Metabolism of Palmitate in Isolated Working Hearts From Spontaneously Diabetic "BB" Wistar Rats

Gary D. Lopaschuk and Helen Tsang

Myocardial fatty acid metabolism was studied in spontaneously-diabetic "BB" Wistar rats. The study involved 4 groups: control Wistar rats, nondiabetic littermates of "BB" Wistar rats, insulin-treated diabetic "BB" rats, and diabetic "BB" rats in which insulin treatment was removed 24 hours prior to study (uncontrolled diabetes). Hearts were perfused for 30 minutes as isolated working hearts in perfusate containing 1.2 mM (1-¹⁴C)-palmitate bound to 3% albumin, and 11 mM glucose. Palmitate oxidative rates, calculated as micromoles palmitate oxidized per gram dry weight per minute, were significantly decreased in both diabetic groups (0.447 ± 0.043 and 0.528 ± 0.038 in uncontrolled diabetic and treated diabetic versus 0.584 ± 0.032 and 0.629 ± 0.033 in nondiabetic littermate and control rats, respectively). This decrease was accompanied, however, by a significant decrease in the heart rate of these 2 groups when compared with control or nondiabetic animals. If the decreased heart function in the diabetic animals was accounted for, no decrease in palmitate oxidative rates occurred, suggesting that fatty acid oxidative metabolism is not impaired in the diabetic myocardium. In the uncontrolled diabetic rats, an increased rate of palmitate incorporation into myocardial triglycerides was seen compared with treated diabetic, nondiabetic littermates, and control rats (8.5 ± 0.3 µmol/g dry wt/30 min versus 4.8 ± 0.3, 5.9 ± 0.7, and 5.7 ± 0.3, respectively). Myocardial levels of coenzyme A were elevated in the uncontrolled diabetic rats compared with all other groups (647 ± 25 nmol/g dry wt versus 484 ± 27, 508 ± 56, and 534 ± 9, in treated diabetic, nondiabetic, and control rats, respectively). Combined with earlier studies in which high coenzyme A levels in control hearts also resulted in an increased rate of palmitate incorporation into triglycerides, the data suggest that high levels of myocardial coenzyme A contribute to the accumulation of myocardial triglycerides seen in poorly controlled diabetes. Similarly, contrary to earlier reports, a decrease in the rate of fatty acid oxidation was not observed in diabetic rat hearts. (Circulation Research 1987;61:853–858)
diabetic rat (streptozotocin or alloxan). These animals will survive without insulin treatment and probably are most representative of the non-insulin dependent diabetic. The spontaneously diabetic "BB" Wistar rat is a strain of rat that becomes spontaneously diabetic at 3 months of age due to an acute inflammation and necrosis of the pancreatic islet cells. These animals must subsequently be treated with insulin to survive. They represent a good experimental model of insulin-dependent diabetes mellitus, and it is for this reason that this animal model is used in this study.

Clearly, a better understanding of the relation between mechanical function and the metabolism of fatty acids in the diabetic heart is needed. In this study, oxidation of palmitate (measured by $^{14}$CO$_2$ production from $^3$C-labelled palmitate), incorporation of fatty acids into the endogenous triglyceride pool, and heart function were measured in working hearts from spontaneously diabetic "BB" Wistar rats. The effect of acute removal of insulin treatment on myocardial fatty acid metabolism was also determined.

Materials and Methods

Animals

Spontaneously diabetic "BB" Wistar rats were bred and maintained at the animal care facility of the Hospital for Sick Children in Toronto, Canada. This facility maintains an extensive colony of "BB" rats, now in its 21st generation. At approximately 3 months of age, 60% of these animals spontaneously became diabetic and were subsequently administered daily insulin injections. The dose (1.5 to 2.5 U/day s.c.) was chosen so that no glycosuria or ketonuria occurred. Urine glucose and ketone content, as well as body weight, of both diabetic and nondiabetic littersmates were monitored daily. Animals were used approximately 100 days following the onset of diabetes. In some animals, insulin treatment was removed 24 hours prior to use (uncontrolled diabetes). Nondiabetic littersmates were used as controls as well as weight-matched normal Wistar rats (control). Serum samples were collected from all animals at the time of killing. Serum glucose levels were determined using a Sigma glucose kit (Sigma Chemical Co., St. Louis, Mo.). Serum free fatty acid content was determined as described previously.

Heart Perfusions

Hearts from sodium pentobarbital-anesthetized rats were excised, and the aorta and pulmonary veins were cannulated as described previously. After a 10-minute washout perfusion as a Langendorff preparation, hearts were perfused with recirculated Krebs-Henseleit buffer (pH 7.4) containing 2.5 mM Ca$^{2+}$, 11 mM glucose, and 1.2 mM (1-$^4$C)-palmitate bound to 3% bovine serum albumin. During this perfusion, the hearts were allowed to perform mechanical work, and rates of (1-$^4$C)-palmitate oxidation were determined by measurement of steady-state $^{14}$CO$_2$ production. The perfusate was recirculated through the apparatus, which was closed to ambient air, and was oxygenated by use of an oxygenator with a large surface area in contact with 95% O$_2$-5% CO$_2$ gas mixture. Perfusion was continued for 30 minutes under conditions of low cardiac work (10 cm H$_2$O preload; 60 mm Hg afterload), and rates of palmitate oxidation were determined between 10 and 30 minutes, when rates of $^{14}$CO$_2$ production had reached a steady state.

For measurement of $^{14}$CO$_2$, the gas mixture entered the perfusion apparatus at the top of the closed oxygenated chamber, exited the chamber through an outlet tube, and was bubbled through a 1 M methylbenzothionium hydroxide trap to collect gaseous $^{14}$CO$_2$. Perfusates, used to determine $^{14}$CO$_2$ present as bicarbonate, were removed with a syringe directly from the system without exposure to air and were placed in a 25-ml stoppered flask with a center well containing methylbenzothionium hydroxide. The sample of perfusate was acidified by addition of 9N H$_2$SO$_4$ (1 ml), the flask was shaken for 1 hour, and the methylbenzothionium hydroxide trap was placed in ACS scintillation cocktail to be counted. Both heart rate and systolic pressure development were monitored throughout the perfusion period.

At the end of the perfusion, hearts were freeze-clamped with Wollenberger clamps that had been cooled to the temperature of liquid nitrogen, and the hearts were used for determining tissue levels of metabolites.

Assay of Coenzyme A Esters

Frozen ventricular tissue was powdered using a porcelain mortar and pestle maintained at liquid nitrogen temperatures. Approximately 200 mg of frozen tissue powder was used to determine the dry weight:wet weight ratio. Extraction of CoA was as previously described.

Measurement of Metabolic Intermediates

Tissue lipids were extracted as previously described. Neutral lipids were separated from phospholipids using the methods of Bowyer and King. Triglycerides were separated from other neutral lipids as previously described, using Baker Si50-PA (19C)-Silica Gel plates (Phillipsburg, N.J.) and a solvent system that consisted of isooctane:diethylether:acetic acid (74:24:2 vol/vol/vol). Measurement of (1-$^4$C)-palmitate incorporation into triglycerides and phospholipids was as previously described. Triglyceride content and phospholipid content was determined by measuring free fatty acid content of saponified samples.

Results

Characteristics of the Spontaneously Diabetic "BB" Wistar Rat

The spontaneously diabetic "BB" Wistar rats used in this study were bred as described in "Materials and Methods." These rats were maintained on daily Lente insulin injections for 100 days prior to experimentation. A dose of insulin, 1.5–2 units, was chosen such that glycosuria and ketonuria did not occur. Some
Heart Function in the Spontaneously Diabetic "BB" Wistar Rat

To assess heart function in these rats, hearts were perfused as working hearts using 1.2 mM palmitate and 11 mM glucose as the energy substrates. As shown in Table 2, heart rate was significantly depressed in all "BB" rats compared with weight-matched controls. The decrease in heart rate that was evident in the nondiabetic littersmates may be a characteristic of this strain of rat. To some extent, the lower heart rates are compensated for by an increase in peak systolic pressure development.

If insulin treatment is removed from diabetic rats for a 24-hour period, a significant decrease in heart rate occurs compared with the insulin-treated diabetic. This is reflected in a decrease in the heart rate-peak systolic pressure product. This demonstrates, using carbon substrates the heart sees in vivo, that removal of insulin for even short periods can result in a depression in heart function.

Table 1. Characteristics of Spontaneously Diabetic "BB" Wistar Rats

<table>
<thead>
<tr>
<th>Condition</th>
<th>Serum glucose (mM)</th>
<th>Serum FFA (μM)</th>
<th>Duration of diabetes (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 9)</td>
<td>14.6 ± 0.8</td>
<td>0.28 ± 0.06</td>
<td>. . .</td>
</tr>
<tr>
<td>Nondiabetic littermate (n = 4)</td>
<td>13.3 ± 0.8</td>
<td>0.36 ± 0.03</td>
<td>. . .</td>
</tr>
<tr>
<td>Diabetic insulin-treated (n = 6)</td>
<td>27.7 ± 4.5</td>
<td>0.56 ± 0.09</td>
<td>94 ± 16</td>
</tr>
<tr>
<td>Diabetic uncontrolled (n = 9)</td>
<td>37.2 ± 1.3</td>
<td>3.22 ± 0.6</td>
<td>109 ± 8</td>
</tr>
</tbody>
</table>

Control animals consisted of weight-matched non-"BB" Wistar rats. Nondiabetic littersmates consisted of age-matched "BB" Wistar rats that did not spontaneously become diabetic at 3 months of age.

Insulin-treated diabetic rats received daily subcutaneous injections of Lente insulin at a dose sufficient to prevent glycosuria and ketonuria. Serum glucose and ketones were monitored daily. Uncontrolled diabetic rats consisted of insulin-treated "BB" diabetic rats in which insulin treatment was stopped 24 hours prior to use. Values are mean ± SEM.

Effect of Diabetes on Fatty Acid Metabolism

To determine what effect diabetes has on fatty acid metabolism, diabetic "BB" Wistar rats were perfused as working hearts with (1-14C)-palmitate bound to 3% bovine serum albumin, 11 mM glucose. Hearts were perfused at a constant 10 cm H2O preload and a 60 mm Hg hydrostatic afterload.

Values are mean ± SEM. *Significantly different from control, p<0.05, measured by analysis of variance followed by Student's t test. †Significantly different from all other groups, p<0.05.

Table 2. Heart Function in Spontaneously Diabetic "BB" Wistar Rats Perfused With 1.2 mM Palmitate

<table>
<thead>
<tr>
<th>Condition</th>
<th>Heart rate (HR) (beats/min)</th>
<th>Peak systolic pressure (PSP) (mm Hg)</th>
<th>HR x PSP (× 10^-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 6)</td>
<td>288 ± 14</td>
<td>93 ± 3</td>
<td>26.7 ± 1.0</td>
</tr>
<tr>
<td>Nondiabetic littermate (n = 6)</td>
<td>223 ± 8*</td>
<td>107 ± 3</td>
<td>24.1 ± 0.6*</td>
</tr>
<tr>
<td>Diabetic insulin-treated (n = 5)</td>
<td>213 ± 10*</td>
<td>105 ± 5</td>
<td>22.2 ± 0.5*</td>
</tr>
<tr>
<td>Diabetic uncontrolled (n = 7)</td>
<td>186 ± 5*†</td>
<td>107 ± 6</td>
<td>20.0 ± 1.0†</td>
</tr>
</tbody>
</table>

All hearts received a 10-minute washout perfusion followed by a 30-minute perfusion as working hearts with Krebs-Henseleit buffer containing 1.2 mM (1-14C)-palmitate bound to 3% bovine serum albumin, 11 mM glucose. Hearts were perfused at a constant 10 cm H2O preload and a 60 mm Hg hydrostatic afterload.

Values are mean ± SEM. *Significantly different from control, p<0.05, measured by analysis of variance followed by Student's t test. †Significantly different from all other groups, p<0.05.
insulin-treated diabetic rats, although to a much lesser extent than the uncontrolled diabetic rats. This increase may be a reflection of the slightly elevated serum fatty acids seen in these animals.

CoA levels were also measured in these hearts because CoA levels are important determinants as to the fate of myocardial fatty acids.1 Insulin treatment of diabetic rats maintained myocardial CoA levels within normal limits. However, removal of insulin for 1 day resulted in a significant increase in CoA levels. CoA levels did not increase further in hearts obtained from diabetic rats 48 hours following insulin removal (676 ± 71 nmol/g dry wt). If the dose of insulin necessary to prevent glucosuria and ketonuria was halved for 10 days in diabetic rats, myocardial CoA levels were only slightly elevated (590 ± 3 nmol/g dry wt). For this reason, only 24-hour uncontrolled diabetic rats were used to study fatty acid metabolism. Accompanying the increase in CoA levels in diabetic rats 24 hours after removal of insulin treatments was a significant increase in the rate at which (1-14C)-palmitate was incorporated into the triglyceride pool. This occurred even in the presence of high existing levels of myocardial triglycerides. Previous work has demonstrated that raising CoA levels in control hearts subsequent to perfusion with 1.2 mM palmitate also results in a significant increase in the rate of palmitate incorporation into triglycerides (raising CoA levels from 537 ± 14 to 679 ± 29 nmol/g dry wt resulted in an increase in palmitate incorporation into triglycerides from 8.4 ± 1.5 to 16.9 ± 1.0 μmol g dry wt/30 minutes). These data suggest that removal of insulin from diabetic rats for even short periods of time can increase CoA synthesis resulting in an increased rate of incorporation of fatty acids into the myocardial triglyceride pool.

Discussion

In this study, we report changes that occur in palmitate metabolism in the diabetic rat myocardium. Myocardial CoA levels significantly increase in spontaneously diabetic “BB” Wistar rats within 24 hours of the removal of insulin treatment. This is accompanied by an increase in the rate in which palmitate is incorporated into a greatly expanded myocardial triglyceride pool. Fatty acid oxidative rates are not

![FIGURE 1. Palmitate oxidation in spontaneously diabetic “BB” Wistar rats. All hearts received a 10-minute washout perfusion followed by a 30-minute perfusion as working hearts with Krebs-Henseleit buffer containing 1.2 mM (1-14C)-palmitate bound to 3% bovine serum albumin, 11 mM glucose. Hearts were perfused at a constant 10 cm H2O preload and a 60 mm Hg hydrostatic afterload. Steady-state 14CO2 production was measured between 10 and 30 minutes. Values are mean ± SEM of at least 6 hearts in each group. * Significantly different from control; †significantly different from control and nondiabetic littermate.](image)

![FIGURE 2. Palmitate oxidation as a function of work in spontaneously diabetic rat hearts. Hearts are the same as those used in Figure 1. Palmitate oxidative rates were normalized for differences in the heart rate-peak systolic pressure development product in these hearts. Values are the mean ± SEM of at least 6 hearts in each group.](image)

Table 3. Effect of Insulin Added Directly to the Perfusion Buffer on Palmitate Oxidation in Control and Spontaneously Diabetic “BB” Wistar Rats

<table>
<thead>
<tr>
<th>Condition</th>
<th>Palmitate oxidation 14CO2 production (μmol/g dry wt/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Control</td>
<td></td>
</tr>
<tr>
<td>− insulin (n = 6)</td>
<td>0.629 ± 0.033</td>
</tr>
<tr>
<td>+ insulin (n = 5)</td>
<td>0.483 ± 0.070*</td>
</tr>
<tr>
<td>B. Uncontrolled diabetic</td>
<td></td>
</tr>
<tr>
<td>− insulin (n = 8)</td>
<td>0.447 ± 0.043*</td>
</tr>
<tr>
<td>+ insulin (n = 6)</td>
<td>0.480 ± 0.039*</td>
</tr>
</tbody>
</table>

Hearts were perfused as described in Table 2. Insulin, when present, was added to the perfusion medium at a concentration of 300 μU/ml. Values are mean ± SEM. *Significantly different than the control in the absence of insulin, p<0.05.
It has long been known that triglyceride levels are markedly elevated in the hearts of diabetics. Elevated serum levels of free fatty acids and triglycerides have been suggested to account for this increase in myocardial triglycerides. Increases in myocardial long chain acyl CoA levels and a resultant inhibition of triglyceride hydrolase may also contribute to this increase in triglycerides. We are proposing that a third possible contributing factor is an increase in myocardial CoA levels that occurs in the diabetic. We have previously demonstrated that elevating CoA levels in the normal heart results in a significant increase in the rate at which fatty acids are incorporated into triglycerides. This probably occurs as a result of an increase in the ratio of cytosolic CoA to carnitine, resulting in the shunting of exogenous fatty acids away from mitochondrial oxidation and toward triglyceride synthesis. In this study, we demonstrate that a similar situation arises in diabetic rats if insulin treatment is removed for even short periods of time. Removal of insulin resulted in a significant decrease in heart rate in these fatty acid perfused hearts.

It should be noted that only palmitate metabolism was studied in these diabetic rats. One must be careful in extrapolating results with palmitate to include all fatty acids, especially in these uncontrolled diabetic rats, which have a markedly expanded endogenous triglyceride pool. Rosen et al. have demonstrated, however, that palmitate is the predominant fatty acid that accumulates in the myocardium of the diabetic. It would appear justified, therefore, to use palmitate to study fatty acid metabolism in the diabetic since it is readily oxidized by the heart. A number of experimental studies using the isolated working heart have demonstrated the presence of diabetic cardiomyopathies in humans and chemically induced diabetic rats. These functional studies, however, have been performed using glucose as the primary substrate for oxidation.
Finally, we demonstrate that fatty acid oxidation rates depressed in spontaneously diabetic "BB" Wistar rats are also demonstrated to be significantly decreased if insulin treatment is removed. We demonstrate that heart rate is significantly decreased if insulin treatment is removed. In their study, however, the control hearts also appeared to have a depressed function in the presence of fatty acids, a situation that is not seen in the working rat heart. In our study, removing insulin treatment from spontaneously diabetic "BB" Wistar rat hearts perfused with fatty acids resulted in a decrease in heart rate, with no change in peak systolic pressure development. These changes parallel what is seen in glucose perfused rat hearts 48 hours after inducing diabetes with alloxan. It would be desirable, in future studies, to determine what effect various substrates have on heart function in these "BB" rats.

In summary, we have characterized fatty acid metabolic changes that occur in the spontaneously diabetic rat. We demonstrate that removal of insulin treatment from diabetic rat hearts results in an increased rate of fatty acid incorporation into endogenous triglycerides, probably as a result of an increase in myocardial CoA levels. We also demonstrate that heart rate is significantly depressed in spontaneously diabetic "BB" Wistar rats and is further decreased if insulin treatment is removed. Finally, we demonstrate that fatty acid oxidation rates are not decreased in the diabetic rat and that apparent decreases in oxidative metabolism are attributable to the depression in heart function.

References


Key Words • coenzyme A • fatty acid oxidation • triglyceride accumulation • diabetes
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