Impact of altered substrate utilization on cardiac function in isolated hearts from Zucker diabetic fatty rats

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The goal of this study was to determine whether changes in cardiac metabolism in Type 2 diabetes are associated with contractile dysfunction or impaired response to ischemia. Hearts from Zucker diabetic fatty (ZDF) and lean control rats were isolated and perfused with glucose, lactate, pyruvate, and palmitate. The rates of glucose, lactate, pyruvate, and palmitate oxidation rates and glycolysis were determined during baseline perfusion and low-flow ischemia (LFI; 0.3 ml/min for 30 min) and after LFI and reperfusion. Under all conditions, ATP synthesis from palmitate was increased and synthesis from lactate was decreased in the ZDF group, whereas the contribution from glucose was unchanged. During baseline perfusion, the rate of glycolysis was lower in the ZDF group; however, during LFI and reperfusion, there were no differences between groups. Despite these metabolic shifts, there were no differences in oxygen consumption or ATP production rates between the groups under any perfusion conditions. Cardiac function was slightly depressed before LFI in the ZDF group. Thus, the increase in cardiac fatty acid utilization that occurs with diabetes is proposed to be detrimental because fatty acids are less-efficient fuels that consume more oxygen per molecule of ATP produced than glucose. Indeed, there is a theoretical 12–14% increase in efficiency of ATP production in shifting from 100% palmitate oxidation to 100% glucose oxidation (35); however, such extreme metabolic shifts are unlikely to be physiologically relevant. In a recent study (24) using physiological substrate mixtures including lactate and pyruvate as well as glucose and palmitate, we estimated that decreasing fatty acid oxidation from ~60 to ~5% of total energy production would not significantly alter the efficiency of ATP production. Another explanation for the adverse effects of increased fatty acid oxidation is that it leads to dissociation between glucose oxidation and glycolysis to result in “excess” proton production and increased intracellular acidity (23, 27). However, this concept is based on studies where glucose and palmitate were the sole available substrates despite the fact that it is well established in both in vivo and in vitro studies that in heart, lactate is more readily oxidized than glucose (10, 11, 18, 19, 24, 25, 45). It is also worth noting that we have shown in a streptozotocin (STZ)-induced model of diabetes that the decrease in cardiac carbohydrate oxidation was attributable solely to decreased lactate oxidation with no change in glucose oxidation (10, 11). These data suggest that the relationship between glycolysis and glucose oxidation may not be important when physiologically relevant carbohydrate mixtures are used. Furthermore, King et al. (20) reported that fatty acids actually improved recovery of function in diabetic hearts after ischemia, possibly compensating for impaired carbohydrate metabolism. Also in a recent study (5), normalization of cardiac fatty acid oxidation in hearts from diabetic db/db mice did not improve contractile function. Thus the role of increased fatty acid utilization in mediating the adverse effects of diabetes on the heart is far from settled.

One limitation regarding our understanding of the effects of diabetes on cardiac metabolic regulation and its impact on the response to ischemic injury is that with a few notable exceptions (1, 5), most of the available data are based on models.
of acute, uncontrolled, insulin-deficient diabetes, which may not reflect the situation in the majority of patients with diabetes. In addition, the majority of studies of the effect of diabetes on cardiac metabolism have almost exclusively focused on long-chain fatty acids such as palmitate and glucose as the sole substrates despite the potential importance of other substrates such as lactate and pyruvate. This is partly because with traditional radioisotope methods, it is possible to quantify the metabolic fluxes of only two substrates in a single experiment, one $^3$H labeled and the other $^{14}$C labeled. However, using $^{13}$C nuclear magnetic resonance (NMR) glutamate isotopomer analysis, it is possible to determine the metabolic fate of up to four substrates in a single experiment (31), which facilitates the use of more complex and physiologically relevant substrate mixtures. We have recently used this approach (25) to better understand the effect of insulin on the regulation of cardiac metabolism or impaired response to ischemia and reperfusion. Thus, insulin-induced changes in metabolism were associated with contractile dysfunction or impaired response to ischemia and reperfusion.

METHODS

Animals. Experiments were approved by the Institutional Animal Care and Use Committee, and protocol followed the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, 1996). Male Zucker diabetic fatty rats (ZDF/Gmi-fa/fa) were obtained from Charles River Laboratories (Genetic Models; Indianapolis, IN). Age-matched lean littermates (ZDF/Gmi-+/fa or +/+) were used as controls. All animals were housed in a temperature-controlled room (21 ± 2°C) with a 12:12-h light-dark cycle; lights were on at 6 AM and off at 6 PM. All studies were performed during the light cycle.

Water and rat diet were available ad libitum. As previously described, all animals were maintained on Purina 5008 diet as recommended by the supplier (49). Although this diet is ~30% higher in fat content by weight than the standard rodent diet used at our institution (Harlan Teklad 22.5 rodent diet W), it is still a relatively low-fat diet with fat contributing only 16.7% of the calories. This contrasts with diets used in rodent studies of diet-induced obesity (7). These data, multiplied by the arteriovenous lactate concentration difference and by coronary flow rates, were used to determine the rates of exogenous [3-13C]lactate uptake and unlabeled glycolytic lactate efflux.

Isolated heart preparations. Hearts were isolated from 12-wk-old fed rats and perfused at 37°C as previously described (11, 12, 24, 25) with buffer that contained 3% fatty acid-free BSA, 5 mM glucose, 0.32 mM sodium palmitate, 1.0 mM sodium lactate, 0.1 mM sodium pyruvate, 0.5 mM glutamine, and 50 μU/ml insulin. Substrate concentrations were chosen to mimic the resting, nondiabetic fed state. Cardiac function was monitored via a fluid-filled balloon placed in the left ventricle that was connected to a pressure transducer, and end-diastolic pressure (EDP) was set to 5 mmHg by adjusting the balloon volume. Hearts were perfused under a constant perfusion pressure of 75 mmHg. All hearts were equilibrated for 30 min; after the first 10 min, hearts were paced at 310 beats/min for the remainder of the experiments.

Experimental protocol. At the end of equilibration, hearts from the control and ZDF groups were subjected to one of three different protocols as follows: 1) baseline perfusion, whereby hearts were perfused under the same conditions for another 30 min ($n = 5$ control and ZDF animals); 2) LFI, where coronary flow rate was reduced to 0.3 ml/min for 30 min ($n = 4$ control and $n = 5$ ZDF animals); and 3) reperfusion, where after 30 min of LFI, coronary flow was restored to achieve a perfusion pressure of 75 mmHg, which was maintained for an additional 60 min ($n = 5$ control and ZDF animals). During the last 30 min of each protocol, hearts were perfused with [U-$^{13}$C]palmitate, [2-13C]pyruvate, and [3-13C]lactate at the same concentrations used during the equilibration period; glucose remained unlabeled.

Coronary effluent samples were collected before ischemia, every 5 min during ischemia, every 1 min during the first 5 min of reperfusion, and after 30 and 60 min of reperfusion. Oxygen consumption (MV0$_2$) was determined as previously described (25).

After 30 min of perfusion with $^{13}$C-labeled substrates, hearts were freeze-clamped and weighed, and both hearts and pulmonary artery effluents were extracted with perchloric acid (11, 24, 25), freeze dried, and redissolved in a potassium phosphate buffer (pH 7.5, 50 mM) with $^2$H$_2$O solvent for subsequent analysis by $^{13}$C and $^2$H NMR spectroscopy as described (see Analysis of $^{13}$C NMR glutamate isotopomer and determination of substrate oxidation rates and Determination of lactate efflux and uptake rates). Analysis of serum metabolites and effluent lactate concentrations. Blood samples were collected immediately after decapitation of all animals, and serum levels of glucose, free fatty acid (FFA), insulin, and leptin were measured as previously described (49). Lactate concentrations in the perfusate and coronary effluent were determined using enzymatic colorimetric methods.

Analysis of $^{13}$C NMR glutamate isotopomer and determination of substrate oxidation rates. $^{13}$C NMR spectra of heart extracts were collected, and [1-13C]glutamate isotopomer analyses were performed as previously described (24, 25) to determine the fraction of total acetyl-CoA entering the tricarboxylic acid cycle that originated from unlabeled, [1,2-13C]-[2-13C]-, and [1-13C]acetetyl-CoA, which originated from unlabeled glucose or glycogen, [U-$^{13}$C]palmitate, [3-13C]lactate, and [2-13C]pyruvate, respectively. Additional details of this analysis can be found elsewhere (24, 25, 31). These data were combined with measurements of tissue MV0$_2$ to determine the absolute oxidation rates as previously described (25, 30).

Determination of lactate efflux and uptake rates. $^2$H NMR spectroscopy was used to determine the fraction of lactate in the effluent that was unlabeled, which resulted from metabolism of either exogenous glucose or glycogen, and the fraction that was [3-13C]lactate, present in the perfusate (7). These data, multiplied by the arteriovenous lactate concentration difference and by coronary flow rates, were used to determine the rates of exogenous [3-13C]lactate uptake and unlabeled glycolytic lactate efflux.

Sarcolemmal palmitate transport. All animals were housed in a temperature-controlled room (21 ± 2°C) with a 12:12-h light-dark cycle. Water and standard rat diet were available ad libitum. Animals were anesthetized with an injection of pentobarbital sodium (6–8 mg/100 g of body wt ip) before all experimental procedures. While rats were under anesthesia, the hearts were removed. Giant sarcolemmal vesicles were then immediately prepared from fresh hearts as we have described elsewhere (28, 29).

Sarcolemmal palmitate uptake studies were performed using giant vesicles from hearts (28, 29). After vesicle preparation, 40 μl of 0.1% BSA in KCl-MOPS, which contained 15 μM unlabeled and 0.3 μCi radiolabeled [3H]palmitate and 0.06 μCi [14C]mannitol, was added to 40 μl of vesicle suspension. After 15 s, palmitate uptake was terminated by addition of 1.4 ml of ice-cold KCl-MOPS, 2.5 mM MgCl$_2$, in 0.1% BSA, and the sample was centrifuged at 12,000 rpm for 1 min. The supernatant was discarded, and radioactivity was determined in the tip of the tube.

Statistics. All data are presented as means ± SE. Paired t-tests and one-way and repeated-measures ANOVA were used where appropriate combined with Scheffé’s post hoc test. A P value of <0.05 was considered significant.
RESULTS

Animal characteristics and cardiac function. Body weight, heart weight, and serum levels of glucose, FFA, insulin, and leptin in the two groups are summarized in Table 1 and are consistent with previously published data on ZDF rats (37, 49, 52) as well as with the Type 2 diabetic phenotype.

Spontaneous heart rate was significantly reduced in the ZDF group compared with control animals (136 ± 12 and 231 ± 7 beats/min, respectively; \( P < 0.05 \)). Although these heart rates are low compared with many perfused rat heart studies, they are in agreement with our earlier study of these animals, which showed an age-dependent decline in heart rate in both groups that was most pronounced in the ZDF group (49). After pacing, the rate-pressure product (RPP, defined as the left ventricular developed pressure or the peak systolic pressure minus the EDP multiplied by the heart rate) and \(+dP/dt\) values were \(10–15\%\) lower in the ZDF group compared with control animals, but there was no difference in \(-dP/dt\) value between the groups (Fig. 1A). The onset of LFI led to a rapid cessation of contractile function and a gradual increase in EDP that is characteristic of the development of contracture (Fig. 1B); these changes were greater in ZDF than control rats (\( P < 0.05 \)). During LFI, values for coronary flow normalized to heart weight were \(0.24 \pm 0.01\) and \(0.23 \pm 0.01\) ml·min\(^{-1}·g\(^{-1}\) in control and ZDF groups, respectively; thus the greater contracture in the ZDF group was not due to lower coronary flow. After 60 min of reperfusion, RPP, \(+dP/dt\), and \(-dP/dt\) values were significantly decreased compared with baseline levels in the control but not the ZDF group (Fig. 1C). There was no difference in left ventricular developed pressure between groups at the end of reperfusion (data not shown), but EDP was significantly higher in the control group compared with the ZDF group (\(25 \pm 4\ vs. \ 9 \pm 5\) mmHg; \( P < 0.05 \)).

There was no difference in \(MV_{O2}\) between the two groups under any perfusion condition (Table 2). During LFI, \(MV_{O2}\) decreased by \(\sim 97\%\), which is consistent with the reduction in coronary flow. To estimate the metabolic efficiency of contractile function, RPP (which is an index of cardiac work in this model) was divided by \(MV_{O2}\) (Table 2). There was no difference in efficiency between the control and ZDF groups at baseline or during reperfusion.

Lactate metabolism. Using arteriovenous differences in lactate concentration, we determined net lactate uptake and efflux during the different perfusion conditions. During baseline perfusion, there was net lactate uptake by both the control and ZDF groups (Fig 2A), and as expected during LFI, there was a marked increase in net lactate release. Interestingly, at the end of reperfusion, in perfusions with baseline conditions, there was net lactate release rather than uptake; however, it was significantly lower than during LFI. There were no differences in net lactate uptake or release between control and ZDF groups under any perfusion conditions.

We have previously reported that because uptake and oxidation of exogenous lactate occur simultaneously with glycolytic lactate efflux, arteriovenous lactate measurements may not accurately reflect glycolytic lactate efflux (7). Therefore, we used the NMR analysis of lactate enrichment in the coronary effluent to quantify the absolute rates of glycolytic lactate efflux (Fig. 2B) and exogenous lactate uptake (Fig. 2C). At baseline, the rates of both glycolytic lactate efflux and exoge-
nous lactate uptake were significantly depressed in the ZDF compared with the control group; however, during LFI, there were no differences in lactate uptake or efflux between groups. At the end of reperfusion, in contrast with baseline perfusion, there was no difference in the rate of lactate efflux between groups; however, the rate of lactate uptake was significantly lower in the ZDF group.

**Substrate oxidation.** The $^{13}$C NMR spectra from control hearts perfused under baseline and LFI conditions are shown in Fig. 3. These spectra demonstrate as previously reported (25) that under normoxic and LFI conditions, oxidation of exogenous $^{13}$C-labeled substrates was sufficient for $[^{13}C]$glutamate isotopomer analysis. Combining the $[^{13}C]$glutamate isotopomer analysis with MV$_{O2}$ measurements, we calculated the rates of palmitate, lactate, pyruvate, and glucose/glycogen oxidation under all perfusion conditions (Fig. 4). Under baseline perfusion conditions, palmitate oxidation was significantly increased and total carbohydrate oxidation was decreased in the ZDF compared with the control group, which is consistent with reports using the db/db mouse model of Type 2 diabetes (1, 2, 5) as well as previous findings after STZ-induced diabetes (6). It is interesting, however, that when the contributions of lactate, pyruvate, and glucose oxidation to total carbohydrate oxidation are examined, it is clear that contrary to accepted wisdom (6), glucose oxidation is not impaired in the diabetic group. Rather, the decrease in carbohydrate oxidation is almost entirely due to a reduction in lactate oxidation with a small but significant decrease in pyruvate oxidation. During LFI, there was significant oxidation of all substrates, although the absolute rates were markedly decreased due to the ~97% reduction in coronary flow and oxygen delivery. However, in contrast with baseline perfusion, during LFI, total carbohydrate oxidation was not significantly impaired in the ZDF group. On reperfusion, the oxidation of all substrates was significantly lower compared with baseline perfusion in both groups; however, palmitate oxidation was still significantly increased and lactate oxidation decreased in the ZDF group compared with the control group.

It should be noted that with the $^{13}$C-labeled mixture used in this study, we could not differentiate between oxidation of exogenous glucose or endogenous glycogen. However, in hearts from normal Sprague-Dawley rats, we have recently shown that glycogen oxidation was undetectable during baseline perfusion, whereas glycogen oxidation was approximately twofold higher than glucose oxidation in LFI (25).

It has been proposed (23, 27) that increased oxidation of fatty acids leads to dissociation between glucose oxidation and glycolysis, which may be detrimental. Therefore, using glucose oxidation measurements and lactate efflux data, we calculated the ratio of glucose oxidation to total glycolytic flux in the control and ZDF groups (Table 2). During LFI, there was a significant decrease in this ratio in both groups as would be expected due to the marked decrease in glucose oxidation during LFI. Although this ratio was slightly higher in the ZDF groups under all perfusion conditions, these differences were not significant. Sample size calculations suggest that under LFI conditions, we would detect a significant difference between the ZDF and control groups (i.e., $P < 0.05$) using a two-tailed $t$-test with 80% power, if the sample size were increased to eight in each group. However, similar calculations with the baseline and reperfusion data suggest sample sizes of 25–50 would be required to detect any significant difference.

**ATP synthesis.** From the lactate efflux and substrate oxidation rates, we calculated the oxidative, nonoxidative, and total ATP synthesis rates for both groups under the different perfusion conditions (Table 3). During baseline perfusion, there were no differences in oxidative ATP production between the control and ZDF groups; however, nonoxidative ATP production was significantly depressed in the ZDF group as evidenced by lower lactate efflux rates. Not surprisingly, during LFI, the oxidative ATP yield was markedly reduced in both groups compared with baseline; however, there was no difference between the two groups. It is noteworthy that despite an ~97% reduction in flow, oxidative ATP production remained an important source of energy production, which is consistent with our recent report (25) on substrate oxidation during LFI. During reperfusion, oxidative and total ATP production values were decreased compared with baseline, whereas nonoxidative ATP production was unchanged.

The relative contributions of palmitate and total carbohydrate to total ATP synthesis are summarized in Fig. 5. These data clearly demonstrate the increased utilization of fatty acids

### Table 2. $MV_{O2}$, assessment of contractile efficiency, and ratio of glucose oxidation to glycolysis during baseline perfusion, low-flow ischemia, and reperfusion

<table>
<thead>
<tr>
<th>Perfusion</th>
<th>$n$, No. of Rats</th>
<th>$MV_{O2}$, $\mu$mol/g•min$^{-1}$</th>
<th>RPP/$MV_{O2}$ × 1,000, mmHg•$\mu$mol$^{-1}$·g$^{-1}$</th>
<th>RPP/ATP Synthesis × 1,000, mmHg•$\mu$mol$^{-1}$·g$^{-1}$</th>
<th>Glucose Oxidation/ Glycolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control rats</td>
<td>16, 5*</td>
<td>5.6 ± 0.4</td>
<td>7.1 ± 0.5</td>
<td>1.4 ± 0.1</td>
<td>0.31 ± 0.05</td>
</tr>
<tr>
<td>ZDF rats</td>
<td>14, 5</td>
<td>5.3 ± 0.3</td>
<td>6.9 ± 0.4</td>
<td>1.4 ± 0.1</td>
<td>0.39 ± 0.04</td>
</tr>
<tr>
<td><strong>Low-flow ischemia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control rats</td>
<td>9, 4†</td>
<td>0.12 ± 0.01‡§</td>
<td>8.1 ± 0.2</td>
<td>1.6 ± 0.1</td>
<td>0.21 ± 0.04</td>
</tr>
<tr>
<td>ZDF rats</td>
<td>9, 4</td>
<td>0.12 ± 0.01‡§</td>
<td>7.7 ± 0.2</td>
<td>1.6 ± 0.1</td>
<td>0.25 ± 0.04</td>
</tr>
<tr>
<td><strong>Reperfusion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control rats</td>
<td>5</td>
<td>3.2 ± 0.5‡</td>
<td>8.1 ± 0.2</td>
<td>1.6 ± 0.1</td>
<td>0.21 ± 0.04</td>
</tr>
<tr>
<td>ZDF rats</td>
<td>5</td>
<td>3.7 ± 0.3‡</td>
<td>7.7 ± 0.2</td>
<td>1.6 ± 0.1</td>
<td>0.25 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± SE. Contractile efficiency was assessed by dividing rate-pressure product (RPP, in mmHg/min) by either oxygen consumption ($MV_{O2}$) or total ATP synthesis rate (in $\mu$mol/g•min$^{-1}$). *Of the 16 experiments where $MV_{O2}$ and RPP were measured during baseline perfusion, the rates of ATP synthesis, glucose oxidation, and glycolysis were determined under the same conditions in only 5 experiments; the remainder were subject to low-flow ischemia (LFI) or LFI and reperfusion. †Of the 9 experiments where $MV_{O2}$ was measured during LFI, rates of ATP synthesis, glucose oxidation, and glycolysis were determined in only 4; the remainder were subject to reperfusion. During LFI, RPP was zero; therefore, RPP/$MV_{O2}$ and RPP/ATP synthesis values were not determined. ‡$P < 0.05$ vs. baseline; §$P = 0.087$ vs. control.

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ALTERED SUBSTRATE UTILIZATION IN ISOLATED ZDF RATS

**DISCUSSION**

In this study, we used $^{13}$C glutamate isotopomer analysis to characterize for the first time the impact of Type 2 diabetes on glucose, lactate, pyruvate, and palmitate oxidation during baseline perfusion, LFI, and reperfusion. We found that contrary to accepted wisdom, there was no difference in glucose oxidation between control and diabetic ZDF groups under any conditions; however, consistent with an earlier study in an insulin-deficient model of diabetes (11), the observed decrease in carbohydrate oxidation in the ZDF group was attributable entirely to a reduction in lactate oxidation. The primary defect in glucose utilization in the ZDF group was in glycolytic lactate production rather than oxidation. Consistent with reports from studies on hearts of db/db diabetic mice (1, 2), there was an increase in fatty acid oxidation in the ZDF group under baseline conditions. This was maintained during LFI and reperfusion and was associated with increased rates of sarcolemmal fatty acid transport. However, although baseline function was modestly impaired in the ZDF group, this was not associated with mechanisms commonly proposed to account for the detrimental effects of increased fatty acid oxidation, namely, decreased cardiac efficiency or a change in the relationship between glucose oxidation and glycolysis, despite the increase in fatty acid oxidation. In contrast with a recent report on the db/db mouse model of Type 2 diabetes (1), we found that there was no increase in susceptibility to ischemia-reperfusion injury in the ZDF group.

That diabetes leads to altered regulation of cardiac metabolism is well established (9); however, the role of these metabolic changes in the development of contractile dysfunction and subsequent diabetic cardiomyopathy remains unresolved. The effect of diabetes on cardiac substrate oxidation is typically characterized as decreased glucose oxidation and increased fatty acid oxidation (26) as recently reported by Belke et al. (2), who studied hearts from obese, diabetic db/db mice. They reported that palmitate oxidation was approximately twofold higher in the diabetic group, which agrees well with our results (see Fig. 4A). Belke et al. also reported that glycolytic rates were decreased by ~30–40%, which is also consistent with the decrease in lactate efflux rates we found in the ZDF group (see Fig. 2B). Additionally, they found that glucose oxidation was depressed in the diabetic group, which is in contrast with our results (see Fig. 4); however, the only substrates available in those studies were glucose and palmitate. Although we found no difference in glucose oxidation rates between ZDF and control groups under any perfusion conditions, lactate oxidation was markedly decreased, which resulted in lower total carbohydrate oxidation. Interestingly, the shift toward increased dependence on palmitate for ATP production and decreased lactate utilization in the ZDF group was maintained during both LFI and reperfusion. We cannot rule out the possibility that a more prolonged period of diabetes might lead to decreased glucose as well as lactate oxidation.

In an earlier study (12) that used similar methods to examine the effects of Type 2 diabetes on cardiac carbohydrate utilization under normal perfusion conditions, we found that the $^{13}$C labeling of lactate and alanine was significantly reduced in the ZDF group, which is consistent with the lower glycolytic lactate efflux seen here. We also showed that there was a reduction in total pyruvate dehydrogenase (PDH) flux that was for ATP synthesis and decreased carbohydrate utilization in the ZDF group under each of the three perfusion conditions; however, the differences are more pronounced under baseline and reperfusion conditions. These data also reinforce that the decreased contribution of carbohydrates to total ATP synthesis in the ZDF group is due primarily to a reduction in lactate oxidation, whereas the contribution of glucose to ATP production is similar between the control and ZDF groups under all of the perfusion conditions.

**Fatty acid transport activity.** The increase in fatty acid oxidation in the ZDF group was associated with increased rates of sarcolemmal palmitate transport in membrane vesicles ($1.5 \pm 0.1$ vs. $1.1 \pm 0.1$ pmol·s$^{-1}$·mg protein$^{-1}$; $P < 0.05$).

![Fig. 2. Rates of net lactate uptake and net release determined from arteriovenous concentration differences (A). Lactate release during LFI was determined from lactate release during the 30-min LFI plus the first 4 min of reperfusion. Rates of lactate efflux (B) and lactate uptake were determined using arteriovenous concentration differences combined with measurements of $^{13}$C lactate enrichment (C). *$P < 0.05$ compared with control; †$P < 0.05$ compared with respective groups at baseline. In all groups, $n = 5$ rats except control LFI, where $n = 4$ rats.](http://ajpheart.physiology.org/)

**A**

**Net lactate uptake or release rates**

![Graph A](http://ajpheart.physiology.org/)

**B**

**Absolute lactate efflux rate**

![Graph B](http://ajpheart.physiology.org/)

**C**

**Absolute lactate uptake rate**

![Graph C](http://ajpheart.physiology.org/)
associated with a decrease in the activation of PDH. However, the contribution of palmitate to total tricarboxylic acid cycle flux was substantially higher in the lean control group compared with the control group in this study, and the decrease in PDH flux in the ZDF group was due to decreased glucose oxidation rather than lactate oxidation (12). The reasons for these discrepancies are unknown; however, the pattern of substrate utilization in the control group in this study is more consistent with our recent studies (24, 25) of hearts from Sprague-Dawley rats than with the control group from our earlier study (12). Thus it is likely that the differences in the results from the two studies can be attributed to the conditions that led to the unusually high palmitate utilization in both the control and ZDF groups in an earlier study. However, nominally, the perfusion conditions were the same in both series of experiments; thus unfortunately, at this time we cannot provide a definitive explanation to account for the discrepancies between the two studies.

Selective inhibition of lactate rather than glucose oxidation in heart was previously reported in an acute insulin-deficient model of diabetes (10, 11) but not in a model of Type 2 diabetes. In our earlier study (10), after 1 wk of STZ-induced diabetes, we found that inhibition of lactate oxidation was not accompanied by decreased expression of monocarboxylate transporter-1 (MCT-1) expression or lactate dehydrogenase isoform expression (10); however, after longer periods of STZ-induced diabetes, MCT-1 expression in heart was reduced by ~30% (17). It was also shown that MCT-1 expression is reduced in skeletal muscle from obese, nondiabetic Zucker rats (38). Thus although our earlier observations (10) indicate that decreased MCT-1 and lactate dehydrogenase expression are not prerequisites for impaired lactate oxidation, we cannot rule out the possibility that they may be reduced in the ZDF group and could be contributing factors to the reduction in lactate oxidation.

Increased dependence on fatty acids for energy production concomitant with decreased glucose oxidation is frequently cited as one of the factors contributing to the development of contractile dysfunction in diabetes (36, 46), at least in part because of the theoretical increase in oxygen required for ATP production from fatty acids compared with glucose (35). More recently it was also proposed (23, 27) that increased fatty acid oxidation leads to dissociation between glucose oxidation and glycolysis, which increases the susceptibility to ischemic injury due to increased lactate production and proton accumulation. However, in these experiments, where physiologically relevant concentrations of lactate were provided in addition to glucose, the increase in fatty acid oxidation was associated with decreased lactate and not decreased glucose oxidation. Furthermore, this increase in fatty acid oxidation was not associated with any change in MV\textsubscript{O2} or cardiac efficiency and did not significantly alter the relationship between glucose oxidation and glycolysis (see Table 2). It is also noteworthy that although function was slightly depressed in the ZDF group under baseline conditions, the increase in fatty acid oxidation was not associated with an impaired response to ischemia.

It is possible that the impact of increased fatty acid oxidation on cardiac efficiency and the relationship between glucose oxidation and glycolysis may be specific to conditions where glucose is the only carbohydrate present. Alternatively, that cardiac efficiency was not impaired and that the tolerance to ischemia and reperfusion was not impaired despite the increase in fatty acid oxidation may reflect an adaptive response to the Type 2 diabetic metabolic milieu in the ZDF group. For example, hearts from insulin-resistant ob/ob mice exhibited significantly higher cardiac function compared with the wild-type group when perfused under conditions designed to mimic the insulin-resistant state (32), which suggests that they were better adapted to this metabolic milieu. However, with respect to cardiac efficiency, it should be noted that although theoretically there is a ~12% decrease in the amount of oxygen required for ATP synthesis when shifting from 100% palmitate oxidation to 100% glucose oxidation (35), such extreme metabolic shifts are unlikely to occur under physiological condi-

Fig. 3. \(^{13}\text{C} \) NMR spectra of heart extracts after perfusion with \(^{13}\text{C} \)-labeled substrates.
tions. Indeed, we found that when lactate and pyruvate were available for oxidation as well as glucose and palmitate, decreasing fatty acid oxidation from 60% to 5% of total energy production did not alter the efficiency of ATP production (24). Thus although increased concentrations of exogenous fatty acids result in a marked increase in basal myocardial oxygen requirements (4, 32), this increase is too large to be accounted for by differences in efficiency of ATP production (4). Consequently, if (as was the case here) exogenous fatty acid concentrations were kept constant, then shifts in the relative contribution of fatty acids to oxidative energy metabolism would not be expected to lead to measurable changes in cardiac efficiency.

The differences in the ratio of glucose oxidation to total glycolytic flux were not significantly different between the control and ZDF groups; however, sample-size analysis indicated that a modest increase in sample size in the LFI groups might have proven this ratio significantly higher in the ZDF group. Because an increase in glucose oxidation relative to glycolysis has been associated with improved recovery from ischemia, this could account for the functional recovery seen in the ZDF group (see Fig. 1). However, an increase in this ratio under conditions where fatty acid oxidation is increased (see group.

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Table 3. Total, oxidative, and nonoxidative ATP production during baseline perfusion, LFI, and reperfusion

<table>
<thead>
<tr>
<th>Perfusion</th>
<th>Total, ( \mu \text{mol} \text{min}^{-1} \text{g}^{-1} )</th>
<th>Oxidative, ( \mu \text{mol} \text{min}^{-1} \text{g}^{-1} )</th>
<th>Nonoxidative, ( \mu \text{mol} \text{min}^{-1} \text{g}^{-1} )</th>
<th>Oxidative, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control*</td>
<td>27±2</td>
<td>26±1</td>
<td>1.4±0.1</td>
<td>95±1</td>
</tr>
<tr>
<td>ZDF*</td>
<td>24±1</td>
<td>23±1</td>
<td>1.0±0.1‡</td>
<td>96±0</td>
</tr>
<tr>
<td>LFI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control†</td>
<td>0.9±0.0§</td>
<td>0.5±0.0§</td>
<td>0.4±0.1§</td>
<td>57±1§</td>
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<tr>
<td>ZDF*</td>
<td>0.9±0.1§</td>
<td>0.6±0.0§</td>
<td>0.3±0.0§</td>
<td>66±2§</td>
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<tr>
<td>Reperfusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control*</td>
<td>16±2§</td>
<td>15±2§</td>
<td>1.4±0.1</td>
<td>91±2§</td>
</tr>
<tr>
<td>ZDF*</td>
<td>18±1§</td>
<td>17±1§</td>
<td>1.4±0.3</td>
<td>92±1§</td>
</tr>
</tbody>
</table>

Values are means ± SE; *\( n = 5 \) or †\( n = 4 \) rats. ‡\( P < 0.05 \) vs. control; §\( P < 0.05 \) vs. baseline.

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Fig. 4. Oxidation rates of palmitate, total carbohydrate (CHO), lactate, pyruvate, and glucose during baseline (A), LFI (B), and reperfusion (C). *\( P < 0.05 \) compared with control; †\( P < 0.05 \) compared with respective groups during baseline. In all groups \( n = 5 \) rats except control LFI, where \( n = 4 \) rats.

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Fig. 5. Relative (percent) contributions of substrates to total ATP production at baseline (A) and during LFI (B) and reperfusion (C). *\( P < 0.05 \) compared with control; †\( P < 0.05 \) compared with baseline. In all groups \( n = 5 \) rats except control LFI, where \( n = 4 \) rats.
Figs. 4 and 5) appears superficially to be counterintuitive in the context of studies that have shown that an increase in this ratio is associated with decreased fatty acid oxidation. Here the tendency for the ratio of glucose oxidation to glycolysis to be higher in the ZDF group during LFI appears to be due to a combination of a slight decrease in glycolysis and a small increase in glucose oxidation relative to the control group. A decrease in glycolysis could be beneficial during ischemia because of a reduction in lactate accumulation and thus reduced acidosis; however, during LFI, increased glycolysis has been associated with improved rather than worsened recovery of function (13). Given the relatively small changes in glucose oxidation and glycolysis seen here, it would be premature to attribute the increased tolerance to ischemia and reperfusion on such subtle alterations in metabolic fluxes.

Zhou et al. (52) recently reported that in obese ZDF rats, the development of contractile dysfunction was associated with accumulation of triglycerides, increased ceramide content, and myocyte apoptosis. These data suggest that abnormalities in nonoxidative fatty acid metabolism and associated lipotoxicity may be more important than increased fatty acid oxidation in mediating the adverse effects of diabetes on heart. The increase in cardiac fatty acid oxidation associated with diabetes is usually attributed to increased activity of carnitine palmitoyl transferase-1 (CPT-1) secondary to reduced acetyl-CoA carboxylase activity (26); however, Luiken et al. (28) reported that fatty acid translocase/CD36 was elevated in cardiac muscle after STZ-induced diabetes. This is consistent with our observation in a similar model of diabetes that at a palmitate concentration of 0.1 mM, fatty acid oxidation was markedly increased, whereas at 1 mM, it was significantly decreased compared with nondiabetic groups (11). Here we found that the increase in fatty acid oxidation in the ZDF group was associated with increased palmitate transport in plasma membrane vesicles, which is similar to previous reports (3) from studies of skeletal muscle from obese Zucker rats. These results combined with earlier studies (11, 28) suggest that the increase in myocardial fatty acid oxidation in diabetes may be a consequence of increased fatty acid transport into the myocyte in addition to altered regulation of CPT-1. If so, it is possible that the detrimental effects of altered lipid metabolism in diabetes may be a result of fatty acid uptake being in excess of its disposal rate via oxidation, thereby leading to increased triglyceride and ceramide levels. Clearly, additional studies are needed to better understand the regulation of fatty acid uptake into heart and the subsequent partitioning between oxidative and nonoxidative metabolic pathways.

It is important to note that in these experiments, hearts were removed from their in vivo metabolic and neurohumoral environments and perfused under identical conditions. This is consistent with the majority of studies investigating the role of altered metabolism in mediating the effects of diabetes on cardiac function including other studies using models of Type 2 diabetes. Nevertheless, we cannot rule out that the use of higher fatty acid or glucose concentrations such as those found in the ZDF group could have affected either baseline cardiac function or the responses to ischemia and reperfusion. However, it should be noted that in an earlier study using an STZ-model of diabetes, we found that increasing the exogenous fatty acid concentration from 0.1 to 1.0 mM had no effect, adverse or otherwise, on contractile function in hearts from either normal or diabetic rats. Another factor to consider is that the workload in isolated perfused hearts is less than that found in vivo. With the use of MV$_{O_2}$ as an index of total cardiac work, hearts in this study were working at a level of approximately one-third of that seen at rest in vivo in rats (22). Although increases in workload clearly increase the rates of substrate oxidation, they do not alter the relative contributions of substrates to energy production (19). Nevertheless, if the maximal energy production capacity of the diabetic heart is compromised, then the reduced energy demand in the isolated heart could tend to minimize differences in contractile function compared with the in vivo environment.

In conclusion, for the first time, we have evaluated the impact of Type 2 diabetes on the metabolic fluxes of lactate, pyruvate, glucose, and palmitate at physiologically relevant concentrations under baseline conditions, during LFI, and after reperfusion in the isolated perfused heart. We have shown that similar to insulin-deficient models of diabetes (10, 11), the primary defect in cardiac carbohydrate oxidation is in lactate oxidation and not glucose oxidation; the main defect in glucose metabolism was in glycolytic lactate production. Of particular significance, we found that despite the increase in palmitate oxidation, there was no change in cardiac efficiency and no alteration in the relationship between glucose oxidation and glycolysis. Consequently, the results from this study suggest that the commonly proposed mechanisms for diabetes-induced contractile dysfunction might be relevant only when glucose and palmitate are the sole available substrates. The increased oxidation of palmitate was associated with increased sarcolemmal palmitate transport rates, which supports a lipotoxicity-mediated mechanism as responsible for the adverse effects of diabetes on heart.

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GRANTS

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