Hepatic Glucose Cycling Does Not Contribute to the Development of Hyperglycemia in Zucker Diabetic Fatty Rats

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Hepatic glucose cycling, whereby glucose is taken up by the liver, partially metabolized, then recycled to glucose, makes a substantial contribution to the development of hyperglycemia in IDDM. This stimulation of glucose cycling appears to be associated with elevated rates of fatty acid oxidation. Whether hepatic glucose cycling also contributes to the development of hyperglycemia in NIDDM is unclear. Using a model of NIDDM, the Zucker diabetic fatty (ZDF) rat, we determined whether glucose cycling was enhanced. Hepatocytes from ZDF rats exhibited higher rates of glucose phosphorylation and glycolysis, but there was no increase in the rate of cycling between glucose and glucose-6-phosphate or between glycolytically derived pyruvate and glucose. Despite the increased rates of glycolysis, the production of CO_2 in liver cells from ZDF rats was no different from rates measured in cells from control animals. Instead, there was a large increase in the accumulation of lactate and pyruvate in the ZDF liver cells. The addition of 2-bromopalmitate, an inhibitor of fatty acid oxidation that inhibited glucose cycling in hepatocytes from IDDM rats, had no effect on glucose cycling in cells from ZDF rats. We therefore conclude that, unlike in IDDM, hepatic glucose cycling does not contribute to the development of hyperglycemia in the NIDDM Zucker rat. *Diabetes* 48:342–346, 1999

Hepatic glucose cycling makes a substantial contribution to the development of hyperglycemia in streptozotocin-induced insulin-dependent diabetic rats (1). Cycling between glucose and glucose-6-phosphate (the G/G-6-P cycle), whereby glucose is taken up by the liver, phosphorylated, and subsequently dephosphorylated, is enhanced. In addition, cycling between glycolytically derived pyruvate and glucose (the G/P cycle) is also substantially higher in diabetic rats. As a result of the increased activity of these cycles, 80% of the glucose taken up by liver cells from diabetic rats is eventually recycled to the extracellular medium, compared with only 40–50% in hepatocytes from normal rats. The stimulation of G/G-6-P and G/P cycling is at least partly the result of the elevated rates of fatty acid oxidation in liver cells from diabetic rats (1).

Whether hepatic glucose cycling also contributes to the development of hyperglycemia in NIDDM is unclear. There is evidence that humans with NIDDM have increased rates of G/G-6-P cycling (2,3), but studies with an animal model of NIDDM and obesity, the Zucker diabetic fatty (ZDF) rat, have produced contradictory results. Chatzidakis et al. (4) reported no difference in whole animal rates of G/G-6-P cycling in ZDF rats and their lean, nondiabetic littermates. In contrast, Wade (5) reported that rates of G/P cycling are higher in obese than lean Zucker rats.

The obesity and NIDDM that characterize the ZDF rat are the result of a mutation in the leptin receptor (6). Since the isolation and characterization of the leptin gene in 1994 (7), a substantial body of evidence has accumulated to suggest that leptin plays a pivotal role in the regulation of appetite, metabolic rate, and body composition (8,9). Leptin is produced almost exclusively by white adipose tissue, and its concentration in the plasma is proportional to the size of the adipose tissue stores (10–12), suggesting that leptin may act as a "lipostat" signal (7). As a consequence of the inability to respond to leptin (13), the ZDF rat is hyperphagic and obese and invariably develops NIDDM (14). In addition, hepatocytes isolated from ZDF rats exhibit increased lipogenesis, glycogen synthesis, and glycolysis and decreased gluconeogenesis, glycogenolysis, and fatty acid oxidation (15). The ZDF rat is also hyperinsulinemic, insulin resistant, and hypertriglyceridemic (14).

The mechanism whereby a defect in the leptin signaling pathway induces NIDDM in the ZDF rat is uncertain, and the contribution of hepatic glucose cycling to the development of hyperglycemia in ZDF rats has not been determined. Therefore, we compared the activities of the G/G-6-P cycle and the G/P cycle in hepatocytes from lean and ZDF rats. Because the stimulation of hepatic glucose cycling measured in hepatocytes from IDDM rats was associated with elevated rates of fatty acid oxidation (1), we also examined whether glucose cycling in hepatocytes from ZDF rats was dependent on fatty acid oxidation.

RESEARCH DESIGN AND METHODS

Materials. [U-^{14}C]glucose and high-performance liquid chromatography (HPLC)-purified [2-^{3}H]glucose and [6-^{3}H]glucose were obtained from New England Nuclear (Beverly, MA). DL-2-Bromopalmitate, which was bound to defatted albumin (16), was purchased from Fluka (Buchs, Switzerland). All enzymes and cofactors required for the enzymatic determination of metabolites were from Boehringer Mannheim (Mannheim, Germany). Other chemicals were of the

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Received for publication 28 April 1998 and accepted in revised form 30 October 1998.

G-6-P, glucose-6-phosphate; ZDF, Zucker diabetic fatty.

TABLE 1
Body weight, blood glucose, triacylglycerol, and ketone body (acetoacetate and 3-hydroxybutyrate) concentrations in 12-week-old ZDF and lean Zucker rats

	Weight (g)	Glucose (mmol/l)	Triacylglycerol (mmol/l)	Ketones (mmol/l)
Lean Zucker rats	247.2 ± 10.6	7.62 ± 0.67	0.20 ± 0.02	0.81 ± 0.13
ZDF rats	368.3 ± 24.9*	13.80 ± 1.12*	3.02 ± 0.50*	0.35 ± 0.14*

Data are means ± SE for four animals in each group. *P < 0.05 vs. lean Zucker rats.

highest grade commercially available. Zucker rats were obtained from Monash University Animal Services (Melbourne, Australia).

Liver cell isolation and incubation. All procedures using animals were approved by the animal ethics committees of the University of Queensland and the Flinders University of South Australia. Twelve-week-old lean and obese Zucker rats were fasted for 24 h and anesthetized with 60 mg/kg pentobarbital (Nembutal) before hepatocyte preparation. Isolated liver cells were prepared as described (17), a modification of the method of Berry and Friend (18). Hepatocytes isolated from lean Zucker rats were designated lean hepatocytes or lean liver cells, and those from ZDF rats, ZDF liver cells or ZDF hepatocytes. Liver cells (~100 mg wet wt) were incubated in a final volume of 2 ml with 40 mmol/l glucose containing 1 μCi [2-^{3}H]glucose or 1 μCi [6-^{3}H]glucose and 0.5 μCi [U-^{14}C]glucose. Such an elevated concentration of glucose was used because the $S_{0.5}$ of glucokinase for glucose is raised in in vitro preparations (19). In experiments in which $^{14}CO_2$ generation was measured, duplicate incubations were carried out in sealed vials, perchloric acid was injected through the seal at the end of the incubation period, and $^{14}CO_2$ was collected in phenethylamine (0.5 ml) for subsequent counting. Measurements of glucose, lactate, pyruvate, acetoacetate, and 3-hydroxybutyrate were carried out by standard enzymatic techniques (20) on a Cobas FARA analyzer (Roche, Basel).

Measurement of substrate cycling. Radiolabeled products of glucose metabolism (lactate, pyruvate, amino acids, and water) were separated from glucose by ion exchange chromatography (21,22). Radiolabeled glycogen was measured as described previously (23). Rates of glucose phosphorylation were measured as the sum of ^3H_2O released from [2-^{3}H]glucose plus the amount of tritiated glycogen formed (21). The tritiated glycogen measurement was included to decrease the error resulting from incomplete equilibration between G-6-P and fructose-6-phosphate (22,24). Glycolytic rates were measured using [6-^{3}H]glucose and were determined from the sum of tritium recovered in water, lactate, pyruvate, and amino acids (21). The rate of G/G-6-P cycling was calculated from the difference between the rates of glucose phosphorylation and total [^{3}H]glucose metabolism (measured as the sum of the rate of glycolysis and the rate of incorporation of tritium, derived from [6-^{3}H]glucose, into glycogen) (21). The rate of glycolysis measured with [6-^{3}H]glucose was higher than the rate of accumulation of [^{14}C]glycolytic products (lactate, pyruvate, amino acids, and CO_2) (21). This discrepancy reflects the recycling of glycolytic products back to glucose (G/P cycle) (25–28). In all experiments, there was >95% recovery of isotope. Because hepatocytes from lean and obese Zucker rats store varying quantities of lipids, results of this study have been expressed as micromoles per gram fat-free dry weight. Fat-free dry weights were prepared by carrying out an initial precipitation with perchloric acid, then extracting lipids from the pellet with chloroform/methanol.

Treatment of blood samples. Blood samples were drawn from the inferior vena cava at the time of hepatocyte preparation, and glucose and ketone body concentrations were measured on acid-precipitated, neutralized extracts of plasma. Triacylglycerol concentrations in plasma were measured with a Hitachi 917 using an enzymatic colorimetric test from Boehringer Mannheim.

All results are expressed as the mean ± SE and statistical analyses were carried out using Student's t test for unpaired data.

RESULTS

Development of diabetes in ZDF rats. At 5 weeks of age, ZDF rats and their lean littermates had similar body weights and blood glucose concentrations (body wt 141 ± 8 g in ZDF [n = 4] and 141 ± 11 g in lean rats [n = 4]; blood glucose 6.75 ± 0.68 mmol/l in ZDF [n = 4] and 6.02 ± 0.50 mmol/l in lean rats [n = 4]). By 8 weeks of age, ZDF rats had become hyperglycemic (blood glucose 10.79 ± 1.43 mmol/l [n = 4]) in contrast with lean rats, which maintained a blood glucose concentration of 5.40 ± 0.62 mmol/l (n = 3; P < 0.05 vs. ZDF rats). ZDF rats were significantly heavier than their lean littermates (225 ± 13 g [n = 4] and 172 ± 9 g [n = 4], respectively; P < 0.05). To ensure that diabetes was fully established, the rats used in these studies were 12 weeks of age.

At 12 weeks, ZDF rats were about 50% heavier than lean littermates (Table 1) and displayed the characteristics of NIDDM. The ZDF rats were hyperglycemic with fasting blood glucose concentrations twice those measured in lean rats and plasma triacylglycerol concentrations that were 15-fold higher than those in lean animals. In contrast, ketone body concentrations were significantly lower in the ZDF rats.

Endogenous metabolism. Hepatocytes isolated from 12-week-old ZDF rats contained 15 times more glycogen than cells from lean animals (Table 2). This glycogen was metabolized to glucose and lactate plus pyruvate in ZDF cells, whereas in hepatocytes from lean animals there was no net synthesis of glucose or lactate plus pyruvate. Endogenous ketone body synthesis was threefold higher in lean hepatocytes than in ZDF liver cells, whereas ATP concentrations were similar in both cell types.

Hepatic glucose metabolism. To compare the hepatic metabolism of glucose in ZDF and lean rats, hepatocytes from fasted animals were incubated with 40 mmol/l [2-^{3}H], [6-^{3}H], or [U-^{14}C]glucose for 60 min. The amount of glucose that was phosphorylated was significantly greater in ZDF hepatocytes compared with cells from lean rats (Table 3). Similarly, glycolysis and the accumulation of glycolytic products was also much greater in hepatocytes from ZDF rats. Even

TABLE 2
Production of glucose, ketone bodies (acetoacetate and 3-hydroxybutyrate), and lactate plus pyruvate (Lac + Pyr) and the concentrations of ATP and glycogen in hepatocytes from 24 h–fasted ZDF and lean Zucker rats

	Glucose	Ketones	ATP	Glycogen	Lac + Pyr
Lean hepatocytes	0 ± 0	128 ± 30.4	9.23 ± 3.06	33.7 ± 19.2	2.40 ± 2.94
ZDF hepatocytes	286 ± 15.8*	42.8 ± 16.7*	11.3 ± 1.8	483 ± 107*	80.3 ± 12.9*

Data are means ± SE for four experiments in each case. Data for glucose, ketone body, and Lac + Pyr production are expressed as micromoles per gram fat-free dry weight per hour, and for ATP and glycogen concentrations, as micromoles per gram fat-free dry weight. *P < 0.05 vs. lean hepatocytes.

though there was no significant increase in the overall rates of glucose cycling, the proportion of G-6-P and glycolytically derived pyruvate that was recycled to glucose was lower in ZDF hepatocytes. The production of $^{14}CO_2$ from [U-^{14}C]glucose was similar in hepatocytes from ZDF and lean Zucker rats. In contrast, the accumulation of lactate plus pyruvate was much greater in ZDF hepatocytes. The rate of incorporation of both [U-^{14}C]glucose and [6-^{3}H]glucose into glycogen was similar in hepatocytes from ZDF and lean rats; however, the rate of synthesis of glycogen from [U-^{14}C]glucose was about twice that of [6-^{3}H]glucose in both cell types.

To study the possible interaction of fatty acid oxidation with glucose metabolism, ZDF hepatocytes were incubated with glucose in the absence and presence of an inhibitor of carnitine acyl transferase, 2-bromopalmitate (29). The inhibitor had no effect on glucose phosphorylation, glycolysis, the accumulation of glycolytic products, or flux through the G/G-6-P or G/P cycles. The inhibitor did, however, substantially reduce the rate of production of $^{14}CO_2$ from [U-^{14}C]glucose, but had no effect on the accumulation of lactate plus pyruvate. The addition of 2-bromopalmitate also had no effect on the rate of incorporation of either [U-^{14}C]glucose or [6-^{3}H]glucose into glycogen.

DISCUSSION

Relationship between leptin deficiency and NIDDM. The ZDF rat, which carries a mutation in the leptin receptor, is a well-established model of obesity and NIDDM. It is unclear whether the inability of the animal to respond to leptin directly precipitates hyperglycemia or whether hyperglycemia is secondary to perturbations in lipid metabolism. For example, the high concentrations of triacylglycerol that accumulate within pancreatic β-cells in ZDF rats may be cytotoxic and may induce insulin resistance and subsequently NIDDM (30–32).

In humans, there is also a well-established association between obesity and the acquisition of NIDDM, although the molecular mechanism and the role of leptin in this association also remain to be elucidated. A number of studies have concluded that hepatic glucose cycling plays a role in the development of hyperglycemia in humans with NIDDM (2,3,33), although this is not always observed (34). It has not been demonstrated whether the defect in the leptin signaling pathway in ZDF rats is associated with alterations in the rate of hepatic glucose cycling, with reports of both an increase (5) and a decrease (4) in glucose cycling. We therefore set out to determine whether increased hepatic glucose cycling could contribute to the development of hyperglycemia in ZDF rats.

Comparison of metabolism in ZDF and lean Zucker rats. At 12 weeks of age, the symptoms of obesity and NIDDM were well established in the ZDF rat (Table 1). Examination of basal metabolism in hepatocytes from ZDF and lean Zucker rats confirmed that hepatic glycogen stores were expanded in ZDF hepatocytes and were not depleted after 24-h fasting (Table 2). Such resistance of glycogen stores to fasting has been reported previously (15). The increased production of glucose, lactate, and pyruvate in ZDF hepatocytes relative to lean controls suggests that the ZDF hepatocytes were primarily using these glycogen stores for energy. It is unlikely that gluconeogenesis contributed significantly to the accumulation of glucose seen in these incubations. There was no evidence that hepatocytes from lean rats were able to carry out gluconeogenesis in the absence of added substrate (Table 2), and rates of gluconeogenesis are reported to be lower in ZDF than lean Zucker rats (15). Hepatocytes from lean animals with much reduced glycogen reserves synthesized ketone bodies at a higher rate than the ZDF rats, suggesting that lean rats relied on endogenous fatty acid oxidation, rather than glycogen breakdown, to meet basal energy requirements. As ATP concentrations were similar in the two cell types, there is no evidence that the fatty phenotype compromises cellular energy production.

Glucose cycling. Glucose phosphorylation was increased in ZDF hepatocytes relative to control cells, suggesting that glucokinase activity is increased in these animals (Table 3). The increased production of G-6-P was associated with an increase in flux through the glycolytic pathway, but the activity of the G/G-6-P cycle was unchanged. Indeed, the relative proportion of G-6-P formed that was recycled to glucose was about 30% lower in ZDF hepatocytes. The inability of ZDF hepatocytes to increase G/G-6-P cycling, despite the twofold increase in the production of glucose G-6-P, suggests that the capacity of glucose-6-phosphatase may be rate-limiting in

TABLE 3
Results of hepatocytes from lean Zucker rats and ZDF rats after incubation with 40 mmol/l glucose with or without 2-bromopalmitate

	Glucose alone		Glucose + 2-bromopalmitate
	Lean hepatocytes	ZDF hepatocytes	ZDF hepatocytes
Glucose phosphorylation	325 ± 82*	578 ± 58*	643 ± 53
Glycolysis	185 ± 43	386 ± 31*	431 ± 52
Glycolytic products	121 ± 28	322 ± 9*	328 ± 17
G/G-6-P cycle	110 ± 30 (34)	138 ± 24 (24)	192 ± 8 (30)
G/P cycle	64 ± 16 (35)	80 ± 12 (21)	103 ± 35 (24)
Incorporation of [6-^{3}H]glucose into glycogen	32.1 ± 13.7	46.7 ± 22.3	53.1 ± 17.1
Incorporation of [U-^{14}C]glucose into glycogen	60.4 ± 24.9	112.8 ± 24.9	103.7 ± 40.1
$^{14}CO_2$	417 ± 94	456 ± 48	69 ± 14†
Lactate plus pyruvate synthesis	144 ± 32	500 ± 28*	531 ± 58

Data are means ± SE for four experiments in each case. Results are expressed as micromoles per gram fat-free dry weight per hour. For G/G-6-P cycle, data in parentheses are the percent of phosphorylated glucose recycled; for G/P cycle, data in parentheses are the percent of glucose molecules metabolized to pyruvate and subsequently recycled to glucose. *P < 0.05 vs. lean hepatocytes; †P < 0.05 vs. ZDF hepatocytes without 2-bromopalmitate.

ZDF rats. In addition, there was no significant difference in the rate of incorporation of either [U-^{14}C]glucose or [6-^{3}H]glucose into glycogen, suggesting that there is not a generalized upregulation of all pathways of G-6-P metabolism in ZDF rats; rather, glycolysis is preferentially increased. The discrepancies observed between the rate of incorporation of [U-^{14}C]glucose or [6-^{3}H]glucose into glycogen probably reflect the activity of the indirect pathway of glycogen synthesis (35). As tritium is lost during the intramitochondrial metabolism of [^{3}H]pyruvate derived from [6-^{3}H]glucose, the synthesis of glycogen from [6-^{3}H]glucose represents only the direct incorporation of glucose into glycogen. In contrast, glycogen synthesis from [U-^{14}C]glucose can occur via both the direct and indirect routes. In both lean and ZDF rats, glycogen synthesis via the indirect route appeared to account for about half of the total glycogen synthesis.

Despite the increased glycolytic flux observed in ZDF hepatocytes, the production of CO_2 and the activity of the G/P cycle was not enhanced in those cells relative to controls. Instead, there was an increased accumulation of lactate plus pyruvate. Both the oxidation of pyruvate to CO_2 and the G/P cycle require the uptake of pyruvate into the mitochondria, whereas the production of lactate plus pyruvate is a cytoplasmic process. This suggests that the capacity of ZDF cells to transport pyruvate into the mitochondria may be rate-limiting, perhaps as a result of the finite capacity of the pyruvate translocator, which has been shown to regulate the rate of uptake of pyruvate into the mitochondria (36,37). Alternatively, the capacity of either pyruvate dehydrogenase or pyruvate carboxylase to metabolize pyruvate may be limiting in ZDF hepatocytes.

In hepatocytes from streptozotocin-induced insulin-dependent diabetic rats, ~80% of the glucose taken up into liver cells was eventually recycled to glucose (1). In contrast, ZDF hepatocytes recycled <40% of the glucose that entered the cell. Hepatocytes from lean rats recycled ~50% of the glucose molecules that were taken up. Therefore, it appears that the hyperglycemia observed in ZDF rats is not associated with increased rates of hepatic glucose cycling.

Effect of fatty acid metabolism. 2-Bromopalmitate inhibited both G/G-6-P and G/P cycling in IDDM rats, leading to an increase in glycolytic flux and the accumulation of glycolytic products (1). These observations led us to propose that elevated rates of endogenous fatty acid oxidation stimulate glucose cycling in IDDM rats. 2-Bromopalmitate had no significant effect on glucose cycling, glycolysis, or glucose phosphorylation in hepatocytes from ZDF rats, implying that fatty acid oxidation played no role in regulating the rate of flux through these pathways. These results are similar to those observed in hepatocytes from normal rats (1). The inability of 2-bromopalmitate to affect these pathways may be because endogenous fatty acid oxidation (measured as ketone body production) is lower in ZDF hepatocytes than in control cells (Table 2). The inhibitor reduced $^{14}CO_2$ production from glucose, however, an effect not seen in hepatocytes from insulin-dependent rats, although such an inhibition was observed in normal cells (1). The mechanism of this effect of 2-bromopalmitate is unclear.

The results reported in this article demonstrate that, in contradiction to studies on humans with NIDDM, hyperglycemia in ZDF rats is not associated with an increase in hepatic glucose cycling. Indeed, the capacity of the liver to dispose of glucose as lactate plus pyruvate is enhanced in these animals.

ACKNOWLEDGMENTS

This work was partially funded by the National Health and Medical Research Council of Australia, the Flinders University of South Australia Research Budget, and Flinders 2000.

We gratefully acknowledge the skilled technical assistance of R. Norris, D. Doherty, and E. Williams.

REFERENCES

1. Henly DC, Phillips JW, Berry MN: Suppression of glycolysis is associated with an increase in glucose cycling in hepatocytes from diabetic rats. *J Biol Chem* 271:11268–11271, 1996
2. Rooney DP, Neely RD, Beatty O, Bell NP, Sheridan B, Atkinson AB, Trimble ER, Bell PM: Contribution of glucose/glucose 6-phosphate cycle activity to insulin resistance in type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 36:106–112, 1993
3. Efendic S, Wajngot A, Vranic M: Increased activity of the glucose cycle in the liver: early characteristic of type 2 diabetes. *Proc Natl Acad Sci U S A* 82:2965–2969, 1985
4. Chatzidakis C, Lazarus DD, Stein TP: Substrate cycling in lean and obese Zucker rats. *Int J Obes Relat Metab Disord* 18:287–293, 1994
5. Wade AJ: Glucose metabolism and recycling of radioactively labelled glucose in the Zucker genetically obese rat (*fa/fa*). *Biochem J* 186:161–168, 1980
6. Phillips MS, Liu Q, Hammond HA, Dugan V, Hey PJ, Caskey CJ, Hess JF: Leptin receptor missense mutation in the fatty Zucker rat. *Nat Genet* 13:18–19, 1996
7. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM: Positional cloning of the mouse obese gene and its human homologue. *Nature* 372:425–432, 1994
8. Flier JS: Leptin expression and action: new experimental paradigms. *Proc Natl Acad Sci U S A* 94:4242–4245, 1997
9. White DW, Tartaglia LA: Leptin and OB-R: body weight regulation by a cytokine receptor. *Cytokine Growth Factor Rev* 7:303–309, 1996
10. Mohamed Ali V, Pinkney JH, Panahloo A, Goodrick S, Coppack SW, Yudkin JS: Relationships between plasma leptin and insulin concentrations, but not insulin resistance, in non-insulin-dependent (type 2) diabetes mellitus. *Diabet Med* 14:376–380, 1997
11. Oksanen L, Ohman M, Heiman M, Kainulainen K, Kaprio J, Mustajoki P, Koivisto V, Koskenvuo M, Janne OA, Peltonen L, Kontula K: Markers for the gene ob and serum leptin levels in human morbid obesity. *Hum Genet* 99:559–564, 1997
12. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, Caro JF: Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 334:292–295, 1996
13. Yamashita T, Murakami T, Iida M, Kuwajima M, Shima K: Leptin receptor of Zucker fatty rat performs reduced signal transduction. *Diabetes* 46:1077–1080, 1997
14. Shafrir E: Animal models of non-insulin-dependent diabetes. *Diabetes Metab Rev* 8:179–208, 1992
15. McCune SA, Durant PJ, Jenkins PA, Harris RA: Comparative studies on fatty acid synthesis, glycogen metabolism, and gluconeogenesis by hepatocytes isolated from lean and obese Zucker rats. *Metabolism* 30:1170–1178, 1981
16. Chen RF: Removal of fatty acids from serum albumin by charcoal treatment. *J Biol Chem* 242:173–181, 1967
17. Berry M, Edwards A, Barritt G: *Isolated Hepatocytes: Preparation, Properties and Application*. Burdon R, Van Knippenber P, Eds. Amsterdam, Elsevier, 1991
18. Berry MN, Friend DS: High-yield preparation of isolated rat liver parenchymal cells: a biochemical and fine structural study. *J Cell Biol* 43:506–520, 1969
19. Bontemps F, Hue L, Hers HG: Phosphorylation of glucose in isolated rat hepatocytes: sigmoidal kinetics explained by the activity of glucokinase alone. *Biochem J* 174:603–611, 1978
20. Bergmeyer HU: *Methods in Enzymatic Analysis*. 3rd ed. Bergmeyer H, Bergmeyer J, Grassl M, Eds. Weinheim, Germany, Verlag Chemie, 1983
21. Berry MN, Phillips JW, Henly DC, Clark DG: Effects of fatty acid oxidation on glucose utilization by isolated hepatocytes. *FEBS Lett* 319:26–30, 1993
22. Clark DG, Rognstad R, Katz J: Isotopic evidence for futile cycles in liver cells. *Biochem Biophys Res Commun* 54:1141–1148, 1973
23. Phillips JW, Clark DG, Henly DC, Berry MN: The contribution of glucose cycling to the maintenance of steady-state levels of lactate by hepatocytes during glycolysis and gluconeogenesis. *Eur J Biochem* 227:352–358, 1995
24. Katz J, Wals PA, Rognstad R: Glucose phosphorylation, glucose-6-phosphatase, and recycling in rat hepatocytes. *J Biol Chem* 253:4530–4536, 1978

25. Hue L: The role of futile cycles in the regulation of carbohydrate metabolism in the liver. *Adv Enzymol Relat Areas Mol Biol* 52:247–331, 1981
26. Clark DG, Filsell OH, Topping DL: Effects of fructose concentration on carbohydrate metabolism, heat production and substrate cycling in isolated rat hepatocytes. *Biochem J* 184:501–507, 1979
27. Clark D, Lee D, Rognstad R, Katz J: Futile cycles in isolated perfused rat liver and in isolated rat liver parenchymal cells. *Biochem Biophys Res Commun* 67:212–219, 1975
28. Lahtela JT, Wals PA, Katz J: Glucose metabolism and recycling by hepatocytes of OB/OB and ob/ob mice. *Am J Physiol* 259:E389–E396, 1990
29. Declercq PE, Falck JR, Kuwajima M, Tyminski H, Foster DW, McGarry JD: Characterization of the mitochondrial carnitine palmitoyltransferase enzyme system. I. Use of inhibitors. *J Biol Chem* 262:9812–9821, 1987
30. Ohneda M, Inman LR, Unger RH: Caloric restriction in obese pre-diabetic rats prevents beta-cell depletion, loss of beta-cell GLUT 2 and glucose incompetence. *Diabetologia* 38:173–179, 1995
31. Shimabukuro M, Koyama K, Chen G, Wang MY, Trieu F, Lee Y, Newgard CB, Unger RH: Direct antidiabetic effect of leptin through triglyceride depletion of tissues. *Proc Natl Acad Sci U S A* 94:4637–4641, 1997
32. Lee Y, Hirose H, Ohneda M, Johnson JH, McGarry JD, Unger RH: Beta-cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: impairment in adipocyte-beta-cell relationships. *Proc Natl Acad Sci U S A* 91:10878–10882, 1994
33. Vranic M: Banting Lecture: glucose turnover: a key to understanding the pathogenesis of diabetes (indirect effects of insulin). *Diabetes* 41:1188–1206, 1992
34. Butler PC, Rizza RA: Contribution to postprandial hyperglycemia and effect on initial splanchnic glucose clearance of hepatic glucose cycling in glucose-intolerant or NIDDM patients. *Diabetes* 40:73–81, 1991
35. Newgard CB, Moore SV, Foster DW, McGarry JD: Efficient hepatic glycogen synthesis in refeeding rats requires continued carbon flow through the gluconeogenic pathway. *J Biol Chem* 259:6958–6963, 1984
36. Halestrap AP: Stimulation of pyruvate transport in metabolizing mitochondria through changes in the transmembrane pH gradient induced by glucagon treatment of rats. *Biochem J* 172:389–398, 1978
37. Kummel L: Mitochondrial pyruvate carrier: a possible link between gluconeogenesis and ketogenesis in the liver. *Biosci Rep* 7:593–597, 1987

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