Cardiac carbohydrate metabolism in Zucker diabetic fatty rats

John C. Chatham\textsuperscript{a,}\textsuperscript{*}, Anne-Marie L. Seymour\textsuperscript{b}

\textsuperscript{a}Center for NMR Research & Development, University of Alabama at Birmingham, CNIR Building, 828 Eighth Court South, Birmingham, AL 35294, USA
\textsuperscript{b}Department of Biological Sciences, University of Hull, Cottingham Road, Hull HU6 7RX, UK

Received 5 September 2001; accepted 4 March 2002

Abstract

Objective: The aim of this study was to test the hypothesis that, shortly after the development of Type-2 diabetes, alterations in cardiac carbohydrate metabolism precede the onset of abnormalities in systolic function. Methods: Hearts from 11-week-old Zucker diabetic fatty (ZDF) rats and age matched controls were perfused in the isovolumic Langendorff mode with \textsuperscript{13}C-labeled glucose, lactate and pyruvate and unlabeled fatty acids. \textsuperscript{13}C-Nuclear magnetic resonance glutamate isotopomer analysis was carried out to determine the contributions of substrates to energy production. Results: The ZDF group was hyperglycemic and the relative flux through pyruvate dehydrogenase (PDH) was significantly depressed compared to lean controls. In the lean group, lactate, pyruvate and glucose contributed 64\%\,6\%, 24\%\,6\% and 11\%\,6\%, respectively, to total pyruvate oxidation. In the ZDF group, the contribution of glucose both to total pyruvate oxidation and to tissue lactate and alanine formation was significantly depressed. Cardiac function assessed by the rate-pressure product was similar in both groups. The fraction of active PDH was decreased in the ZDF group compared to controls (p<0.025). Conclusions: These results highlight significant changes in cardiac carbohydrate metabolism shortly after the development of hyperglycemia in a model of Type 2 diabetes in the absence of overt changes in systolic function. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Cardiomyopathy; Contractile function; Diabetes; Energy metabolism; Glycolysis

1. Introduction

Diabetes is linked to obesity, dyslipidemia and hypertension [1–3], which are associated with increased risk for coronary artery disease, cardiac hypertrophy and heart failure [3–5]. However, there is growing evidence to support the concept of a specific diabetic cardiomyopathy independent of other risk factors for heart disease [6]. Both Type-1 and Type-2 diabetic patients have been shown to have significantly reduced left ventricular end diastolic volume and impaired diastolic filling compared to non-diabetics [7,8]. Impaired isovolumic relaxation [9,10] as well as impaired exercise responses in ejection fraction [11,12] have been reported in otherwise healthy diabetic patients. Even in young (~20 year old) Type-1 diabetic patients, Kimball et al. [13] reported significant alterations in cardiac contractility. Despite the apparent similarities in contractile abnormalities seen in both Type-1 and Type-2 diabetic patients it is not known whether the cardiomyopathy is the same in both conditions.

In animal models of diabetes, alterations in metabolism, protein synthesis, calcium handling and contractile proteins [14] may be contributing factors to the development of contractile dysfunction. For example, decreased myocardial Ca\textsuperscript{2+}-ATPase activity has been reported as has depressed sarcoplasmic reticulum Ca\textsuperscript{2+} uptake following insulin deficient diabetes (see Ref. [15] for a review). Ren and Davidoff showed that, at the single cell level, diabetes prolongs both myocyte contraction and relaxation [16]. However, some of the earliest changes following the induction of diabetes are at the level of myocardial substrate utilization. Consequently, it has been proposed

\textsuperscript{*}Corresponding author. Tel.: +1-205-934-0240; fax: +1-205-934-7367.
E-mail address: jchatham@uab.edu (J.C. Chatham).

Time for primary review 29 days.
that these metabolic adaptations may be causally related to the development of contractile dysfunction and increased susceptibility to ischemic injury [17,18].

The majority of studies on the effects of diabetes on the heart have focused on models of uncontrolled Type-1 diabetes. In such studies, the animals are severely insulinopenic, hyperglycemic and frequently ketotic and experiments are typically carried out after several weeks of diabetes when both metabolic and contractile abnormalities exist [14]. Consequently, despite the many diabetic studies describing changes in cardiac energy metabolism, it has been difficult to demonstrate that these alterations actually contribute to the depressed contractile function. Furthermore, approximately 90% of diabetic patients have Type-2 diabetes [19], and are hyperinsulinemic and hyperglycemic although ketosis is usually absent. Type-2 diabetes is characterized by a gradual onset, with a period of insulin resistance, consisting of hyperinsulinemia and euglycemia preceding the onset of hyperglycemia. This is clearly quite distinct from the rapid onset of Type-1 diabetes.

However, to date, there have been remarkably few studies of cardiac metabolism and function in models of Type-2 diabetes. Belke et al., reported both functional and metabolic abnormalities in hearts from obese, Type-2 diabetic db/db mice [20]. They demonstrated that increasing GLUT4 expression improved both contractile function and normalized glucose and palmitate metabolism in the db/db mice. This provides evidence to support a metabolic basis for the development of diabetic cardiomyopathy. Glucose was the only carbohydrate source provided in these experiments despite the fact that lactate is a major source of energy for contractile function. Therefore, it is unclear whether this improvement in function would be seen with more physiological substrate mixtures.

The Zucker diabetic fatty (ZDF) rat is a well-characterized model of Type-2 diabetes which is hyperphagic, obese, hyperlipidemic, hyperinsulinemic and hyperglycemic [21,22]—features typical of the human disease. Up to about 6 weeks of age, male ZDF rats are obese and insulin resistant, but are euglycemic; hyperglycemia starts to develop around 6 weeks of age and, by 10–12 weeks of age, a relatively stable level of hyperglycemia is established [21,22]. Cardiac function has been shown to be significantly depressed in 20-week-old ZDF rats but not at 7 weeks of age [23]. Thus, these animals provide a good model to investigate the effects of the development of Type-2 diabetes on cardiac metabolism and function.

The aim of this study, therefore, was to test the hypothesis that shortly after the development of diabetes in ZDF rats, alterations in cardiac carbohydrate metabolism precede the onset of contractile dysfunction. To test this hypothesis, we used 13C-nuclear magnetic resonance (NMR) glutamate isotopomer analysis to determine the relative contributions of glucose, lactate and pyruvate to cardiac energy metabolism in isolated perfused hearts from 11-week-old ZDF rats and lean age matched controls.

2. Methods

2.1. Animals

The investigation conforms to the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Male Zucker diabetic fatty rats (ZDF/Gmi-fa/fa) and age matched lean (+/−) litter mates were obtained from Genetic Models, Indianapolis, IN, USA.

2.2. Isolated perfused heart experiments

2.2.1. Heart preparation

Fed rats were anesthetized with ketamine (100 mg/kg i.p.), heparinized (500 U/100 g i.p.), and decapitated. Hearts from lean and 11-week-old ZDF rats were excised and perfused in a modified Langendorff mode with Krebs–Henseleit bicarbonate buffer equilibrated with 95% O2–5% CO2 (38°C, pH 7.4) as previously described [24,25]. The buffer contained 3% bovine serum albumin (BSA, essentially fatty acid free, Sigma, St. Louis, MO, USA) and the following components (mM): NaCl 118, KCl 4.8, MgSO4 1.2, CaCl2 1.4, KH2PO4 1.2, NaHCO3 25, glucose 5, sodium lactate 1, sodium pyruvate, 0.1, sodium palmitate 0.4 and insulin (50 μU/l).

Contractile function was determined via a fluid filled balloon inserted into the left ventricle via the mitral valve and connected to a Gould P23Db pressure transducer on a four-channel chart recorder (Gould RS 3400, Cleveland, OH, USA). The balloon volume was inflated to obtain a left ventricular end diastolic pressure of 5 mmHg. Coronary flow was adjusted to maintain perfusion pressure at 75 mmHg. Measurements of cardiac function, including coronary flow, heart rate and developed pressure were recorded every 10 min through out perfusion with 13C-labeled substrates. The data are reported as an average over the duration of perfusion with 13C-labeled substrates. After a 20 min equilibration period the perfusion medium was switched to buffer containing the following 13C-labeled substrates: [U-13C]glucose; [3-13C]lactate; [2-13C]pyruvate and unlabeled palmitate; the concentrations of all the substrates were kept constant. After 45 min of perfusion, hearts were freeze clamped with Wollenberger tongs pre-cooled in liquid nitrogen. Freeze-clamped hearts were extracted with 6% perchloric acid as previously described [25].

2.2.2. 13C-NMR spectroscopy

High-resolution 13C-NMR spectra were collected using a Bruker 500-MSL NMR spectrometer equipped with a 11.75 T magnet using a commercial 10 mm probe as previously described [24,25]. The relative contributions of glucose, lactate and pyruvate to the total acetyl-CoA pool entering the tricarboxylic acid (TCA) cycle were determined from glutamate isotopomer analysis [26], using
Carbon-13 will then be transferred to TCA cycle intermediates and subsequently to alanine formation relative to lactate. In addition to being oxidized by PDH, pyruvate can also be metabolized via pyruvate dehydrogenase (PDH), which will form [1,2-13C], [2-13C]- and [1-13C]acetetyl-CoA, respectively. The palmitate is unlabeled and thus when metabolized will produce unlabeled acetyl-CoA. For the purposes of this analysis we have assumed that fatty acids, either exogenous palmitate or endogenous triglycerides, are the only source of unlabeled acetyl-CoA; however, we cannot rule out a small contribution from unlabeled pyruvate resulting from glycogenolysis.

Using glutamate isotopomer analysis, we determined the fraction of total acetyl-CoA entering the TCA cycle that originates from unlabeled, [1,2-13C]-, [2-13C]- and [1-13C]acetetyl-CoA which reflects the relative contributions of palmitate, glucose, lactate and pyruvate to acetyl-CoA formation. Fatty acids are converted to acetyl-CoA via β-oxidation, which, in Fig. 1A, is represented by $F_b$. The formation of acetyl-CoA from pyruvate occurs via PDH and is represented by $F_{pdh}$, which reflects the sum of the contributions from [U-13C]pyruvate, [3-13C]pyruvate or [2-13C]pyruvate. In other words, $F_{pdh}$ represents the flux of all 13C-labeled pyruvate through PDH relative to total TCA cycle flux. By definition $F_{pdh} + F_b = 1$.

In addition to metabolism via PDH, pyruvate can also enter the TCA cycle via pyruvate carboxylase. The 13C-labeling pattern in glutamate is different if 13C-labeled pyruvate is metabolized via this route. Thus, analysis of the glutamate isotopomer distribution enables us to determine not only the flux through PDH but also the relative flux through pyruvate carboxylase, represented by $F_{pcx}$ in Fig. 1A. $F_{pcx}$ represents the total flux of all 13C-labeled pyruvate through pyruvate carboxylase relative to the total TCA cycle flux.

As noted above $F_{pdh}$ and $F_{pcx}$ are composed of three different 13C-labeled pyruvate species, resulting from the metabolism of exogenous [U-13C]glucose, [3-13C]lactate and [2-13C]pyruvate, represented by $F_{gl}$, $F_{la}$ and $F_{py}$, respectively, in Fig. 1A. We have assumed that unlabeled pyruvate is negligible, therefore, by definition: $F_{gl} + F_{la} + F_{py} = 1$. In other words, $F_{gl}$ is the fraction of tissue pyruvate that originated from [U-13C]glucose, which is subsequently metabolized via PDH (or pyruvate carboxylase). $F_{la}$ and $F_{py}$ are the fractions of tissue pyruvate originating from [3-13C]lactate and [2-13C]pyruvate, respectively, which are subsequently metabolized.

2.2.3. Glutamate isotopomer analysis

The different fluxes that can be determined by glutamate isotopomer analysis with the substrates used in these experiments are shown in Fig. 1A. Metabolism of the exogenous 13C-labeled substrates will lead to the formation of [U-13C]pyruvate, [3-13C]pyruvate or [2-13C]pyruvate which, when metabolized via pyruvate dehydrogenase (PDH), will form [1,2-13C]-, [2-13C]- and [1-13C]acetetyl-CoA, respectively. The palmitate is unlabeled and thus when metabolized will produce unlabeled acetyl-CoA. For the purposes of this analysis we have assumed that fatty acids, either exogenous palmitate or endogenous triglycerides, are the only source of unlabeled acetyl-CoA; however, we cannot rule out a small contribution from unlabeled pyruvate resulting from glycogenolysis.

Using glutamate isotopomer analysis, we determined the fraction of total acetyl-CoA entering the TCA cycle that originates from unlabeled, [1,2-13C]-, [2-13C]- and [1-13C]acetetyl-CoA which reflects the relative contributions of palmitate, glucose, lactate and pyruvate to acetyl-CoA formation. Fatty acids are converted to acetyl-CoA via β-oxidation, which, in Fig. 1A, is represented by $F_b$. The formation of acetyl-CoA from pyruvate occurs via PDH and is represented by $F_{pdh}$, which reflects the sum of the contributions from [U-13C]pyruvate, [3-13C]pyruvate or [2-13C]pyruvate. In other words, $F_{pdh}$ represents the flux of all 13C-labeled pyruvate through PDH relative to total TCA cycle flux. By definition $F_{pdh} + F_b = 1$.

In addition to metabolism via PDH, pyruvate can also enter the TCA cycle via pyruvate carboxylase. The 13C-labeling pattern in glutamate is different if 13C-labeled pyruvate is metabolized via this route. Thus, analysis of the glutamate isotopomer distribution enables us to determine not only the flux through PDH but also the relative flux through pyruvate carboxylase, represented by $F_{pcx}$ in Fig. 1A. $F_{pcx}$ represents the total flux of all 13C-labeled pyruvate through pyruvate carboxylase relative to the total TCA cycle flux.

As noted above $F_{pdh}$ and $F_{pcx}$ are composed of three different 13C-labeled pyruvate species, resulting from the metabolism of exogenous [U-13C]glucose, [3-13C]lactate and [2-13C]pyruvate, represented by $F_{gl}$, $F_{la}$ and $F_{py}$, respectively, in Fig. 1A. We have assumed that unlabeled pyruvate is negligible, therefore, by definition: $F_{gl} + F_{la} + F_{py} = 1$. In other words, $F_{gl}$ is the fraction of tissue pyruvate that originated from [U-13C]glucose, which is subsequently metabolized via PDH (or pyruvate carboxylase). $F_{la}$ and $F_{py}$ are the fractions of tissue pyruvate originating from [3-13C]lactate and [2-13C]pyruvate, respectively, which are subsequently metabolized.

2.2.4. Alanine and lactate isotopomer analysis

In addition to being oxidized by PDH, pyruvate can also
be converted to alanine via alanine aminotransferase, and lactate via lactate dehydrogenase. As shown in Fig. 1B, [3-13C]lactate will be metabolized to [3-13C]alanine in the heart and [U-13C]glucose metabolism will result in [U-13C]alanine, generating singlet and doublet C3-resonances of alanine, respectively. Thus, the ratio of the C3 doublet of alanine to the total intensity of the C3-resonance represents the contribution of exogenous glucose to tissue alanine formation relative to exogenous lactate. Similar isotopomer analysis of the C3-lactate resonance enabled us to assess the relative contribution of glucose to lactate formation. Overall, isotopomer analysis of the C3-resonances of alanine and lactate provides an index of the metabolism of [U-13C]glucose via glycolysis.

2.3. Determination of PDH activity

2.3.1. Tissue preparation

Fed rats were anesthetized with ketamine-HCl (50 mg/kg, i.p.); decapitated and hearts were rapidly excised and freeze clamped with liquid nitrogen-cooled Wollenberger tongs. Frozen heart tissue was ground into a fine powder using a mortar and pestle cooled to liquid nitrogen temperature and extracted and assayed as described previously [27].

Serum was prepared from blood samples collected at the time of death by centrifugation at 600 g for 10 min at 4 °C. Concentrations of glucose, free fatty acids and triglycerides were determined using spectrophotometric assay kits from Sigma and Boehringer Mannheim.

2.3.2. Enzyme assays

Different extraction buffers were used to determine the fraction of PDH in the active form (PDHa), and the total activity (PDHt), as previously described [27].

Pyruvate dehydrogenase activity was assayed by adding 25–50 μl of PDHt extracts or 50–200 μl of PDHa extract to 0.95 ml containing 50 mM HEPES (pH 7.2), 1 mM MgCl2, 0.08 mM EGTA, 1 mM DTT, 4 mM rotenone, 1.67 mM NAD, 0.1 mM co-enzyme A, 0.2 mM thiamine pyrophosphate and 16.7 mM lactate and 2 U lactate dehydrogenase (LDH) as previously described [27].

Oxoglutarate dehydrogenase (OGDH) activity was assayed by adding 20–50 μl of tissue extract to 1.0 ml of medium containing 50 mM KH2PO4, 10 mM MgSO4, 5 mM MgCl2, 1 mM EDTA, 1 mM DTT, 2 mM rotenone, 2 mM NAD+, 150 mM co-enzyme A, 20 mM thiamine pyrophosphate, 10 mM 2-oxoglutarate and 2 mM ADP [27]. Citrate synthase activity was assayed in Tris–HCl buffer (50 mM, pH 8.1) containing 0.1 mM acetyl-CoA, 0.5 mM oxaloacetate and 0.2 mM dithiobisnitrobenzoic acid (DTNB; Ellman’s reagent) as previously described [28]. The reaction was initiated by the addition of 10 μl of tissue extract to 1 ml of the assay mixture and the release of CoA was followed by its reaction with DTNB at 412 nm.

2.4. Statistical analysis

All data are presented as means±S.E.M. An unpaired t-test was used for two-way comparisons. Analyses were performed with Statview statistical software (Abacus Concepts, Berkeley, CA, USA). A p value of less than 0.05 was considered significant.

3. Results

As expected, the ZDF animals were significantly heavier than the age matched lean control group (Table 1). However, heart weight and heart-to-body weight ratio were not different between the two groups. Serum glucose concentrations were ~threefold higher in the ZDF rats and triglycerides were increased more than eightfold (Table 1). These results are consistent with previously reported data for 11-week-old ZDF rats [21–23]. Although free fatty acid levels were ~40% higher in the ZDF group this difference did not reach statistical significance. No differences in coronary flow, developed pressure or rate pressure product were observed between the ZDF and lean groups (Table 2).

The fluxes through PDH relative to total TCA cycle flux (Fpdh) for the lean and ZDF groups are shown in Fig. 2A. In the lean control group Fpdh is ~10% of total TCA cycle flux and this is decreased by 30–40% in the ZDF group (p=0.066). The relative flux through β-oxidation, Fβ, was increased from 90.6±1.3 in controls to 93.9±1.2 in the

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Body weight</th>
<th>Heart weight</th>
<th>Heart/body weight</th>
<th>Glucose (mM)</th>
<th>Fatty acids (mM)</th>
<th>TG (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean (n=5)</td>
<td>310±6</td>
<td>1.26±0.21</td>
<td>4.0±0.6</td>
<td>8.9±0.3</td>
<td>0.26±0.04</td>
<td>0.68±0.24</td>
</tr>
<tr>
<td>ZDF (n=5)</td>
<td>366±7*</td>
<td>1.24±0.06</td>
<td>3.4±0.2</td>
<td>26.9±1.9*</td>
<td>0.36±0.03</td>
<td>5.67±0.55*</td>
</tr>
</tbody>
</table>

Data presented as means±S.E.M.

* p<0.0001.
Table 2  
Cardiac function of hearts from 11-week-old ZDF rats and lean controls averaged over the 45 min perfusion with $^{13}$C-labeled substrates

<table>
<thead>
<tr>
<th></th>
<th>Coronary flow (ml/min/g)</th>
<th>Developed pressure (mmHg)</th>
<th>Rate pressure product* (mmHg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean ($n=3$)</td>
<td>7.3±0.4</td>
<td>115±3</td>
<td>22,300±3,800</td>
</tr>
<tr>
<td>ZDF ($n=5$)</td>
<td>8.9±0.8</td>
<td>115±1</td>
<td>26,600±2,000</td>
</tr>
</tbody>
</table>

Data presented as means±S.E.M.

*Rate pressure product=heart rate×developed pressure.

ZDF group ($p=0.066$). There was no difference in the flux through pyruvate carboxylase ($F_{pcx}$) between the groups. In control hearts, the ratio of $F_{pdh}/F_{pcx}$ was 0.93±0.01 (Fig. 2B), (similar to a previous report by Comte et al. [29]), whereas in the ZDF group, the ratio was significantly decreased (Fig. 2B). This reduction highlights a decrease in the flux through PDH relative to pyruvate carboxylase.

As noted earlier $F_{pdh}$ and $F_{pcx}$ represent the flux of all $^{13}$C-labeled pyruvate through those pathways. Three different $^{13}$C-labeled pyruvate species contributed to the tissue pyruvate pool, resulting from the metabolism of exogenous [U-$^{13}$C]glucose, [3-$^{13}$C]lactate and [2-$^{13}$C]pyruvate. The relative contributions of these substrates to the pyruvate subsequently metabolized via PDH and pyruvate carboxylase are shown in Fig. 3. In the lean group, glucose ($F_{gl}$) contributed 11±1% to total pyruvate oxidation, exogenous pyruvate ($F_{py}$) and lactate ($F_{la}$) contributed 24±3 and 64±3%, respectively (Fig. 3). In the ZDF group $F_{gl}$ was reduced by more than 35% ($p<0.03$); $F_{py}$ and $F_{la}$ were not significantly different from the lean group.

The relative contributions of glucose to both tissue lactate and alanine formation are depressed in the ZDF group (Fig. 4). This is consistent with the decrease in contribution of glucose to total pyruvate oxidation in the ZDF group.

![Fig. 2. (A) Fluxes through PDH ($F_{pdh}$) and pyruvate carboxylase ($F_{pcx}$) relative to total TCA cycle flux; (B) the ratio of $F_{pdh}/F_{pcx}$ in 11-week-old lean ($n=3$) and ZDF ($n=5$) rats. *$=p<0.005$ vs. lean group.](image)

![Fig. 4. Percentage of C$_3$-lactate and C$_3$-alanine originating from [U-$^{13}$C]glucose in 11-week-old lean ($n=3$) and ZDF ($n=5$) rats. †$=p<0.004$; *$=p<0.001$ vs. lean group.](image)

4. Discussion

Studies into the effects of diabetes on cardiac metabolism have, almost exclusively, used models of acute, uncontrolled, insulin-deficient diabetes [30]. Since ~90% of diabetic patients have Type-2 diabetes, the significance of such studies in understanding the pathogenesis of cardiac dysfunction in diabetes is unclear. In this study, we have shown for the first time that, in a model of Type-2
diabetes, significant alterations in oxidative and non-oxidative cardiac carbohydrate metabolism are present in the absence of overt changes in systolic function.

There have been very few reports on the impact of the more clinically relevant Type-2 diabetes. Kuo et al. [30] reported a decrease in cardiac PDH activity in genetically diabetic db/db mice. Unfortunately, the severity of the diabetic state was not reported and it was unclear whether total or active PDH was being determined. More recently Belke et al. [20] have reported marked changes in cardiac glucose and palmitate metabolism in ~3-month-old db/db mice as well as a significant reduction in cardiac work. These alterations were very similar to those previously reported in models of Type-1 diabetes. The db/db mice used in Belke’s study exhibited a very severe diabetic state with serum glucose levels almost fivefold higher than control mice and free fatty acid concentrations more than double; the body weight of the db/db mice were over 50% higher than controls. In contrast, in the ZDF rats used in this study, glucose was only threefold higher and fatty acids less that 40% higher than the lean group and there was only ~20% increase in body weight. It should also be noted that db/db mice are hyperglycemic at a very young age, whereas the ZDF rats only develop hyperglycemia after ~6 weeks of age [21,22]. Thus, the difference between the results reported here and those on the db/db mice [20] may be accounted for by differences in both the severity and duration of the diabetes.

To our knowledge there has been only one study of cardiac function in ZDF animals [23]. Using echocardiography, Zhou et al. [23] demonstrated a significant decrease in contractility in 20-week-old ZDF rats but normal function in 7-week-old animals. Therefore, in combination with our data it would appear that overt contractile dysfunction develops between 12 and 20 weeks of age in ZDF rats consistent with the development of diabetic cardiomyopathy. In isolated myocytes from insulin resistant, but non-diabetic Zucker fatty rats Ren et al. demonstrated significant alterations in excitation contraction coupling [31]. The indices of contractile function used in our study would not be sensitive enough to detect such subtle alterations in contractility. Thus, while we were unable to detect an overt decrease in cardiac function in 11-week-old ZDF rats, it is possible that more subtle contractile abnormalities may exist. It is possible that alterations in function might become apparent at higher workloads and may require the use of more sensitive indices of contractility such ±dP/dt. Another potential explanation for the difference between the isolated cell studies and the data from the intact heart is that in the latter alterations in the extracellular matrix may mask or compensate for myocyte dysfunction.

We have shown that, in hearts from hyperglycemic ZDF rats, there is a significant decrease in flux through PDH relative to that through pyruvate carboxylase (Fig. 2B) and this is associated with a ~40% decrease in the fraction of PDH in the active form (Fig. 5C). These decreases in carbohydrate oxidation and PDH activity in the heart are much less marked than those typically seen in uncontrolled Type-1 diabetes [14]. After 6 weeks after induction of Type-1 diabetes, cardiac glucose oxidation is typically reported to be only 10–30% of that in control [32–34]. Even after only 1 week of insulin-deficient diabetes, we found that the contribution of carbohydrates to energy production was reduced to ~50% of the non-diabetic group [24,35]. Furthermore, in 6 weeks diabetic animals, not only was PDHa/PDHT ~six times lower than controls but there was also a decrease in total PDH activity [27], with a concomitant reduction of 40–50% in OGDH activity, potentially a reflection of decreased mitochondrial density. In contrast, in this study with ZDF rats, no differences in PDHt, OGDH or citrate synthase activity were observed compared to the lean group. This difference between Type-1 diabetic animals and the ZDF rats most likely reflects the fact that uncontrolled insulin-deficient diabetes is a much more severe metabolic stress than that in 11-week-old Type-2 diabetic ZDF rats. This further highlights the need to investigate cardiac metabolism and physiology in more clinically relevant models of diabetes.

13C-NMR analysis has demonstrated a decrease in total pyruvate flux through PDH (F_{PDH}) relative to pyruvate carboxylase and a corresponding increase in flux through β-oxidation (F_{β}) and a reduction in the contribution of glucose to total pyruvate oxidation (F_{py}) (see Fig. 6). The contributions of pyruvate and lactate (F_{py} and F_{la}) to total pyruvate oxidation were unchanged. This is in contrast to our recent studies of myocardial lactate metabolism, following one-week of Type-1 diabetes, where lactate
oxidation was inhibited to a greater extent than glucose oxidation [24,25]. The decrease in the contribution of glucose to tissue alanine and lactate formation (Fig. 4) in the ZDF group is consistent with the reduction in $F_{\text{gl}}$ determined by glutamate isotopomer analysis. Overall, these data suggest that in the ZDF group the contribution of glucose to tissue pyruvate was decreased relative to the contributions of exogenous lactate and pyruvate, consistent with a reduced flux through glycolysis. Thus, the development of hyperglycemia in this model of Type-2 diabetes results in significant changes in both the flux from glucose to pyruvate as well as in total pyruvate oxidation.

It should be noted that while the alterations in glucose metabolism are significant, fatty acid oxidation still provides ~90% of the acetyl-CoA entering the TCA cycle in both control and ZDF groups. Thus, the decrease in glucose metabolism represents a relatively small alteration in energy production. The mechanisms by which such changes in substrate utilization may contribute to the development of contractile dysfunction are as yet unclear. However, in the uncontrolled Type-1 model of diabetes, we have demonstrated multiple defects in both carbohydrate and long chain fatty acid metabolism that appear to limit energy production [24,25]. We have also shown that the contractile dysfunction can be reversed by the addition of hexanoate, a medium-chain fatty acid [35]. Thus, based on these studies one might expect that as the duration of diabetes in the ZDF rats increases the derangement in metabolism become progressively worse and may ultimately affect contractile function. Furthermore, there is increasing evidence that stimulating carbohydrate oxidation improves functional recovery following ischemia and reperfusion [36]. Therefore, the impairment in glucose metabolism observed here, may play a major role in the enhanced sensitivity to ischemic injury and the increased mortality in diabetic patients following myocardial infarction [37,38].

An important consideration in the interpretation of the results from this study is whether obesity and insulin resistance in the absence of diabetes may contribute to the development of metabolic and contractile abnormalities. In non-diabetic, obese Zucker rats contractile function does not appear to be altered at 9 weeks of age but may be impaired in older animals (i.e., ≥5 months) [39,40]. In isolated perfused hearts from 12 to 14-week-old non-diabetic, insulin resistant JCR:LA (cp/cp) corpulent rats, Lopaschuk and Russell reported altered contractile response to calcium and insulin but found a significant increase in the rate of glucose oxidation compared to lean controls [41]. These studies would suggest that the impairment in carbohydrate metabolism, seen here, was not a result of insulin resistance and obesity alone. However, it has been proposed that insulin resistance may be a consequence rather than a cause of hyperlipidemia [42]. Given that increased circulating lipids will decrease cardiac carbohydrate oxidation we cannot rule out the possibility that the altered regulation of cardiac carbohydrate metabolism reported here could also be an adaptive response to chronic dyslipidemia and insulin resistance. Clearly additional studies on the consequences of insulin resistance and obesity on cardiac function and metabolism are required.

In studies of cardiac metabolic regulation glucose is frequently the only carbohydrate source considered. However, in vivo both exogenous lactate and pyruvate are available as potential sources of energy [43–45]. To our knowledge there have been no studies that have investigated the relative contributions of glucose, lactate and pyruvate to myocardial energy production. We found that exogenous lactate represented ~60% of the pyruvate entering the TCA cycle ($F_{\text{p}}$) in both lean and diabetic groups (Fig. 4). This is consistent with reports identifying lactate as a significant source of energy for the heart in vivo [46–49]. Our results provide further evidence that lactate rather than glucose is the predominant carbohydrate oxidized by the myocardium. Furthermore, even though pyruvate was present at a concentration 50 times lower than glucose it contributed 2–3 times more to total pyruvate oxidation than glucose. These results are in good agreement with the work of Jeffrey et al. [50] and highlight the fact that glucose appears to contribute relatively little to oxidative energy production in myocardium when present in a substrate mixture designed to mimic the in vivo milieu.

These results demonstrate, for the first time, that, in a model of Type-2 diabetes, similar to that seen in the majority of patients with diabetes, changes in cardiac metabolism occur in the absence of overt changes in systolic function. This supports the hypothesis that alterations in the regulation of energy metabolism contribute to the development of diabetic cardiomyopathy. The effects of Type-2 diabetes on the myocardium are clearly much less severe than those previously reported in models of uncontrolled Type-1 diabetes, which have to date provided...
the basis for our investigations of diabetes-induced cardiac dysfunction. Further studies are clearly necessary in older diabetic animals to determine whether the progression of the metabolic abnormalities is associated with the onset of contractile dysfunction. Studies in younger pre-diabetic ZDF rats will also be necessary to assess whether insulin resistance in the absence of hyperglycemia is associated with alterations in metabolic regulation. Therefore, we believe that the data presented here demonstrate that the ZDF rat represents an excellent model for the investigation of the pathogenesis of diabetic cardiomyopathy in Type-2 diabetes.

Acknowledgements

This work was supported in part by grants from National Heart Lung and Blood Institute, NIH (HL48789, HL67464) and the American Heart Association (Grant in Aid 0050545N) to J.C.C. and by a North Atlantic Treaty Organization Collaborative Research Grant (CRG 940624) and a British Heart Foundation Project Grant (PG/96097) to A.-M.L.S.

References


