CARNITINE PALMITOYL TRANSFERASE-I INHIBITION IS NOT ASSOCIATED WITH CARDIAC HYPERTROPHY IN RATS FED A HIGH-FAT DIET

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SUMMARY

1. Cardiac lipotoxicity is characterized by hypertrophy and contractile dysfunction and can be triggered by impaired mitochondrial fatty acid oxidation and lipid accumulation. The present study investigated the effect of dietary fatty acid intake alone and in combination with inhibition of mitochondrial fatty acid uptake with the carnitine palmitoyl transferase (CPT)-I inhibitor oxfenicine. Long-chain fatty acids activate peroxisome proliferator-activated receptors (PPAR), thus mRNA levels of PPAR target genes were measured.

2. Rats were untreated or given the CPT-I inhibitor oxfenicine (150 mg/kg per day) and were fed for 8 weeks with either: (i) standard low-fat chow (10% of energy from fat); (ii) a long-chain saturated fatty acid diet; (iii) a long-chain unsaturated fatty acid diet; or (iv) a medium-chain fatty acid diet (which bypasses CPT-I). High-fat diets contained 60% of energy from fat.

3. Cardiac triglyceride content was increased in the absence of oxfenicine in the saturated fat group compared with other diets. Oxfenicine treatment further increased cardiac triglyceride stores in the saturated fat group and caused a significant increase in the unsaturated fat group. Despite elevations in triglyceride stores, left ventricular mass, end diastolic volume and systolic function were unaffected.

4. The mRNA levels of PPAR-regulated genes were increased by the high saturated and unsaturated fat diets compared with standard chow or the medium chain fatty acid chow. Oxfenicine did not further upregulate PPARα target genes within each dietary treatment group.

5. Taken together, the data suggest that consuming a high-fat diet or inhibiting CPT-I do not result in cardiac hypertrophy or cardiac dysfunction in normal rats.

Key words: fatty acids, heart, hypertrophy, lipotoxicity, mitochondria, oxfenicine, peroxisome proliferator-activated receptors.

INTRODUCTION

Long-chain fatty acids are the primary metabolic fuel for the heart and also are ligands for the peroxisome proliferator-activated receptors (PPARs), which induce expression of genes encoding enzymes that regulate fatty acid metabolism (Fig. 1). Results from studies in transgenic mice and rat models of obesity and diabetes suggest that the accumulation of lipids and related intermediates (e.g. ceramides) in cardiomyocytes can result in cardiac lipotoxicity, resulting in impaired systolic contractile function, an increase in the end diastolic volume of the left ventricle (LV) and cardiac hypertrophy. Conversely, we observed recently that when rats consumed a diet high in long-chain fatty acids there was upregulation of the mRNA of PPAR-regulated genes, but no change in the maximal activity or protein expression of medium-chain acyl-CoA dehydrogenase (a marker enzyme of the β-oxidation pathway). Moreover, there were no adverse consequences on cardiac mass, cardiomyocyte apoptosis, contractile function or LV end diastolic volume, suggesting that a high-fat diet alone does not cause any gross toxicity in the heart.

The combined effects of dietary fatty acid intake and CPT-I inhibition on the expression of genes regulated by PPARα, cardiac hypertrophy and contractile function has not been investigated. The goal of the present study was to assess the interaction among CPT-I inhibition and dietary composition of fatty acids on the expression of PPARα-regulated genes, LV mass and chamber size and LV contractile function. Rats were either untreated or treated with the CPT-I inhibitor oxfenicine and were fed one of four diets for 8 weeks: (i) standard low-fat rat chow (SC; 10% of calories from fat as 3% unsaturated and 7% saturated fatty acids); (ii) high saturated fat diet (SAT); (iii) high unsaturated fat diet (UNSAT); or (iv) high medium-chain

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fatty acid diet (MED). All high-fat diets contained 60% of total calories from fat. We hypothesized that inhibition of CPT-I with oxfenicine increases tissue triglyceride accumulation, upregulates the mRNA of genes regulated by PPARs and results in LV hypertrophy and cardiac dysfunction in rats fed a diet high in long-chain fatty acids compared with either standard low-fat chow or high-fat chow comprised of medium-chain fatty acids (Fig. 1). We expected that CPT-I inhibition would be more detrimental in animals fed a long-chain saturated fat diet than those on the unsaturated fat diet owing to greater accumulation of lipid byproducts in the cytosol and that CPT-I inhibition would have no effect in the group fed the medium-chain fatty acid diet because these fatty acids enter the mitochondria independent of CPT-I. Cardiac function and LV pressure were assessed by echocardiography and cardiac mRNA levels were determined by quantitative real-time polymerase chain reaction (PCR). In addition, plasma leptin and adiponectin levels were determined, as well as free fatty acids and triglycerides.

**METHODS**

All procedures were conducted according to the Guidelines for the Care and Use of Laboratory Animals (NIH publication No. 85-23) and were approved by the Institutional Animal Care and Use Committee of the Case Western Reserve University. Male Wistar rats were obtained at 8–9 weeks of age (Harlan, Indianapolis, IN, USA) and were maintained on a reverse 12 h light–dark cycle. Pretreatment echocardiography and tail-cuff blood pressure measurements were performed at 9–10 weeks of age; diets and oxfenicine treatment were commenced at 10–11 weeks of age. All measurements were performed with the investigator blinded to treatment.

Initial pretreatment measurements were made for systolic arterial blood pressure by tail-cuff, LV function by echocardiography and body mass. Rats were then divided into eight groups: (i) standard low-fat rat chow (SC); (ii) SC + oxfenicine; (iii) high saturated fat diet (SAT); (iv) SAT + oxfenicine; (v) high unsaturated fat diet (UNSAT); (vi) UNSAT + oxfenicine; (vii) medium-chain fatty acid diet (MED); and (viii) MED + oxfenicine. The dose of oxfenicine was 200 mg/kg/day, which has been shown to cause cardiac hypertrophy in rats fed standard rat chow when administered for prolonged periods (1–2 years), but not with 3 months of dosing. Data from untreated SC, SAT and UNSAT groups were recently reported in a separate publication.

The composition of the SC was 10% of total energy from fat (3% from unsaturated fatty acids and 7% saturated fatty acids), 20% protein and 70% carbohydrate (Harland Teklad, Indianapolis IN, USA), as reported previously. All high-fat diets were custom-manufactured by Research Diets (New Brunswick, NJ, USA) and contained 60% of the total energy from fat, 20% protein and 20% carbohydrate. The percentage of the total fatty acid content that was palmitate, stearate, oleate and linoleate was 29, 64, 6 and 1%, respectively, in the SAT chow and 21, 24, 16 and 40%, respectively, in the UNSAT chow, as determined by gas chromatography–mass spectrometry. The MED diet was comprised of essential long-chain fatty acid as in the SC, with the addition of octanoate as a triglyceride to bring the total energy from fat to 60%.

Rats were maintained on the diets for 8 weeks. Left ventricular function was then evaluated by echocardiography, as described previously, at 2–7 h into the dark phase of the light–dark cycle. Briefly, rats were anaesthetized with isoflurane 1.2–2.0% by mask and two-dimensional guided M-mode, two-dimensional and Doppler echocardiographic studies of aortic and transmural flows were performed from parasternal and foreshortened apical windows. On a subsequent day, rats were anaesthetized with 1.5–2% isoflurane and LV pressure was measured through a high-fidelity 3.5 Fr pressure transducer (Millar Instruments, Houston, TX, USA) inserted via the right carotid artery and recorded digitally, as described previously. Blood (3 mL) was drawn from the inferior vena cava and the LV was removed quickly. In order to get the maximum dietary effect on the mRNA response, terminal studies were performed between 3 and 6 h after the start of the dark phase of the daily light–dark cycle.

**Biochemical measurements**

Plasma free fatty acid and triglyceride concentrations and tissue triglyceride were measured using enzymatic spectrophotometric assays. The plasma concentration of insulin and serum levels of leptin and adiponectin were assayed by ELISA immunoassay (ALPCO Diagnostics, Salem, NH, USA). Myocardial activities of citrate synthase (CS) and medium-chain acyl-CoA dehydrogenase (MCAD) were measured spectrophotometrically, as described previously. All tissue measurements are expressed per g wet mass. Standard RNA was made for all assays by the T7 polymerase method (Ambion, Austin, TX, USA), using total RNA isolated from rat hearts. The correlation between the number of PCR cycles required for the fluorescent signal to reach a detection threshold (Ct) and the amount of standard was linear over at least a five-log range of RNA for all assays. The mRNA for atrial natriuretic peptide (ANP) and PPAR-regulated genes pyruvate dehydrogenase kinase 4 (PDK4), uncoupling protein 3 (UCP3) and MCAD were measured on frozen powdered LV tissue using quantitative reverse transcription–PCR (RT-PCR), as described in detail elsewhere. The mRNA concentration was normalized to cyclophilin, which was not different among the experimental groups (data not shown).

**Statistical analysis**

Two-way ANOVA was used to assess the effects of diet and oxfenicine, with post hoc analysis performed using the Bonferroni test for multiple comparisons. Data are presented as the mean±SEM and P < 0.05 was considered significant.
RESULTS

Left ventricular and body mass

There were no differences in body mass, or gain in body mass, over 8 weeks among the SC, SAT, UNSAT, and MED groups, with or without oxfenicine treatment; however, there was significantly less weight gain in the SC group compared with the SAT and UNSAT groups (Table 1). Left and right ventricular mass were not affected by diet or treatment with oxfenicine (Table 1).

Cardiovascular measurements

Arterial systolic blood pressure and heart rate did not differ among groups (Table 2). Cardiac function, as determined from LV pressure and echocardiographic measurements, was also unaffected by diet and treatment with oxfenicine (Tables 2, 3).

Lipid and hormone measurements

The plasma free fatty acid concentration was significantly higher in the SAT and UNSAT groups compared with the MED group, and that in the UNSAT group was significantly higher than that in the SC group for the untreated animals (Table 4). Treatment with oxfenicine lowered plasma free fatty acid concentrations in the SC, SAT, and UNSAT groups and the oxfenicine-treated SC group had lower plasma free fatty acid concentrations than the oxfenicine-treated UNSAT group (Table 4). The plasma triglyceride concentration was significantly lower in the UNSAT group compared with the untreated SAT and UNSAT groups and in the untreated UNSAT group compared with the oxfenicine-treated SC group (Table 4).

As shown in Fig. 2, the cardiac triglyceride concentration was increased significantly in the SAT group compared with the SC and MED groups and in the SAT group compared with all other groups in the untreated and oxfenicine-treated animals, respectively. Oxfenicine had no effect on cardiac triglyceride content in the SC, UNSAT, and MED groups, but significantly increased cardiac triglyceride content in the SAT group (Fig. 2).

Serum insulin concentration was not affected by oxfenicine treatment, but was significantly lower in the SAT group compared with the SC group for both untreated and oxfenicine-treated animals (Table 4). Serum leptin in untreated rats was significantly lower in the SAT and MED groups compared with the SC group. Treatment with oxfenicine significantly lowered serum leptin in the SAT group only. Serum adiponectin levels were not affected by either diet or oxfenicine treatment (Table 4).

Cardiac mRNA and enzyme activities

Expression of ANP, a marker of cardiac hypertrophy and heart failure, did not differ among groups (Fig. 3). Within each dietary group,
Table 3  Echocardiography results after 8 weeks on the different diets

<table>
<thead>
<tr>
<th></th>
<th>SC</th>
<th>SC + oxfenicine</th>
<th>SAT</th>
<th>SAT + oxfenicine</th>
<th>UNSAT</th>
<th>UNSAT + oxfenicine</th>
<th>MED</th>
<th>MED + oxfenicine</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV end diastolic area (cm²)</td>
<td>0.69 ± 0.03</td>
<td>0.68 ± 0.03</td>
<td>0.77 ± 0.05</td>
<td>0.70 ± 0.02</td>
<td>0.68 ± 0.03</td>
<td>0.71 ± 0.04</td>
<td>0.73 ± 0.04</td>
<td>0.70 ± 0.02</td>
</tr>
<tr>
<td>LV end systolic area (cm²)</td>
<td>0.24 ± 0.02</td>
<td>0.24 ± 0.04</td>
<td>0.30 ± 0.04</td>
<td>0.21 ± 0.01</td>
<td>0.23 ± 0.02</td>
<td>0.23 ± 0.03</td>
<td>0.25 ± 0.04</td>
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<tr>
<td>Area of FS (%)</td>
<td>0.65 ± 0.02</td>
<td>0.65 ± 0.02</td>
<td>0.62 ± 0.02</td>
<td>0.70 ± 0.02</td>
<td>0.67 ± 2</td>
<td>0.69 ± 0.03</td>
<td>0.66 ± 0.04</td>
<td>0.64 ± 0.01</td>
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<td>Ejection fraction (%)</td>
<td>87 ± 1</td>
<td>87 ± 2</td>
<td>83 ± 2</td>
<td>89 ± 2</td>
<td>90 ± 2</td>
<td>87 ± 2</td>
<td>84 ± 2</td>
<td>85 ± 2</td>
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<tr>
<td>MPI</td>
<td>0.40 ± 0.02</td>
<td>0.40 ± 0.02</td>
<td>0.43 ± 0.02</td>
<td>0.45 ± 0.01</td>
<td>0.43 ± 0.03</td>
<td>0.41 ± 0.02</td>
<td>0.38 ± 0.01</td>
<td>0.40 ± 0.01</td>
</tr>
<tr>
<td>Velocity of CS (s)</td>
<td>7.04 ± 0.26</td>
<td>7.50 ± 0.53</td>
<td>6.91 ± 0.36</td>
<td>8.44 ± 0.64</td>
<td>8.55 ± 0.65</td>
<td>7.60 ± 0.56</td>
<td>6.57 ± 0.43</td>
<td>7.02 ± 0.33</td>
</tr>
<tr>
<td>CI (mL/min per kg)</td>
<td>91 ± 5</td>
<td>96 ± 7</td>
<td>110 ± 6</td>
<td>111 ± 10</td>
<td>89 ± 4</td>
<td>111 ± 10</td>
<td>96 ± 18</td>
<td>90 ± 5</td>
</tr>
<tr>
<td>RWT</td>
<td>0.45 ± 0.01</td>
<td>0.48 ± 0.02</td>
<td>0.40 ± 0.02</td>
<td>0.45 ± 0.01</td>
<td>0.46 ± 0.02</td>
<td>0.47 ± 0.03</td>
<td>0.44 ± 0.01</td>
<td>0.46 ± 0.02</td>
</tr>
</tbody>
</table>

There were no significant differences among groups fed standard low-fat rat chow (SC; 10% of calories from fat as 3% unsaturated and 7% saturated fatty acids), a high saturated fat diet (SAT), a high unsaturated fat diet (UNSAT) or a high medium-chain fatty acid diet (MED).

LV, left ventricle; FS, fractional shortening; MPI, myocardial performance index; CS, circumferential shortening; CI, cardiac index; RWT, relative wall thickness.

Table 4  Plasma lipid and hormones concentrations

<table>
<thead>
<tr>
<th></th>
<th>SC</th>
<th>SC + oxfenicine</th>
<th>SAT</th>
<th>SAT + oxfenicine</th>
<th>UNSAT</th>
<th>UNSAT + oxfenicine</th>
<th>MED</th>
<th>MED + oxfenicine</th>
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<tbody>
<tr>
<td>Plasma</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>FFA (mmol/L)</td>
<td>0.42 ± 0.04</td>
<td>0.26 ± 0.02*</td>
<td>0.52 ± 0.04</td>
<td>0.41 ± 0.05*</td>
<td>0.61 ± 0.05*††</td>
<td>0.49 ± 0.03*††</td>
<td>0.33 ± 0.03</td>
<td>0.41 ± 0.05</td>
</tr>
<tr>
<td>Triglyceride (mg/mL)</td>
<td>1.52 ± 0.16</td>
<td>1.49 ± 0.37</td>
<td>1.31 ± 0.12</td>
<td>1.03 ± 0.13</td>
<td>0.57 ± 0.06*††</td>
<td>0.46 ± 0.07††</td>
<td>0.94 ± 0.12</td>
<td>0.88 ± 0.15</td>
</tr>
<tr>
<td>Insulin (pmol/mL)</td>
<td>317 ± 46</td>
<td>294 ± 71</td>
<td>102 ± 25†</td>
<td>97 ± 16†</td>
<td>178 ± 34</td>
<td>233 ± 73</td>
<td>161 ± 49</td>
<td>228 ± 64</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
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<tr>
<td>Adiponectin (µg/mL)</td>
<td>5.7 ± 1.0</td>
<td>6.0 ± 0.8</td>
<td>4.7 ± 0.8</td>
<td>6.4 ± 1.1</td>
<td>6.1 ± 0.9</td>
<td>4.8 ± 0.7</td>
<td>5.9 ± 1.1†</td>
<td>5.6 ± 1.0</td>
</tr>
<tr>
<td>Leptin (pg/mL)</td>
<td>4.9 ± 0.8</td>
<td>2.7 ± 0.5*</td>
<td>2.0 ± 0.2†</td>
<td>2.0 ± 0.2</td>
<td>4.4 ± 0.4</td>
<td>4.0 ± 0.6</td>
<td>2.9 ± 0.5†</td>
<td>2.1 ± 0.2</td>
</tr>
</tbody>
</table>

Data are the mean±SEM in groups fed standard low-fat rat chow (SC; 10% of calories from fat as 3% unsaturated and 7% saturated fatty acids), a high saturated fat diet (SAT), a high unsaturated fat diet (UNSAT) or a high medium-chain fatty acid diet (MED). *P < 0.05 for diet compared with diet + oxfenicine. Within untreated groups, †P < 0.05 for SAT and/or UNSAT compared with MED and ††P < 0.05 for UNSAT compared with SAT; ‡P < 0.05 for MED compared with SC. ¶P < 0.05 for SAT and/or UNSAT compared with SC; §P < 0.05 for SAT and/or UNSAT compared with MED.

Table 5  Activities of citrate synthase and medium-chain acyl-CoA dehydrogenase

<table>
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<th></th>
<th>SC</th>
<th>SC + oxfenicine</th>
<th>SAT</th>
<th>SAT + oxfenicine</th>
<th>UNSAT</th>
<th>UNSAT + oxfenicine</th>
<th>MED</th>
<th>MED + oxfenicine</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS (µmol/g per min)</td>
<td>164 ± 7</td>
<td>173 ± 3</td>
<td>181 ± 7</td>
<td>193 ± 9</td>
<td>164 ± 6</td>
<td>191 ± 4*</td>
<td>180 ± 8</td>
<td>169 ± 6</td>
</tr>
<tr>
<td>MCAD (µmol/g per min)</td>
<td>13.7 ± 0.6</td>
<td>14.2 ± 0.5</td>
<td>12.2 ± 0.4</td>
<td>15.0 ± 0.4*</td>
<td>12.7 ± 0.4</td>
<td>14.6 ± 0.5*</td>
<td>14.8 ± 0.6†</td>
<td>13.9 ± 0.6</td>
</tr>
<tr>
<td>MCAD/CS activity</td>
<td>0.084 ± 0.003</td>
<td>0.082 ± 0.004</td>
<td>0.069 ± 0.004††</td>
<td>0.079 ± 0.004*</td>
<td>0.080 ± 0.002</td>
<td>0.075 ± 0.003</td>
<td>0.080 ± 0.002</td>
<td>0.082 ± 0.002</td>
</tr>
</tbody>
</table>

Data are the mean±SEM in groups fed standard low-fat rat chow (SC; 10% of calories from fat as 3% unsaturated and 7% saturated fatty acids), a high saturated fat diet (SAT), a high unsaturated fat diet (UNSAT) or a high medium-chain fatty acid diet (MED). *P < 0.05 compared with the untreated group on the same diet; †P < 0.05 compared with untreated SAT and UNSAT groups; ‡P < 0.05 compared with untreated SC and UNSAT groups.

CS, citrate synthase; MCAD, medium-chain acyl-CoA dehydrogenase.

treatment with oxfenicine had no effect on mRNA levels, except for a significant increase in the expression of PDK4 in the UNSAT group (Fig. 3). Regarding the mRNA expression of PPARα-regulated genes in the groups that were not treated with oxfenicine: (i) UCP3 was significantly increased in the SAT and UNSAT groups compared with the SC group, whereas UCP3 expression in the MED group did not differ from that in the SC group; (ii) PDK4 was increased only in the SAT group compared with the SC group; and (iii) MCAD was increased in the SAT group compared with both the SC and MED groups. The oxfenicine-treated groups showed the following differences in mRNA expression with the various dietary treatments: (i) UCP3 expression was significantly increased in the UNSAT group compared with the SC and MED groups; (ii) expression of PDK4 was significantly increased in the SAT and UNSAT groups compared with the SC group; and (iii) expression of MCAD was significantly increased in the UNSAT group compared with the
MED group, in the SAT and UNSAT groups compared with the MED group and in the SAT group compared with the SC group.

Citrate synthase activity was modestly, but significantly, increased by 16% with treatment with oxfenicine in the UNSAT group, whereas CS activity in untreated animals was not differently affected by diet (Table 5). The activity of MCAD in untreated animals was significantly lower in the SAT and UNSAT groups compared with the MED group. The activity of MCAD was modestly and significantly increased by treatment with oxfenicine in both the UNSAT and SAT groups only. Treatment with oxfenicine significantly increased the ratio of MCAD activity to CS activity in the SAT group only and the ratio of MCAD/CS activity was lower in untreated animals in the SAT diet group only compared with the SC group.

**DISCUSSION**

Extensive studies in transgenic mice and rat models of obesity and type II diabetes suggest that cardiac lipotoxicity can develop when there is either chronic exposure to high plasma free fatty acid and/or triglycerides,5–7,23,24 accelerated uptake and esterification of fatty acids1–4 or impaired mitochondrial fatty acid oxidation.25–27 Conversely, lipotoxicity is not consistently observed in models of type II diabetes in rats28 or with feeding a high-fat diet8,16 and there was no gross evidence of toxicity with a high-fat diet comprised of unsaturated fatty acids compared with saturated fatty acid.29–31 The results of the present study suggest that there are no adverse effects on cardiac function during 8 weeks of feeding with a high-fat diet comprised of either long-chain saturated or unsaturated fatty acids. Furthermore, increased cardiac lipid accumulation with CPT-I inhibition in the saturated fat-fed animals did not have any adverse effect on cardiac mass or contractile function. Taken together, the data suggest that consuming a high-fat diet or inhibiting CPT-I for 2 months does not result in cardiac hypertrophy or cardiac dysfunction.

It has been observed previously that cardiac mRNA levels for PPARα/PPARβ/δ target genes are upregulated by exposure of the heart to fatty acids16,32,33 and by CPT-I inhibition.11,12 Although we

![Fig. 2](image-url)  
**Fig. 2** Cardiac triglyceride content in untreated (■) and oxfenicine-treated (□) rats fed standard low-fat rat chow (SC; 10% of calories from fat as 3% unsaturated and 7% saturated fatty acids), a high saturated fat diet (SAT), a high unsaturated fat diet (UNSAT) or a high medium-chain fatty acid diet (MED). There was a main effect for diet and treatment. *P < 0.05 for SAT compared with SAT + oxfenicine. Within untreated groups, †P < 0.05 for SAT compared with MED and SC. Within oxfenicine-treated groups, ‡P < 0.05 for SAT compared with all other groups and §P < 0.05 for UNSAT compared with MED.

![Fig. 3](image-url)  
**Fig. 3** mRNA expression in left ventricular tissue of (a) atrial natriuretic peptide (ANP) and the peroxisome proliferator-activated receptor (PPAR) α-regulated genes (b) uncoupling protein (UCP) 3, (c) pyruvate dehydrogenase kinase 4 (PDK4) and (d) medium-chain acyl-CoA dehydrogenase (MCAD) after 8 weeks of dietary treatment in untreated (■) and oxfenicine-treated (□) rats fed standard low-fat rat chow (SC; 10% of calories from fat as 3% unsaturated and 7% saturated fatty acids), a high saturated fat diet (SAT), a high unsaturated fat diet (UNSAT) or a high medium-chain fatty acid diet (MED). Values are normalized to cyclophilin mRNA and expressed as a percentage of the mean of the SC group. There was a main effect for diet only on UCP3, PDK4 and MCAD mRNA expression. *P < 0.05 for UNSAT untreated compared with the oxfenicine-treated group. Within untreated and oxfenicine-treated groups: †P < 0.05 for SAT compared with SC; †P < 0.05 for SAT compared with MC; and §P < 0.05 for UNSAT compared with MED.
observed an increase in the expression of PPAR target genes with feeding a diet high in long-chain fatty acids, we did not consistently observe a further increase with oxfenicine. Oxfenicine treatment increased cardiac triglyceride stores in the saturated and unsaturated fat groups, presumably due to accumulation of fatty acyl groups in the cytosol secondary to the reduced transport into the mitochondrial matrix. One would postulate that this would result in enhanced ligand activation of PPARα/PPARβ/δ and greater transcription of target genes, but this effect was only observed for PDK4 mRNA in the UNSAT group. One possible explanation for this could be that oxfenicine alters the well described fourfold diurnal oscillation in mRNA levels for PDK4 in the heart\(^9\) and that we did not kill the animals at the peak of mRNA levels for PPAR-regulated genes in the oxfenicine-treated groups. Rats were housed on a reverse light–dark cycle and tissue was harvested at the time of the peak in the mRNA levels for genes that are regulated by PPAR.\(^{18,34}\) It is interesting to note that the activity of MCAD was significantly increased by oxfenicine treatment in the SAT and UNSAT groups, but not in the SC or MED groups, which suggests that there was greater MCAD protein expression with CPT-I inhibition. Additional work is needed in order to understand the effects of CPT-I inhibition on the regulation of transcript and translation for proteins involved in myocardial fatty acid metabolism.

We observed major differences in cardiac triglyceride contents between the long-chain saturated, long-chain unsaturated and medium-chain saturated fatty acid diets. The lack of accumulation of triglyceride in the MED group was expected, because medium-chain fatty acids are not converted to triglyceride. The lower cardiac triglyceride content in the UNSAT groups compared with the SAT groups corresponded to a lower plasma triglyceride, consistent with the concept put forth by Goldberg \(et\ al\). that plasma triglycerides are an important regulator of myocardial lipid uptake and storage.\(^{4,35,36}\) Our \textit{in vivo} observation of greater triglyceride storage with a saturated fat diet compared with an unsaturated fat diet runs counter to the \textit{in vitro} observations of Listenberger \(et\ al\.), who reported that oleate supplementation resulted in triglyceride accumulation and was well tolerated, but palmitate was not readily incorporated into triglyceride and caused apoptosis.\(^{37}\) Furthermore, Listenberger \(et\ al\.) observed that addition of unsaturated fatty acids to the medium rescued palmitate-induced apoptosis by routing palmitate into the triglyceride pool and away from ceramide formation. The SAT diet used in the present study contained a small amount of oleate and linoleate (6 and 1% of total fatty acids, respectively),\(^{16}\) which may have been sufficient to protect the heart from the potentially damaging effects of palmitate.

We observed reductions in circulating fatty acid and insulin levels among the various treatment groups; however, serum adiponectin concentrations were unaffected. Although serum insulin concentrations in the fed state were reduced with the SAT diet, one should interpret this observation with caution because insulin secretion is highly dependent on the absorption of dietary carbohydrate. Eating behaviour was not monitored or controlled in the present study and only a single time-point was measured; thus, it is not possible to draw conclusions regarding the effects of the various diets on insulin levels. Oxfenicine treatment resulted in a modest reduction in plasma free fatty acid levels in the SC, SAT and UNSAT groups; however, the same caveats for insulin apply here. More extensive studies on the time-course of circulating insulin and fatty acid levels following food consumption are needed before the effects of diet and CPT-I inhibition are understood. There were no differences in the \(\text{derived hormone adiponectin among groups. Recent studies demonstrate a key role in adiponectin in limiting cardiac hypertrophy in mice subjected to pressure overload,}^{38}\) however, the results of the present study clearly demonstrate that adiponectin is not grossly affected by the high-fat diets used or by treatment with oxfenicine.

It is important to note that the results of the present study are limited to the particular age and strain of rat and the CPT-I inhibitor that was used. We did not observe cardiac hypertrophy with oxfenicine in the present study, as has been observed with administration of etomoxir for 5 weeks in 16-week-old Wistar rats.\(^9\) In addition, CPT-I inhibition can clearly have profound positive effects on ventricular morphology and cardiac function under conditions of chronic cardiac stress, such as with rapid ventricular pacing in dogs\(^39\) or pressure overload in rats.\(^40\) Future studies should evaluate the effects of extended duration of treatment with various CPT-I inhibitors under both normal and pathological conditions.

In summary, the present investigation demonstrated that consumption of a high-fat diet comprised primarily of either long-chain saturated or unsaturated fatty acids did not result in cardiac hypertrophy or contractile dysfunction, even in conjunction with CPT-I inhibition. This lack of effect was observed even when cardiac triglyceride content was elevated. Taken together, the data suggest that consuming a high-fat diet or inhibiting CPT-I does not result in cardiac lipotoxicity in normal rats. One cannot rule out that, in models of diabetes or impaired mitochondrial function, increased fatty acid availability may exacerbate contractile dysfunction and hypertrophy.

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Cardiac effects of high-fat diets

119


