RESEARCH

Cardiovascular Therapeutics

Trimetazidine Reduces Endogenous Free Fatty Acid Oxidation and Improves Myocardial Efficiency in Obese Humans

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Keywords
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SUMMARY

Introduction: The metabolic modulator trimetazidine (TMZ) has been suggested to induce a metabolic shift from myocardial fatty acid oxidation (FAO) to glucose utilization, but this mechanism remains unproven in humans. The oxidation of plasma derived FA is commonly measured in humans, whereas the contribution of FA from triglycerides stored in the myocardium has been poorly characterized. **Aims:** To verify the hypothesis that TMZ induces a metabolic shift, we combined positron emission tomography (PET) and magnetic resonance spectroscopy (^{1}H-MRS) to measure myocardial FAO from plasma and intracellular lipids, and myocardial glucose metabolism. Nine obese subjects were studied before and after 1 month of TMZ treatment. Myocardial glucose and FA metabolism were assessed by PET with ^{18}F-fluorodeoxyglucose and ^{11}C-palmitate. ^{1}H-MRS was used to measure myocardial lipids, the latter being integrated into the PET data analysis to quantify myocardial triglyceride turnover. **Results:** Myocardial FAO derived from intracellular lipids was at least equal to that of plasma FAs (P = NS). BMI and cardiac work were positively associated with the oxidation of plasma derived FA ($P \leq 0.01$). TMZ halved total and triglyceride-derived myocardial FAO (32.7 ± 8.0 to 19.6 ± 4.0 μmol/min and 23.7 ± 7.5 to 10.3 ± 2.7 μmol/min, respectively; $P \leq 0.05$). These changes were accompanied by increased cardiac efficiency since unchanged LV work (1.6 ± 0.2 to 1.6 ± 0.1 Watt/g × 10^2, NS) was associated with decreased work energy from the intramyocardial triglyceride oxidation (1.6 ± 0.5 to 0.4 ± 0.1 Watt/g × 10^2, $P = 0.036$). **Conclusions:** In obese subjects, we demonstrate that myocardial intracellular triglyceride oxidation significantly provides FA-derived energy for mechanical work. TMZ reduced the oxidation of triglyceride-derived myocardial FAs improving myocardial efficiency.

Introduction

The metabolic modulator trimetazidine (TMZ) may improve myocardial energy metabolism by inhibiting FAO and promoting glucose metabolism. This speculation originates from in vitro and animal studies in which TMZ partially inhibits the beta-oxidation enzyme 3-keto-CoA-thiolase [1,2]. Our recent PET study in patients with dilated cardiomyopathy is the only investigation in which FAO was measured during TMZ treatment, which improved cardiac efficiency and function, accompanied by a modest decline in plasma FAO rate constant [3]. However, the role of intracellular lipids was not considered, glucose uptake was not measured, and patients had severe heart failure with metabolic and medication-related confounders. To our knowledge, there are no data about the reciprocal modulation of FA and glucose metabolism by TMZ, and the consequences on work efficiency in humans.

Obesity is an independent predictor of heart failure [4,5]. There is compelling evidence that myocardial metabolic toxicity due to obesity and insulin resistance is implicated in the pathogenesis of the related cardiac complications [6]. We and others have recently documented the occurrence of cardiac steatosis in obese and type 2 diabetic patients, correlating with cardiac dimensions and function [7,8]. PET studies in humans have shown that myocardial FA oxidation is elevated in insulin resistant obese individuals [9]. This technique measures the influx of FAs from the circulation and gives the relative fractions (rate constants) of substrate moving in and out of triglyceride stores, but it cannot estimate the absolute amounts of intracellular lipid turnover. Estimation of the latter requires that the PET-derived rate constants are multiplied by the intracellular mass of FAs, as quantified by ^{1}H-MRS. Of note, myocardial triglycerides in lean individuals are 10 μmol/g (= 30 μmol/g of FAs) [7] which is greater more than twice the hourly uptake of FAs from blood (about 4 μmol/g/h of triglycerides or 12 μmol/g/h of FAs, calculated from Peterson et al.) [9]. Obesity trebles the size of this pool, which may account for a remarkable overflow of FA to mitochondria in overweight subjects [7].

The aims of our study were to estimate the contributions of plasma and intracellular lipids to myocardial FAO, and to test whether TMZ provokes a metabolic shift from myocardial FA metabolism to glucose uptake, which may influence efficiency of work in obese individuals. We hypothesized that triglyceride stores represent a significant source of oxidative energy in the heart of obese individuals, and that the inhibition of endogenous FAO by TMZ shifts myocardial metabolism towards glucose utilization. This hypothesis was tested by combining compartmental modeling of PET data with measurements obtained with ^{1}H-MRS in obese individuals before and after 1 month of TMZ treatment.

Material and Methods

Subjects

Nine nondiabetic, obese subjects were recruited by local advertisement. Inclusion criteria were body mass index (BMI) $\geq$27 kg/m^2, age 20–75 years, no chronic diseases, no substance abuse, normal physical examination and a stable weight and diet for the past 3 months. Written informed consent was obtained from all subjects after providing detailed information on the nature, purpose, and potential risks of the study. The study protocol was conform with the principles outlined in the Declaration of Helsinki and was approved by the Ethics Committee of the Hospital District of Southwest-Finland.

Study Design

Routine blood testing was performed during the screening visit, together with a 75 g oral glucose tolerance test to exclude undiagnosed diabetes mellitus. Patients underwent PET imaging sessions using [^{18}F]-fluorodeoxyglucose ([^{18}F]FDG) and [^{11}C]-palmitate to determine myocardial glucose and FA metabolism, MR imaging to assess left ventricular (LV) dimensions and function, and proton MRS to quantify the content of triglycerides and creatine in the LV wall. After baseline examinations, a 4-week treatment with TMZ (35 mg daily, Vastarel®, Laboratoires Servier France, Neuilly-sur-Seine, France) was started. Diet counseling was provided for the standardization of caloric intake, and patients were instructed to maintain their current lifestyle. After 2 weeks of treatment, an additional visit took place during which compliance was confirmed, a clinical examination performed, and blood samples drawn. The PET and MR imaging examinations were repeated at the end of the treatment.

MRI and ^{1}H-MRS Studies

A Philips 1.5T Gyroscan Intera CV Nova Dual MR scanner (Philips Medical Systems, Best, The Netherlands) was used for cardiac MR and ^{1}H-MRS, as previously described [7]. LV mass, end diastolic volume (EDV), end systolic volume (ESV) and ejection fraction (EF) were measured from continuous short axis slices by using the balanced field echo sequence [7]. Cardiac output (CO) and stroke volume (SV) were computed from ESV, EDV, and heart rate [10]. LV work was calculated as the product of mean arterial blood pressure and CO, and then multiplied by 0.133 and divided by LV mass to obtain units of joules/min/g. The cardiac index was calculated as the ratio between CO and body surface area. Peripheral vascular resistance was calculated as the ratio of mean blood pressure (mmHg) to CO (mL/min).

The water, triglyceride and creatine signals in the MR spectra were analyzed with the user-independent LCModel software [11]. For calculation of myocardial triglyceride concentrations, signal amplitudes were corrected for different T_2 decay and molar concentrations of ^{1}H nuclei in triglycerides and water [12,13]. Myocardial triglyceride content was defined as triglyceride in relation to the total weight of myocardial tissue [14,15]. Using the same MR spectra, creatine signals around 3.0 ppm were analyzed and myocardial creatine concentration was calculated as the creatine-to-water ratio [16].

PET Studies

Each study was performed after an 8–10 h overnight fast. PET images were obtained by either the GE PET-CT scanner (Discovery VCT, GE Medical systems, Milwaukee, USA) or the Siemens/CTI HR+ PET scanner (Siemens Inc, Knoxville, TN, USA). The positron emitting tracers [^{18}F]FDG and [^{11}C]-palmitate were produced as previously described [17,18]. Two catheters were inserted, one in an antecubital vein for injection of the radiolabeled tracer, another in the opposite antecubital vein for blood sampling. A heating pad was used to arterialize venous blood at the sampling site. Dynamic imaging of the thoracic area was performed after intravenous administration of the radiopharmaceutical. Each patient underwent PET studies both with [^{11}C]-palmitate (295 $\pm$ 48 MBq) and [^{18}F]FDG (156 $\pm$ 8 MBq) in separate days, both before and after treatment, to quantify myocardial FA and glucose metabolism, respectively. During the [^{11}C]-palmitate scan, arterialized venous blood was withdrawn at 10, 20, 30, 40 and 50 min to assess the fraction of unchanged versus metabolized radiopharmaceutical [19].

All data were corrected for dead time, decay, and measured photon attenuation. Images were reconstructed with standard algorithms. Tissue and input time activity curves (TACs) were obtained

from reconstructed PET images, by drawing horseshoe regions of interest (ROI) to cover the LV myocardium on 3–4 transaxial planes and a small ROI in the LV cavity to obtain blood concentrations over time.

[^{18}F]FDG-TACs were used for calculation of the myocardial fractional extraction rate of [^{18}F]FDG by using graphical analysis. Myocardial glucose uptake (MGU) was computed as the product of fractional extraction rate (1/min) and plasma glucose concentration (mmol/L), and divided by a lumped constant term of 1.0, as previously described [20]. [^{11}C]-palmitate-TACs were analyzed with two recognized procedures to measure myocardial FFA uptake (MFU), FFA oxidation, FFA esterification, and oxidation of stored FAs:

(a) Compartmental model analysis was implemented by using the model proposed by de Jong et al. for the estimation of plasma free FA (FFA) oxidation, esterification, and uptake rates [21]. The model, which is shown in Figure 1(A), is composed of three tissue compartments, representing free [^{11}C]-palmitate, [^{11}C]-palmitate bound in complex lipids, and [^{11}C]-breakdown products. The movement of radioactivity between compartments is described by four rate constant terms, as given in Figure 1(A). The differential equations describing changing tracer concentrations in the model compartments are

$$dC_1/dt = C_a(t)xK_1 - C_1(t)x(k_2 + k_3) \quad (1)$$

$$dC_2/dt = C_1(t)xk_2 \quad (2)$$

$$dC_3/dt = C_1(t)xk_3 - C_3(t)xk_4 \quad (3)$$

and the total concentration of [^{11}C]-palmitate in the myocardium at a given time can be defined by the sum of the tracer in each compartment and in the tissue blood fraction

$$\text{Myocardium}(t) = (1 - S_{1v})xC_1(t) + C_2(t) + C_3(t) + C_b(t)xS_{1v}, \quad (4)$$

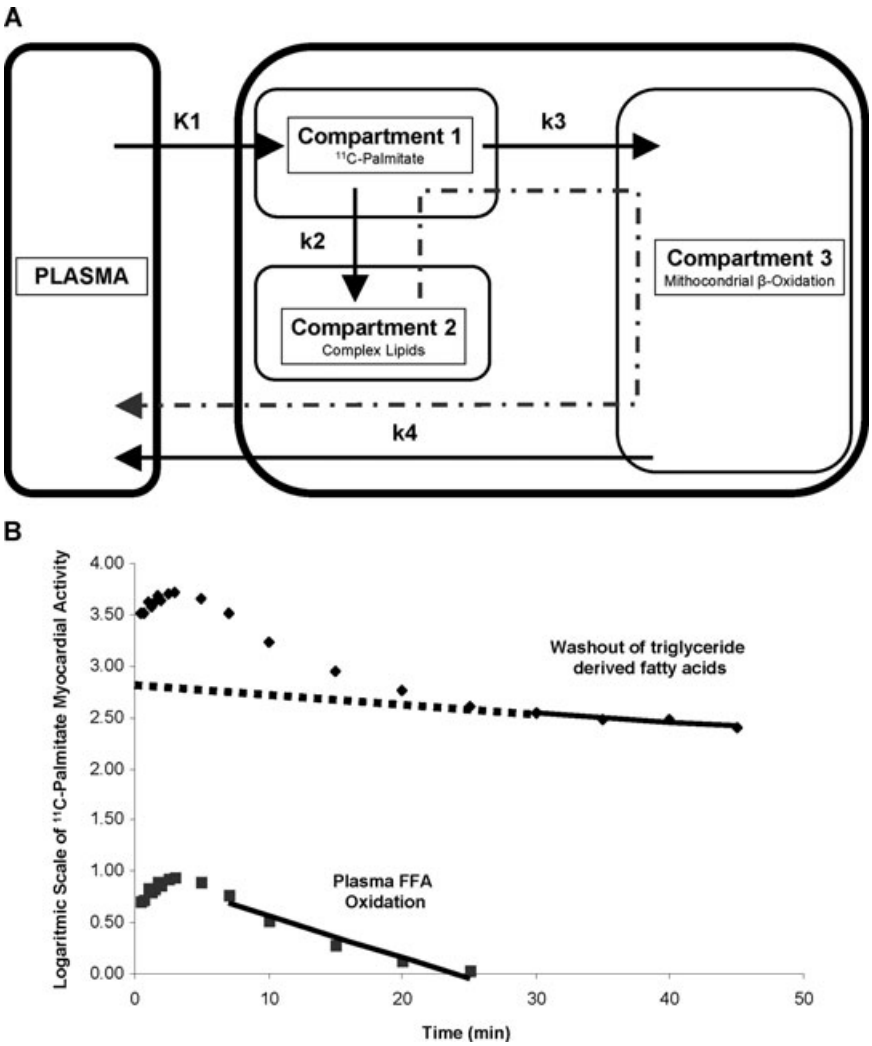

Figure 1 Kinetics of [^{11}C]-palmitate in the heart as analyzed through (A) compartmental modeling, or (B) bi-exponential fitting. The tissue curve in "B" shows distinct declining rates in the initial and later phase of the myocardial washout phase. The dashed gray arrow in "A" indicates slow fatty acid turnover and washout through oxidation.

where $C_b(t)$ is the blood tracer concentration, as derived from the left ventricle cavity ROI interpolated through a spline function, $C_a(t)$ is the plasma concentration of unchanged [^{11}C]-palmitate, that is, discounted for metabolites, S_{lv} represents the fraction of arterial activity into the myocardial tissue, and C_n is the concentration of tracer in each tissue compartment. The rate constant k_4 was fixed to be equal to k_3, as suggested [21].

The system of differential equations was solved numerically, by using the commercial software SAAM II (Simulation Analysis and Modeling II, version 1.2.1, SAAM Institute). The rate constants obtained were used together with plasma (P) FFA concentration measurements (C_{FFA}) to derive respective substrate flux rates, by assuming steady-state, according to Bergmann et al. [22].

$$\text{P-FFA esterification rate} = C_{FFA}(\mu\text{mol/mL}) x (K_1 x k_2)/(K_1 + k_2 + k_3)\,(1/\text{min}) \quad (5)$$

$$\text{P-FFA oxidation rate} = C_{FFA}(\mu\text{mol/mL}) x (K_1 x k_3)/(K_1 + k_2 + k_3)\,(1/\text{min}) \quad (6)$$

$$\text{P-FFA uptake rate} = \text{esterification} + \text{oxidation rates}\ (\mu\text{mol/mL min}) \quad (7)$$

(b) Bi-exponential fitting was applied to the myocardial [^{11}C]-Palmitate TACs (Figure 1B). The two washout rate coefficients have been used to represent FA oxidation rate constants. The initial and rapid descending part of the curve is commonly interpreted as the immediate oxidation of plasma derived FFA [23], whereas the later and slower tissue activity decline represents the oxidation of FAs after they have been transferred in and out of the myocardial triglyceride pool [24]. Since the triglyceride pool was measured by ^{1}H-MRS in the current study, we were able to estimate the absolute oxidative flux of triglyceride derived FAs (triglyceride:FA, 3:1) by using the following equation

$$\text{Oxidation of stored FAs} = \text{Cardiac lipid}(\mu\text{mol/g}) \times 3 \times \text{rate constant}\ (1/\text{min}) \quad (8)$$

Energy Provision and LV Work

We used PET and MR data to estimate the amount of the energy potentially provided by the substrates utilized in the heart. The rates of plasma FFA oxidation, triglyceride FA oxidation, and glucose uptake were multiplied by 129 or 36 (ATP net molecular yields for FAs and glucose, respectively) and then by 30.5 (joules/mol of ATP) in order to translate respective energy production into the same units used for work estimations (watts/g of tissue) [25,26]. These measured energy production values were compared against the measured LV forward work. This allows us to estimate the energy wasting due to an inefficient mechanical-metabolic coupling and the effect of TMZ on it.

Biochemical Analysis

All laboratory specimens were drawn after a 12-h fasting period. Fasting plasma glucose was determined using the glucose oxidase method (Analox Glucose analyzer, GM9). Insulin was measured using electrochemiluminescence immunoassay (Roche Modular E170 analyzer; Roche Diagnostics GmbH, Mannheim, Germany). Serum FFA were measured enzymatically (Wako Nefa C kit; Wako Chemicals GmbH, Germany). Among biohumoral determinations, we included also plasma concentrations of apelin-12, determined using a commercially available enzyme immunoassay without extraction (Phoenix Pharmaceuticals, Burlingame, CA, USA) according to manufacturer's instructions. This assay employs an immunoaffinity purified rabbit antibody specific for apelin 1–12. The antibody has 100% cross-reactivity to apelin 1–12, 1–13, and 1–36. Each sample was assayed in duplicate. Plasma concentrations were obtained by the interpolation of the dose–response curves computed using a four-parameter logistic function [27].

Statistical Analysis

All data are presented as means ± SEM. Differences in paired data were evaluated using the nonparametric Wilcoxon signed rank test. Regression analyses were carried out according to standard techniques. *P*-values ≤ 0.05 were deemed statistically significant. The *a priori* power analysis was based on our previous study [3], and showed that in a two tailed paired test with alpha error of 0.05, a 15% significant change in plasma FAO could be detected with a power of 0.81 in a group of eight subjects.

Results

All patients completed the 1-month treatment with TMZ. One patient had to be excluded from the paired evaluation due to his poor compliance with the diet counseling, as assessed by food intake questionnaires (data not shown). Table 1 shows baseline biochemical and hemodynamic characteristics.

Table 1 Subjects' characteristics

Sex (male/female)	4/5		
Age (years)	54 ± 3		
	Baseline	After Treatment	*P*
Body mass (kg)	88.8 ± 3.6	86.5 ± 3.8	0.017
Body fat (%)	38 ± 2	38 ± 1	0.202
BMI (kg/m^2)	31.2 ± 1.1	30.9 ± 1.2	0.017
Biochemical			
fS-FFA (mmol/L)	0.62 ± 0.05	0.7 ± 0.05	0.208
fP-glucose (mmol/L)	5.24 ± 0.11	5.45 ± 0.22	0.671
fS-insulin (mU/L)	8.11 ± 1.44	8.38 ± 1.50	0.270
Apelin-12 (ng/mL)	0.34 ± 0.04	0.26 ± 0.03	0.068
Leptin (ng/mL)	27 ± 9	35 ± 13	0.327
Hemodynamic			
Heart rate (bpm)	63 ± 3	63 ± 2	0.611
Systolic BP (mmHg)	135 ± 8	138 ± 9	0.932
Diastolic BP (mmHg)	85 ± 5	86 ± 3	0.483
RPP (mmHg/min)	8748 ± 970	8621 ± 695	0.889

BMI, body mass index; fP, fasting plasma; fS, fasting serum; BP, blood pressure; and RPP, rate-pressure product.
Notes: Values are mean ± SEM for number of patients. Baseline n = 9, After Treatment and *P* values n = 8.

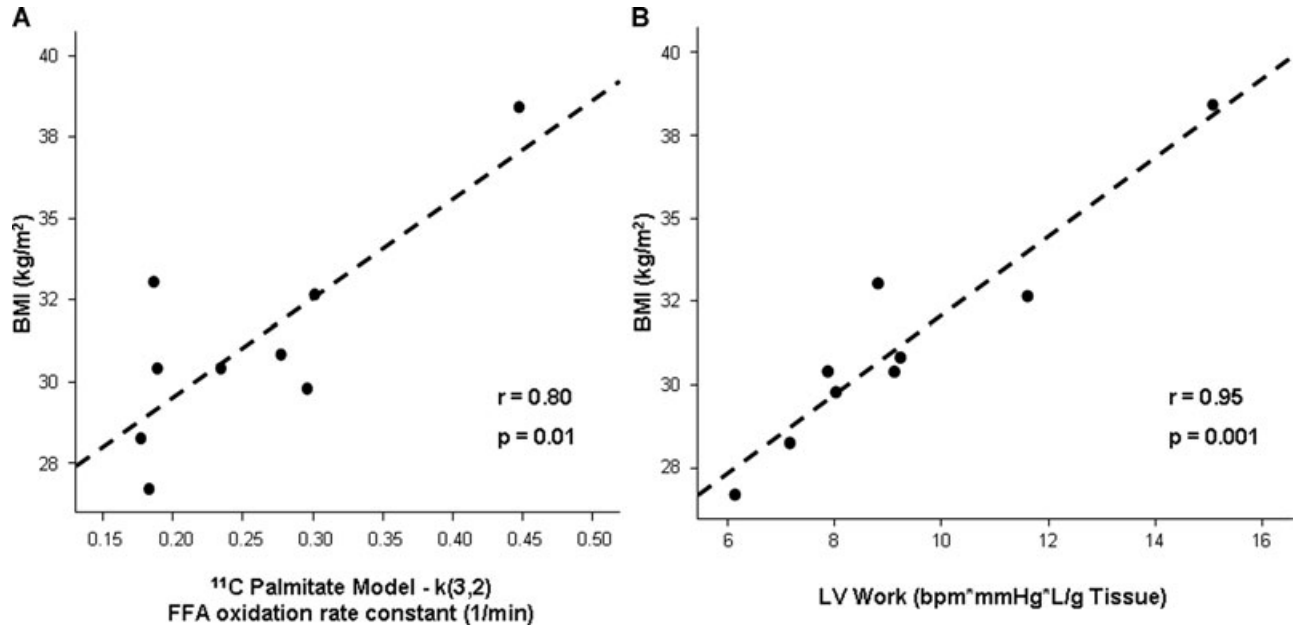

Figure 2 Positive relationships between BMI and (A) plasma fatty acid oxidation or (B) LV work.

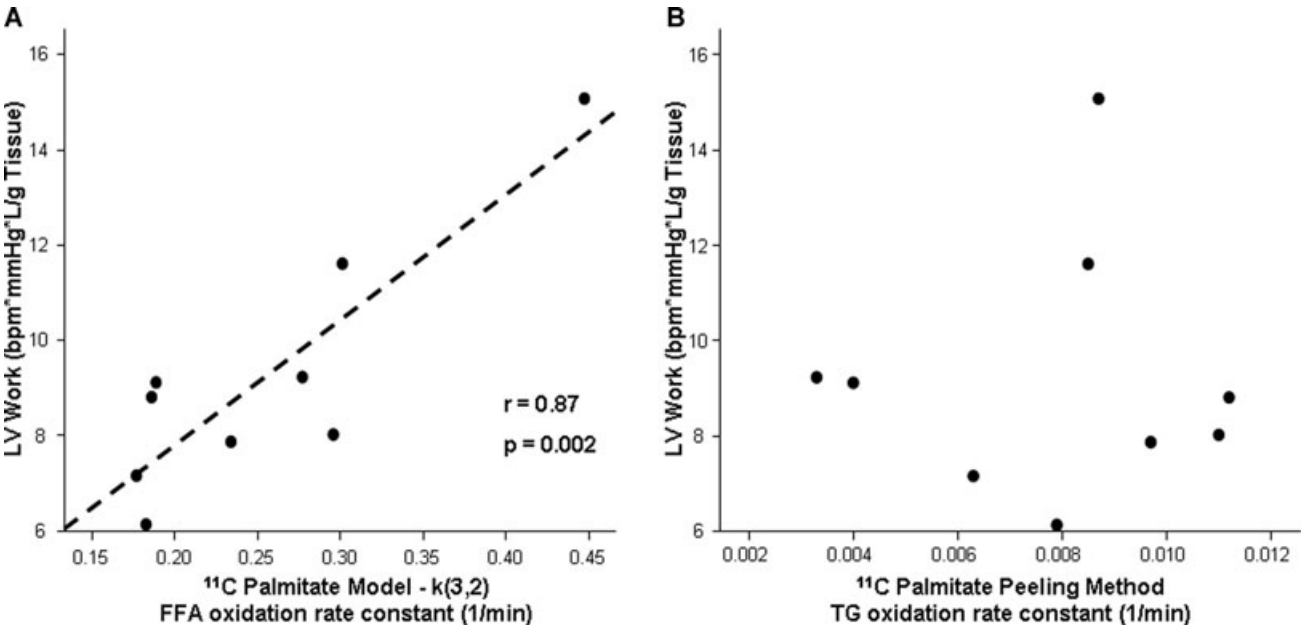

Figure 3 Relationships between LV Work and (A) plasma fatty acid oxidation (positive) or (B) intracellular triglycerides oxidation (absent).

The BMI correlated positively with the cardiac oxidation rate of plasma FFA (r = 0.80, $P = 0.01$, Figure 2A) and with LV work (r = 0.95, $P = 0.001$, Figure 2B). LV work was also positively associated to the plasma FFA oxidation rate (r = 0.87, $P = 0.002$, Figure 3A) but not with the oxidation rate from intracellular triglyceride stores (Figure 3B).

EF and SV increased slightly, though significantly after the treatment ($P = 0.01$) (Table 2), and the end systolic, but not EDV was decreased ($P = 0.028$, $P = 0.123$, respectively). The other MR parameters did not change. Proton MRS data showed that the cardiac creatine-to-water ratio was significantly decreased after the treatment period ($P < 0.03$), whereas myocardial triglyceride content was unchanged (Table 2).

The measured parameters of myocardial substrate metabolism are summarized in Table 2. MGU and MFU did not differ from baseline to end of treatment. The rate of oxidation of plasma derived FAs and the esterification of FAs into triglycerides were not affected by TMZ. However, the oxidation of FAs derived from

Table 2 Left ventricle structure, function, and metabolism before and after treatment

	Baseline	After Treatment	*P*
MRI Measurements and Indexes			
Ejection Fraction (%)	66.0 ± 2.0	69.7 ± 1.7	**0.012**
Stroke Volume (mL)	98.1 ± 4.2	106.5 ± 3.7	**0.012**
Cardiac Output (L/min)	6.46 ± 0.30	6.60 ± 0.31	0.310
End Diastolic Volume (mL)	150 ± 9	154 ± 8	0.123
End Systolic Volume (mL)	52 ± 6	47 ± 5	**0.028**
Left Ventricle Mass (g)	95 ± 9	95 ± 9	0.575
Peripheral Vascular Resistance (mmHg × min/L)	18.26 ± 1.10	17.30 ± 1.22	0.401
Left Ventricle Work (bpm mmHg L)	655 ± 48	685 ± 47	0.401
^{1}H-MRS Measurements			
Heart Lipid Content (%)	0.80 ± 0.24	0.70 ± 0.18	0.889
Creatine/water ratio × 10^{3}	1.27 ± 0.26	0.82 ± 0.24	**0.025**
PET Metabolism			
MGU, μmol/(min g)	0.035 ± 0.015	0.046 ± 0.037	0.401
MFU, μmol/(min g)	0.11 ± 0.01	0.11 ± 0.01	0.327
Plasma FFA Oxidation Rate, μmol/(min g)	0.095 ± 0.009	0.096 ± 0.009	0.575
Plasma FFA Esterification Rate, μmol/(min g)	0.011 ± 0.002	0.012 ± 0.002	0.779
Intracellular FA Oxidation Rate, μmol/(min g)	0.24 ± 0.08	0.10 ± 0.02	**0.036**
Intracellular FA Oxidation Rate Coefficient (1/min)	0.008 ± 0.001	0.005 ± 0.001	**0.025**
LV Total FA Oxidation Rate (μmol/min)	32.7 ± 8.1	19.7 ± 4.0	**0.05**

MGU, indicates Myocardial Glucose Uptake Rate; MFU, Myocardial FFA Uptake Rate; FFA, Free Fatty Acids; FA, Fatty Acids.
Note: n = 8, Values are mean ± SEM. *P* values ≤ 0.05 were considered statistically significant (Wilcoxon Paired *t*-test). Significant values are shown in bold text.

intracellular stores and, subsequently, the total myocardial FA oxidation were decreased by 40% and 57%, respectively. The relative contribution of plasma and intracellular FAs to myocardial oxidation was shifted in favor of the former, from 41% to 54% and from 59% to 46%, respectively ($P < 0.04$) (Figure 4). Total FFA oxidation was measured by two different methods (bi-exponential fitting and compartmental modeling); the values were significantly correlated ($r = 0.71$, $P = 0.03$).

The comparison between LV forward work and the energy production from substrate oxidation at baseline and after treatment is shown in Figure 5. After 1 month of treatment, the two variables leveled off, that is, the LV work did not change significantly, but the overall work potentially deriving from substrate use was decreased, mostly due to the reduced oxidation of lipid stores.

Discussion

This study aimed to dissect the contributions of plasma and intracellularly bound FA to myocardial FA oxidation in obese individuals, and to investigate whether the hypothesized action of TMZ to shift myocardial metabolism from the utilization of FA to that of glucose occurs in humans.

Subjects with obesity release more FAs from peripheral adipose tissue into the circulation [28], associated with an expanded myocardial intracellular triglyceride pool [7]. Enhanced FA oxidation is associated with mitochondrial respiratory chain uncoupling, free radical formation, oxygen wasting, and impaired LV efficiency, that is, inability to match the amount of substrate consumed to the work output [29]. In our study, obesity was not severe, and circulating FA and intracellular lipid levels were quite normal. However, the observed correlation between BMI and FA oxidation or cardiac work is compatible with previous evidence [9].

One important finding of this study was that, in our obese individuals myocardial triglycerides represented a major source of FAs that underwent oxidation. To the best of our knowledge, this is the first human study with quantification of cardiac metabolism to include the turnover of intracellular triglycerides obtained by exploiting information derived from two complementary imaging techniques. Two methods have been commonly adopted in the literature to quantify FA metabolism from [^{11}C]-palmitate PET data. We have implemented the compartmental model suggested by de Jong et al. [21] to calculate fluxes of plasma derived FFA oxidation, esterification, and uptake [21]. In our hands, the FFA oxidation rate was strongly correlated with that obtained with the alternative bi-exponential fitting method [23]. Thus, from the latter we used the second exponential of the myocardial [^{11}C]-palmitate washout curve to represent the rate constant for the mobilization and oxidation of cardiac lipids. This rate constant was multiplied by the cardiac triglyceride concentration, as obtained with proton MRS, to give absolute flux of triglyceride oxidation in the heart. The washout values obtained in this study are consistent with those of Kisrieva-Ware et al. [24], and they are in the order of 10^{-3}, that is, approximately thirty times smaller than the corresponding rate constant of plasma FA oxidation [21]. These numbers may suggest that triglyceride metabolism is negligible under normal fasting and resting conditions [24]. However, it should be kept in mind that rate constants represent only the fraction of substance moving between compartments, whereas the absolute flux depends on the size of the source pool. Concentrations

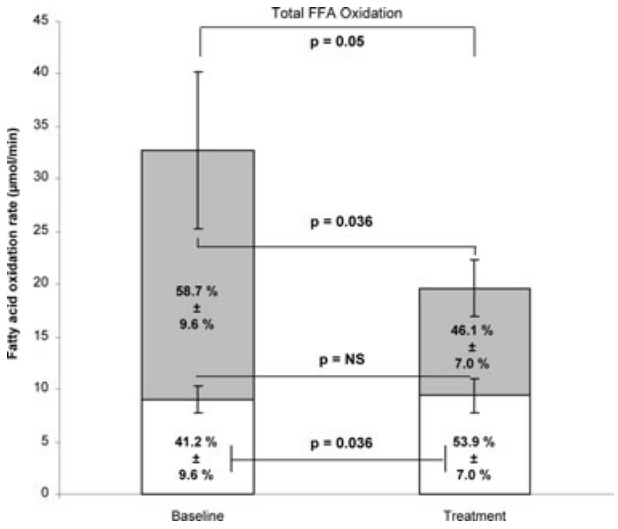

Figure 4 Myocardial plasma FFA oxidation (white bars) and intra-cardiac lipid oxidation (gray bars), at baseline and after treatment, showing significant changes in total and triglyceride derived fatty acid oxidation.

of plasma FFA and cardiac triglyceride FAs are typically around 0.4–0.7 μmol/g in blood versus >20 μmol/g of tissue, a ratio of approximately 30 or more in favor of the latter, thus balancing out the above difference in rate constants.

Therefore, the most salient finding of the study was that the oxidation of endogenous lipids was quantitatively relevant as compared to the plasma FAs in our overweight individuals, representing almost 60% of the total myocardial oxidation of FAs. This finding is consistent with the recent evidence that the myocardial triglyceride pool is dynamic [30,31]. In support of this figure, we estimated the amounts of substrates that would be required to fulfil the LV work measured with MRI. Our data indicate that the full oxidation of substrates extracted from plasma would not provide sufficient energy, unless the contribution of intracellular stores is taken into account.

Our data are the first to evaluate if TMZ provokes inverse changes in FA and glucose metabolism in a drug-naïve human model. Concordant with previous studies, TMZ led to an improvement in cardiac efficiency. The proposed action of TMZ is that it lessens FA metabolism in skeletal [3] and cardiac muscle [32] by partial inhibition of mitochondrial ß-oxidation, resulting in decreased insulin resistance with a shift in substrate utilization from FA to glucose. We document a remarkable suppression of total and endogenous myocardial FA oxidation by the drug in our obese individuals. The reduction in triglyceride oxidation without a change in the cardiac lipid content suggests the diversion of FAs into a different cellular pool, in line with increased phospholipid synthesis in the myocardium [33]. Via this route, and other mechanisms mediated by the decline in FA oxidative metabolism, TMZ may consolidate mitochondrial stability and function [34,35]. The inhibition of FA oxidation induced by TMZ was accompanied by a compensatory enhancement in MGU, though this increment did not achieve statistical significance. Stronger inhibitors of FA metabolism have been previously used. Etomoxir suppresses the activity of carnitine palmitoyltransferase I (CPT-I) to carry FA from the cytosol into mitochondria. In a recent study, its use in living rats was not accompanied by any changes in FA uptake or oxidation, or glucose uptake under basal or maximally stimulated metabolic states [36]. A more recent investigation confirms that the effects produced by etomoxir *in vitro* are not reproduced *in vivo* [37]. Furthermore, the use of etomoxir has been

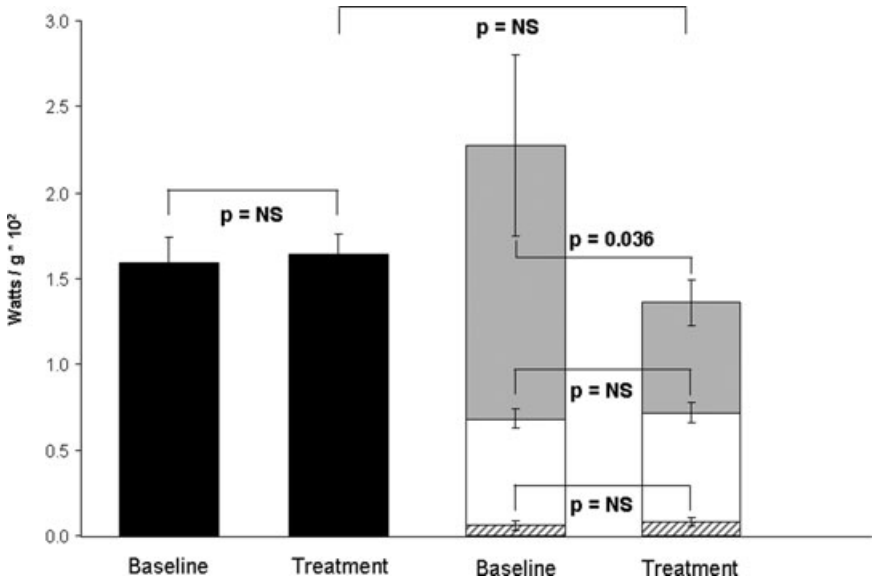

Figure 5 Left ventricular work as assessed by MRI (black bars), and maximum work attainable from the measured substrate utilization rates, namely glucose uptake (broken bars), plasma FFA oxidation (white bars), and triglyceride FA oxidation rates (gray bars). The graph demonstrates that baseline substrate uptake tended to exceed the work performed by the organ, whereas the two processes achieved tighter coupling after treatment.

associated with the risk of hypertrophy [36,38], increase in heart lipid content [39], oxidative stress [40], and hepatotoxicity, leading to the interruption of a clinical trial [41]. An alternative inhibitor of CPT-I is perhexiline. Initially developed as antianginal drug, it has been recently employed in patients with heart failure. Perhexiline inhibits both CPT-I and CPT-II causing an improvement in myocardial function and skeletal muscle energetics but it has been associated with potential peripheral neuropathic [42] and hepatotoxic effects, and for these reasons requires monitoring of plasma levels [43]. Ranolazine shares with TMZ the *in vitro* evidence of a shift in muscle substrate preference from FA to glucose [44], it was previously believed to be an activator of piruvate dehydrogenase [45], but subsequently an *in vivo* study suggested that the drug might not inhibit FA oxidation [46], nonetheless the mechanism of action remains to be elucidated [47]. Overall, the recent literature suggests that *in vitro* studies do not prove the *in vivo* actions, supporting the rationale of this study. Our data are in line with the above reports, in showing that TMZ is similar to other FA oxidation inhibitors in causing limited or no changes in the oxidation of plasma FA and uptake of glucose. However, our data disclose an important effect of TMZ in reducing FA oxidation from endogenous sources. This process was not determined in previous studies and this may justify the controversial or negative findings.

Notably, the pretreatment rate of substrate utilization exceeded, though not significantly, the strict mechanical work requirements, suggesting oxygen wasting. The metabolism of FAs was reduced for the same amount of work, suggesting a tighter metabolic–mechanical coupling and greater LV efficiency. Taken together, our findings of a reduction in FAO and a small compensatory change in glucose utilization by the heart indicate a decrease in the global amount of substrate derivable energy, in accord with the evidence of creatine depletion. This energy sparing effect may result from the multiple TMZ effects on mitochondria [34,35].

Study Limitations

The main limitations were the lack of a placebo group and blinding. Though we have previously shown that placebo treatment for 3 months did not alter cardiac function and metabolism in heart failure patients [3], this finding may not be directly extrapolated to the subjects of the current study. Thus, this study only assessed the association between treatment with TMZ and subsequent effects, especially in reference to the previously surmised metabolic shift in myocardial metabolism. Our data support this action, primarily on the reduction of FA oxidation. We selected obese individuals to guarantee sufficient magnitude of FA oxidation and lipid content to maximize the likelihood of detecting a reduction in cardiac FA metabolism. Our findings document that changes in myocardial metabolism have effects on cardiac efficiency. The limited number of subjects, the inter-subject variability and the low rates of fasting glucose uptake may have weakened the significance of changes in glucose metabolism. In addition, we used a TMZ dose that was half as compared to the therapeutic dose utilized in our previous study in heart failure patients. This was because we preferred to act with some caution in healthy subjects; however, the dose of TMZ may have been suboptimal to delineate all of the relevant metabolic effects of the drug, especially in reference to the change in glucose uptake. Despite the instructions not to change their lifestyle and diet, patients experienced a slight decline in the average body weight. We cannot establish whether this was a drug effect or placebo effect. However, changes in body weight did not correlate with, and unlikely accounted for the changes in metabolic variables. Finally, though a slight change in cardiac function was observed, these subjects had normal baseline function and the study design was not targeted towards this process.

Conclusions

This work introduces for the first time the complementary use of molecular imaging modalities, namely MRS and PET, thereby making the absolute flux of triglyceride derived FAs accessible for direct measurement. In the heart of obese subjects, the myocardial triglyceride pool provided a major contribution to the total substrate oxidation. The inhibition of this process by TMZ was accompanied by an increase in LV work efficiency in obese individuals.

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Conflict of interest

None declared.

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