Malonyl-CoA in Skeletal Muscle and Liver of Streptozotocin-Diabetic Rats (43290)

I. M. ELAYAN, D. C. CARTMILL, C. B. ECKERSELL, J. WILKIN, AND W. W. WINDER[1]
Department of Zoology, Division of Physiology and Anatomy, Brigham Young University, Provo, Utah 84602

Abstract. **Malonyl-CoA, the inhibitor of carnitine palmitoyl transferase I, has been examined in this study in the muscle and liver of diabetic rats. Male Sprague-Dawley rats were rendered diabetic with streptozotocin (6 mg/100 g body wt). The gastrocnemius/plantaris muscles and liver samples were frozen at liquid nitrogen temperature. Muscle malonyl-CoA was 1.8 ± 0.2 pmol/mg in control rats and 1.5 ± 0.2 pmol/mg in the diabetic rats. This difference was not statistically significant. Liver malonyl-CoA of control rats was 8.6 ± 0.8 pmol/mg, in comparison to 4.3 ± 0.6 pmol/mg in diabetic rats. In the liver, high concentrations of malonyl-CoA inhibit fatty acid oxidation and ketogenesis. Failure of malonyl-CoA to decline in muscle in the diabetic may be responsible in part for the diversion of fatty acids to the liver, thereby enhancing hepatic fatty acid oxidation and ketogenesis.** [P.S.E.B.M. 1991, Vol 198]

In both the liver and muscle, malonyl-CoA is a potent inhibitor of carnitine palmitoyltransferase I, the enzyme responsible for the transfer of fatty acids from the outer surface of the inner mitochondrial membrane into the mitochondrial matrix, where fatty acid oxidation occurs (1–3). In the liver, malonyl-CoA content decreases in response to conditions in which plasma glucagon and fatty acids are elevated, plasma insulin is depressed, and liver glycogen is depleted. The decline in liver malonyl-CoA allows enhanced fatty acid oxidation and ketogenesis (1). These are the conditions imposed by fasting, exercise, and diabetes mellitus (1, 4–6). Malonyl-CoA is also present in muscle (2). Much less is known, however, about the regulation of muscle malonyl-CoA and its role in the control of muscle metabolism. Malonyl-CoA has been reported to decrease in skeletal muscle in response to fasting and exercise (2, 7, 8), but no data are available concerning the effect of insulin deficiency. In this study, we have examined the effect of experimental diabetes mellitus on levels of malonyl-CoA in skeletal muscle and liver.

Materials and Methods

Animal Care. Male Sprague-Dawley rats (Sasco, Omaha, NE) were housed in individual cages in a temperature- (19–21°C) and light-controlled (12:12-hr light:dark cycle) room. Three days before the day of sacrifice, jugular catheters were implanted while the rats were under light ether anesthesia. Streptozotocin was slowly injected via the catheter at this time. Streptozotocin (6 mg/100 g body wt) was dissolved in 0.5 ml of 0.1 *M* citrate buffer (pH 4.5) immediately prior to injection. Control rats were injected with the same volume of citrate buffer without addition of streptozotocin.

On the day of sacrifice, rats were anesthetized by intravenous injection of sodium pentobarbital while at rest. Special care was taken to avoid disturbance of the rats. Sacrifice times were between 10:00 and 11:30 AM in order to avoid variation due to diurnal changes.

Collection of Blood and Tissues. As soon as the rats were anesthetized, the gastrocnemius/plantaris muscles and a piece of liver were isolated and frozen at liquid nitrogen temperature. Blood was collected as rapidly as possible via the abdominal aorta. Half a milliliter was added to a tube containing 2 ml of 10% $HClO_4$ for the determination of blood glucose and 3-hydroxybutyrate. The remaining blood was heparinized and used for fatty acid determination. All tubes were kept on ice and were centrifuged immediately after the collection of blood. Plasma samples and perchloric acid extracts were stored at −20°C until time of analysis. Muscle and liver samples were stored at −70°C until analyzed.

Analytical Methods. Malonyl-CoA was determined by the method described by McGarry *et al.* (6) on neutralized 10% perchloric acid extracts of muscle

[1] To whom correspondence and requests for reprints should be addressed at Zoology Department, 545 WIDB, Brigham Young University, Provo, UT 84602.

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and liver. This assay measures malonyl-CoA-dependent incorporation of tritium-labeled acetyl-CoA into fatty acids. The reaction is catalyzed by fatty acid synthetase enzyme isolated earlier by the procedure of Linn (9).

For the cAMP assay, muscle and liver samples were ground to powder under liquid nitrogen. The powder (100 mg) was homogenized in 2 ml of 6% trichloroacetic acid. A 0.5-ml aliquot was extracted six times with 6 vol of water-saturated ethyl ether after addition of 0.02 ml of 5 N HCl. The extracts were evaporated to dryness and redissolved in 50 m*M* sodium acetate (pH 4). The method of Gilman (10) was utilized for cAMP determination.

Fructose 2,6-bisphosphate (F2,6-P2) was determined in neutralized 0.05 *N* NaOH extracts by the method of Van Schaftingen and Hers (11, 12). Liver and muscle glycogen were determined by the anthrone method (13).

Perchloric acid extracts were neutralized and analyzed for glucose (14) and 3-hydroxybutyrate (15).

Results are expressed as mean ± SE. Statistical significance of differences between data from diabetic and control rats was determined by use of Student's *t* test (two-tailed, non-paired).

Results

Food intake during the 18 hr before sacrifice was 18 ± 2.3 g for the diabetic group and 26 ± 0.6 g for the control group.

Data in Table I show that streptozotocin-treated rats were severely diabetic. Blood glucose was increased 326% above control values. Blood 3-hydroxybutyrate and plasma free fatty acids were markedly increased in diabetic rats compared with controls.

Liver malonyl-CoA decreased 50% in response to streptozotocin injection (Table II). This change was accompanied by a significant increase in liver cAMP and significant decreases in F2,6-P2 and glycogen (Table II).

No significant change was observed in gastrocnemius muscle malonyl-CoA in response to experimental diabetes (Table III). Neither cAMP or F2,6-P2 were different in muscle of diabetic rats compared with controls. Glycogen was slightly lower in the gastrocnemius muscle of the diabetic rats (Table III).

Table I. Blood Glucose, Blood 3-Hydroxybutyrate, and Plasma FFA in Control and Diabetic Rats[a]

	Control rats	Diabetic rats[b]
Blood glucose (m*M*)	6.1 ± 0.2	19.9 ± 0.5
Blood 3-hydroxybutyrate (m*M*)	0.05 ± 0.01	2.9 ± 0.5
Plasma FFA (m*M*)	0.12 ± 0.01	0.49 ± 0.06

[a] Values are means ± SE; $n = 13$ for controls and 14 for diabetics.
[b] Significantly different from value of control rats, $P < 0.001$.

Table II. Liver Malonyl-CoA, cAMP, Fructose 2,6-Bisphosphate, and Glycogen in Control and Diabetic Rats[a]

	Control rats	Diabetic rats[b]
Malonyl-CoA (pmol/mg)	8.6 ± 0.8	4.3 ± 0.6
cAMP (pmol/mg)	0.32 ± 0.01	0.69 ± 0.11
F2,6-P2 (pmol/mg)	9.6 ± 0.9	0.4 ± 0.20
Glycogen (μmol/g)	313 ± 22	63 ± 14

[a] Values are means ± SE; $n = 13$ for control rats and 14 for diabetic rats.
[b] Significantly different from value of control rats, $P < 0.001$.

Table III. Gastrocnemius Muscle Malonyl-CoA, cAMP, Fructose 2,6-Bisphosphate, and Glycogen in Control and Diabetic Rats[a]

	Control rats	Diabetic rats
Malonyl-CoA (pmol/mg)	1.8 ± 0.2	1.5 ± 0.2
cAMP (pmol/mg)	0.19 ± 0.01	0.22 ± 0.01
F2,6-P2 (pmol/mg)	1.9 ± 0.4	2.0 ± 0.4
Glycogen (μmol/g)	41.2 ± 2.5	32.8 ± 2.4[b]

[a] Values are means ± SE; $n = 13$ for control rats and 14 for diabetic rats.
[b] Significantly different from value of control rats, $P < 0.05$.

Discussion

In the liver, malonyl-CoA plays an important role in the regulation of fatty acid oxidation and ketogenesis. This compound is both an intermediate in the pathway for fatty acid synthesis and an allosteric modulator important for regulation of fatty acid oxidation and ketogenesis (1). Carnitine palmitoyl transferase I, the enzyme responsible for the transfer of fatty acyl-CoA into the mitochondria, is inhibited by a physiologic concentration of malonyl-CoA (1). When glucose is present in abundance and is being converted to fatty acids by the liver, malonyl-CoA is elevated, thus preventing oxidation of the fatty acids at the same time as they are being synthesized. A low glucagon to insulin ratio, induced by elevated glucose following a carbohydrate meal, is the hormonal signal for maintaining high hepatic malonyl-CoA levels (1). When the glucagon to insulin ratio increases as a result of fasting, exercise, or diabetes, hepatic cAMP increases with consequent reduction in fructose 2,6-bisphosphate and malonyl-CoA formation (1, 16–19). The reduction in F2,6-P2 serves to channel metabolic flux in the gluconeogenic direction and to decrease glycolysis and fatty acid synthesis. The decline in malonyl-CoA allows enhanced fatty acid oxidation and ketogenesis. The increase in hepatic cAMP, and the decline in hepatic F2,6-P2 and malonyl-CoA seen in the diabetic rats of this study, confirms previous findings (1, 6, 20).

Malonyl-CoA (an intermediate in fatty acid synthesis in the liver) can be detected in skeletal muscle, which is considered to be a nonlipogenic tissue (2, 7,

8). Malonyl-CoA is a potent inhibitor of carnitine palmitoyltransferase I in skeletal muscle (2, 3). Little is known of the route of synthesis and the regulation of malonyl-CoA in muscle. Recent studies demonstrate that malonyl-CoA concentration decreases in skeletal muscle in response to fasting (2) and exercise (7, 8). A muscle enzyme with several properties similar to hepatic and heart acetyl-CoA carboxylase and which could be responsible for synthesis of malonyl-CoA has recently been detected in this laboratory (submitted for publication). It is not clear whether this enzyme is influenced by cAMP-dependent protein kinase phosphorylation, similar to the liver enzyme (18).

Since fasting and exercise result in a decline in malonyl-CoA content of both liver and skeletal muscle, the failure to observe a decrease in both tissues in response to streptozotocin-induced diabetes was surprising. This indicates that insulin deficiency by itself will not cause a drop in muscle malonyl-CoA. It is also apparent that simply increasing plasma free fatty acid (FFA) does not cause a decline in malonyl-CoA. A previous study in this laboratory indicates that glucose infusion partially attenuates the decline in muscle malonyl-CoA during exercise (21). It is possible that the markedly elevated blood glucose was responsible for the prevention of a change in muscle malonyl-CoA in response to diabetes.

Hagenfeldt (22) has reported that in diabetic patients, with blood ketones 3–4 m*M*, the leg fractional uptake of FFA at rest is decreased compared with normal control patients. The respiratory quotient has also been reported to be higher in resting diabetic patients than in normal patients, indicating a lower rate of fat oxidation (23). Wahren *et al.* (23) attributed the lower fractional uptake of FFA by the leg to elevated blood ketone body concentration, with consequent enhancement of muscle ketone oxidation. The present study suggests that another possible reason for the reduced fractional uptake of FFA by the diabetic leg may have been due to failure of malonyl-CoA to decline, despite elevated FFA. The FFA would, therefore, be shunted to the liver to serve as substrate for ketogenesis rather than oxidized by the muscle.

Malonyl-CoA decreases in the liver but not in the skeletal muscle of diabetic rats. Failure of malonyl-CoA to decrease may contribute to reduced oxidation of fatty acids by skeletal muscle, thereby shunting fatty acids to the liver for ketogenic substrate.

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