Shift in metabolic substrate uptake by the heart during development of alloxan-induced diabetes

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Inhibition of endothelial nitric oxide (NO) synthase (eNOS) is associated with an increase in glucose uptake by the heart. We have already shown that Type I diabetes also causes a decrease in eNOS protein expression and altered NO control of both coronary vascular resistance and oxygen consumption. Therefore, we predict that the increase in plasma glucose and the reduction in eNOS protein expression and altered NO control of coronary vascular resistance and oxygen consumption together would result in a large increase in cardiac glucose uptake. Arterial (A) and coronary sinus (C) plasma levels of glucose, free fatty acid (FFA), β-hydroxybutyrylic acid (β-HBA), and lactate were measured, and myocardial uptake was calculated before and at week 1, 2, 3, and 4 of alloxan-induced diabetes. The heart of healthy dogs consumed FFA (19.2 ± 2.6 μg/min) and lactate (19.7 ± 3.4 μmol/min). Dogs in the late stage of diabetes (at week 4) had elevated arterial β-HBA concentrations (1.6 ± 0.7 μmol/l) that were accompanied by an increased β-HBA uptake (0.3 ± 0.2 μmol/min). In contrast, myocardial lactate (−4.8 ± 3.0 μmol/min) and FFA uptake (2.5 ± 1.9 μg/min) were significantly reduced in diabetic animals. Despite a marked hyperglycemia (449 ± 25 mg/dl), the heart did not take up glucose (−7.9 ± 4.1 mg/dl). Over the last several years we have focused considerable attention on nitric oxide (NO) as a modulator of myocardial substrate utilization. Recchia and co-workers (11) demonstrated that inhibition of NO synthesis by Nω-nitro-L-arginine (L-NNA) in conscious dogs resulted in a reduced FFA uptake by the heart. In contrast, glucose and lactate uptake increased significantly after blockade of NO synthesis. This was paralleled by an elevation in the respiratory quotient, indicating a switch from FFA to carbohydrate oxidation, and all of these changes could be immediately reversed by a NO donor. However, the NO synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME) was shown to decrease FFA oxidation and increase glucose oxidation without changing the activity of key enzymes of glycolysis or β-oxidation (12). Inhibition of NO synthase in normal dogs and a reduction in NO bioavailability due to a downregulation of endothelial NO synthase (eNOS) protein expression in dogs with chronic heart failure were found to be associated with an increased glucose uptake by the heart (10–12). Furthermore, we have demonstrated that coronary eNOS protein expression and eNOS-derived NO are diminished in a canine model of chronic Type I diabetes (19). However, FFA, lactate, glucose, and β-HBA uptake were not assessed as a function of NO production in that study. Numerous studies investigating substrate uptake and oxidation have been conducted over the last sev-

Address for reprint requests and other correspondence: T. H. Hintze, Dept. of Physiology, New York Medical College, Valhalla, NY 10595 (E-mail: Thomas_Hintze@nymc.edu).
eral years comparing diabetic with healthy patients or animals. To our knowledge, no data are available about the exact time course of changes in the myocardial substrate uptake during the development of Type I diabetes and also the late stage of the disease, when NO production is reduced. We hypothesize that the increase in plasma glucose and the reduced NO bioavailability in late-stage diabetes together would result in an enhanced glucose uptake by the heart. Therefore, the aim of this study was 1) to investigate arterial FFA, lactate, glucose, and β-HBA concentration; and 2) to determine the myocardial uptake of those substrates in conscious dogs during the development of Type I diabetes.

**MATERIALS AND METHODS**

The protocols were approved by the Institutional Animal Care and Use Committee of New York Medical College. They conform to the guiding principles for the use and care of laboratory animals of the National Institute of Health and the American Physiological Society.

*Surgical procedure and instrumentation.* Male mongrel dogs (*n* = 7), weighing 23–33 kg, were premedicated with acepromazine maleate (1 mg/kg body wt im), anesthetized with pentobarbital sodium (25 mg/kg body wt iv), intubated, and then ventilated with room air. A thoracotomy was performed in the fifth intercostal space by use of sterile surgery techniques. A catheter (Tygon, Cardiovascular Instruments; Wakefield, MA) was placed into the descending thoracic aorta for measurement of arterial pressure and collection of arterial blood samples. A second catheter was inserted into the coronary sinus with the tip leading away from the right atrium to collect coronary sinus blood samples. A solid-pressure gauge (model P6.5, Konigsberg Instruments, Pasadena, CA) was placed in the left ventricle through the apex for measurement of left ventricular (LV) systolic pressure (LVSP). The left circumflex coronary artery was isolated and cleared of fat and connective tissue, and a Doppler flow transducer was placed around the artery for measurement of blood flow. The wires and catheters were run subcutaneously to the intrascapular region, the chest was closed, and the pneumothorax was reduced. For 6 days after surgery, antibiotics were given, and the dogs were allowed to recover fully. Ten days after surgery, the dogs were trained to lie quietly on the laboratory table.

*Hemodynamic recordings.* The aortic catheter was connected to a strain-gauge transducer (P23 ID, Statham; Rahway, NJ) for measurement of aortic pressure. LV pressure was measured with the previously implanted pressure gauge. The first derivative of LV pressure (LV dP/dt) was calculated by using an operational amplifier (LM 324, National Semiconductor; Newark, NJ), and triangular wave signals with known slope were substituted for the pressure signals to calibrate the differentiator directly. Left circumflex coronary blood flow (CBF) was measured with a pulsed Doppler flowmeter (model 100, Triton Instruments; San Diego, CA). Mean CBF and mean arterial pressure (MAP) were derived from a 2-Hz low-pass filter. Heart rate (HR) was monitored from the LV pressure-pulse interval using a cardiotachometer (model 9857B, Beckmann Instruments; Newark, NJ). All hemodynamics were recorded continuously on an eight-channel chart recorder (Gould RS 3800, National Instruments; Rahway, NJ). The triple product, an index of mechanically related cardiac oxygen consumption, was calculated as LVSP multiplied by dP/dt max and HR.

*Induction of diabetes.* After the control experiment, the dogs were injected with alloxan monohydrate (40 mg/kg body wt iv) over 1 min, prepared as a 5% solution in citrate buffer (pH 4.0–4.5) (19). Plasma glucose levels were measured again 3 days after the first alloxan administration. Dogs with plasma glucose levels <200 mg/dl were reinfused with alloxan monohydrate (60 mg/kg body wt iv). Only dogs with fasting blood glucose levels above 200 mg/dl on day 7 after the first alloxan injection were studied.

*Western blot analysis.* Tissue from the LV free wall was harvested from dogs in end-stage diabetes and from dogs that had received alloxan but did not develop diabetes. The tissue was pulverized in liquid nitrogen and resuspended in lysing buffer containing protease inhibitors (4). After sonification for 60 s, samples were centrifuged at 5,000 rpm for 10 min, and protein concentration was determined as previously described (4). Briefly, 100 μg of protein were separated on a 7.5% SDS-polyacrylamide-gel (SDS-PAGE), followed by electronic transfer to a polyvinylidene fluoride membrane (PVDF, Amersham; Piscataway, NY). Human umbilical vein endothelial cells were loaded as a positive control for eNOS. Only proteins were incubated with a 1:50 dilution of a monoclonal anti-eNOS antibody (Affinity BioReagents; Golden, CO), at 4°C overnight. The bound primary antibody was detected by a peroxidase-coupled anti-mouse antibody (dilution 1:2,000, Amersham) followed by a chemiluminescent reaction using luminol (SuperSignal West Pico, Pierce; Rockford, IL). Afterward, the membrane was exposed to a film, and bands were analyzed by densitometry as previously published (4).

*Blood analysis and cardiac metabolites.* Blood samples were collected from the aorta and the coronary sinus in syringes containing either EDTA or heparin and immediately stored on ice. Blood was withdrawn slowly from the coronary sinus to avoid contamination with right atrial blood. Blood gases were measured using a blood-gas analyzer (model 1306, Instrumentation Laboratory; Lexington MA), and hemoglobin was analyzed by a CO-Oximeter (model 482, Instrumentation Laboratory). PO2 was multiplied by 0.003 to obtain the concentration of oxygen dissolved in plasma and added to the measured O2 content to calculate total oxygen content of the blood (vol/vol). Hematorcrits were obtained by centrifugation. Blood samples were centrifuged 15 min at 6,000 rpm. Plasma glucose and β-HBA concentration were determined by a spectrophotometric enzymatic assays (Sigma; St. Louis, MO) according to the instructions of the manufacturer. Total lactate was measured in serum deproteinized with ice-cold 1 M perchloric acid (1:2 vol/vol) using an enzymatic assay (Sigma). Total FFA concentration was determined spectrophotometrically (Kontron Instruments) in plasma after centrifugation of EDTA-treated blood samples with the use of a calorimetric assay (NEFA C kit, Wako Pure Chemical Industries; Richmond, VA). The arterial-coronary sinus concentration differences of oxygen, lactate, glucose, β-HBA, and FFA were multiplied by the mean CBF assumed as double of the mean flow measured in the left circumflex coronary artery to calculated cardiac uptake. Respiratory quotient and cardiac production of CO2 were calculated as previously described (11).

The concentration of nitrite/nitrate in plasma was measured using the method established in our laboratory previously (19). All data in the tables, text, and figures are presented as mean values ± SE. Statistical analysis was performed using commercially available software (SigmaStat 2.03, SPSS; San Rafael, CA). Body weight, hemodynamics, blood gases, arterial substrate
levels, substrate uptake at different time points, and plasma NOx levels were compared using a one-way repeated measures ANOVA followed by Tukey’s test to evaluate statistical differences between the time points. Furthermore, one-way ANOVA followed by Tukey’s test was applied to determine whether substrate uptake at different time points significantly differ from zero. eNOS protein expression in the myocardium of dogs in end-stage diabetes and dogs that received alloxan but did not develop diabetes was compared by a t-test. Linear regression analysis was performed to determine the relationship among lactate, plasma NOx, FFA uptake, and β-HBA uptake, respectively. A P value of <0.05 was considered statistically significant.

RESULTS

Body weight. Dogs had a body weight of 28 ± 2 kg before alloxan injection. At week 1 of diabetes, the dogs had already lost 9% of their weight (25 ± 2 kg), lost 14% at week 2 (24 ± 2 kg), lost 19% at week 3 (23 ± 2 kg), and finally at week 4 lost 22% (22 ± 2 kg), respectively (P < 0.001 vs. control). Furthermore, dogs had clinical signs of diabetes, i.e., polyuria and polydipsia.

Myocardial eNOS expression and concentration of nitrate/nitrite in plasma. We found a 33% reduction of eNOS protein in the myocardium of the diabetic dogs at week 4 after alloxan injection (164,850 ± 22,495 arbitrary units) compared with the myocardium of dogs that received alloxan but did not develop diabetes (245,000 ± 10,312 arbitrary units, P = 0.05). This reduction in eNOS protein expression during the development of diabetes was accompanied by a gradual decrease in plasma NOx from 1.69 ± 0.29 μM before diabetes to 1.55 ± 0.33 μM at week 1, to 1.42 ± 0.28 μM at week 2, to 1.18 ± 0.20 μM at week 3, and to 1.10 ± 0.13 μM at week 4 of diabetes (P ≤ 0.05 vs. before diabetes).

Cardiac metabolism. Arterial blood glucose levels before and after alloxan injection are shown in Table 1. Induction of diabetes mellitus increased arterial glucose levels by 190% at week 1, by 235% at week 2, by 230% at week 3, and by 354% at week 4, respectively (P < 0.05 vs. control). Despite the marked elevation in arterial plasma glucose concentrations up to 450 mg/dl in some dogs, the heart did not consume significant amounts of glucose at any time (Fig. 1A). Furthermore, arterial concentrations of FFA were increased by 95% at week 1 of diabetes compared with control (Table 1, P < 0.05). This elevation was even more pronounced at week 2. At week 3 of diabetes, arterial FFA levels began to fall, and at 4 wk, levels were not significantly different from control (Table 1).

Table 1. Cardiac substrate concentration in time course of diabetes

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial glucose, mg/dl</td>
<td>99 ± 4</td>
<td>287 ± 24‡</td>
<td>332 ± 34‡</td>
<td>326 ± 14‡</td>
<td>449 ± 25‡</td>
</tr>
<tr>
<td>CS glucose, mg/dl</td>
<td>99 ± 3</td>
<td>282 ± 23‡</td>
<td>345 ± 32‡</td>
<td>331 ± 16‡</td>
<td>461 ± 28‡</td>
</tr>
<tr>
<td>Arterial lactate, mmol/l</td>
<td>1.33 ± 0.17</td>
<td>1.35 ± 0.26</td>
<td>1.03 ± 0.20</td>
<td>1.37 ± 0.37</td>
<td>1.68 ± 0.31</td>
</tr>
<tr>
<td>CS lactate, mmol/l</td>
<td>1.07 ± 0.12</td>
<td>1.19 ± 0.17</td>
<td>0.92 ± 0.12</td>
<td>1.17 ± 0.16</td>
<td>1.72 ± 0.23</td>
</tr>
<tr>
<td>Arterial FFA, meq/l</td>
<td>0.73 ± 0.05†</td>
<td>1.42 ± 0.25†</td>
<td>1.48 ± 0.21†</td>
<td>1.32 ± 0.13*</td>
<td>1.23 ± 0.06</td>
</tr>
<tr>
<td>CS FFA, meq/l</td>
<td>0.48 ± 0.04</td>
<td>1.25 ± 0.25†</td>
<td>1.15 ± 0.12†</td>
<td>1.11 ± 0.16*</td>
<td>1.13 ± 0.11†</td>
</tr>
<tr>
<td>Arterial β-HBA, mg/dl</td>
<td>16.4 ± 7.1</td>
<td>70.8 ± 28.6</td>
<td>90.0 ± 25.9</td>
<td>219.2 ± 86.0*</td>
<td>226.6 ± 95.5*</td>
</tr>
<tr>
<td>CS β-HBA, mg/dl</td>
<td>12.3 ± 7.5</td>
<td>42.9 ± 17.1</td>
<td>57.5 ± 20.9</td>
<td>154.4 ± 56.5*</td>
<td>171.3 ± 76.7*</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 7 dogs. CS, coronary sinus; FFA, free fatty acid; β-HBA, β-hydroxybutyric acid. *P < 0.05 vs. control; †P < 0.01 vs. control; ‡P < 0.001 vs. control.

Fig. 1. A: changes in myocardial glucose uptake in conscious dogs before (control) and at week 1, 2, 3, and 4 of diabetes. B: changes in myocardial free fatty acid (FFA) uptake in conscious dogs before (control) and at week 1, 2, 3, and 4 of diabetes. *P < 0.05 vs. control; †P < 0.05 vs. 0; ‡P < 0.001 vs. 0.
significantly during the development of diabetes. At week 1 and 2 of diabetes, the heart consumed only 9.29 ± 7.52 and 3.60 ± 4.01 μmol/min lactate, respectively (Fig. 2A). At week 3 and 4 of diabetes, the heart did not take up lactate at all (week 3: 1.05 ± 2.87 μmol/min, week 4: 4.84 ± 3.02 μmol/min; \( P < 0.05 \) vs. control, Fig. 2A).

Almost no \( \beta \)-HBA was detectable in the plasma of healthy dogs (Table 1), and the heart did not utilize significant amounts of \( \beta \)-HBA. One week after induction of diabetes, arterial \( \beta \)-HBA plasma concentrations were elevated by 330%, at week 2 by 450%, at week 3 by 1,240% \( (P < 0.05 \) vs. control), and at week 4 by 1,280% \( (P < 0.05 \) vs. control), respectively (Table 1). This increase in the arterial plasma concentrations of \( \beta \)-HBA during the development of diabetes was accompanied by a significant myocardial \( \beta \)-HBA uptake at week 4 (Fig. 2B). To determine whether \( \beta \)-HBA replaces FFA and lactate as a fuel of the heart, linear regression analysis was performed. It revealed that an increased \( \beta \)-HBA uptake was closely associated with a decrease in myocardial lactate uptake \( (r = -0.94, P < 0.05, \text{Fig. 3A}) \) and a reduction in FFA uptake \( (r = -0.98, P < 0.01, \text{Fig. 3B}) \).

To assess whether the change in NO bioavailability during the development of diabetes impacts on myocardial substrate uptake, a regression analysis was applied. We found a close inverse linear correlation between plasma NOx levels and \( \beta \)-HBA uptake \( (r = -0.91, P < 0.05, \text{Fig. 4A}) \), indicating that a decrease in the NO production is associated with an increase in the myocardial \( \beta \)-HBA uptake during the development of diabetes. In contrast, the reduction in NO production was associated with a decline in the myocardial uptake of FFA \( (r = 0.94, P < 0.05, \text{Fig. 4B}) \).

Fig. 2. A: changes in myocardial lactate uptake in conscious dogs before (control) and at weeks 1, 2, 3, and 4 of diabetes. *\( P < 0.05 \) vs. control; **\( P < 0.01 \) vs. control; †\( P < 0.05 \) vs. 0. B: changes in myocardial \( \beta \)-hydroxybutyric acid (\( \beta \)-HBA) uptake in conscious dogs before (control) and at weeks 1, 2, 3, and 4 of diabetes. *\( P < 0.05 \) vs. control; **\( P < 0.01 \) vs. control; †\( P < 0.05 \) vs. 0.

Fig. 3. A: correlation between \( \beta \)-HBA and lactate uptake in conscious dogs during the development of diabetes. B: correlation between \( \beta \)-HBA and FFA uptake in conscious dogs during the development of diabetes. Filled circles, different time points of measurement from control to week 4 of diabetes.

Fig. 4. A: correlation between plasma NOx levels and \( \beta \)-HBA uptake in conscious dogs during the development of diabetes. B: correlation between NOx levels and FFA uptake in conscious dogs during the development of diabetes. Filled circles, different time points of measurement from control to week 4 of diabetes.
Blood gases. Arterial pH, PO$_2$, PCO$_2$, and oxygen content did not change during the development of diabetes (Table 2). However, dogs were characterized by a significant reduction of the hematocrit at week 4 of diabetes compared with control (Table 2, $P < 0.05$).

Myocardial O$_2$ consumption and respiratory quotient. Cardiac oxygen consumption and respiratory quotient are presented in Table 3. Cardiac oxygen consumption was decreased by 11% at week 1 of diabetes, by 33% at week 2 ($P < 0.05$ vs. control), by 33% at week 3 ($P < 0.05$ vs. control), and by 37% at week 4 ($P < 0.05$ vs. control), respectively. However, myocardial O$_2$ consumption (MV.O$_2$) per beat did not change during the entire study period (Table 3).

To determine whether the shift in the uptake of cardiac substrates was related to their oxidation, the respiratory quotient (RQ) of the heart was calculated for the control and diabetic state. However, RQ did not significantly change during the development of diabetes.

Hemodynamics. The hemodynamic data are shown in Table 4. LVSP and LV dP/dt did not change from control during diabetes. MAP decreased gradually, and this reduction reached statistical significance at week 4 of diabetes (Table 4). Moreover, at week 2 of diabetes, heart rate and mean CBF were significantly diminished by 37% and 50%, respectively, compared with control ($P < 0.05$). However, neither MAP nor mean CBF dropped further within the last 2 wk of the study. The triple product, an index of mechanically related cardiac MV.O$_2$, tended to decrease, but this did not reach statistical significance.

**Discussion**

The following major findings emerge from this study investigating the changes in substrate uptake during the development of diabetes in conscious dogs: despite markedly elevated arterial glucose concentrations in diabetic dogs, the heart did not take up glucose at any time. Arterial FFA concentrations increased during diabetes, but myocardial FFA uptake was reduced at week 3 and 4 after alloxan injection. Arterial lactate concentrations remained constant over the entire study period, but lactate uptake of the heart was diminished at week 3 and 4 of diabetes. Arterial concentrations of β-HBA increased shortly after the onset of diabetes and reached the maximum at week 4. The elevation of plasma β-HBA was paralleled by a significant β-HBA uptake of the heart. Furthermore, β-HBA uptake was inversely correlated with FFA and lactate uptake, suggesting that β-HBA replace FFA and lactate as substrates of the myocardium in diabetes. This trade in substrates leads to no change in calculated RQ.

**Diabetes and substrate metabolism.** To our knowledge, this is the first study investigating the changes in arterial substrate concentration and substrate uptake of the heart in conscious chronically instrumented dogs during the development of diabetes over a period of 4 wk. Numerous studies in the past have been focused on the acute effects of diabetes on myocardial substrate metabolism in anesthetized dogs. In 1973 Wiener and Spitzer (18) found that 3 days after alloxan injection, dog hearts did not extract significant amounts of glucose, despite a remarkable increase in the arterial glucose concentration. Arterial lactate concentrations were also unchanged compared with normal dogs, and the heart did not utilize lactate consistently at this early stage of diabetes. In contrast, arterial FFA concentration and FFA turnover were elevated by 100% compared with normal dogs. However, the rate of FFA oxidation did not significantly differ between control and diabetic dogs (18). Furthermore, a moderate ketosis was detectable in the diabetic animals. The earliest time point of measurement in our study was day 7 of diabetes. At this time, dogs exhibited significantly elevated arterial glucose concentrations that were not accompanied by a consistent glucose uptake of the heart. The unchanged lactate concentration and the slight ketosis in our study are also in accord with the previous results. However, we found a significant myocardial lactate uptake before diabetes that was diminished at 3 wk of diabetes. The differences with respect to lactate uptake might be explained by the use of

**Table 2. Arterial pH, PO$_2$, and PCO$_2$, CaO$_2$, and hematocrit in conscious dogs during development of diabetes**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.37 ± 0.01</td>
<td>7.36 ± 0.01</td>
<td>7.39 ± 0.01</td>
<td>7.37 ± 0.02</td>
<td>7.35 ± 0.01</td>
</tr>
<tr>
<td>PO$_2$, mmHg</td>
<td>93 ± 1</td>
<td>90 ± 2</td>
<td>89 ± 3</td>
<td>93 ± 3</td>
<td>87 ± 4</td>
</tr>
<tr>
<td>PCO$_2$, mmHg</td>
<td>38 ± 1</td>
<td>36 ± 1</td>
<td>36 ± 2</td>
<td>36 ± 2</td>
<td>36 ± 2</td>
</tr>
<tr>
<td>CaO$_2$, %</td>
<td>15.8 ± 0.9</td>
<td>14.2 ± 0.6</td>
<td>14.7 ± 0.3</td>
<td>14.8 ± 0.7</td>
<td>13.9 ± 0.8</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>38 ± 2</td>
<td>33 ± 1</td>
<td>35 ± 1</td>
<td>35 ± 1</td>
<td>32 ± 2*</td>
</tr>
</tbody>
</table>

Data are means ± SE; $n = 7$ dogs. CaO$_2$, arterial O$_2$ content. *$P < 0.05$ vs. control.

**Table 3. RQ and MV.O$_2$ during development of diabetes**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>RQ</td>
<td>0.76 ± 0.05</td>
<td>0.79 ± 0.09</td>
<td>0.78 ± 0.07</td>
<td>0.80 ± 0.04</td>
<td>0.77 ± 0.09</td>
</tr>
<tr>
<td>MV.O$_2$, ml/min</td>
<td>4.34 ± 0.49</td>
<td>3.91 ± 0.21</td>
<td>2.95 ± 0.31*</td>
<td>2.94 ± 0.36*</td>
<td>2.77 ± 0.45†</td>
</tr>
<tr>
<td>MV.O$_2$/beat, ml/beat × 10</td>
<td>0.50 ± 0.06</td>
<td>0.56 ± 0.06</td>
<td>0.48 ± 0.07</td>
<td>0.49 ± 0.07</td>
<td>0.45 ± 0.07</td>
</tr>
</tbody>
</table>

Data are means ± SE; $n = 7$ dogs. RQ, respiratory quotient; MV.O$_2$, myocardial O$_2$ consumption. *$P < 0.05$ and †$P < 0.001$ vs. control.
pentobarbital to anesthetize the animals in the previous study, because pentobarbital is known to affect the cardiovascular system in dogs (8). Wiener and Spiteri (18) infused ketone bodies intravenously to mimic the effects of advanced diabetes. The resulting increase in plasma ketones was accompanied by a further reduction in FFA extraction by the heart. This effect was even more pronounced in our conscious dogs when the arterial $\beta$-HBA concentrations and uptake reached $\sim 15$ times the control values at week 4 of diabetes resulting in a marked decline of FFA uptake by the heart.

To our knowledge, only one study in the past examined the metabolic alterations at two different time points after alloxan injection in dogs (6). Kraupp and co-workers (6) showed that $\sim 17$ days after alloxan injection dogs had significantly increased arterial FFA concentrations but FFA uptake by the heart remained unchanged compared with control. The arterial levels as well as the uptake of hydroxybutyrate and acetoacetate, the two main ketone bodies, were significantly elevated in the diabetic animals. However, despite a threefold higher arterial glucose concentration after alloxan, the heart of the diabetic animals did not utilize significant amounts of glucose. Additionally, arterial lactate concentrations were not different in diabetic and normal animals (6). In our investigation, we could confirm the increase in the arterial serum concentrations of glucose, FFA, and $\beta$-HBA in diabetic dogs. We also found an augmented utilization of ketone bodies and a reduced lactate uptake by the diabetic heart. However, in our study, diabetic dog hearts consumed significantly less FFA at day 21 of diabetes compared with the nondiabetic state. The disparate results might be explained by the differences in the study design. Whereas Kraupp et al. (6) summarized three different animals that were investigated at days 5, 16, and 30 of diabetes in the anesthetized state in one group and compared them with a group of normal dogs, our conscious dogs were studied every week. Our longitudinal study design with a larger group size makes it easier to detect differences between the pre-diabetic and the diabetic state, e.g., for the FFA uptake. Furthermore, we were able to assess differences in the arterial substrate concentrations and substrate uptake by the heart every week during the development of diabetes, whereas the study of Kraupp and co-workers (6) did not.

Possible causes for the metabolic alteration in the diabetic heart. From our data, it appears that arterial FFA and $\beta$-HBA concentrations are increased in diabetes due to enhanced lipolysis over time. In contrast to previous studies, we found a reduction in FFA uptake in the diabetic state. This may be explained by the dramatic elevation of ketone bodies normally not seen in human diabetes. Hydroxybutyric acids can be directly converted into acetyl-CoA units without using the $\beta$-oxidation. Acetyl-CoA is further converted by the acetyl-CoA decarboxylase in malonyl-CoA that represents an effective inhibitor of carnitine palmitoyl transferase I (CPT-I), a part of an enzyme complex that is necessary to transfer the acyl moiety from the fatty acyl-CoA into the mitochondrion. An increased uptake of $\beta$-HBA, the conversion into malonyl-CoA with the consequence of an CPT-I inhibition, might reflect one cause of the reduced FFA uptake into the mitochondrion. This provides one explanation why ketone bodies seem to replace FFA as substrates of the heart and may account for the impaired FFA uptake in our model of diabetes. In diabetic dogs, regardless of whether HBA or FFA is metabolized by the heart, both pathways would result in increased cellular citrate and acetyl-CoA concentrations that are known to inhibit lactate and glucose utilization (5, 17).

Previously, we had shown that plasma NOx levels were significantly reduced at week 3 of diabetes. The impaired NO production was attributed to a decreased gene expression and protein formation of eNOS and accompanied by an impaired endothelium-dependent vasodilatation (12). We confirm these previous results regarding the decrease in the plasma levels of NOx in the present study. Moreover, we are showing that myocardial eNOS expression is also decreased in end-stage diabetes. Besides the impact of NO on myocardial respiration, it exerts additional effects on substrate utilization of the heart. Recchia et al. (10) clearly demonstrated that attenuation of myocardial NO release during the development of pacing-induced heart failure was accompanied by a shift in myocardial substrate utilization from FFA to glucose. This effect could be mimicked in normal dogs by the injection of a nonselective NOS inhibitor and was fully reversible after administration of a NO donor (11). The modulating effect of eNOS-derived NO on myocardial glucose utilization was further confirmed by Tada et al. (15). They showed only a moderate glucose uptake by hearts

Table 4. Changes in hemodynamics during development of diabetes

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVSP, mmHg</td>
<td>127 ± 4</td>
<td>145 ± 5</td>
<td>133 ± 7</td>
<td>133 ± 6</td>
<td>127 ± 4</td>
</tr>
<tr>
<td>LV $dP/dt$, mmHg/s</td>
<td>2,971 ± 206</td>
<td>3,297 ± 232</td>
<td>3,354 ± 190</td>
<td>3,353 ± 238</td>
<td>2,959 ± 224</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>101 ± 2</td>
<td>102 ± 2</td>
<td>98 ± 3</td>
<td>96 ± 2</td>
<td>93 ± 4*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>89 ± 4</td>
<td>73 ± 6</td>
<td>65 ± 5*</td>
<td>64 ± 6*</td>
<td>66 ± 11*</td>
</tr>
<tr>
<td>CBF, ml/min</td>
<td>39 ± 4</td>
<td>34 ± 2</td>
<td>26 ± 3*</td>
<td>26 ± 3*</td>
<td>25 ± 4*</td>
</tr>
<tr>
<td>Triple product, mmHg$^{2} \times 10^{6}$</td>
<td>34.4 ± 4.1</td>
<td>31.6 ± 2.5</td>
<td>27.8 ± 2.4</td>
<td>26.7 ± 3.9</td>
<td>24.1 ± 5.9</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 7 dogs. LVSP, left ventricular systolic pressure; $dP/dt$ first derivative of LV pressure; MAP, mean arterial pressure; HR, heart rate; CBF, coronary blood flow. *P < 0.05.
in normal mice, whereas eNOS knockout mice consumed much higher amounts of glucose. Treatment with L-NAME to block eNOS activity significantly increased myocardial glucose utilization in normal mice but did not change glucose utilization in eNOS knockout mice. Therefore, it is conceivable that the heart of diabetic dogs also switches from FFA to glucose utilization when cardiac NO production decreases. In fact, at week 4 of diabetes when FFA uptake was severely impaired, not only were plasma NO\textsubscript{2} concentrations significantly reduced but myocardial eNOS protein expression was significantly diminished compared with a nondiabetic state. Moreover, we found that the gradual decrease in plasma NO\textsubscript{2} levels was accompanied by a decline in FFA uptake and an increase in β-HBA uptake during the development of diabetes. However, the altered FFA uptake was not paralleled by an elevated glucose or lactate uptake in diabetic animals as it was in heart failure (10). Furthermore, the RQ of the heart did not increase during the development of diabetes and makes it therefore unlikely that carbohydrates were used as a fuel. Because the heart favors ketones as an energy supply and ketones are available in excess in untreated diabetes, the heart does not switch to glucose utilization despite high plasma glucose levels and an impaired cardiac NO production. On the contrary, ketones seem to partially replace FFA as well as lactate despite increased arterial FFA (Fig. 3). However, in heart failure and eNOS knockout mice, arterial ketone concentrations are negligible excluding the use of ketone bodies as a myocardial energy supply.

Hemodynamics, M\textsubscript{VO}\textsubscript{2}, and RQ. In our study the time course for changes in baseline hemodynamics and coronary circulation was also observed. In accord with previously published data (19), we found a reduction in MAP and CBF over time. Furthermore, the heart rate was significantly reduced in conscious diabetic dogs at week 4 of diabetes. In contrast, LVSP and dP/dt\textsubscript{max}, measures of systolic function, were unchanged over the study period. The triple product, an estimate of cardiac work, tended to decrease during the development of diabetes; however, this did not reach statistical significance.

In contrast, the M\textsubscript{VO}\textsubscript{2}, a measure of mechanically related oxygen consumption, was significantly diminished at week 3 and 4 compared with the nondiabetic state. However, the M\textsubscript{VO}\textsubscript{2} normalized per beat remained constant over the entire study period, suggesting that the reduction in M\textsubscript{VO}\textsubscript{2} was due to bradycardia. In summary, our data provide evidence that the efficiency of the heart in terms of the ratio of mechanically related energy to M\textsubscript{VO}\textsubscript{2} in diabetic dogs is not altered.

Study limitations. In the present study we measured arterial concentrations and myocardial substrate uptake of FFA, β-HBA, lactate, and glucose in conscious dogs before and during the development of alloxan-induced diabetes mellitus. Our longitudinal study design and the used methods do not allow a measurement of substrate oxidation at any time. However, the absence of a marked glucose uptake despite increased arterial plasma concentrations and the significant reduction in lactate uptake at week 3 and 4 of diabetes together with the unchanged RQ makes it unlikely that carbohydrates play an important role as myocardial fuel in alloxan-induced diabetes. In our study we found an increase in myocardial β-HBA uptake during the development of diabetes, indicating that β-HBA replaces FFA as a fuel. This notion is further supported by the inverse correlation between β-HBA and FFA uptake. However, no matter whether FFA or β-HBA is oxidized by the heart, the RQ remains the same. Furthermore, HBA uptake was inversely related to lactate uptake, suggesting that lactate is only of minor importance as a substrate for the heart in diabetes. Because only a small part of myocardial energy supply is derived from glycolysis and lactate uptake, this might explain why the shift in substrate utilization from lactate to β-HBA is not accompanied by a detectable change in RQ.

Finally, the relevance of these studies to our understanding of cardiac function and metabolism should be pointed out. In our study and those previously conducted in humans (1, 3), there is no evidence of glucose uptake by the diabetic heart. Rather the heart takes up ketone bodies. In vitro studies incubating cardiac and other parenchymal cells only with glucose as a model of diabetes are not reasonable. Coincubation with ketone bodies would better reflect the pathophysiological circumstances in vivo.

DISCLOSURES

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