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 (l-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 /umol/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 $^{13}$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 littermates 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 littermates 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-$^{14}$C)-palmitate bound to 3% bovine serum albumin. During this perfusion, the hearts were allowed to perform mechanical work, and rates of (1-$^{14}$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 oxygencerated chamber, exited the chamber through an outlet tube, and was bubbled through a 1 M methylbenzothonium hydroxide trap to collect gaseous $^{14}$CO$_2$. Perfusate samples, 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 methylbenzothonium 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 methylbenzothonium 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-$^{14}$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
characteristics of these rats are presented in Table 1. Unfortunately, insulin treatment of the diabetic rats did not maintain normal serum glucose levels. This may be a reflection of the fact that animals were killed in the morning, before the daily dose of insulin was administered. Regardless, serum free fatty acid levels were only slightly higher than control. If insulin treatment was removed one day prior to study, both serum glucose and serum free fatty acids were markedly elevated. Serum glucose and fatty acids remained high in diabetic rats if insulin treatment was withheld for 48 or 72 hours. By 48 hours, however, animals became severely ketotic, and their physical condition deteriorated rapidly. For this reason, only diabetic rats in which insulin treatment was removed for 24 hours were used. As shown in Table 1, rats were used approximately 100 days after the onset of diabetes.

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>
<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 1.2 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.

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 1.2 mM (1-14C)-palmitate bound to 3% bovine serum albumin, 11 mM glucose. Oxidative rates of palmitate were then determined by quantitative measurement of 14CO2 production since 11 mM glucose was present in all perfusion solutions. In hearts from uncontrolled diabetic rats, however, oxidative rates of palmitate were now significantly depressed compared with both control rats and nondiabetic littersmates (Figure 1). This was based on calculations of 14CO2 production/g dry heart tissue/minute. Since palmitate oxidative rates are markedly dependent on the work performed by the heart, oxidative rates were also calculated per unit of heart work. As shown in Figure 2, palmitate oxidative rates were not depressed if normalized for heart rate–peak systolic pressure differences. This suggests that palmitate oxidative rates are not depressed in hearts from either insulin-treated or uncontrolled diabetic rat hearts but simply decrease as a function of the work performed by the heart.

Palmitate oxidative rates were also determined in hearts in which insulin was added directly to the perfusion medium (Table 3). In control hearts, insulin (500 μU/ml) resulted in a significant decrease in palmitate oxidation. This probably reflects an increase in glucose oxidation since 11 mM glucose was present in all perfusion solutions. In hearts from uncontrolled diabetic rats, however, insulin added to the perfusion medium did not result in a further decrease in oxidative rates. In fact, oxidative rates of palmitate were now similar in both control hearts and uncontrolled diabetic hearts perfused with insulin.

In addition to palmitate oxidation, other parameters of fatty acid metabolism were measured (Table 4). Confirming what has been shown in other diabetic animal models, myocardial triglyceride levels were markedly elevated in diabetic rats. The increase in triglyceride levels was most evident in the uncontrolled diabetic rat. Triglyceride levels were also elevated in...
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.8 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 triglycerides (raising CoA levels 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.

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 500 μU/ml. Values are mean ± SEM. *Significantly different than the control in the absence of insulin, p<0.05.
decreased in either the insulin-treated diabetic rats or diabetic rats in which insulin treatment has been removed. Although overall oxidative rates were reduced if expressed as palmitate oxidized per heart weight, this could be explained by a decrease in the work performed by the diabetic rat hearts. Removal of insulin treatment for 24 hours in diabetic rats resulted in a significant decrease in heart rate in these fatty acid perfused hearts.

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 increase in myocardial CoA levels accompanied by an increase in the rate at which exogenous fatty acids are incorporated into triglycerides. This increase in the rate of fatty acid incorporation into triglycerides occurs independent of serum fatty acid concentrations since all hearts were perfused with the same concentration of palmitate. Interestingly, even though myocardial triglycerides were markedly elevated in the uncontrolled diabetic rat, fatty acids were still incorporated into triglycerides at a significantly faster rate. These results confirm our original hypothesis that elevating myocardial CoA levels results in fatty acids being directed toward triglyceride synthesis.

Although the diabetic heart is more dependent on fatty acid oxidation it was not clear if actual oxidative rates of fatty acid are decreased in these hearts. If oxidative metabolism is depressed, it is conceivable that the diabetic heart would not be able to meet the energy demands of the heart at high workloads or under stressful conditions. Kreisberg reported a decrease in oxidation of triglycerides in Langendorff perfused hearts, which he attributed to an increased utilization of endogenous lipids. Paulson and Crass, also using Langendorff perfused hearts, demonstrated that fatty oxidation was unchanged or enhanced in the diabetic rat, depending on the substrate used. Neither of these studies, however, assessed heart function in these diabetic animals. This is important since oxidative rates of fatty acid are extremely dependent on the work performed in the heart. Chen et al also did not see a decrease in fatty acid oxidation in isolated myocytes from diabetic rats, but the inability to measure myocyte contractile activity also complicates these studies. In our study, we did notice a decrease in fatty acid oxidative rates in spontaneously diabetic “BB” Wistar rats perfused with a concentration of fatty acid seen in vivo. If we account for the lower heart work performed by these hearts, however, no change in fatty acid oxidation occurs. It is interesting that oxidative rates of exogenous fatty acid were similar, even in hearts containing high levels of endogenous triglycerides (uncontrolled diabetics). If, as suggested by other workers, an enhanced myocardial turnover of triglycerides is occurring in the diabetic rats, then overall oxidative rates of fatty acids may actually increase in the diabetic myocardium.

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...
sole perfusion substrate. It must be considered that exogenous substrate supply and utilization by the diabetic heart contribute to the functional changes. For instance, Fields et al demonstrated that diabetic rabbit hearts are more sensitive to possible deleterious effects of high fatty acids. 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.\(^5\) 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.\(^6\) 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 rats 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

Metabolism of palmitate in isolated working hearts from spontaneously diabetic "BB" Wistar rats.
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