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No Correlation Between Changes in Fatty Acid-binding Protein Content and Fatty Acid Oxidation Capacity of Rat Tissues in Experimental Diabetes

J. H. VEERKAMP,* H. T. B. VAN MOERKERK, J. VAN DEN BORN

Departments of Biochemistry and Nephrology, University of Nijmegen, Nijmegen, The Netherlands

Fatty acid-binding protein is considered to play an important role in fatty acid oxidation. Since diabetes mellitus causes marked changes of this latter metabolic process, we compared the effect of this pathological condition on both parameters in a comparative investigation of different rat tissues. Palmitate oxidation capacity and content of fatty acid-binding protein were determined in liver, heart and quadriceps muscle from rats with 2-week streptozotocin-induced diabetes mellitus and controls. In liver homogenates fatty acid oxidation capacity increased by 90%, but their content of fatty acid-binding protein decreased by 35%. Fatty acid oxidation capacity of heart and quadriceps muscle and fatty acid-binding protein content of quadriceps muscle did not change, but fatty acid-binding protein content of heart muscle doubled. Long-term diabetes (8 months) had a similar effect on content of this protein. In summary, changes of fatty acid oxidation capacity do not appear to correlate with fatty acid-binding protein content during the development of diabetes. This does not preclude other functions of fatty acid-binding proteins in regulation of lipid metabolism and processes in which fatty acids play a modulatory role. Copyright © 1996 Elsevier Science Ltd.

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INTRODUCTION

Diabetes mellitus represents a serious disturbance of homeostasis of glucose and fatty acid metabolism in many tissues of the body. The absence of insulin in insulin-dependent diabetes mellitus of man and streptozotocin-induced diabetes of rats causes an abnormal high lipolysis leading to very high plasma free fatty acid levels (McGarry, 1994). Glucagon action on the liver, in the absence of restraint by insulin, activates by a decrease of malonyl-CoA content hepatic carnitine acyltransferase I and in this way hepatic fatty acid oxidation and ketogenesis (McGarry and Foster, 1980). The high levels of plasma free fatty acids appear to increase triacylglycerol synthesis in the diabetic heart

and impair together with insulin lack myocardial glucose oxidation (Randle *et al.*, 1988; Saddik and Lopaschuk, 1994; Wall and Lopaschuk, 1989). These changes are closely linked to the accumulation of various acyl-CoA and acylcarnitine esters (Feuvray *et al.*, 1979). The heart of the diabetic rat applies almost entirely fatty acid oxidation as its source of ATP, but oxidation of exogenous and endogenous palmitate was not elevated (Wall and Lopaschuk, 1989). Fatty acid oxidation is increased in perfused muscle of diabetic dogs (Spitzer and Hori, 1969) and incubated diaphragm of diabetic rats (Stearns *et al.*, 1979).

Fatty acid-binding proteins (FABP) are supposed to play an important role in the cellular uptake and targeting of fatty acids (Veerkamp *et al.*, 1993; Veerkamp, 1995). A relation between fatty acid oxidation capacity and cytosolic FABP content appears to exist in heart and various muscles of man and rat (Peeters

*To whom all correspondence should be addressed.

Abbreviation: FABP, fatty acid-binding protein.

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et al., 1989). The same was observed for various tissues of control and clofibrate-treated rats (Glatz *et al.*, 1988). Other tissues as brain and adrenals do not show this relation, presumably due to involvement of FABP in other pathways of fatty acid metabolism (Veerkamp and Van Moerkerk, 1993). Rat liver showed concurrent changes at treatment with peroxisomal proliferators and high-fat diet and with sex, but not during postnatal development and at starvation (Veerkamp and Van Moerkerk, 1993). Heart and skeletal muscle FABP content increase at postnatal development, but are stable in most physiological conditions investigated (Veerkamp and Van Moerkerk, 1993).

Primary deficiencies of FABP have not been detected up to now. Only secondary deficiencies have been described. A lot of interest, however, has evolved around the relation of FABP and insulin resistance. Indications have been obtained for a genetic linkage on chromosome 4 between the locus of the human intestinal FABP gene and a locus related to insulin action (Prochazka *et al.*, 1993). Recently, a threonine-alanine substitution in the human intestinal FABP was found to be associated with increased fat oxidation *in vivo* and insulin resistance (Baier *et al.*, 1995). No association was, however, observed for the intestinal FABP-Thr allele with non-insulin dependent diabetes mellitus. Others found no evidence for a major genetic influence of the intestinal FABP locus on the development of non-insulin-dependent diabetes mellitus (Humphreys *et al.*, 1994).

The effect of insulin-dependent diabetes on FABP content has been studied in investigations on separate rat tissues (Brandes and Arad, 1993; Carey *et al.*, 1994; Glatz *et al.*, 1994), but never in comparative investigations in combination with fatty acid oxidation capacity and in long-term diabetes. In this study, we report observations on the effect of 2 weeks' streptozotocin-induced diabetes on the fatty acid oxidation capacity and the FABP content of rat liver, heart and skeletal muscle. We also investigated the FABP content in these rat tissues after 8 months of diabetes, to look for long-term effects.

MATERIALS AND METHODS

Animals and experimental design

After an overnight fast, male Wistar-Münich or Lewis rats (160–180 g body

weight) were made diabetic by i.v. injection of 55 mg streptozotocin/kg body weight [freshly prepared in 0.1 M citrate buffer (pH 4.5), 25 mg/ml]. Time- and age-matched rats that did not receive STZ served as controls. In one series of experiments, control and diabetic Wistar-Münich rats were sacrificed 11–13 days after injection. All diabetic animals had an increased urine production, glucosuria and significantly increased blood glucose levels (above 25 mM), whereas the control animals had blood glucose concentrations of about 5 mM. In two other series, groups of diabetic and control rats of both strains were followed for 8 months for morning blood glucose, body weight, urine production, and albuminuria (Van den Born *et al.*, 1995). Diabetic animals were treated 3 times a week with a low dose (1.2 I.U.) of bovine insulin to maintain blood glucose levels around 25 mM. The body weight of experimental rats was 230 g after 8 months in contrast to 350 g for control rats.

Assays

Cytosols (100,000 g supernatants) of liver, heart, and quadriceps muscle were prepared from 5% homogenates in 10 mM K-phosphate/154 mM KCl (pH 7.4) as described (Veerkamp and Van Moerkerk, 1986). Specific antisera against rat liver FABP or rat heart FABP type were used to determine the cytosolic FABP content by ELISA in liver, and heart and quadriceps muscle, respectively (Paulussen *et al.*, 1989). The antibodies were coupled to horseradish peroxidase (Nakane and Kawaoi, 1974). Total and peroxisomal fatty acid oxidation rates were determined in tissue homogenates in the absence and presence of 10 μ M rotenone/36 μ M antimycin A, respectively. The substrate was 120 μ M [1- 14 C]palmitate bound to albumin (molar ratio 5:1). Other details were previously given (Veerkamp and Van Moerkerk, 1986). Citrate synthase activity was measured according to the procedure of Shepherd and Garland (1969) in sonicated homogenates. Protein content of cytosols was determined according to Lowry *et al.* (1951) with bovine serum albumin as standard. The FABP standards in ELISA are based on quantitative amino acid analysis. All data are expressed as means \pm SD.

Table 1. Effect of diabetes on fatty acid oxidation capacity, citrate synthase activity, and FABP content of rat tissues

Tissue	Condition	Palmitate oxidation rate (nmol/min.g)	Citrate synthase activity (mU/g)	FABP content (nmol/mg protein)
Liver	diabetic	630 ± 184*	22.7 ± 1.1*	0.92 ± 0.15*
	control	331 ± 67	15.5 ± 2.1	1.52 ± 0.17
Heart	diabetic	1117 ± 122	111 ± 9	1.42 ± 0.10*
	control	948 ± 288	111 ± 8	0.70 ± 0.12
M. quadriceps	diabetic	156 ± 22	16.1 ± 1.2	0.73 ± 0.08
	control	122 ± 36	20.3 ± 3.9	0.81 ± 0.08

Assays were performed 11–13 days after injection of streptozotocin (55 mg/kg). Values are mean ± SD for six animals.

* $P < 0.01$.

RESULTS

Diabetes was confirmed by measurement of glucosuria and blood glucose levels. Diabetic animals sacrificed after 11–13 days of streptozotocin injection showed less increase of body weight in spite of higher food intake. This period was taken to obtain a stable metabolic state. A period of 8 months was taken to observe the effects of chronic diabetes as in man. In this longitudinal study diabetic animals showed marked renal pathology (Van den Born *et al.*, 1995).

Our data for control rats are in accordance with previous results (Paulussen *et al.*, 1989; Veerkamp and Van Moerkerk, 1986). The total palmitate oxidation capacity measured in liver homogenates showed a 90% increase in the 11–13 days diabetic rats (Table 1). Simultaneously, the citrate synthase activity increased by 46% and the peroxisomal (antimycin-insensitive) oxidation rate (data not presented) by only $15 \pm 4\%$. This indicates that the volume and/or number of mitochondria and the specific mitochondrial fatty acid oxidation rate (rate per U citrate synthase) are higher in the diabetic rat liver. The palmitate oxidation rates and the citrate synthase activity did not change significantly in heart and quadriceps muscle of diabetic rats (Table 1). The FABP content of the tissue cytosols showed a marked decrease for liver and a 100% increase for heart of 11–13 day

diabetic rats. Quadriceps muscle did not exhibit a significant change of FABP content. After 8 months of diabetes the two investigated rat strains showed similar changes of the FABP content in liver and heart, but no change in skeletal muscle (Table 2). The effects were, however, less than those after 11–13 days, due to changes of the control values. These changes are presumably related to maturation and ageing.

DISCUSSION

Diabetes like fasting causes a marked increase of mitochondrial fatty acid oxidation in perfused rat liver (McGarry *et al.*, 1975), hepatocytes (Witters and Trasko, 1979), liver homogenates (MacDonald and Swan, 1992; this work) and mitochondria (Harano *et al.*, 1972). An increase of the carnitine palmitoyltransferase I activity (Brady *et al.*, 1985; Cook and Gamble, 1987; Harano *et al.*, 1972), a decrease of its malonyl-CoA sensitivity (Brady *et al.*, 1985; Cook and Gamble, 1987) and a decrease of malonyl-CoA concentration (McGarry *et al.*, 1978) are the main factors responsible for this phenomenon. Peroxisomal fatty acid oxidation increased only slightly like in starvation (Veerkamp and Van Moerkerk, 1986), in contrast to mitochondrial oxidation. Others found a 1.3-fold and no increase of peroxisomal oxidation in rat liver after 10–21 days of diabetes (Kawashima *et al.*, 1983; Thomas *et al.*, 1989).

Table 2. Effect of chronic diabetes on FABP content of rat tissues

Strain	Condition	Liver	Heart	M. quadriceps
Wistar-Münich	diabetic (5–13)	0.83 ± 0.16	1.70 ± 0.11*	0.71 ± 0.12
	control (5)	0.90 ± 0.02	1.47 ± 0.16	0.72 ± 0.09
Lewis	diabetic (7)	0.65 ± 0.07**	1.59 ± 0.13*	0.71 ± 0.05*
	control (4)	1.08 ± 0.09	1.34 ± 0.16	0.62 ± 0.08

The period of streptozotocin-induced diabetes was 8 months.

Values (mean ± SD) are given in nmol/mg cytosolic protein for the number of rats indicated between parenthesis.

* $P < 0.05$, ** $P < 0.01$.

Fatty acid oxidation by the heart appears to be unaffected by diabetes or starvation (Veerkamp and Van Moerkerk, 1986). The presence of chronic diabetes (6–14 weeks) had no effect on oxygen consumption and palmitate oxidation in perfused rat heart and cardiomyocytes (Chen *et al.*, 1984; Murthy *et al.*, 1990; Wall and Lopaschuk, 1989). Our data on the fatty acid oxidation capacity of heart homogenates and the slightly increased or constant activity of 3-hydroxyacyl-CoA dehydrogenase (Chen *et al.*, 1984; Glatz *et al.*, 1994) are in accordance with these observations. Only one group reported an increase of myocardial fatty acid oxidation in diabetic rats (MacDonald *et al.*, 1992). Diabetes also had no effect on the palmitate oxidation capacity of quadriceps muscle (Table 1). Others found an increase in diabetic rat soleus, but not in psoas and extensor digitorum longus muscles (MacDonald *et al.*, 1992). $^{14}\text{CO}_2$ production from [^{14}C]palmitate was increased in incubated hemidiaphragm from diabetic rats (Stearns *et al.*, 1979), but $^{14}\text{CO}_2$ represents only a part of the ^{14}C -labelled oxidation products at short incubation periods (Veerkamp *et al.*, 1986). Acute diabetes (2–3 d) caused an increase of fatty acid uptake and oxidation by skeletal muscle of dogs *in vivo* (Spitzer and Hori, 1969). 3-Hydroxyacyl-CoA dehydrogenase activity increased in rat soleus, plantaris, white and red gastrocnemius muscles and diaphragm 10–12 weeks after induction of diabetes (Chen and Iannuzzo, 1982). The unchanged citrate synthase activity also observed by others (Chen and Iannuzzo, 1982; Chen *et al.*, 1984; Glatz *et al.*, 1994) indicates that the mitochondrial content of heart and skeletal muscles was unaffected by diabetes. One group reported, however, reduced activities of citrate synthase and other mitochondrial enzymes of rat skeletal muscles after 12–16 weeks of diabetes (Kainulainen *et al.*, 1994). Starvation did also not change the palmitate oxidation capacity of rat heart and quadriceps muscle (Veerkamp and Van Moerkerk, 1986) and their citrate synthase activity (Caterson *et al.*, 1982).

The FABP content is influenced by hormonal conditions, especially in the liver. Gonadal hormones, glucocorticoids, thyroid hormones and insulin regulate liver FABP level (Brandes and Arad, 1983; Nakagawa *et al.*, 1994; Ockner *et al.*, 1979). The FABP content in rat liver cytosol measured by oleic acid binding was decreased in streptozotocin-diabetes rates

(Brandes and Arad, 1983; Nakagawa *et al.*, 1994), to a comparable extent (by 52%) as in our diabetic rats. A correlation was observed between the level of hepatic FABP and the incorporation of [^{14}C]oleic acid into triacylglycerol for control, diabetic and adrenalectomized rats (Nakagawa *et al.*, 1994). The 40% decrease of liver FABP at an increase of fatty acid oxidation capacity by 90% in diabetic rat liver is in contrast with the slight (2%) decrease of FABP content at an increase of fatty acid oxidation capacity by 72% in starved rat liver (Veerkamp and Van Moerkerk, 1986). High-fat diet and peroxisomal proliferators enhance both liver FABP content and fatty acid oxidation (Veerkamp and Van Moerkerk, 1993). These data strengthen previous conclusions that FABP is involved in liver in more processes of fatty acid metabolism than fatty acid oxidation (e.g. synthesis of triacylglycerols, phospholipids and eicosanoids), and presumably also acts as a more universal binding protein of hydrophobic ligands (Veerkamp *et al.*, 1993; Veerkamp, 1995).

An increase of rat heart FABP content was also observed in rats with insulin- and with non-insulin-dependent diabetes mellitus (Glatz *et al.*, 1994). The increase in the former group (32%) was less than in our comparable, diabetic animals. In contrast with results on red quadriceps muscle (Carey *et al.*, 1994) we did not find a marked increase of the FABP content of total quadriceps muscle of diabetic (this report) and 1- or 3-day-starved (Paulussen *et al.*, 1989; Veerkamp and Van Moerkerk, 1993) rats. An increase of FABP mRNA was observed in red quadriceps (Carey *et al.*, 1994), but not in heart and psoas muscle (Sakai *et al.*, 1995) of diabetic rats. These differences may be related to the proportion of slow oxidative fibres of the muscle samples. The highest concentration of heart FABP type is present in heart and red skeletal muscles as soleus and diaphragm and its concentration corresponds to the proportion of slow-oxidative fibres in other muscles (Claffey *et al.*, 1987; Crisman *et al.*, 1987; Paulussen *et al.*, 1989; Veerkamp *et al.*, 1990; Vork *et al.*, 1991). In these muscle cells, FABP expression appears to be induced by diabetes, but this induction is not accompanied by an increase of the mitochondrial capacity of citrate synthase activity and fatty acid oxidation.

Fatty acids induce the expression of adipocyte FABP *in vitro* (Amri *et al.*, 1991). This molecule may be involved in the fatty acid flux into the

adipocyte during feeding (lipogenic conditions) and out of this cell during fasting (lipolytic) conditions. Although streptozotocin-induced diabetes is characterized by an increase in fatty acid mobilization and a loss of triacylglycerol stores in rat adipose tissue, it caused a large decrease in the transcription of the adipocyte FABP and in the levels of adipocyte FABP mRNA and protein (Melki and Abumrad, 1993). These changes were suggested to be related to insulin deficiency generating a cellular state similar to that of the preadipocyte. About other tissues few data is known on the effect of diabetes on FABP. The levels of heart type FABP and its mRNA are significantly decreased in aorta, but the heart FABP mRNA level was the same in kidney of streptozotocin-diabetic rats (Sakai *et al.*, 1995).

In conclusion, streptozotocin-induced insulin-dependent diabetes mellitus causes changes of FABP content which do not correlated with changes of fatty acid oxidation capacity in rat liver, heart and skeletal muscle or of lipolysis in rat adipose tissue. The absence of a correlation suggests FABP is not playing a direct role in regulation of fatty acid oxidation during diabetes. Clearly these studies do not rule out functions for FABPs in other pathways of lipid metabolism, e.g. synthesis of triacylglycerols, phospholipids and eicosanoids. In addition, they may modulate the effect of fatty acids on metabolic enzymes, ion channels and gene activity.

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