Preferential inhibition of lactate oxidation relative to glucose oxidation in the rat heart following diabetes

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Abstract

Objective: Alterations in myocardial metabolism occur early after the onset of diabetes suggesting that they may play a role in the development of cardiac dysfunction. Inhibition of myocardial pyruvate dehydrogenase (PDH), glucose transport and glycolysis have all been reported following diabetes. In vivo lactate is also a potential source of energy for the heart and its oxidation should not be affected by changes in glucose transport and glycolysis. Therefore, the objective of this study, was to test the hypothesis that following diabetes the inhibition of glucose oxidation would be greater than the inhibition of lactate oxidation.

Methods: Hearts from control and one-week-old diabetic rats were perfused with [1-13C]glucose (11 mmol/l) alone, [1-13C]glucose plus lactate (0.5 mmol/l) or glucose plus [3-13C]lactate (0.5 or 1.0 mmol/l) as substrates. Glucose and lactate oxidation rates were determined by combining 13C-NMR glutamate isotopomer analysis of tissue extracts with measurements of oxygen consumption.

Results: In diabetic hearts perfused with glucose alone, glucose oxidation was decreased compared to controls (0.31 ± 0.08 vs. 0.71 ± 0.11 μmoles/min/g wet weight; p < 0.05). Surprisingly, in hearts perfused with glucose plus 0.5 mmol/l lactate, there was no difference in glucose oxidation between control and diabetic groups (0.20 ± 0.05 vs. 0.16 ± 0.04 μmoles/min/g wet weight respectively). However, under these conditions lactate oxidation was markedly reduced in the diabetic group (0.89 ± 0.18 vs. 0.24 ± 0.05 μmoles/min/g wet weight; p < 0.05). At 1.0 mmol/l lactate oxidation was still significantly depressed in the diabetic group. Conclusion: There was a greater decrease in lactate oxidation relative to glucose oxidation in hearts from diabetic animals. These results demonstrate that diabetes leads to a specific inhibition of lactate oxidation independent of its effects on pyruvate dehydrogenase. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Cardiovascular disease is a major factor contributing to the marked increased rate of morbidity and mortality seen in patients with diabetes [1]. The incidence of heart failure is up to eight-fold higher in the diabetic population compared to non-diabetics [2] and there is a three to six-fold increase in mortality associated with coronary heart disease [3]. Although diabetes is associated with hypertension, obesity and hyperlipidemia which are independent risk factors for heart disease, both clinical and experimental evidence is accumulating to support the concept of a specific diabetic cardiomyopathy. Mustonen et al. reported abnormal cardiac function in diabetic patients, otherwise free of clinical signs of cardiovascular disease [4]. They concluded that the most likely explanation for their findings was an effect of diabetes on the myocardium itself. Lehto et al., reached similar conclusions in their investigation of increased mortality rates in diabetic patients following myocardial infarction [5]. Kannel and colleagues have suggested that diabetes is a specific cause of congestive heart failure independent of other risk factors for heart disease [6–8].

In animal models, diabetes results in numerous alterations in the myocardium, including changes in the contractile proteins, in Ca2+-ATPases and intermediary metabo-
lism, all of which could contribute to the development of cardiac dysfunction. Interestingly, alterations in metabolism appear to precede changes in protein synthesis and expression as well as the development of functional and structural changes of the myocardium. This suggests that changes in energy metabolism may play an important role in the development of diabetes-induced cardiac dysfunction [9–11]. Investigations into the effects of diabetes on myocardial substrate selection and utilization, have typically focused on glucose and fatty acids as the substrates of interest (see Refs. [12,13] for reviews). This is despite the fact that in the intact animal the heart will use a variety of substrates for energy production, including lactate and ketone bodies. Under in vivo conditions lactate has been shown to be a significant source of energy for the heart [14,15]. For example, Drake and colleagues have shown that the extraction rate of lactate by the heart is proportional to its circulating concentration and can provide up to 87% of substrate oxidation [15]. Despite its potential importance as an energy source for the heart very few investigations have examined the effect of diabetes on myocardial lactate oxidation.

Glucose and lactate have a common primary entry point into the TCA cycle via the conversion of pyruvate to acetyl-CoA by pyruvate dehydrogenase (PDH). Thus, it might be anticipated that glucose and lactate oxidation would be regulated by similar mechanisms. Furthermore, decreased PDH activity is one of the major consequences of diabetes on the heart [12] and is presumed to be the main rate limiting step in glucose oxidation. Consequently, one might expect that diabetes would have a similar effect of decreasing both glucose and lactate oxidation rates. On the other hand diabetes has been reported to decrease the rates of glucose transport, glucose phosphorylation and glycolysis [12]. Therefore, it is possible that following diabetes, lactate oxidation could be affected to a lesser extent than glucose. If this were the case, then given the availability of lactate as a substrate, this might limit the metabolic and physiological consequences of decreased glucose oxidation on the heart in vivo.

The purpose of this study, therefore, was to determine the relative contributions of glucose and lactate to myocardial energy production under normal conditions and following diabetes. We tested the hypothesis that following diabetes the inhibition of glucose oxidation would be greater than the inhibition of lactate oxidation. The hypothesis was tested using isolated hearts from normal and diabetic rats perfused with a mixture of $^{13}$C-labeled glucose and lactate. $^{13}$C-NMR spectroscopy was used to determine the relative contribution of both lactate and glucose to the total acetyl-CoA pool entering the TCA cycle [16]. Hearts were isolated from the animals in order to minimize effects of systemic differences between control and diabetic animals on substrate utilization. This enabled us to control the exogenous substrates available for energy production. Fatty acids are an important source of energy for the heart; however, they also inhibit carbohydrate oxidation by decreasing flux through PDH. In addition, the relative impact of physiological concentrations of fatty acids on the metabolism of pyruvate by PDH in hearts from control and diabetic animals is poorly understood. Therefore, in order to eliminate extraneous factors that can modulate PDH activity, exogenous fatty acids were not included as substrates in these experiments.

2. Methods

The investigation conforms with 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). Diabetes was induced in male, Sprague–Dawley rats (270–320 g) with streptozocin (STZ, 60 mg/kg, i.v.; Upjohn Laboratories, Kalamazoo, Michigan) as previously described [17]. One week after treatment with STZ, the rats were sacrificed and hearts were perfused as described in Section 2.1. The dosage of STZ was chosen to produce a moderate hyperglycemia and mild ketosis.

2.1. Isolated rat heart preparation

Rats were anesthetized with ketamine (100 mg/kg i.p.), heparinized (500 U/100 g i.p.), and decapitated. Blood was collected for serum glucose, triglyceride, β-hydroxybutyrate and lactate measurements. Hearts were quickly excised and perfused on modified Langendorff preparation with Krebs–Henseleit bicarbonate buffer equilibrated with 95% O$_2$, 5% CO$_2$ (38°C, pH 7.4). The buffer contained the following components (mmol/l): NaCl 118, KCl 4.8, Mg$_2$SO$_4$ 1.2, CaCl$_2$ 1.25, KH$_2$PO$_4$ 1.2, Na$_2$HPO$_4$ 25, glucose 11±lactate/pyruvate 0.5/0.05. Pyruvate was added to the perfusate to produce a physiologic ratio of 10:1 for lactate/pyruvate, in order to minimize perturbations of the cytosolic redox state that would occur with lactate alone [18]. (Glucose plus lactate/pyruvate is abbreviated to just glucose plus lactate in the rest of the text.) A balloon was inserted into the left ventricle via the mitral valve and connected to a Gould P23Db pressure transducer for continuous measurement of left ventricular contractile function. The balloon volume was adjusted to obtain a left ventricular end diastolic pressure of 5 mm/Hg. A second transducer was connected to the arterial perfusion line for the continuous monitoring of perfusion pressure and flow was adjusted to maintain perfusion pressure at 75 mm/Hg.

2.2. Physiological measurements

Left ventricular pressure (LVDP) and heart rate (HR) were measured continuously throughout the experiment; cardiac performance was assessed using the rate pressure product (RPP=LVDP×HR). Samples of the perfusate and coronary effluent were collected at 20-min intervals for
determination of coronary flow and myocardial oxygen consumption (MVO$_2$; Radiometer Copenhagen ABL3 pH/blood gas analyzer, Copenhagen, Denmark). MVO$_2$ was calculated as the product of arterio–venous oxygen content difference and coronary flow.

2.3. Preparation of tissue extracts

The frozen hearts were pulverized into a fine powder with a mortar and pestle under liquid N$_2$ and then homogenized in 6% perchloric acid using a Tekmar Tissuemizer (Tekmar Company, Cincinnati, OH) for 1 min. The homogenate was centrifuged at 17 500 $g$ for 8 min at 4°C. The supernatant was neutralized with KOH. One portion of supernatant was put aside for measurements of tissue glutamate and lactate content, while the other portion was freeze-dried. The lyophilized heart extract was dissolved in 2.5 ml D$_2$O, buffered with 50 mmol/l potassium phosphate (pH 7.2), and filtered through 0.2-$\mu$m syringe filter (Corning Glass Works, Corning NY). prior to $^{13}$C-NMR measurements (see Section 2.7 for more details). One portion of the frozen powder was left for measurement of tissue glucose and glycogen contents.

2.4. Measurement of serum substrate concentrations

The serum triglyceride and β-hydroxybutyrate levels were measured by kits purchased from Sigma Chemical (St. Louis, MO). Serum glucose and lactate concentration were measured by Glucose/lactate Analyzer (YSI Glucose & Lactate Analyzer 2400, Ohio).

2.5. Measurements of lactate dehydrogenase activity

Preliminary studies showed that hearts taken directly from the animal had consistently higher lactate dehydrogenase (LDH) activity compared with Langendorff-perfused hearts, presumably due to the presence of red blood cells in the vascular space. As a result, the $K_m$ and $V_{max}$ for LDH were measured in control ($n=5$) and diabetic hearts ($n=6$) following 30 min of perfusion with glucose as sole substrate. Tissue was homogenized with phosphate buffer [19] containing (mmol/l): KH$_2$PO$_4$ 100; ADP 1; Glutathione, 10; EDTA 10; at pH 7.2, followed by 10-min centrifugation (9150 $g$ 4°C). The LDH activity was measured in the supernatant at room temperature and all assays were carried out within 4 h after homogenization and centrifugation. The reaction buffer contained (mmol/l): Tris base 112, KCl 100, β-NAD 6.5, at pH 9.3 [20]. The final lactate concentration used to start the reaction ranged from 0.32 to 100 mmol/l and the $K_m$ and $V_{max}$ of LDH for lactate determined.

2.6. Determination of monocarboxylic acid transporter (MCT1) and LDH expression

MCT1 and LDH were measured as previously described [21,22] using proteins isolated from the same hearts used for determination of LDH activity. Western blotting of MCT1 was carried out using a polyclonal anti-peptide antibody similar to that used by Garcia et al. [23]. MCT1 was detected using an enhanced chemiluminescence detection method by exposing the membranes to film (Hyperfilm-ECL; Amersham) at room temperature. MCT-1 protein band densities were obtained by scanning the blots on a densitometer connected to a Macintosh LC computer with appropriate software.

LDH isozymes were separated and detected as described previously [21,24]. The isozymes were separated by agarose gel electrophoresis at 100 V for 30 min and stained with a solution containing 208 mmol/l Li-t-lactate, 5.6 mmol/l NAD+, 2.4 mmol/l p-nitroblue tetrazolium chloride and 0.33 mmol/l phenazine methosulphate (Paragon, Lactate Dehydrogenase Isoenzyme Electrophoresis Kit, Beckman). LDH isozyme bands were scanned for densitometric readings as described above. H-LDH and M-LDH subunit concentrations were calculated from the absorbance for the LDH isoforms according to the formula provided by Thorling and Jensen [25].

2.7. NMR spectroscopy

High resolution $^{13}$C-NMR spectra were collected using a Bruker 500-MSL NMR spectrometer equipped with 11.75 T magnet using a commercial 10-mm probe as described previously [26]. Magnetic field homogeneity was optimized by observing the water signal using the $^1$H decoupling coil. Spectra were collected with a sweep width of 25 KHz, 60° pulse width and a 3-s relaxation delay. $^1$H-decoupling was carried out during acquisition only. Under these conditions, the relative intensities of $C_2$, $C_3$, and $C_4$ glutamate, and the isotopomer distribution were indistinguishable from those collected under fully relaxed conditions; however, data acquisition was significantly shortened. The relative contributions of lactate and glucose to the total acetyl-CoA pool entering the TCA cycle were determined from glutamate isotopomer analysis [16] using software developed and kindly provided by Dr. Mark Jeffrey (TCAcalc™; University of Texas Southwestern Medical Center).

2.8. Experimental protocol

The experimental protocol is summarized in Fig. 1. After approximately 30 min of equilibration, the perfusion medium was switched to buffer containing $^{13}$C-labeled substrates and perfused for an additional 60 min. With [1-$^{13}$C]glucose as the sole substrate (Series 1), the relative contribution of glucose to total substrate oxidation in the absence of any other exogenous substrates was determined. Hearts perfused with [1-$^{13}$C]glucose and unlabeled lactate (Series 2) were used to determine the contribution of glucose to acetyl-CoA entry into the TCA cycle, in the presence of lactate. Perfusion of hearts with [3-$^{13}$C]lactate
was not significantly different between the untreated controls and diabetic rats. In Table 1(b) it can be seen that in the diabetic group, serum glucose concentration was three times higher than that of untreated controls, ranging from 26 to 40 mmol/l compared with 5 to 11 mmol/l in controls. Serum β-hydroxybutyrate and triglycerides increased 18- and 11-fold respectively in diabetic rats compared with control. There was no significant difference in serum lactate concentrations between the two groups. Lactate levels were higher than those typically found in the resting state presumably as a result of treatment with heparin and ketamine prior to sacrifice and/or a consequence of decapitation. Hall et al. [27] showed that in diabetic pigs arterial lactate concentrations were not significantly different from non-diabetic animals despite a three to four-fold increase in glucose concentrations. These data are consistent with STZ-treated rats being diabetic, and are similar to our previous work [17,26] as well as others [28].

### 3.2. Cardiac function

Fig. 2 shows HR, LVDP, RPP, and MVO₂ after 20-min perfusion with ¹³C-labeled substrate for all groups. In all groups there was a small but significant decrease in RPP over the duration of perfusion with ¹³C-labeled substrates (12–20% decline over 60 min; *p*<0.05, ANOVA with repeated measures). ANOVA with repeated measures also demonstrated significant differences between the control and diabetic groups perfused under the same conditions. Since all groups exhibited the same behavior, functional data from only a single time point are presented for clarity. LVDP and RPP were both significantly lower in the diabetic groups compared to the appropriate controls. Interestingly in control hearts perfused with glucose and lactate as substrates, LVDP and RPP were significantly lower compared to perfusion with glucose alone. There were no between- or within-group differences in MVO₂.

The fact that function was depressed in the diabetic group and yet oxygen consumption was not significantly different suggests that there might be a decrease in energy efficiency in the diabetic group. To determine whether this might be the case, energy efficiency was estimated by dividing the

### Table 1

(a) Body weight, heart weight and heart weight to body weight ratio and (b) serum concentrations of glucose, lactate, β-hydroxybutyrate, and triglyceride. Data expressed as Mean±SEM.

<table>
<thead>
<tr>
<th></th>
<th>Body weight (g)</th>
<th>Heart weight (g)</th>
<th>Heart/body ×10³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=27)</td>
<td>316±4</td>
<td>1.82±0.03</td>
<td>5.2±0.1</td>
</tr>
<tr>
<td>Diabetes (n=18)</td>
<td>249±7*</td>
<td>1.3±0.03</td>
<td>5.3±0.2</td>
</tr>
</tbody>
</table>

(b) Glucose (mmol/l) | Lactate (mmol/l) | β-Hydroxybutyrate (mmol/l) | Triglyceride (mmol/l) |
<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=15)</td>
<td>10.3±0.4</td>
<td>2.8±0.3</td>
<td>0.20±0.03</td>
</tr>
<tr>
<td>Diabetes (n=21)</td>
<td>33.4±1.0*</td>
<td>3.7±0.5</td>
<td>3.56±0.61*</td>
</tr>
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* = p<0.05 vs. control.
incorporation into the glutamate and aspartate pools as approximately 29% of acetyl-CoA was derived from diabetic hearts except for a marked reduction of C-label details). In control hearts perfused with glucose and lactate, a transamination of the TCA cycle intermediates from [1-C]glucose into glutamate and aspartate, from TCA cycle was derived from the exogenous C-labeled alanine are all clearly evident. Incorporation of the label substrate, approximately 85% of acetyl-CoA entering the natural abundance taurine, dioxane (standard), lactate and In control hearts perfused with glucose as the sole substrates to acetyl-CoA entering the TCA cycle. Similar spectra were obtained in extracts from either glycogen or triglycerides (see below for more detail on the different possible glutamate isotopomers). In Fig. 4, only the glutamate-C4 resonances are shown for control and diabetic hearts under the different perfusion conditions. In all these spectra the glutamate resonance consists of a singlet and a doublet, and the differences between the experiments is apparent by differences in the relative intensities of these peaks. The singlet results from the C4-glutamate labeled with carbon-13 at C4 only (i.e., no label at the C3 or C5 position); whereas the doublet arises when the C3- and C5-carbons of glutamate are both labeled with carbon-13 but the C4-carbon is unlabeled (i.e., the C4D4 doublet [16]). When using substrates such as [1-13C]glucose, which leads to the production of [2-13C]acetyl-CoA and unlabeled acetyl-CoA, or [3-13C]lactate which produces only [2-13C]acetyl-CoA there is no enrichment of the C3-glutamate. Consequently, under the conditions in these experiments the C4-glutamate resonance can only consist of a singlet and/or a doublet. As the enrichment of substrate entering the TCA cycle increases, the probability that both the C1- and C2-carbons of glutamate will be enriched increases and thus the proportion of the C4D4 increases relative to the singlet. Additional detail on the different possible glutamate isotopomers can be found in the work of Malloy and co-workers (for example see Ref. [16]).

When glucose is present as the sole substrate, the C4D4 doublet in the diabetic heart is lower than in the control heart (Fig. 4, upper panel), which is consistent with a decrease in glucose oxidation following diabetes as previously reported [17,26]. Entry of 13C-labeled glucose into the TCA cycle in both control and diabetic groups was significantly decreased when unlabeled lactate was present (middle panel). When the label was on lactate rather than glucose (lower panel), there was significant incorporation of the label into glutamate in control hearts consistent with the relatively large amount of lactate oxidation relative to glucose. However, the satellites of glutamate C4 were much lower in the diabetic hearts than in the controls, indicating decreased lactate oxidation. These spectra were analyzed along with the C2- and C3-glutamate isotopomers as described by Malloy et al. [16] and the contribution of 13C-labeled substrates to acetyl-CoA entering the TCA cycle were calculated and the results presented in Fig. 5.

In control hearts perfused with glucose as the sole substrate, approximately 85% of acetyl-CoA entering the TCA cycle was derived from the exogenous 13C-labeled glucose (see Fig. 5). In the diabetic group this decreased significantly to about 50%, consistent with our previous studies [26]. The remaining unlabeled acetyl-CoA (14 and 50% respectively for control and diabetics) originates from either glycogen or triglycerides (see below for more details). In control hearts perfused with glucose and lactate, approximately 29% of acetyl-CoA was derived from...
Fig. 3. High resolution $^{13}$C-NMR spectra of the extract from a control heart perfused with 11 mmol/l [1-13C] glucose for 60 min. Spectra were zero filled to 64 K and processed with a 2-Hz exponential filter. Dioxane (Diox) is standard located at 67.4 ppm. The glucose and dioxane resonances have been truncated for clarity. $\beta$-Glu, $\alpha$-Glu=$\beta$-anomer and $\alpha$-anomer of [1-13C]glucose; Diox=dioxane standard; C2-Glu=[2-13C]glutamate; C2-Asp=[2-13C]aspartate; Tau=natural abundance taurine; C3-Asp=[3-13C]aspartate; C4-Glut=[4-13C]glutamate; C3-Glut=[3-13C]glutamate; Lac=[3-13C]lactate; Ala=[3-13C]alanine.

glucose (from [1-13C]glucose plus unlabeled lactate experiments—Fig. 1, Series 2) and 60% from lactate ([3-13C]lactate plus unlabeled glucose experiments—Fig. 1, Series 3). Interestingly in diabetic hearts perfused with the same combination of substrates, the contribution of glucose to acetyl-CoA was not decreased relative to controls. However there was an almost three-fold decrease in the contribution from lactate. It is noteworthy that the addition of lactate did not affect the contribution of endogenous substrates to energy production in either group.

Although the analysis of glutamate-labeling patterns provides a measurement of the relative contribution of lactate and glucose to acetyl-CoA entry into the TCA cycle, it does not provide actual rates of substrate oxidation which are dependent on cardiac work and basal energy demands. Oxygen consumption (MVO$_2$) directly reflects the rate of total substrate oxidation, and mitochondrial electron transport. It is possible therefore to combine the relative contributions of lactate and glucose with the measurement of MVO$_2$ to calculate the actual rates of glucose and lactate oxidation. In hearts perfused with glucose alone, glucose oxidation rates were $0.71 \pm 0.11$ and $0.31 \pm 0.08$ mmol/min/g ($p<0.05$) in control and diabetic groups respectively; indicating a marked inhibition of glucose oxidation. The rates of glucose and lactate oxidation in hearts perfused with both substrates are shown in Fig. 6a and these data clearly demonstrate a profound inhibition of lactate but not glucose oxidation in the diabetic group. In other words with both glucose and lactate as substrates the decrease in carbohydrate oxidation following diabetes is due to a decrease in lactate but not glucose oxidation.

In order to determine the effects of a higher concentration of exogenous lactate on the contributions of lactate to energy production, lactate and pyruvate concentrations were increased to 1 and 0.1 mmol/l respectively. A comparison of lactate oxidation rates at 0.5 and 1.0 mmol/l lactate are presented in Fig. 6b. It can be seen that in the control group lactate oxidation rates increased by ~50%, whereas in the diabetic group oxidation rates increased almost three-fold but were still significantly decreased compared to the control. As a result of the greater increase in lactate oxidation in the diabetic group at 1.0 mmol/l, the rate of lactate oxidation was a little less than 50% of the control rate compared to ~25% of the control level at 0.5 mmol/l. It is interesting to note that although the relative increase in lactate oxidation was clearly different between the two groups the absolute oxidation rates increased by about the same amount, i.e., ~0.4 mmol/min/g in both groups. The increase in lactate concentration had no effect on contractile function in either control or diabetic groups. For example the RPP in the control group was 24 700±1300 and 26 400±1200 mmHg/min with 0.5 mmol/l and 1.0 mmol/l lactate.
Fig. 6. (a) Rates of glucose and lactate oxidation in control and diabetic hearts perfused with both glucose (11 mmol/l) and lactate (0.5 mmol/l) as substrates. Oxidation rates calculated from the data in Fig. 5 and rates of oxygen consumption. (b) Rates of lactate oxidation in control and diabetic hearts perfused with glucose (11 mmol/l) plus 0.5 or 1.0 mmol/l lactate; note that the lactate oxidation data from Fig 6a is repeated here in order to provide a direct comparison between the oxidation rates at the two lactate concentrations. Means±SEM from three to six experiments. *p<0.05 vs. control. ‡p<0.05 vs. 0.5 mmol/l lactate. respectively; in the diabetic group the RPP was 14 100±1900 and 16 800±3700 with 0.5 and 1.0 mmol/l lactate respectively.

In the control group, the addition of 1.0 mmol/l exogenous lactate, decreased the relative contribution of glucose to energy production to 20.3±1.0% (p<0.05 compared to 0.5 mmol/l lactate). The relative contribution of lactate increased to 78.2±2.4% (p<0.05 compared to 0.5 mmol/l lactate); thus the increase in lactate oxidation can be accounted for by both a decrease in the contribution of glucose and a decrease in the contribution of endogenous substrates to energy production. The contribution of glucose to energy production with 1 mmol/l lactate as substrate was not determined in the diabetic group due to signal-to-noise constraints. The contribution of lactate to total energy production increased from 21.8±4.3% at 0.5 mmol/l lactate to 59.3±6.9% with 1.0 mmol/l (p<0.05); this was still significantly depressed compared to the control group.

The K<sub>m</sub> and V<sub>max</sub> of LDH for lactate were measured in myocardial tissue homogenates in order to determine whether changes in lactate oxidation in the diabetic group could be due to an alteration in enzyme function. The maximal activity of LDH was 82.3±6.2 and 72.8±3.6 μmoles/min/g for control (n=5) and diabetic (n=6) groups respectively and the K<sub>m</sub> for lactate was 4.0±0.5 and 3.2±0.4 mmol/l respectively. These differences were not significant. Determination of MCT1 expression and LDH isoform distribution showed that there were also no
significant differences between control and diabetic groups (Table 2).

Myocardial glycogen content was measured in both control and diabetic hearts under four conditions: (1) in hearts immediately following sacrifice; (2) after 30 min of perfusion with glucose alone; (3) after an additional 60-min perfusion with glucose alone and (4) after an additional 60-min perfusion with glucose plus lactate (Fig. 7). In the control groups, glycogen content was the same under the four conditions. In hearts from diabetic animals, the initial glycogen content was three times higher than that of controls; however, there was a 55% decrease in glycogen content following 30 min of perfusion. Thus at the time when perfusion with 13C-labeled substrates started there were no differences in glycogen content between control and diabetic groups. Following an additional 60 min of perfusion with glucose alone, there was a further decrease in glycogen content in the diabetic group; however, there was no change after perfusion for 60 min with glucose plus lactate.

4. Discussion

Glucose and lactate have a common primary entry point into the TCA cycle via the conversion of pyruvate to acetyl-CoA by PDH. Regulation of flux through PDH is typically considered to be the principal site for the control of carbohydrate oxidation. Since inhibition of PDH is one of the major consequences of diabetes on the heart [12], one might expect that diabetes would lead to similar decreases in both glucose and lactate oxidation. Alternatively, because of the reported decreases in glucose transport, phosphorylation and glycolysis in the diabetic heart [12], lactate oxidation might be affected to a lesser extent than glucose. Surprisingly, neither of these outcomes was observed in this study. We found that glucose oxidation in diabetic hearts perfused with both glucose and lactate as substrates was the same as controls. In contrast, there was a three to four-fold decrease in lactate oxidation (Figs. 5 and 6). In other words, in hearts perfused with glucose and lactate as substrates, diabetes does not result in a decrease in glucose oxidation. Instead, the decreased carbohydrate entry into the TCA cycle is entirely due to an inhibition in the conversion of lactate to pyruvate. We believe that these results demonstrate for the first time that diabetes leads to a specific inhibition of lactate oxidation independent of its effects on pyruvate dehydrogenase.

Despite the potential importance of lactate as an energy source for the heart there have been very few investigations into the effects of diabetes on myocardial lactate metabolism. In heart homogenates from diabetic dogs, lactate oxidation was found to be decreased by 54%. However, lactate was the sole substrate and there was no measurement of glucose oxidation [29] and the results were interpreted as being consistent with an inhibition of PDH. Chen and co-workers found that diabetes decreased both lactate and glucose oxidation to a similar extent in myocytes isolated from control and four week diabetic rats [30]. As the duration of diabetes increases, there is an increasing inhibition of glucose oxidation [12]; thus, it is possible that with longer periods of diabetes the differences between glucose and lactate oxidation observed here decrease. More recently Hall and colleagues [27] examined myocardial substrate uptake in vivo in diabetic swine. They found that glucose uptake was decreased by almost 60% in the diabetic group; however, net lactate uptake was more than 90% lower following diabetes. Since net lactate uptake reflects rates of lactate oxidation [31] these results, are consistent with our observation that diabetes has a more pronounced effect on myocardial lactate oxidation than glucose oxidation.

The decreased lactate oxidation relative to glucose oxidation in the diabetic heart, indicates that the conversion of lactate to pyruvate is inhibited; this could occur at the level of lactate transport or LDH. However there were no differences between control and diabetic groups for either the $V_{max}$ or $K_{m}$ for lactate. There was also no difference in LDH isoform distribution between control and diabetic groups (Table 2). This suggests that the decrease in lactate oxidation was not due to alterations in LDH. We also found that there was no difference in the
expression of the lactate transporter MCT1 in the two groups (Table 2) which suggests that the defect in lactate oxidation is not at the site of entry into the cell. However, it should be emphasized that we have not measured MCT1 activity only the protein content. It is possible that diabetes leads to some post-translational modification of the protein itself which may decrease activity. Alternatively, changes in membrane composition could also affect its activity.

Increasing lactate concentration from 0.5 to 1.0 mmol/l resulted in about a 50% increase in lactate oxidation in the control group, whereas in the diabetic group, lactate oxidation rates were more than doubled. Thus, in diabetic hearts perfused with 0.5 mmol/l lactate, the rate of lactate oxidation was only 27% of control, and increased to 45% at 1.0 mmol/l lactate. This would be consistent with the decreased flux from lactate to pyruvate not being a consequence of altered protein expression or activity but possibly a result of increased cytosolic pyruvate concentration or altered redox state. Based on simple principles of mass action, an increase in cytosolic pyruvate following diabetes might be expected to decrease the flux from lactate to pyruvate. It has also been shown that the H_2-isozyme of LDH (the predominant isozyme in heart) is inhibited by pyruvate [32]; thus, an increase in cytosolic pyruvate could decrease the rate of formation of pyruvate from lactate via this mechanism. However, Ramasamy et al. [33] found no difference in tissue pyruvate concentrations in perfused hearts from control and diabetic rats.

An increase in the cytosolic ratio of NADH/NAD^+ could also lead to a decrease in the flux through LDH. Puckett and Reddy [34] reported a decrease in the malate–aspartate shuttle activity in mitochondria from diabetic rats, which could result in an increase in the cytosolic NADH/NAD^+ ratio. Recently, an increase in the ratio of lactate to pyruvate in hearts from diabetic rats has been reported [33]. This would also be consistent with an increase in the cytosolic NADH/NAD^+ ratio. Metabolism of glucose through the polyol pathway leading to the accumulation of sorbitol has been implicated as playing a role in the development of many of the complications of diabetes [35]. One of the potential consequences of flux through this pathway is an increase in oxidation of sorbitol to fructose by sorbitol dehydrogenase resulting in an elevation in the ratio of NADH/NAD^+ in the cytosol [35,36]. There is evidence that diabetes-related vascular and neural dysfunction can be related to increased sorbitol oxidation and thus possibly associated with an increase in cytosolic NADH/NAD^+ [36]. Recent studies have also shown that inhibition of the polyol pathway may mediate diabetes related cardiac dysfunction, possibly by decreasing the NADH/NAD^+ ratio [33,37,38].

Based on the relative concentrations of exogenous glucose (11 mmol/l) and lactate (0.5 and 1.0 mmol/l), the amount of glucose-derived pyruvate should exceed lactate-derived pyruvate by a factor of 44 or 22 respectively, if there was simple competition at the level of pyruvate. However, in control hearts perfused with 0.5 mmol/l lactate, lactate-derived pyruvate contributed more than twice as much acetyl-CoA to the TCA cycle than glucose-derived pyruvate and more than four times as much with 1 mmol/l lactate. This indicates that under normal conditions lactate exerts a strong inhibitory effect on the metabolism of glucose to pyruvate either at the level of glucose transport [39–41] or glycolysis [42]. It is possible, therefore, that in the diabetic heart the decrease in lactate oxidation could be due to a decrease in the inhibitory effect of lactate on the flux from glucose to pyruvate. This would increase the formation of glucose-derived pyruvate relative to the formation of lactate-derived pyruvate which would have the same consequence on the relative contributions of glucose and lactate to acetyl-CoA formation as a specific inhibition of the flux from lactate to pyruvate. However, if this were the case this would indicate an increase in glycolysis which would be counter to the work of Randle and colleagues and [39,43] as well as Gamble and Lopaschuk [44] who have reported decreased glycolysis in the diabetic heart.

In all experimental groups there was a contribution of endogenous substrates to acetyl-CoA formation ranging from 10–15% in the control group and ~50% in the diabetic group. The endogenous substrates could be glycogen, triglycerides or amino acids resulting from proteolysis. It is unlikely that proteolysis contributes significantly since the rate of proteolysis required to contribute only 10% of acetyl-CoA entry into the TCA cycle is at least one order of magnitude greater than that measured in the perfused heart [45]. In control hearts there were no differences in glycogen content in any of the groups examined suggesting that there is no breakdown of glycogen. Consequently, it is unlikely that the contribution from endogenous substrates is due to glycogenolysis. Thus, in the control group at least, triglycerides are the most likely source of unlabeled substrate entry into the TCA cycle in control hearts.

In the diabetic group, the results are a little less clear, as there was a marked decrease in glycogen during the first 30 min of perfusion and a further decrease in glycogen during the next 60 min with glucose alone. However with glucose plus lactate as substrate there was no further decrease in glycogen content (Fig. 7). The marked breakdown of glycogen during the first 30 min of perfusion is consistent with the reported increased sensitivity of diabetic hearts to glycogenolysis [46]. The glycogen content at `baseline' is at the end of 30 min of perfusion with unlabeled substrates and immediately prior to perfusion with 13C-labeled substrates for an additional 60 min. There was no further change in glycogen content in the diabetic group perfused with glucose plus lactate. Therefore similar to the control hearts, triglycerides rather than glycogen appear to be the principal source of unlabeled substrate entry into the TCA cycle in the diabetic group. This would be consistent with other studies that have demonstrated a significant increase in endogenous fatty acid utilization in the diabetic heart resulting from breakdown in triglycerides.
This is also in agreement with recent studies from our laboratory in which we found no significant decrease in triglyceride content in hearts from control hearts but an almost 80% decrease in the diabetic group perfused with glucose and lactate as substrates [50]. In the diabetic group with glucose as the only substrate, the glycogen level is significantly lower than baseline at the end of the perfusion period and thus we cannot completely rule out a contribution from glycogen to acetyl-CoA formation in that series of experiments.

The concentration of lactate used in many of the experiments reported here (i.e., 0.5 mmol/1) was lower than that typically found at rest in vivo (i.e., ~1 mmol/1) and much lower than that measured here in animals following sacrifice (Table 1). The hypothesis we set out to test was that following diabetes the inhibition of glucose oxidation would be greater than lactate oxidation, we therefore chose a concentration of lactate that would provide us with a measurable amount of both glucose and lactate oxidation. If we had used much higher concentrations of lactate we would not have been able to reliably determine glucose oxidation as was the case in the diabetic group at 1 mmol/1 lactate. While this does not alter the principal observations in this study it does limit the extrapolation of these results to the in vivo situation. It should also be noted that insulin was not present in these experiments, which also would not be the case in vivo. However, Hall and colleagues [27] showed that in diabetic pigs in vivo there was a greater decrease in myocardial lactate uptake compared to glucose uptake. While they did not measure substrate oxidation rates, their results are consistent with the observations reported here.

We have shown that in hearts perfused with glucose and lactate as substrates, the marked decrease in exogenous carbohydrate oxidation is due to a decrease in lactate rather than glucose oxidation. In other words, in addition to a decrease in flux through PDH, these data demonstrate for the first time that diabetes also affects the regulation of pyruvate formation from glucose and lactate. It is proposed that the decrease in lactate oxidation is most likely a result of an increase in the cytosolic NADH/NAD⁺ ratio rather than alterations at the level LDH or MCT1. Although these studies were carried out in the isolated heart without insulin and exogenous fatty acids the results are consistent with in vivo measurements of myocardial glucose and lactate uptake in diabetic pigs [27]. These results also indicate that investigations into myocardial substrate selection and utilization should consider lactate independently from glucose and not simply as another pyruvate source.

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