceted and has been found in both cardiac and skeletal muscle (29). Nuutela et al. (22) demonstrated that heart and arm skeletal muscle glucose uptakes are inversely related to serum free fatty acid (FFA) levels and increased FFA flux from adipose tissue to nonadipose tissue, resulting from abnormalities of fat metabolism, and participates in and amplifies many of the fundamental metabolic derangements that are characteristic of the insulin resistance syndrome and type 2 diabetes (17).

In addition, new and interesting findings suggest that raised FFA levels not only impair glucose uptake in heart and skeletal muscle but also cause alterations in the metabolism of vascular endothelium, leading to premature cardiovascular disease (31).

In line with these suggestions, our group (19), by means of positron emission tomography, showed that high FFA levels are to be considered an additional risk for myocardial insulin resistance associated with endothelial dysfunction, in type 2 diabetic patients without cardiovascular disease.

Recently, new agents have raised interest in the treatment of cardiovascular ischemic heart disease or heart failure along with more classical cardiological agents and have been defined as “metabolic modulators,” switching energy substrate preference from fatty acid oxidation to glucose oxidation (29). Among them, trimetazidine has been used with beneficial effects as an antianginal agent. Trimetazidine has a favorable pharmacokinetic profile (13). In healthy volunteers it is rapidly absorbed, with an oral bioavailability for a dose of 40 mg of ~89%. In healthy volunteers after 15 days of treatment with 20 mg given orally twice daily, the Cmax is achieved in 1.7 h, and steady-state levels are reached within 24 h and remain stable. It is a weak protein binding, so it is widely distributed throughout the body and is excreted mainly unchanged (62%) in the urine (>80% in 48 h) The elimination half-life is ~6 h after both single or repeated oral administration. The beneficial effects of trimetazidine can be explained by an inhibition of fatty acid oxidation, secondary to inhibitions of mitochondrial long-chain 3-ketoacyl-CoA thiolase (18). In addition, Fantini et al. (10) showed that trimetazidine is a potent inhibitor of palmitoyl carnitine oxidation in isolated rat heart mitochondria. It is well known that, during and after ischemia, rates of glycolysis are high and glucose oxidation rates are low, with an increase of the production of protons and lactate and a decrease of cardiac efficiency. The use of trimetazidine, inhibiting fatty acid oxidation, will increase glucose oxidation, improve the coupling of glycolysis to glucose oxidation, lessen lactate and proton production, and improve cardiac efficiency (6).

Furthermore, in isolated perfused rat hearts, trimetazidine reverted the harmful effects of increased triglyceride levels, normalizing the impaired myocardial recovery from low-flow ischemia. This mechanism was related to a decrement in myocardial lipid oxidation and reduced citrate release (20).

Few studies have been reported on the long-term beneficial effects of trimetazidine. Vitale et al. (35) demonstrated that 6 mo of trimetazidine treatment was able to ameliorate left ventricular systolic and diastolic function, also improving quality of life in elderly patients with ischemic cardiomyopathy. In type 2 diabetic patients with ischemic cardiomyopathy, our group demonstrated that short-and long-term administration of...
trimetazidine improved left ventricular function, overall glucose metabolism, and glyated hemoglobin. Interestingly, the metabolic effects of trimetazidine were accompanied by a significant decrease of endothelin-1 levels, suggesting a beneficial effect of trimetazidine on endothelial function (11).

An additional interest in the use of trimetazidine in type 2 diabetes might arise from a beneficial effect of this drug on skeletal muscle metabolism, but as far as we know, no data are available on the effect of trimetazidine on forearm glucose and lipid metabolism and on forearm endothelial function in type 2 diabetes. Therefore, the aim of the present study was 1) to evaluate the influence of trimetazidine on muscle forearm glucose oxidation, nonoxidative glycolysis, and lipid oxidation and 2) to investigate the relationship between glucose and lipid muscle forearm metabolism with cGMP and endothelin-1 forearm release and their modifications by trimetazidine in patients with type 2 diabetes mellitus and ischemic cardiomyopathy.

METHODS

Patients. We selected 15 consecutive male diabetic patients (age 64 ± 7 yr, range 52–76 yr) with ischemic cardiomyopathy. Their clinical and metabolic variables are reported in Table 1. For type 2 diabetes mellitus, defined according to the American Diabetic Association criteria, they were treated by diet alone. We choose to study type 2 diabetic patients in diet alone in order to have a specific effect of trimetazidine on forearm glucose metabolism independent of possible interaction with other hypoglycemic agents. Diet was strictly controlled during the study period not only for carbohydrate but also for cholesterol content and remained unchanged throughout the study period, and this could explain the fact that they had near-normal cholesterol and normal HDL cholesterol and triglyceride levels. For cardiac disease, all patients received standard treatment with ACE inhibitors and β-blockers. Patients were considered for study in the presence of documented ischemic heart disease and optimized treatment for ≥12 wk and stable doses for the last 4 wk. Patients were excluded in case of recent acute coronary syndromes (<3 mo), primary valvular disease, severe New York Heart Association functional class (III or IV), high-grade arrhythmias, coronary lesions suitable for revascularization, and/or left ventricular aneurysm. The choice of patients with ischemic heart disease relates to the fact that this is a specific therapeutic indication for trimetazidine in Italy, and recently a beneficial effect of trimetazidine was demonstrated on dysfunctional myocardium in ischemic cardiomyopathy in a long-term study (7). After informed consent was obtained, patients were randomly allocated to a double-blind, placebo-controlled, cross-over parallel study to receive either placebo or trimetazidine (20 mg 3 times daily) for 15 days. During both periods, no changes were made in treatments for diabetic or cardiovascular diseases. Whether the medications for ischemic cardiomyopathy affected metabolic or endothelial variables, the fact of having stable treatments without changes during the whole study period allowed us to evaluate only the effects related to the addition of trimetazidine.

Study design. The morning after the last evening dose of a 15-day oral treatment with 20 mg of trimetazidine given three times daily, patients were admitted to the Metabolic Unit in the morning after an overnight fast to undergo a euglycemic hyperinsulinemic (insulin infusion 66 mU·kg⁻¹·h⁻¹) clamp lasting 120 min. On the morning of each test, a 20-gauge plastic cannula (Abbocath T; Abbott Ireland, Sligo, Ireland) was inserted, in retrograde position, into a dorsal hand vein of one hand and the hand was placed in a Plexiglas box and maintained at 55°C for intermittent sampling of arterialized blood. A 20-gauge plastic cannula was inserted into a large antecubital vein of the same arm for infusions. Another, 18-gauge, plastic cannula was inserted into a large, deep antecubital vein of the controlateral arm for intermittent sampling of deep venous forearm blood. Arterialized and deep venous samples for glucose, FFA, citrate, lactate, pyruvate, alanine, β-OH-butyrate, cGMP, and endothelin-1 measurements were withdrawn at −5, 0, 110, and 120 min.

Blood flow of the proximal forearm was measured immediately after each blood sample by venous occlusion plethysmography at −5, 0, 110, and 120 min. Blood flow was expressed in milliliters per minute per 100 ml of forearm tissue volume.

Forearm balances of the metabolic variables were calculated by using the Fick principle: (arterial blood concentration − deep venous blood concentration) × forearm blood flow.

Forearm glucose oxidation (FGOX) rates were estimated by forearm indirect calorimetry using arterialized and deep venous blood samples obtained at −5, 0, 110, and 120 min, after the start of the test, for the measurements of O₂ and CO₂. Nonoxidative glycolysis was derived as the net balance of lactate, pyruvate, and alanine in glucose equivalents. In addition, forearm glucose storage (glycogen formation) was calculated as the difference between glucose uptake and the sum of glucose oxidation and nonoxidative glycolysis. All these estimates were derived from well-validated methods, as previously published (23, 24). The net forearm balances of cGMP and endothelin-1 were calculated as (dV − A) × F, where A and dV indicate arterial and deep venous cGMP and endothelin-1 concentrations and F is forearm blood flow. Therefore, a positive balance indicates substrate release, whereas a negative balance indicates uptake, as previously demonstrated (21, 27).

Assays. Plasma glucose was measured with a glucose oxidase-based analyzer (Glucose Analyzer 2; Beckman Instruments, Fullerton, CA). Serum insulin levels were assayed with a microparticle enzyme immunoassay (IMX; Abbott Laboratories, Diagnostic Division, Chicago, IL). cGMP and endothelin-1 levels were measured with radioimmunoassay kits (NEN Life Science Products, Boston, MA, and Amersham International, Buckinghamshire, UK, respectively). FFA and serum triglyceride levels were measured using automated enzymatic spectrophotometric techniques adapted to COBAS FARA II (Hoffmann-La Roche, Basel, Switzerland). Samples for intermediary metabolites (lactate, alanine, pyruvate, and β-OH-butyrate) and citrate were assayed using automated enzymatic spectrophotometric methods adapted to COBAS FARA II.

Statistical analysis. Data are reported as mean ± SE. Comparisons between groups were performed using Student’s-paired t-test. Linear regression was calculated using Pearson’s correlation analysis. Two-tailed P < 0.05 was considered statistically significant.
RESULTS

Effects of trimetazidine on metabolic and endothelial parameters in the basal state. Compared with placebo, trimetazidine treatment decreased basal glucose levels by 12% (126 ± 8 vs. 143 ± 11 mg/dl; \( P < 0.05 \)), whereas insulin levels were slightly but not significantly (NS) decreased (11.8 ± 2.0 vs. 12.7 ± 2.1 \( \mu \)U/ml, NS). Accordingly, homeostasis model assessment of insulin resistance (HOMA-IR) levels, an index of fasting insulin resistance, were significantly lower after trimetazidine treatment, suggesting an amelioration of insulin sensitivity (3.67 ± 0.04 vs. 4.48 ± 0.06, \( P < 0.05 \)). On the other hand, no differences were found in the basal levels of triglyceride, FFA, citrate, and lactate in both trimetazidine and placebo treatments (Table 2).

In Figs. 1 and 2, the evaluation of basal forearm glucose and lipid metabolism demonstrated that basal forearm glucose uptake (FGU), glucose oxidation, glucose storage, lipid oxidation, FFA uptake, and citrate release were similar during trimetazidine or placebo treatments. On the other hand, basal nonoxidative glycolysis was significantly lower after trimetazidine (0.098 ± 0.08 vs. 0.331 ± 0.09 \( \mu \)mol·100 ml·min\(^{-1}\), \( P < 0.05 \)).

In Fig. 3 is reported the evaluation of basal and postclamp endothelial parameters. After trimetazidine, basal cGMP levels were significantly higher (3.2 ± 0.4 vs. 1.9 ± 0.4 \( \mu \)mol/l, \( P < 0.05 \)), whereas endothelin-1 levels significantly decreased (11.1 ± 0.5 vs. 13.0 ± 0.8 pg/ml, \( P < 0.05 \)) compared with placebo.

Forearm blood flows were similar in the basal state (2.4 ± 0.2 vs 2.2 ± 0.2 ml/min/100ml forearm tissue volume) while it increased by about 20% after trimetazidine treatment (3.1 ± 0.2 vs 2.6 ± 0.2 ml·100·1 min\(^{-1}\)·min\(^{-1}\) forearm tissue volume, NS).

Effects of trimetazidine on forearm glucose and lipid metabolism and forearm cGMP and endothelin-1 release after the euglycemic clamp. All patients were successfully clamped, with a coefficient of variation below 5%, and insulin levels reached a plateau of 95.1 ± 7.1 and 94.8 ± 7.7 \( \mu \)U/ml after trimetazidine and placebo, respectively.

By the end of the clamp in the group treated with trimetazidine compared with placebo, the M-value (glucose infusion rate at the end of the clamp period) was 4.0 ± 0.4 vs. 3.3 ± 0.4 mg·kg body wt \(^{-1}\)·min\(^{-1}\) (\( P < 0.003 \)). Furthermore, there was

Table 2. Clinical and metabolic variables of 15 type 2 diabetic patients with ischemic cardiomyopathy after 15 days of trimetazidine or placebo treatments

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>TMZ</th>
<th>( P )</th>
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<tbody>
<tr>
<td>Fasting glucose, mg/dl</td>
<td>143±11</td>
<td>126±8</td>
<td>0.05</td>
</tr>
<tr>
<td>Fasting insulin, ( \mu )U/ml</td>
<td>12.7±2.1</td>
<td>11.8±2.0</td>
<td>NS</td>
</tr>
<tr>
<td>Hb A(_1c), %</td>
<td>7.1±0.4</td>
<td>7.1±0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting cholesterol, mg/dl</td>
<td>224±15</td>
<td>215±20</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting HDL cholesterol, mg/dl</td>
<td>48±3</td>
<td>54±5</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting triglycerides, mg/dl</td>
<td>143±15</td>
<td>145±17</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting FFA, mmol/l</td>
<td>0.63±0.05</td>
<td>0.56±0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting lactate, ( \mu )mol/l</td>
<td>791±70</td>
<td>745±61</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting pyruvate, ( \mu )mol/l</td>
<td>99±12</td>
<td>100±11</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting citrate, ( \mu )mol/l</td>
<td>100±13</td>
<td>63±8</td>
<td>0.05</td>
</tr>
<tr>
<td>Fasting β-OH-butrate, ( \mu )mol/l</td>
<td>197±29</td>
<td>116±18</td>
<td>0.05</td>
</tr>
<tr>
<td>Fasting alanine, ( \mu )mol/l</td>
<td>275±27</td>
<td>310±18</td>
<td>NS</td>
</tr>
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</table>

Values are means ± SE. TMZ, trimetazidine; NS, not significant.
a significant increase of FGU by 27.7% (3.58 ± 0.43 vs 2.60 ± 0.56 μmol·100 ml forearm⁻¹·min⁻¹, P < 0.05; Fig. 1). Interestingly, glucose oxidation nearly doubled after trimetazidine (1.80 ± 0.14 vs. 0.84 ± 0.16 μmol·100 ml forearm⁻¹·min⁻¹, P < 0.0001; Fig. 1), whereas no differences were found for glucose storage and nonoxidative glycolysis. In Fig. 2 are reported estimations of forearm lipid metabolism. In particular, after trimetazidine compared with placebo, lipid oxidation was almost completely inhibited (−0.05 ± 0.03 vs. 0.07 ± 0.02 μmol·100 ml forearm⁻¹·min⁻¹, P < 0.05), whereas citrate release decreased by 62% (14.7 ± 6.15 vs. 39.7 ± 10.2 μmol·100 ml forearm⁻¹·min⁻¹, P < 0.05). No significant differences were found when FFA uptake and β-OH-butyrate uptake were measured during both treatments. At the end of the clamp, lactate levels were lower during trimetazidine compared with placebo (870 ± 63 vs. 1,129 ± 100 μmol/l, P < 0.05; data not shown).

Compared with placebo, cGMP levels significantly increased, and endothelin-1 significantly decreased, after the euglycemic clamp (Fig. 3). This was accompanied by a significant increase of forearm cGMP release by 162% (2.36 ± 0.31 vs. 0.91 ± 0.38 μmol·100 ml forearm⁻¹·min⁻¹, P < 0.001). Furthermore, forearm endothelin-1 release was completely abolished (−0.08 ± 0.37 vs. 2.38 ± 0.92 pg·100 ml forearm⁻¹·min⁻¹, P < 0.05).

Relation between forearm glucose and lipid metabolism and forearm release of endothelial vasodilator cGMP and vasocostructor endothelin-1 variables. Forearm glucose oxidation was positively and significantly correlated with forearm cGMP release (r = 0.37, P < 0.04), whereas lipid oxidation was positively and significantly correlated with endothelin-1 release (r = 0.40, P < 0.03). In addition, lipid oxidation directly correlated with citrate release (r = 0.42, P < 0.02) and inversely correlated with glucose oxidation, as expected (r = −0.71, P < 0.0001). There was a significant correlation between citrate release and FFA uptake (r = 0.37, P < 0.05) and between citrate release and lactate levels (r = 0.53, P < 0.05).

DISCUSSION

Type 2 diabetes mellitus is a widely recognized state of whole body, myocardial, and skeletal muscle insulin resistance associated with an elevation of fasting and postprandial FFA levels. It is well known that, in diabetes and insulin-resistant states, the heart uses an excess of FFAs and has reduced metabolism of glucose (38). The glucose-fatty acid cycle hypothesis suggests that an increased availability of FFA increases the tricarboxylic acid cycle activity, leading to intracellular entry of both acetyl-CoA and citrate. To decrease FFA activity, compounds with antilipolytic activities, such as nicotinic acid or nicotinic acid analogs, have been previously used with promising results (23). Unfortunately, due to their short antilipolytic duration, they were not able to determine a chronic decrement in FFA levels and in turn a prolonged improvement in glucose metabolism (34).

Recently Kantor et al. (15) showed that trimetazidine inhibits one of the terminal steps in the β-oxidation pathway, the mitochondrial long-chain 3-ketoacyl A-CoA thiolase, resulting in a switch of energy substrate preference and improving coupling between glycolysis and glucose oxidation.

Until now, the metabolic effects of trimetazidine were shown only on heart. The novelty of the present study is the demonstration that the effects of trimetazidine on β-oxidation are also operating in skeletal muscle in type 2 diabetic patients with ischemic cardiomyopathy. The effects of trimetazidine were determined by three different approaches, first by measuring forearm muscle lipid and glucose oxidation by forearm indirect calorimetry. Fifteen-day trimetazidine treatment was able to increase glucose oxidation while completely inhibiting lipid oxidation and significantly reducing lactate levels, both in the fasting state and after insulin stimulation, suggesting a shift to a better energy supply and also explaining the amelioration of whole body glucose metabolism achieved in patients with type 2 diabetes and cardiomyopathy by Fragasso et al. (11). Second, we measured forearm citrate release as an indirect index of β-oxidation (20), and there was a significant decrease of this index during insulin stimulation when trimetazidine was administered. Third, the measurement of forearm FFA uptake, not different with or without trimetazidine treatment, strongly confirmed the direct inhibition of forearm β-oxidation by trimetazidine.

The fact that FFA uptakes were not different is related, in our opinion, to the fact that in both studies FFA levels fell below 0.1 mmol/l at the end of the clamp. These low levels might have reduced the activation of fatty acid transport protein-1 (FATP1) (acyl-CoA synthetase). In addition, fatty acids taken up through FATP1 are preferentially channeled into triglyceride synthesis and only subsequently hydrolyzed to acyl-CoA for β-oxidation determining an intracellular acyl-CoA pool (14).
Different rates of findings could explain why FFA uptake is not discriminant of total lipid oxidation in skeletal muscle (1, 12). These latter findings that plasma FFA accounted for only 20–50% of the FFA pool combined with indirect calorimetry, have shown that plasma FFA turnover and oxidation in humans. Moreover, the simultaneous acute administration of L-arginine and endothelin-1 levels in patients with cardiovascular syndrome, in type 2 diabetic patients, and in patients with cardiovascular disease (3). In addition, it was demonstrated that chronic endothelin-1 treatment induced insulin resistance, suggesting that a positive feedback system may exist in vivo in which insulin resistance begets more insulin resistance through the endothelin-1 system (36). 

However, the significant correlation between lipid oxidation and endothelin-1 release. This work was supported, in part, by a grant from Italy’s Ministry of Health (RF02-130).

**REFERENCES**


