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

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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-ketoyl-CoA thiolase (18). In addition, Fantini et al. (10) showed that trimetazidine is a potent inhibitor of palmitoylcarnitine 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

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). Nuutila 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).
trimetazidine improved left ventricular function, overall glucose metabolism, and glycated 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 glucose metabolism independent of pos-

tional 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 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: (arterialized 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 intermedi-

ate 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 μU/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 uptake, and citrate release during lipid metabolism demonstrated that basal forearm glucose metabolism at the end of the euglycemic clamp studies (right) in 15 type 2 diabetic patients with cardiomyopathy after 15 days of trimetazidine (TMZ; filled bars) and after 15 days of placebo (empty bars). FGU, forearm glucose uptake; F., forearm; Gluc., glucose; Non Ox., nonoxidative. Values are means ± SE. *P < 0.05; ***P < 0.001.

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 μmol·100 ml⁻¹·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 μmol/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 ml⁻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 μU/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 no change in the basal forearm blood flows.

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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</thead>
<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, μU/ml</td>
<td>12.7 ± 2.1</td>
<td>11.8 ± 2.0</td>
<td>NS</td>
</tr>
<tr>
<td>Hb A₁c, %</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, μmol/l</td>
<td>791 ± 70</td>
<td>745 ± 61</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting pyruvate, μmol/l</td>
<td>99 ± 12</td>
<td>100 ± 11</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting citrate, μmol/l</td>
<td>100 ± 13</td>
<td>63 ± 8</td>
<td>0.05</td>
</tr>
<tr>
<td>Fasting β-OH-butyrate, μmol/l</td>
<td>197 ± 29</td>
<td>116 ± 18</td>
<td>0.05</td>
</tr>
<tr>
<td>Fasting alanine, μmol/l</td>
<td>275 ± 27</td>
<td>310 ± 18</td>
<td>NS</td>
</tr>
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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 vasoconstrictor 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).

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).
Previous studies using [1-14C]palmitate as a tracer of circulating FFA pool combined with indirect calorimetry, have shown that plasma FFA accounted for only 20–50% of the total lipid oxidation in skeletal muscle (1, 12). These latter findings could explain why FFA uptake is not discriminant of different rates of β-oxidation and the citrate release is the best marker of β-oxidation. Data of a complete inhibition of muscle lipid oxidation after trimetazidine confirmatory of previous results of our group (23) using acipimox (to inhibit lipolysis) under conditions of hyperinsulinemia, and the strong inverse correlation between glucose and lipid oxidation at the end of the clamp suggests that glucose was the preferential substrate for the muscle under these circumstances.

During trimetazidine treatment, a 12% decrease in circulating glucose levels in the presence of unchanged insulin levels was accompanied by a 17% increase in muscle glucose disposal, suggesting that this drug improves insulin action not only to heart but also to skeletal muscle, acting as a whole body “metabolic modulator,” as suggested in Refs.16 and 30.

However, new aspects of this drug are presented mainly on the effects on endothelial function. To our knowledge, there are few results on the effects of trimetazidine on endothelin-1 release. New and interesting findings of the present study relate to the evaluation of the forearm release of vasodilator (cGMP) and vasoconstrictor (endothelin-1) factors after 15 days of trimetazidine treatment. The administration of trimetazidine determined a significant increase in circulating cGMP levels, a second messenger of nitric oxide, and in insulin induced-forearm cGMP release associated with a decrement in circulating endothelin-1 levels and in insulin-stimulated forearm endothelin-1 release. Note that forearm glucose oxidation rate positively correlated with cGMP release. On the other hand, a significant correlation was found between lipid oxidation and endothelin-1 release.

Previous studies have shown that an acute or prolonged intravenous insulin administration can simultaneously stimulate both nitric oxide and endothelin-1 release in healthy humans (4, 27). These results are confirmed by an elegant in vitro study performed by Cusi et al. (5), wherein insulin can stimulate both phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways. Through the PI3K-Akt pathway it is possible to induce endothelial nitric oxide synthase (eNOS) activation and in turn stimulate NOS and cGMP activation (33, 39), whereas through the MAPK pathway, endothelin-1 is released.

Previous studies have shown that insulin stimulates cGMP production in blood vessels (39) and in vascular smooth muscle cells (33). More recently, an acute infusion of 0.1 U/kg body wt insulin induced a significant increase in circulating NO levels and forearm cGMP release in humans (25). Furthermore, an acute infusion of l-arginine was able to decrease basal endothelin-1 levels in patients with cardiovascular syndrome X, and the simultaneous acute administration of l-arginine and insulin was able to restore endothelin-1 levels to near-normal values (27). On the other hand, Steinberg et al. (33) demonstrated that, in case of insulin resistance, a state of chronic hyperinsulinemia, impaired insulin-mediated glucose uptake is associated with a defect in insulin-stimulated endothelial vasodilation and increased endothelin-1 levels.

In the present study, the prolonged inhibition of β-oxidation by trimetazidine increased circulating cGMP levels to those previously found in normal subjects (26). Our data are a further demonstration that, when NO availability and activity are improved, there is a significant amelioration of insulin sensitivity and glucose utilization with a positive feedback on insulin action (9, 37). These data are reinforced by the presence of a positive significant correlation between forearm blood flow and muscle glucose uptake during the clamp period.

Conversely, during the state of insulin resistance, insulin signaling via the MAPK pathway remains intact (5), increasing endothelin-1 production (8). It is well known that endothelin-1 is a powerful vasoconstrictor peptide implicated in atherogenesis by stimulating mitogen activation (2), and increased endothelin-1 levels were found in subjects with the metabolic 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).

Our results of decreased endothelin-1 levels in the presence of hyperinsulinemia during the clamp are only apparently contradictory of previous studies, as the major effect of insulin was to restore the balance between vasodilator (cGMP) and vasoconstrictor agents (endothelin-1).

The present study underlines the strict correlation between lipid and glucose metabolism and endothelial function and the possibility of ameliorating glucose metabolism and myocardial and skeletal muscle function improving endothelial activity.

A possible limitation of the study relates to the fact that we did not perform a tracer study to evaluate the effect of trimetazidine not only on muscle glucose uptake but also on hepatic glucose production, but this was beyond the aim of study, even though this important issue deserves further investigation.

The present study was performed in a highly selected group of male type 2 diabetic patients with ischemic cardiomyopathy. Further studies are needed to corroborate the presence of similar beneficial effects of trimetazidine in women with ischemic cardiomyopathy. In addition, other studies are desired to better understand the effects of trimetazidine in the presence of hyperlipemia and in large-scale trials.

In conclusion, we evaluated forearm glucose and lipid metabolism and forearm release of endothelial vasodilator and vasoconstrictor factors during a prolonged inhibition of β-oxidation by trimetazidine. Under these circumstances, insulin induced-forearm glucose oxidation and forearm cGMP release were increased whereas forearm endothelin-1 release was decreased. The effects of trimetazidine at the skeletal muscle add new interest in the use of trimetazidine in type 2 diabetic patients, but the chronic effects of trimetazidine need further evaluation.

GRANTS

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