Impact of 1 wk of diabetes on the regulation of myocardial carbohydrate and fatty acid oxidation

JOHN C. CHATHAM, ZHI-PING GAO, AND JOHN R. FORDER
Division of Nuclear Magnetic Resonance Research, Department of Radiology,
The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Chatham, John C., Zhi-Ping Gao, and John R. Forder. Impact of 1 wk of diabetes on the regulation of myocardial carbohydrate and fatty acid oxidation. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E342–E351, 1999.—The aim of this study was to investigate the effect of increasing exogenous palmitate concentration on carbohydrate and palmitate oxidation in hearts from control and 1-wk diabetic rats. Hearts were perfused with glucose, [3-13C]lactate, and [U-13C]palmitate. Substrate oxidation rates were determined by combining 13C-NMR glutamate isotopomer analysis of tissue extracts with measurements of oxygen consumption. Carbohydrate oxidation was markedly depressed after diabetes in the presence of low (0.1 mM) but not high (1.0 mM) palmitate concentration. Increasing exogenous palmitate concentration 10-fold resulted in a 7-fold increase in the contribution of palmitate to energy production in controls but only a 30% increase in the diabetic group. Consequently, at 0.1 mM palmitate, the rate of fatty acid oxidation was higher in the diabetic group than in controls; however, at 1.0 mM fatty acid oxidation, it was significantly depressed. Therefore, after 1 wk of diabetes, the major differences in carbohydrate and fatty acid metabolism occur primarily at low rather than high exogenous palmitate concentration.

Diabetes is an independent risk factor for cardiovascular disease and is linked to a marked increase in the incidence of heart failure and mortality associated with coronary heart disease (16). There have been reports (24) of abnormal cardiac function in diabetic patients otherwise free of clinical signs of cardiovascular disease. It has been suggested that diabetes is a specific cause of congestive heart failure independent of other risk factors for heart disease (18). Although many factors may ultimately contribute to the development of contractile dysfunction in diabetes, in experimental models of diabetes changes in myocardial metabolism occur very early after the onset of diabetes. It has been proposed that these alterations in energy metabolism may play an important role in the development of cardiac dysfunction in diabetes (30, 35).

Studies of cardiac metabolism in experimental models of diabetes have been carried out for over 30 years. It has been clearly demonstrated, in a wide range of diabetic models, that myocardial carbohydrate oxidation is depressed, at least in part, as a result of decreased activity of pyruvate dehydrogenase (see Ref. 5 for a review). In contrast, the consequences of diabetes on fatty acid metabolism are much less clear. After diabetes, there are reports of decreased fatty acid oxidation (10) as well as either normal (19) or elevated rates of fatty acid oxidation (19). The accumulation of long-chain fatty-acyl CoA in hearts from diabetic animals has been proposed as evidence of decreased fatty acid oxidation due to decreased flux through carnitine-O-palmitoyltransferase I (CPT-I) (30). However, Lopaschuk (19) has proposed that there is an increase in flux through CPT-I after diabetes, leading to a greater reliance on fatty acids for energy production.

One of the limitations of much of the work on myocardial fatty acid oxidation after diabetes is the use of relatively high concentrations of palmitate, typically 1.2 mM (19). The normal in vivo range for serum free fatty acids is ~0.2–0.5 mM for nondiabetic animals depending on the fed state and ~0.7 mM for diabetic animals (25, 27, 28). Furthermore, the only other substrate that is usually present is glucose, despite the fact that lactate can be a significant energy source for the heart (12, 36). It is also worth noting that the majority of studies on the effects of diabetes on fatty acid metabolism have been carried out after 6 wk of diabetes (25, 37), a time when there are already changes in tissue structure and alterations in calcium handling or in the contractile proteins (8). The effects of shorter durations of diabetes on the interaction between fatty acids and carbohydrates have not been extensively studied.

Thus our understanding of the effects of diabetes on the regulation of fatty acid and carbohydrate oxidation is still quite limited. Consequently, the aim of this study was to determine 1) whether the regulation of carbohydrate oxidation by fatty acids was altered after 1 wk of diabetes and 2) whether this relatively short duration of diabetes significantly affected fatty acid oxidation. To address these questions, we used 13C-NMR spectroscopy to determine the contributions of lactate and palmitate to the acetyl-CoA entering the TCA cycle over a range of palmitate concentrations (0–1.0 mM) in hearts from 1-wk diabetic rats. We also combined 13C-NMR glutamate isotopomer analysis of tissue extracts with measurements of oxygen consumption to obtain measurements of substrate oxidation rates.

MATERIALS AND METHODS

Animal experimentation conforms to the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, 1996). Diabetes was induced in 22 male Sprague-Dawley rats (270–320 g) with streptozotocin (STZ; 60 mg/kg iv; Upjohn Laboratories, Kalamazoo, MI) as previously described (7). Untreated age-matched male Sprague-Dawley
rats were used as controls (n = 21). One week after treatment with STZ, the rats were killed and the hearts were isolated and perfused as described in Isolated heart preparation. This dose of STZ resulted in a marked increase in serum glucose (27.1 ± 0.9 vs. 8.6 ± 0.2 mM in untreated controls; P < 0.05) with only a moderate increase in β-hydroxybutyrate (1.97 ± 0.60 vs. 0.21 ± 0.04 mM in controls; P < 0.05). This is consistent with our previous experience with this model of diabetes (6, 7).

Isolated heart preparation. Rats were anesthetized with ketamine (100 mg/kg ip), heparinized (500 U/100 g ip), and decapitated; animals were not fasted before death. Heats were quickly excised and perfused in a modified Langendorff mode with Krebs-Henseleit bicarbonate buffer (see Buffer composition for more detail) equilibrated with 95% O2-5% CO2 (38°C, pH 7.4). Buffer was not recirculated. A balloon was inserted into the left ventricle via the mitral valve and connected to a Gould P23Db pressure transducer for continuous measurement of heart function (see Physiological Measurements) on a four-channel chart recorder (Gould RS 3400, Cleveland, OH). The balloon volume was adjusted to maintain perfusion pressure at 75 mmHg.

Flow rates ranged from 6 to 9 ml·min⁻¹·g wet wt⁻¹ of tissue (see Table 1 for coronary flow rates in all experimental groups).

Buffer composition. The buffer contained 3% BSA (essentially fatty acid free, Sigma, St. Louis, MO) and the following components (in mM): 118 NaCl, 4.8 KCl, 1.2 MgSO4, 1.4 CaCl2, 1.2 KH2PO4, 25 NaHCO3, 5 glucose, 0.5 sodium lactate, 0.05 sodium pyruvate, 0, 0.1, 0.32, or 1 sodium palmitate, and 5 U/l insulin. Sodium palmitate was dissolved in 200 ml of hot water (~70°C) and gradually added to the BSA solution to achieve the final concentrations. The [U-13C]palmitic acid was purchased as the free acid from Cambridge Isotope Laboratories (Cambridge, MA) and was converted to the sodium salt according to the methods of Wolfe et al. (38). Pyruvate was added to the perfusate to produce a lactate-to-pyruvate ratio of 10:1 to minimize perturbations of the cytosolic redox state that would occur with lactate alone. The buffer was filtered through a 0.45-µm low protein binding filter (Millipore, HV type) before use.

The BSA was prepared as a 30% stock solution, which was dialyzed overnight at 4°C against 20 vol of water containing 118 mM NaCl and 2.5 mM CaCl2. This minimized contributions from low-molecular-weight contaminants in the BSA and saturated the calcium-binding sites. After dialysis, the BSA solution was frozen at −20°C before use.

Physiological measurements. Left ventricular developed pressure (LVDP) and its first derivative (dP/dt), as well as coronary flow (CF) and heart rate (HR), were measured continuously throughout the experiment. Perfuse and coronary effluent were sampled at 30-min intervals for determination of myocardial oxygen consumption (MVO2; Radiometer Copenhagen ABL3 pH/blood gas analyzer, Copenhagen, Denmark). MVO2 was calculated as the product of arteriovenous oxygen content difference and coronary flow.

Table 1. Cardiac function in control and diabetic hearts after 20-min perfusion with 13C-labeled buffer

<table>
<thead>
<tr>
<th></th>
<th>Palmitate, mM</th>
<th>n</th>
<th>RPP, (10³ mmHg/min)</th>
<th>HR, beats/min</th>
<th>LVDP, mmHg</th>
<th>dP/dtmax, mmHg/min</th>
<th>dP/dtmin, mmHg/min</th>
<th>CF, ml·min⁻¹·g⁻¹</th>
<th>MVO2, μmol·min⁻¹·g wet wt⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.00</td>
<td>5</td>
<td>24.6 ± 1.6</td>
<td>274 ± 19</td>
<td>91 ± 7</td>
<td>1,808 ± 226</td>
<td>1,440 ± 182</td>
<td>7.6 ± 0.5</td>
<td>3.2 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>0.10</td>
<td>5</td>
<td>23.5 ± 1.9</td>
<td>299 ± 16</td>
<td>80 ± 9</td>
<td>1,856 ± 279</td>
<td>1,360 ± 196</td>
<td>7.9 ± 0.8</td>
<td>3.2 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>0.32</td>
<td>6</td>
<td>27.8 ± 3.2</td>
<td>283 ± 17</td>
<td>99 ± 10</td>
<td>2,107 ± 146</td>
<td>1,620 ± 141</td>
<td>6.4 ± 0.8</td>
<td>3.0 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>1.00</td>
<td>1</td>
<td>28.6 ± 2.3</td>
<td>299 ± 19</td>
<td>97 ± 7</td>
<td>2,192 ± 243</td>
<td>1,696 ± 172</td>
<td>6.5 ± 0.3</td>
<td>2.9 ± 0.2</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>21</td>
<td>26.2 ± 1.2</td>
<td>288 ± 9</td>
<td>92 ± 4</td>
<td>1,996 ± 108</td>
<td>1,533 ± 84</td>
<td>7.0 ± 0.3</td>
<td>3.0 ± 0.2</td>
<td></td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.00</td>
<td>5</td>
<td>18.2 ± 1.6</td>
<td>229 ± 19</td>
<td>80 ± 5</td>
<td>1,744 ± 146</td>
<td>1,324 ± 130</td>
<td>9.0 ± 0.7</td>
<td>3.5 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>0.10</td>
<td>5</td>
<td>19.4 ± 2.5</td>
<td>271 ± 16</td>
<td>71 ± 8</td>
<td>1,520 ± 247</td>
<td>1,080 ± 134</td>
<td>6.6 ± 0.4</td>
<td>2.6 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>0.32</td>
<td>6</td>
<td>20.9 ± 1.7</td>
<td>251 ± 4</td>
<td>84 ± 7</td>
<td>1,827 ± 138</td>
<td>1,320 ± 126</td>
<td>6.6 ± 0.4</td>
<td>2.6 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>1.00</td>
<td>1</td>
<td>16.3 ± 1.0</td>
<td>264 ± 24</td>
<td>63 ± 6</td>
<td>1,400 ± 237</td>
<td>880 ± 135</td>
<td>8.2 ± 0.7</td>
<td>3.4 ± 0.5</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>22</td>
<td>18.7 ± 1.0</td>
<td>254 ± 9</td>
<td>74 ± 4</td>
<td>1,621 ± 99</td>
<td>1,146 ± 74</td>
<td>7.6 ± 0.4</td>
<td>3.0 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 3–6 for myocardial oxygen consumption (MVO2) measurements. Significance as determined by ANOVA with Scheffe’s post hoc test, indicated by P values for comparison with appropriate control group. HR, heart rate; LVDP, left ventricular developed pressure; RPP, rate pressure product (HR × LVDP); CF, coronary flow; MVO2, myocardial oxygen consumption; dP/dtmax, rate of pressure development; dP/dtmin, rate of relaxation.

Downloaded from http://ajpendo.physiology.org/ by 10.220.33.2 on October 14, 2017
described previously (6). Magnetic field homogeneity was optimized by observing the water signal with the 1H-decoupling coil. 2H2O was used as a field frequency lock to minimize any changes in magnetic field over the time of acquisition. Spectra were collected with a sweep width of 25 KHz, 60°-pulse width, and a 3-s relaxation delay. 1H decoupling was carried out during acquisition only. Under these conditions, the relative intensities of C-2, C-3, and C-4 glutamate and the isotope distribution were indistinguishable from those collected under fully relaxed conditions; however, data acquisition was significantly shortened.

Analysis of glutamate isotope distributions. Hearts perfused with unlabeled glucose, [3-13C]lactate, and [U-13C]palmitate, lead to the formation of unlabeled, [2-13C], and [1,2-13C] acetyl-CoA respectively. From acetyl-CoA, the 13C label will be transferred to the various tricarboxylic acid (TCA) cycle intermediates, the concentrations of which are typically below the level of detection by 13C-NMR spectroscopy. However, glutamate is a molecule that is at a high enough concentration (i.e., >1 mM) to be observed and is in equilibrium with the TCA cycle via α-ketoglutarate and aspartate aminotransferase. Thus measurement of 13C-label incorporation into glutamate can be used as an index of entry of 13C-labeled acetyl-CoA into the TCA cycle. With the use of the phenomenon of spin-spin coupling it is possible to determine the relative contribution of different 13C-labeled acetyl-CoA molecules to the overall TCA cycle flux; this has been described in detail by Malloy et al. (21).

Thus, for a specific combination of unlabeled, [2-13C]- and [1,2-13C]-acetyl-CoA entering the TCA cycle, there will be a unique pattern of labeling in glutamate, giving rise to a unique glutamate-splitting pattern. From the analysis of this splitting pattern, it is possible to determine the relative contributions of the different labeled acetyl-CoA molecules to the TCA cycle, which directly reflect the relative contribution of the different substrates to the formation of acetyl-CoA. Analysis of the glutamate isotope distribution was carried out with software developed and kindly provided by Dr. Mark Jeffrey (TCAcalc; Univ. of Texas Southwestern Medical Center; Ref. 21).

Experimental protocol. After ~30 min of equilibration, the perfusion medium was switched to buffer containing 13C-labeled substrates, as shown in Fig. 1, and perfused for an additional 45–60 min. The composition of substrates during the equilibration period and during perfusion with 13C-labeled substrates was identical. The concentrations of lactate and glucose were the same in all experiments, whereas the concentration of palmitate was varied from 0 to 1 mM. Each experimental group consisted of five to six hearts.

An additional series of experiments was carried out to determine the relative contribution of glucose to the TCA cycle in the presence of palmitate. In these experiments, hearts from control (n = 4) and diabetic (n = 5) groups were perfused with [1-13C]glucose (5 mM) and unlabeled lactate (0.5 mM) and palmitate (0.1 mM). The relative contribution of glucose to acetyl-CoA production was then determined by glutamate isotope analysis as described in Analysis of glutamate isotope distribution.

At the end of the perfusion period, hearts were freeze-clamped with Wallenberger tongs precooled to constant temperature (−196°C) in liquid N2. The freeze-clamped tissue was acid extracted for subsequent 13C-NMR studies and metabolite assays as described in Tissue extraction and metabolite assays and NMR spectroscopy.

Statistics. Data are presented as means ± SE throughout. Single comparisons of means were performed with two-tailed Student's t-test. Groups and repeated measurements were compared by ANOVA in combination with Scheffe’s test for post hoc analysis. The analyses were performed with Statview statistical software (Abacus Concepts, Berkeley, CA). Significance was established at P < 0.05.

RESULTS

Cardiac function. The functional parameters after 20 min of perfusion with 13C-labeled buffer are summarized in Table 1; all parameters were stable throughout the experimental protocol. ANOVA showed that the rate pressure product (RPP) (RPP = HR × LVDP), LVDP, HR, dP/dtmax, and dP/dtmin were all significantly depressed in diabetic hearts relative to controls; however, there was no significant interaction with palmitate concentration. There were no significant differences in CF or MVV2 between control and diabetic groups. The fact that cardiac function was depressed and yet oxygen consumption was not different might indicate that there was a decrease in energy efficiency in the diabetic group. As an estimate of efficiency, the RPP was divided by the oxygen consumed for each heart. The combined results for the control and diabetic groups were 5.1 ± 0.3 and 4.9 ± 0.3 × 103 mmHg·mol O2 respectively. ANOVA indicated that there were no significant between-group differences in this index of energy efficiency.

Tissue metabolite concentrations. The tissue content of ATP, PCR, Cr, total Cr (i.e., PCR + Cr) and the ratio of PCR to ATP and PCR to Cr for all the groups are summarized in Table 2. ANOVA showed that ATP and PCR content were significantly depressed in the diabetic group compared with controls; however, there was no significant interaction with palmitate concentration. There were no significant differences in Cr, total Cr content, or PCR-to-ATP ratio in control or diabetic hearts (Table 2). The ATP and PCR concentrations and PCR-to-ATP ratio are consistent with previously published data on both control and diabetic hearts (22, 27). Ye et al. (39) reported that the PCR-to-Cr ratio is a very sensitive indicator of the bioenergetic status of tissue. As can been seen from the grouped data, the PCR-to-Cr ratio appears to be ~20% lower in the diabetic group; however, ANOVA did not reveal any significant between-group differences in this parameter. Tissue lactate appears to reflect the concentration of lactate in the perfusate concentrations and was not significantly different (0.44 ± 0.03 vs. 0.47 ± 0.04 mM for combined data in control and diabetic groups).

Fig. 1. Experimental protocol. U-13C: 13C uniformly labeled palmitate; MV, myocardial oxygen consumption.
FATTY ACID AND LACTATE OXIDATION AFTER DIABETES

Table 2. Concentrations (μmol/g wet wt) of ATP, PCr, Cr, total Cr, and PCr-to-ATP and PCr-to-Cr ratios in control and diabetic rats

<table>
<thead>
<tr>
<th>Palmitate, mM</th>
<th>n</th>
<th>ATP</th>
<th>PCr</th>
<th>Cr</th>
<th>PCr + Cr</th>
<th>PCr-to-ATP Ratio</th>
<th>PCr-to-Cr Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.00</td>
<td>5</td>
<td>3.9±1.0</td>
<td>7.4±0.6</td>
<td>5.0±0.5</td>
<td>12.3±1.0</td>
<td>1.9±0.1</td>
<td>1.5±0.1</td>
</tr>
<tr>
<td>0.10</td>
<td>5</td>
<td>4.1±0.2</td>
<td>8.8±1.1</td>
<td>4.2±0.7</td>
<td>13.0±1.3</td>
<td>2.1±0.2</td>
<td>2.3±0.4</td>
</tr>
<tr>
<td>0.32</td>
<td>6</td>
<td>3.9±0.1</td>
<td>8.0±0.2</td>
<td>3.9±0.3</td>
<td>11.9±0.4</td>
<td>2.0±0.1</td>
<td>2.1±0.1</td>
</tr>
<tr>
<td>1.00</td>
<td>5</td>
<td>3.9±0.1</td>
<td>8.5±0.8</td>
<td>3.5±0.8</td>
<td>11.2±1.3</td>
<td>2.2±0.2</td>
<td>2.9±0.6</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>4.0±0.1</td>
<td>8.2±0.4</td>
<td>4.1±0.3</td>
<td>12.3±0.5</td>
<td>2.1±0.1</td>
<td>2.2±0.2</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.00</td>
<td>5</td>
<td>3.5±1.0</td>
<td>6.3±0.3</td>
<td>4.0±0.4</td>
<td>10.3±0.4</td>
<td>1.8±0.1</td>
<td>1.6±0.2</td>
</tr>
<tr>
<td>(P = 0.0422)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.10</td>
<td>5</td>
<td>3.5±0.2</td>
<td>5.9±0.5</td>
<td>5.1±0.4</td>
<td>11.0±0.7</td>
<td>1.7±0.1</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>(P = 0.0393)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.32</td>
<td>6</td>
<td>3.6±0.2</td>
<td>7.4±0.3</td>
<td>3.8±0.6</td>
<td>11.2±0.7</td>
<td>2.1±0.1</td>
<td>2.2±0.4</td>
</tr>
<tr>
<td>(P = 0.0453)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.00</td>
<td>6</td>
<td>3.2±0.2</td>
<td>7.5±0.1</td>
<td>3.8±0.5</td>
<td>11.3±0.5</td>
<td>2.4±0.2</td>
<td>2.2±0.4</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>3.4±0.1</td>
<td>6.9±0.2</td>
<td>4.2±0.2</td>
<td>11.1±0.3</td>
<td>2.0±0.1</td>
<td>1.8±0.2</td>
</tr>
</tbody>
</table>
| Values are means ± SE. Significance as determined by ANOVA with Scheffe’s post hoc test, indicated by P values for comparison with appropriate control group. Cr, creatine; PCr + Cr, total Cr.

13C-NMR spectroscopy data. The [13C4]glutamate resonances from 13C-NMR spectra of hearts from control and diabetic groups are shown for each palmitate concentration in Fig. 2. In the control group, as the palmitate concentration increases, the C-4 resonance initially becomes more complex due to relatively equal contribution of both [2-13C]- and [1,2-13C]acetyl-CoA to the TCA cycle, derived from [3-13C]lactate and [U-13C16]palmitate, respectively. However, at 1.0 mM palmitate, the C-4 glutamate resonance is less complex compared with 0.32 mM palmitate, reflecting the fact that the majority of acetyl-CoA is now derived from [U-13C16]palmitate with very little contribution from [3-13C]lactate.

The results for the diabetic group show a dramatically different pattern. In the diabetic group, perfused without palmitate, a decrease in label incorporation from [3-13C]lactate compared with control is apparent by the reduced intensity of the doublet. This is consistent with our previous study (9). After the addition of 0.1 mM palmitate, there is a marked increase in label incorporation compared with controls, with relatively little change seen with increasing palmitate concentration.

The relative contributions of lactate and palmitate to the TCA cycle determined from the high resolution 13C-NMR spectra are shown in Fig. 3, A and C. In the control group, it can be seen that as the palmitate concentration increases, the contribution of lactate to the TCA cycle decreases in a linear fashion (r2 = 0.979; P < 0.0001). The effect of palmitate on lactate oxidation in the diabetic group is significantly different compared with control (P < 0.0001; ANOVA control vs. diabetic). In the absence of palmitate, the contribution of lactate to the TCA cycle is significantly decreased. In diabetic hearts, the addition of 0.1 mM palmitate resulted in a 52% decrease in lactate entry into the TCA cycle compared with only a 13% decrease in controls. However, between 0.32 and 1.0 mM palmitate, the relative contribution of lactate to the TCA cycle decreased only 3-fold in the diabetic group, compared with ~10-fold decline in the control group. The contribution of lactate to the TCA cycle is significantly lower than controls at all palmitate concentrations; including 1.0 mM palmitate (control: 3.5 ± 0.3% vs. diabetic: 2.7 ± 0.12%; P < 0.02).

The relative contribution of palmitate to the TCA cycle (Fig. 3C) was linear in both groups with respect to...
exogenous palmitate concentration, but the slopes were significantly different ($P < 0.005$). The effect of increasing exogenous palmitate on palmitate oxidation was also significantly different between the two groups when analyzed with ANOVA ($P < 0.0001$; ANOVA control vs. diabetic). In the control group, there was an almost sevenfold increase in the contribution of palmitate to the TCA cycle when the palmitate concentration was increased from 0.1 to 1.0 mM. In contrast, in the diabetic group, there was only a 30% increase in the contribution of palmitate between 0.1 and 1 mM palmitate. As a result, the contribution of the fatty acid to the TCA cycle in diabetic hearts was significantly higher than controls at low palmitate but was depressed at high palmitate concentration.

Analysis of glutamate-labeling patterns provides a measurement of the relative contribution of lactate and palmitate to the TCA cycle; however, it does not reflect the actual rates of oxidation that are dependent on cardiac work and basal metabolic rate. As cardiac function was depressed in the diabetic group (Table 1), it is possible that the differences in the relative contribution of palmitate and lactate may not reflect changes in substrate oxidation rates. In Fig. 3, B and D, the total lactate and fatty acid oxidation rates for both groups normalized to RPP are shown and the results are similar to the relative contributions of the substrates to energy production. Therefore, even when the lower workload in the diabetic hearts is taken into account, the fatty acid oxidation rates in the diabetic heart are significantly depressed compared with control hearts at 1.0 mM palmitate. These data demonstrate that the differences in substrate oxidation between control and diabetic hearts are not simply a consequence of decreased contractile function.

At all fatty acid concentrations, in both control and diabetic groups, the sum of the relative contributions of lactate and palmitate contributing to acetyl-CoA formation is <100%. This difference represents the contribution of unlabeled substrates entering the TCA cycle via acetyl-CoA. Because glucose is present in the perfusate, the unlabeled substrate entry could be from either exogenous glucose or endogenous sources, such as glycogen or triglycerides. The contribution of unlabeled substrate entry to total TCA cycle flux is shown in Fig. 4. In both control and diabetic groups, as the palmitate concentration increases, the amount of unlabeled acetyl-CoA entering the TCA cycle decreases; however, this is more pronounced in the controls ($P < 0.0001$; control vs. diabetic, ANOVA). We have shown previously that in hearts with glucose and lactate as the only exogenous substrates, that endogenous substrates account for 5–10% of substrate entry into the TCA cycle in control hearts and ~50% in diabetic hearts (9). Therefore, we can estimate that in the control group at 0 mM palmitate, the majority of unlabeled substrate entering the TCA cycle (i.e., ~45%) originates from exogenous glucose. However, in the diabetic group at 0 mM palmitate, only ~25% of unlabeled substrate utilization originates from exogenous glucose.

In control hearts perfused with [1-13C]glucose and unlabeled lactate and palmitate, we found that at 0.1

![Fig. 3. Relative contributions of lactate (A) and palmitate (C) to the TCA cycle for control and diabetic hearts perfused at different palmitate concentrations. Oxidation rates of lactate (B) and palmitate (D) normalized to rate pressure product. Data are means ± SE. *P < 0.05 vs. control.](http://ajpendo.physiology.org/).
mM palmitate, glucose contributed 50.8 ± 4.5% (n = 4) of the acetyl-CoA entering the TCA cycle. In comparison, the unlabeled contribution in hearts perfused with labeled palmitate and lactate was 47.5 ± 1.6% (Fig. 4). In other words, oxidation of exogenous glucose appears to account for all the unlabeled substrate entry in control hearts perfused with 0.1 mM palmitate. Presumably, this is also the case at higher palmitate concentrations. Therefore, at 1.0 mM palmitate, glucose contributes 13.2 ± 3.3% of total acetyl-CoA entering the TCA cycle.

The signal-to-noise ratio of 13C-NMR spectra of extracts from individual diabetic hearts perfused with [1-13C]glucose and unlabeled lactate and palmitate (0.1 mM) was too low to accurately determine the contribution of glucose to acetyl-CoA formation in a single heart. As a result, extracts from five diabetic hearts perfused under these conditions were combined and a single 13C-NMR spectrum was collected. From this spectrum, we estimated that the fraction of acetyl-CoA originating from exogenous glucose was ~13%. Therefore, in the diabetic group, perfused with unlabeled glucose and 13C-labeled lactate and 0.1 mM 13C-labeled palmitate, ~13% of the 36.6 ± 3.7% contribution of unlabeled substrate to acetyl-CoA originates from exogenous glucose. Therefore, the remainder, i.e., ~24% of total acetyl-CoA entry into the TCA cycle, comes from endogenous sources. The contribution of unlabeled substrate is not significantly different between 0.1 and 1.0 mM palmitate (36.6 ± 3.7 vs. 31.3 ± 3.1%). Assuming that the distribution between exogenous glucose and endogenous substrates is also unchanged, we can estimate that at 1.0 mM palmitate, glucose contributes ~11% of acetyl-CoA entering the TCA cycle.

Therefore, at 0.1 mM palmitate, glucose plus lactate contributed ~90% of all acetyl-CoA entering the TCA cycle in control hearts compared with only ~25% in the diabetic group. However, at 1 mM, the relative contributions of glucose plus lactate were ~17 and ~14% in control and diabetic groups, respectively. Therefore, in contrast to the absence or at low concentrations of palmitate, carbohydrate oxidation is not decreased in the diabetic group at 1 mM palmitate.

Myocardial triglyceride content. To determine the primary source of endogenous substrates for acetyl-CoA formation, the triglyceride content of hearts from control and diabetic groups perfused under different conditions was determined (Fig. 5). The triglyceride content in in situ hearts is similar to that previously reported for both control and diabetic hearts (26, 29, 32). In the control group, triglyceride content was significantly decreased compared with the in situ group in the 0.1 mM palmitate group only. In contrast, there was a significant increase in triglyceride content in all of the diabetic groups after perfusion, ranging from a 78% decline in the absence of palmitate to a 42% decrease in the 1.0 mM palmitate group. In the diabetic group, perfusion with 1.0 mM palmitate resulted in a higher triglyceride content compared with hearts perfused without palmitate. There were no differences in the control groups perfused with different palmitate concentrations.

DISCUSSION

These studies demonstrate that the effect of diabetes on myocardial substrate oxidation is complex, being dependent not only on which substrates are available for energy production but also on their respective concentrations. We have shown, for the first time, that the inhibition of myocardial lactate oxidation by fatty acids is much more sensitive after 1 wk of diabetes.

![Fig. 4. Relative contributions of unlabeled substrate to acetyl-CoA entry into the TCA cycle in control and diabetic hearts at different concentrations of palmitate. Data are means ± SE. †P < 0.05 vs. control hearts perfused with 0.32 and 1.0 mM palmitate; ‡P < 0.05 vs. control hearts perfused with 1.0 mM palmitate; §P < 0.05 vs. diabetic hearts perfused under same conditions. *P < 0.05 vs. all other diabetic groups.](http://ajpendo.physiology.org/)

![Fig. 5. Myocardial triglyceride content in hearts from control and diabetic animals, immediately after removal (in situ) or at end of perfusion with different concentrations of palmitate as indicated. Data are means ± SE. *P < 0.05 vs. all other diabetic groups; †P < 0.05 vs. control heart perfused with 0.1 mM palmitate; ‡P < 0.05 vs. diabetic hearts perfused with 0 mM palmitate; §P < 0.05 vs. diabetic hearts perfused under same conditions.](http://ajpendo.physiology.org/)
addition of 0.1 mM palmitate resulted in a 50% decrease in lactate oxidation in the diabetic group compared with <15% decrease in controls. However, at higher concentrations of palmitate, we found that carbohydrate oxidation was not markedly impaired in the diabetic group. Thus the regulation of carbohydrate oxidation by fatty acids is clearly altered after only 1 wk of diabetes.

The relationship between exogenous fatty acid concentration and fatty acid oxidation was also markedly altered in the diabetic group. For the first time, we have shown that after diabetes the oxidation of exogenous fatty acids is much less sensitive to the concentration of fatty acids in the perfusate. After diabetes, a 10-fold increase in exogenous palmitate concentrations resulted in only a 30% increase in the relative contribution of fatty acids to energy production compared with an ~6-fold increase in the control group. When normalized for differences in RPP, the rate of fatty acid oxidation increased by ~60% in the diabetic group compared with a fivefold increase in controls. This study also demonstrated that at low palmitate concentrations, hearts in the diabetic group use exogenous fatty acids for energy production to a much greater extent than the control group, whereas, at high palmitate concentrations, exogenous fatty acid oxidation is significantly lower than controls even when differences in workload are accounted for.

The observation that carbohydrate oxidation in the diabetic group perfused with 1.0 mM palmitate is not different from controls is at odds with much of what has been reported previously on the effects of diabetes on cardiac metabolism. However, the majority of the early work (see Ref. 5 for review) that reported decreased glucose oxidation after only a short period of diabetes was carried out in the absence of fatty acids. Our results are consistent with those studies because glucose and lactate oxidation are depressed in the absence of, or at, low palmitate concentrations. Studies that report impaired glucose oxidation in hearts perfused with both glucose and fatty acids have typically been carried out after periods of diabetes of 6 wk or more (see Ref. 37 for an example). In one of the very few studies to have looked at the consequences of diabetes on lactate and glucose metabolism, Hall et al. (15) found no change in myocardial glucose uptake in vivo but a twofold decrease in lactate uptake after diabetes. However, this study was carried out after 12 wk of diabetes. Therefore, as the duration of uncontrolled diabetes increases, there is a progression in the metabolic abnormalities, especially with respect to carbohydrate oxidation. After 6 wk of diabetes, there is a threefold decrease in total pyruvate dehydrogenase activity (33), which may account for the further impairment of glucose and lactate oxidation after longer periods of diabetes.

Many studies of the interactions between carbohydrate and fatty acid oxidation have used glucose as the sole pyruvate source, despite the fact that lactate can be a major source of energy for the heart in vivo (12, 36). In the absence of fatty acids, diabetes results in a preferential inhibition of myocardial lactate oxidation relative to glucose (9). Interestingly, a similar situation was observed here in the control group at 1.0 mM palmitate. Increasing palmitate from 0.1 to 1.0 mM resulted in a 10-fold reduction in the contribution of lactate to acetyl-CoA formation compared with a <4-fold reduction in the contribution of glucose to energy production. In other words, in control hearts, high concentrations of exogenous palmitate exerted a greater inhibitory effect on lactate oxidation than glucose oxidation relative to low palmitate concentrations. After ischemia and reperfusion, carbohydrate oxidation also shifts away from lactate to glucose oxidation (11). The mechanisms underlying this regulation have yet to be defined; however, it is most likely due to alterations in cytosolic pyruvate concentration and/or cytosolic redox state (9). Regardless of the precise mechanism, it appears as though under conditions where total carbohydrate oxidation is decreased, flux from glucose to pyruvate is maintained relative to flux from lactate to pyruvate.

In the control group, exogenous glucose accounts for all the unlabeled substrate entry in hearts perfused with palmitate. This indicates that there is no significant contribution from endogenous substrates to energy production. This is consistent with the fact that there were no differences in triglyceride content in control hearts at any palmitate concentration. These results are similar to a 13C-NMR study of substrate utilization in working hearts perfused with 0.35 mM palmitate where endogenous substrate utilization contributed only 5–10% of total energy production (17). However, this is in contrast to reports that with 0.4 mmol/l palmitate, endogenous fatty acid oxidation contributed over 46% of substrate used for ATP production (31). The reason for this discrepancy is unclear; however, in 13C-NMR studies, measurement of substrate utilization is typically time averaged over 30–90 min of perfusion after a 20- to 30-min equilibration period. Saddik and Lopaschuk (31) commenced radioisotope measurements of substrate oxidation after only a 10-min equilibration period. It has been shown that myocardial triglyceride content rapidly decreases by 30–40% during the first 30 min of perfusion and then remains fairly stable over the next 60 min (23, 26). Therefore, our measurements and those by Jeffery et al. (17) were made when triglyceride breakdown was minimal, whereas the radioisotope measurements were most likely carried out shortly after the beginning of perfusion when the rate of triglyceride breakdown was still elevated.

In the diabetic group, we estimated that endogenous substrates contribute ~24% to acetyl-CoA formation at 0.1 mM palmitate and that there was no significant change at higher palmitate concentrations. We have previously shown that in hearts from diabetic animals perfused with lactate and glucose, glycogen content did not change during 60 min of perfusion (9). Therefore, it is unlikely that glycogen is a major source for acetyl-CoA in these experiments. Others have shown that there is accelerated triglyceride breakdown in the heart
after diabetes (23, 26, 32). Furthermore, in the diabetic group, triglyceride levels were significantly decreased in all perfusion groups compared with controls. Consequently, the most likely endogenous source of acetyl-CoA in the diabetic group is triglycerides.

Total fatty acid oxidation represents the sum of endogenous and exogenous fatty acids. In the 0.1 mM palmitate control group, there was no significant contribution of endogenous fatty acids to acetyl-CoA. However, in the diabetic group at 0.1 mM palmitate, endogenous fatty acids contributed ~24% of acetyl-CoA production and at 1 mM palmitate, we estimated that this was still ~20%. Therefore, in the diabetic group, at 0.1 mM palmitate, ~75% of the acetyl-CoA entering the TCA cycle originates from fatty acids (24% endogenous and 51% exogenous) compared with only 13% in controls (all exogenous). Surprisingly, in the diabetic group, when palmitate is increased to 1.0 mM, there is only a modest increase in the contribution of fatty acids to acetyl-CoA formation to ~86% (20% endogenous and 66% exogenous) compared with a sixfold increase in the control group (83%, all exogenous fatty acids). Therefore, the biggest difference between control and diabetic groups is the use of fatty acids for energy production at low exogenous palmitate concentrations. This difference in exogenous fatty acid utilization presumably accounts, at least in part, for the significant decrease in lactate oxidation at low palmitate in the diabetic group.

The principal regulation of fatty acid oxidation in the heart is at the level of CPT-I. Flux through CPT-I appears to be controlled by the concentration of malonyl-CoA. In the diabetic heart, it has been suggested that as a result of decreased activity of acetyl-CoA carboxylase, malonyl-CoA levels are depressed and thus there is an increase in flux through CPT-I and an increased dependence on fatty acids for energy production (19). This does not appear to be the case after only 1 wk of diabetes. There is no doubt that at low concentrations of palmitate, fatty acid oxidation is increased. However, at 1.0 mM palmitate, exogenous fatty acid oxidation is depressed compared with controls and total fatty acid oxidation (i.e., endogenous plus exogenous) is similar to controls.

Although an increase in flux through CPT-I could account for an increase in total fatty acid oxidation in the diabetic heart, it is difficult to see how alterations in CPT-I would result in a fourfold increase in exogenous palmitate oxidation at 0.1 mM palmitate. If fatty acid entry into myocytes was diffusion mediated, the rate of entry into the cell would be controlled by the concentration gradient. This gradient is likely to be similar in control and diabetic groups or could be decreased due to the increased breakdown of triglycerides after diabetes (23, 26, 32). Therefore, although total fatty acid utilization could be increased in the diabetic group, the contribution of exogenous palmitate would be similar to or maybe even decreased relative to controls. There is increasing evidence that entry of long-chain fatty acids into cells is a carrier-mediated process (34); for example, fatty acid transport into membrane vesicles correlates with transporter protein content (4, 20). The importance of fatty acid transporters in regulating fatty acid oxidation in the intact heart is not yet known; however, one explanation for the increased exogenous fatty acid oxidation at 0.1 mM palmitate could be an increase in fatty acid transport into the diabetic heart. Berk et al. (2) reported a twofold increase in oleate transport in myocytes from Zucker diabetic fatty rats, possibly due to increased expression of putative fatty acid transport proteins.

Even if there is an increase in fatty acid entry into the cell, this cannot account for the fact that between 0.1 and 1.0 mM palmitate the contribution of fatty acids to energy production hardly changes or that exogenous fatty acid oxidation is significantly decreased at 1.0 mM palmitate. These data would suggest that the rate of oxidation of fatty acids is almost saturated in the diabetic heart at 1.0 mM palmitate and may indicate a decrease in the maximal rate of fatty acid oxidation. This would be consistent with reports of accumulation of long-chain fatty-acyl-CoA in the hearts of diabetic animals (30). An increase in long-chain acyl-CoA esters may have wide-ranging effects on cell function, including changes in regulation of calcium handling, alteration in insulin sensitivity, and possibly activation of peroxisome proliferator-activated receptors (13).

There is no doubt that in these experiments 1 wk of diabetes results in profound changes in the regulation of both carbohydrate and fatty acid oxidation. However, it should be noted that in vivo, in addition to glucose, lactate, and fatty acids, ketone bodies (i.e., acetoacetate and β-hydroxybutyrate) will also be available for energy production. J effrey et al. (17) showed that 0.17 mM acetoacetate, when present in combination with other substrates, contributed 20–30% of total acetyl-CoA production. In uncontrolled diabetes, ketone bodies can be increased more than 10-fold. Therefore, given the profound effect of ketone bodies on both fatty acid and carbohydrate oxidation, it is likely that ketone bodies will be a significant energy source in vivo.

The energy demand in vivo will also be greater than in the isovolumic perfused heart preparation used in these studies, which may have an impact on the regulation of substrate utilization. However, although it is clear that increasing workload increases the actual rates of substrate oxidation, the effect of changes in workload on the relative contributions of different substrates to energy production is uncertain. J effrey et al. (17) showed that in over a twofold range of workloads in the working rat heart there were no significant differences in the contributions of carbohydrates, fatty acids, and ketone bodies to energy production. In contrast, Goodwin et al. (14) reported that an acute increase in carboxylate work after epinephrine stimulation resulted in a greater increase in carbohydrate oxidation compared with fatty acid oxidation. To our knowledge, there have been no studies looking at the effect of altered workload on substrate utilization after diabetes.

Although in vivo, there will be differences in available substrates and workload, this does not affect the fact that after 1 wk of diabetes, there are marked
alterations in the regulation of both fatty acid and carbohydrate oxidation. This study demonstrates that a relatively short period of diabetes leads to complex changes in the regulation of carbohydrate and fatty acid oxidation that are different from those seen after longer periods of diabetes. Whereas carbohydrate oxidation was clearly depressed at low palmitate concentrations, it was not significantly impaired at high palmitate. Conversely, exogenous palmitate oxidation was markedly elevated at low fatty acid concentrations but depressed at high concentrations. We have hypothesized that the increased oxidation of exogenous palmitate at low concentrations may be due in part to an increase in fatty acid transport. Our results also indicate that the rate of fatty acid oxidation may be close to maximum even at low fatty acid concentrations. Given the differences between these studies after 1 wk of diabetes and those after 6 wk, further investigations are warranted to understand the changes in the regulation of fatty acid and carbohydrate oxidation that occur as the duration of diabetes increases.

We would like to thank Dr. A.-M. Seymour for stimulating discussions on this work, which were facilitated by a North Atlantic Treaty Organization Collaborative Research Grant (CRG 940624) awarded to Dr. Seymour. We are also grateful to Dr. L. Becker for helpful comments and advice regarding the manuscript. Streptozotocin was kindly provided by Pharmacia & Upjohn Company, Kalamazoo, MI.

This work was supported by National Heart, Lung, and Blood Institute Grant HL-48789 (to J. C. Chatham) and a national Grant-in-Aid from the American Heart Association with funds contributed in part by the American Heart Association, Maryland Affiliate (to J. R. Forder).

Address for reprint requests and correspondence: J. C. Chatham, Dept. of Radiology, NMR Research Division, 217 Traylor Bldg., The Johns Hopkins School of Medicine, 720 Rutland Ave., Baltimore, MD 21205-2195 (E-mail: jchatham@mri.jhu.edu).

Received 9 November 1998; accepted in final form 20 April 1999.

REFERENCES


