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Role of brown adipose tissue in glucose utilization in conscious pre-obese Zucker rats

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In 16-day-old conscious Zucker rats, at a time when pre-obese *fa/fa* rats were not yet hyperinsulinaemic compared with their lean *Fa/fa* littermates, the whole-body glucose-metabolism rate was decreased by 10% in pre-obese compared with lean pups. The markedly decreased glucose utilization found in brown adipose tissue (BAT) of pre-obese compared with lean pups accounted for at least 70% of the difference in whole-body glucose metabolism observed between the two genotypes. In pre-obese *fa/fa* rats, the 20% decrease in noradrenaline content of BAT reported in this study is consistent with the diminished glucose utilization by this tissue, and further supports the hypothesis of a defect in the sympathetic-nervous-system regulation of BAT metabolism as one of the primary causes for this genetic obesity.

INTRODUCTION

Obesity in Zucker rats, inherited as an autosomal recessive trait, is characterized in the first week of life by enhanced white-adipose-tissue (WAT) growth (Boulangé *et al.*, 1979) and decreased energy expenditure (Planche *et al.*, 1983). This latter abnormality could be partly ascribed to an impaired thermogenic capacity of brown adipose tissue (BAT) (Bazin *et al.*, 1983), the principal source of thermoregulatory heat production in newborn rats.

At the end of the first week of life, pre-obese (*fa/fa*) pups, when compared with their lean (*Fa/fa*) littermates, exhibited an overcapacity for lipogenesis in both WAT and BAT (Bazin & Lavau, 1982; Bazin *et al.*, 1983). At weaning, these anomalies increased dramatically, with concomitant development of hyperinsulinaemia and hyperphagia (York *et al.*, 1981; Stern & Johnson, 1977), which worsen this obesity syndrome.

Glucose is regarded as a predominant precursor for fatty acid synthesis in both WAT and BAT, and a stimulatory role for insulin is well established in these tissues (McCormack & Denton, 1977). In good agreement, hyperinsulinaemic 30-day-old fatty rats were recently shown to display markedly increased glucose uptake *in vivo* in both WAT and BAT (Krief *et al.*, 1988). Furthermore, whole-body glucose turnover was enhanced in these young obese rats compared with their lean littermates, suggesting that 30-day-old *fa/fa* rats respond normally to their hyperinsulinaemia.

In 16-day-old *fa/fa* rats, before the development of hyperinsulinaemia, previous workers reported increased glucose uptake and oxidation in isolated adipocytes, suggesting that a defect in glucose metabolism could be already present before weaning (Guerre-Millo & Lavau, 1987). However, there is no available information *in vivo* either on glucose uptake in peripheral tissues or on body-glucose turnover in suckling Zucker rats.

Therefore the aim of the present study was to measure, in 16-day-old conscious normo-insulinaemic *fa/fa* pups and their *Fa/fa* littermates, glucose uptake *in vivo* in several peripheral tissues (skeletal muscles, BAT, WAT), by the 2-deoxyglucose method; furthermore, we evaluated the physiological importance of these tissues in terms of whole-body glucose metabolism.

MATERIALS AND METHODS

Animals and diet

Animals used in this study were 16-day-old pre-obese Zucker rats (*fa/fa*) and their lean littermates (*Fa/fa*), resulting from male *fa/fa* and female *Fa/fa* crosses. Pups (9–11 per litter) and their mother were housed in polypropylene cages in a temperature-controlled room (22 °C) on a 12 h-light/12 h-dark cycle. Dams had free access to standard laboratory chow (U.A.R., 91600 Epinay sur Orge, France) and tap water.

Whole-body glucose utilization

After very light diethyl ether anaesthesia (1 min), rats were injected via the jugular vein with 100 kBq of [3-^{3}H]glucose (185 GBq/mmol; Amersham France S.A.) in 50 μl of 0.9% NaCl as a bolus (zero time). Animals returned to a conscious state within 1 min after the injection. In order to avoid a cold stress, which has been shown to worsen the defect of energy expenditure which characterizes *fa/fa* pups, they were maintained at an ambient temperature of 30 °C to mimic the nest conditions (Planche *et al.*, 1983). Blood samples ($\sim$ 25 μl) were collected from the cut tip of the tail at selected times (3, 15 and 30 min). Provided that glycaemia was constant, the whole-body glucose metabolism rate could be evaluated from the decay rate of plasma [3-^{3}H]glucose and from the glucose pool in extracellular fluid as previously described (Krief *et al.*, 1988). In each litter, the genotype of pups was diagnosed by plotting inguinal-fat-pad weight versus body weight, and the data fell on two straight lines which differed very significantly by both their slope and intercept (Lavau & Bazin, 1982).

Abbreviations used: BAT, brown adipose tissue; WAT, white adipose tissue.

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Glucose uptake

By using the same experimental procedure as described above, animals were injected as a bolus with 370 kBq of 2-deoxy[1-^{3}H]glucose (630 GBq/mmol; Amersham France S.A.) in 50 μl of saline. At the end of the experiment (30 min), rats were decapitated and several tissues (inguinal WAT, interscapular BAT, diaphragm, anterior and posterior limbs) were rapidly dissected, weighed and immediately frozen in liquid N_2 for further measurement of the content of 2-deoxyglucose 6-phosphate as previously described (Krief *et al.*, 1988). The use of 2-deoxyglucose as a tracer for glucose uptake does not allow determination of absolute tissue glucose uptake, but rather gives a 'glucose metabolic index' (R_g') according to Kraegen's terms (Kraegen *et al.*, 1985).

Several additional litters were used for measurement of plasma non-esterifed fatty acids (NEFA-C kit, Wako Chemicals G.m.b.H., Neuss, Germany), insulinaemia by a radioimmunoasay with rat insulin standard, and noradrenaline content by h.p.l.c. with an electrochemical detector (Waters 460; Millipore, Milford, MA, U.S.A.).

Statistical analysis

All values are presented as means ± S.E.M. The significance of the differences was assessed by Student's *t* test or two-way analysis of variance.

RESULTS AND DISCUSSION

Body composition and blood metabolites

The 16-day-old pre-obese (*fa/fa*) pups weighed about 1.2 g more than their lean (*Fa/fa*) littermates (Table 1). This excess body weight was totally accounted for by the excess of WAT. At this age, WAT represented 5.7% and 1.5% of body weight in *fa/fa* and *Fa/fa* pups respectively. Subcutaneous WAT, with preferential depots in inguinal and dorsal regions as well as around the neck, wrists, ankles and limb articulations, accounted for 75 and 90% of total WAT in lean and pre-obese pups respectively, and was 5 times larger in pre-obese than in lean pups. Conversely, the masses of internal WAT depots (gonadal, perirenal, retroperitoneal and intermuscular) did not differ significantly between the two genotypes. Thus the large difference in WT mass observed between pre-obese and lean rats was primary due to hypertrophy of subcutaneous WAT. The BAT was also heavier (25%) in pre-obese pups. However, if we assume that, in 16-day-old Zucker rats, the lipid content measured in interscapular BAT (33 ± 0.9 versus 45 ± 0.7% in 11 *Fa/fa* versus 7 *fa/fa* pups) was similar in other BAT sites, the fat-free mass did not differ between the two genotypes (191 versus 194 mg, *Fa/fa* versus *fa/fa*). For both *fa/fa* and *Fa/fa* pups, interscapular-BAT weight was one-third of that of the total BAT mass.

Pre-obese rats were slightly but significantly hyperglycaemic (+6%) compared with their lean littermates, and the difference between the two genotypes remained constant throughout the experiment. After light ether anaesthesia, necessary for injection of tracer, basal plasma glucose increased by approx. 15% in both genotypes and remained fairly constant during the experiment, justifying further analysis of data on the basis of a steady-state system.

There was no significant difference in plasma non-esterified fatty acids between lean and pre-obese rats (456 ± 24.4 and 428 ± 27.3 μmol/l respectively).

Table 1. Body weight and WAT and BAT mass and distribution in 16-day-old lean (*Fa/fa*) and pre-obese (*fa/fa*) Zucker rats

WAT and BAT mass and distribution were determined after dissection of the entire tissue in 5 *Fa/fa* and 5 *fa/fa* rats. Values are means ± S.E.M. Numbers of rats were 20 *Fa/fa* and 18 *fa/fa* for measurement of body weight. * $P < 0.05$, ** $P < 0.01$ *Fa/fa* versus *fa/fa*.

		WAT (mg)				BAT (mg)		
			Subcutaneous					
	Body wt. (g)	Total	Inguinal	Other	Other	Total	Interscapular	Other
Lean	26.6 ± 0.29	405 ± 50.7	161 ± 13.8	141 ± 33.9	104 ± 13.7	283 ± 19.9	96 ± 9.7	187 ± 15.1
Obese	27.8 ± 0.25**	1576 ± 108.3**	482 ± 38.3**	936 ± 90.5**	158 ± 29.4	354 ± 20.7*	136 ± 5.1**	218 ± 19.7

Table 2. Glucose metabolic index in muscles, WAT and BAT in 16-day-old lean (*Fa/fa*) and pre-obese (*fa/fa*) Zucker rats

Glucose metabolic indexes were measured per mg wet wt. in inguinal WAT (IWAT), diaphragm (DIAPH), total skeletal-muscular mass of posterior (POST) and anterior (ANT) limbs, and interscapular BAT (IBAT). Glucose metabolic index in whole WAT and BAT was calculated after determination of whole tissue mass by dissecting out the entire tissue (see Table 1). Glucose metabolic index in total muscular mass was extrapolated, by assuming that muscular mass was identical in *Fa/fa* and *fa/fa* rats (Bell & Stern, 1977) and was 28% of body wt. in rats weighing 25 g, according to Miller (1969). It was assumed that glucose metabolic index per mg wet wt. in IWAT, IBAT and limb muscles was representative of total BAT, total WAT and total skeletal-muscular mass respectively. Values are means ± S.E.M. for the numbers of rats in parentheses: ** $P < 0.01$ *fa/fa* versus *Fa/fa*; NS, not significant.

	Glucose metabolic index (ng/min)							
	per mg of tissue					per whole tissue		
	IWAT	DIAPH	POST	ANT	IBAT	Muscle	WAT	BAT
Fa/fa	2.9 ± 0.19 (15)	13.6 ± 1.18 (15)	7.8 ± 0.83 (7)	10.1 ± 1.07 (7)	77 ± 15.2 (15)	67000 (7)	1300 (15)	21600 (15)
fa/fa	3.5 ± 0.35 (12) NS	11.4 ± 1.57 (12) NS	7.7 ± 1.08 (8) NS	9.3 ± 1.22 (8) NS	13 ± 2.4 (12) **	64000 (8)	5500 (12)	4600 (12)

Pre-obese pups were normo-insulinaemic (49 ± 10.6 versus 53 ± 9.2 μ-units/ml, 10 *fa/fa* versus 15 *Fa/fa*).

Kinetics of glucose disposal

The rate of plasma disappearance of tracer in 16-day-old Zucker rats was shown to fit a single exponential function (results not shown) which, after logarithmic transformation of the data, enabled the calculation of the rate constant of disappearance of tracer from plasma (K_p and K'_p for plasma decay of [3-^{3}H]glucose specific radioactivity and 2-deoxy[1-^{3}H]glucose/glucose ratio respective). K_p was 12% lower in pre-obese than in lean pups, i.e. 246 (± 10.8) $\times 10^{-4}$ versus 274 (± 6.0) $\times 10^{-4}$ min^{-1} ($P < 0.05$, 16 *fa/fa* versus 19 *Fa/fa*). In spite of the slight hyperglycaemia in pre-obese pups, the glucose pool (G_p) was not statistically different between the two groups (10.2 ± 0.20 and 9.9 ± 0.20 mg for *fa/fa* and *Fa/fa* respectively). Therefore whole-body glucose metabolism ($R = G_p \cdot K_p$) was also less (10%) in 16-day-old pre-obese than in lean rats (250 ± 11.5 versus 274 ± 9 μg/min; $P < 0.05$, two-way analysis of variance). These data demonstrate, for the first time, the presence of a defect in the glucose-utilization rate of pre-obese rats (−10%) before weaning and the concomitant emergence of hyperinsulinaemia. Thus the increased glucose turnover rate previously reported in post-weaned (Krief *et al.*, 1988) and adult (Wade, 1980) *fa/fa* rats might be a consequence of their hyperinsulinaemia. Taken together, these data emphasize that some metabolic disorders already present in very young *fa/fa* pups could be masked in older rats after development of hyperinsulinaemia.

Uptake of 2-deoxyglucose by tissues

In order to determine which tissues could be responsible for the decreased whole-body glucose utilization displayed by 16-day-old pre-obese rats compared with their lean littermates, we measured 2-deoxyglucose uptake in several glucose-utilizing tissues (Table 2).

In lean *Fa/fa* rats, inguinal adipose tissue was a low glucose-utilizing tissue as compared with muscle. Conversely, interscapular BAT was found to be very active in glucose disposal, with R_g' 8 and 27 times higher than that for skeletal muscle and WAT respectively. In pre-obese *fa/fa* rats, glucose uptake in skeletal muscle and inguinal WAT followed the same pattern as described above for lean rats. In these *fa/fa* rats, interscapular BAT was the only tissue in which glucose uptake was altered, with R_g' 6-fold lower than that of lean rats.

In an attempt to evaluate the relative importance of muscle, WAT and BAT in whole-body glucose metabolism, we extrapolated glucose-metabolic indexes on a mg-wet-wt. basis to whole tissue mass (Table 2). Total muscular mass, which at this age did not differ between lean and pre-obese pups (Bell & Stern, 1977), made the greatest contribution (25%) to whole-body glucose metabolism in both genotypes. WAT of lean pups contributed marginally to whole-body glucose disposal (less than 0.5%). In obese rats, owing to its hyperdevelopment, WAT was found to be the only tissue in which glucose uptake was increased (4-fold). The hyperlipogenic capacity of this tissue, which develops in *fa/fa* rats very early in life (Bazin & Lavau, 1982), could be instrumental in such overutilization of glucose. This hypothesis is in good agreement with the report in isolated adipocytes, showing, in *fa/fa* rats, a preferential orientation of glucose towards incorporation into fatty acids (Guerre-Millo & Lavau, 1987). However, in 16-day-old *fa/fa* rats, total WAT (5.7% of body wt.) was shown to play a minor role in total glucose disposal (2.2%).

In lean rats, BAT was found to play an important role in total body glucose utilization, metabolizing one-third of that disposed of by the total muscular mass. This striking capacity of BAT (1% of body wt.) of lean suckling rats to dispose of large amounts of glucose (8% of whole-body glucose metabolism) indicates that this tissue could play a quantitatively important role in blood glucose homoeostasis, as has been previously suggested for the adult mouse (Cooney *et al.*, 1985).

Conversely, in pre-obese suckling compared with lean rats, the marked decrease in glucose uptake in BAT, calculated as representing 25 mg of glucose spared per day, accounted for 70% of the difference in body glucose

turnover observed between the two genotypes. The interscapular brown fat, from which total BAT glucose uptake was extrapolated, is a thermogenic site of lesser importance than other depots, e.g. perirenal and periaortic (Foster, 1986). This suggests that the contribution of total BAT glucose uptake could be underestimated in both genotypes, thus the decreased glucose uptake in BAT of pre-obese pups might be totally accounted for by the decreased whole-body glucose metabolism observed in *fa/fa* rats.

One explanation for the impaired glucose uptake evidenced in BAT of *fa/fa* rats might be the well-known glucose-sparing effect of non-esterified fatty acids, which has recently been reported to occur also in rat BAT (Saggerson *et al.*, 1988). However, this explanation is invalidated by the fact that 16-day-old *fa/fa* rats had normal plasma contents of non-esterified fatty acids.

It has been previously demonstrated that glucose utilization in BAT is stimulated by noradrenaline, and suggested that glucose could be used as a direct fuel for thermogenesis as well as lipogenic precursors (Cooney *et al.*, 1985). Thus the hypo-utilization of glucose by BAT of pre-obese pups is consistent with the great decrease in thermogenesis which characterized *fa/fa* pups (Bazin *et al.*, 1984) and with the decreased BAT noradrenaline content reported in the present study in pre-obese compared with lean rats (1876 ± 54.4 versus 1479 ± 67.1 pmol; 10 *Fa/fa* versus 10 *fa/fa*, $P < 0.01$). Taken together, these data support the hypothesis that a defect in the regulation of BAT by the autonomic nervous system suggested by previous reports (Levin *et al.*, 1981; Ricquier *et al.*, 1986) is already present in pre-obese pups by 16 days of age.

In addition to its stimulatory effect on glucose utilization in BAT, noradrenaline has also been shown to decrease lipogenesis (Agius & Williamson, 1980; Gibbins *et al.*, 1985; Ebner *et al.*, 1987) and to stimulate lipoprotein lipase activity (Carneheim *et al.*, 1984). Thus, before weaning and the concomitant development of hyperinsulinaemia, all of the abnormalities previously reported in BAT of suckling pre-obese rats [i.e. hyperlipogenesis (Bazin *et al.*, 1983), decreased lipoprotein lipase activity (Boulangé *et al.*, 1979) and thermogenesis (Bazin *et al.*, 1984), as well as the alteration in glucose uptake reported in the present study] could be explained by decreased stimulation of BAT by hypothalamus efferent sympathetic nerves, as suggested in the *ob/ob* mouse and in ventro-median-hypothalamus-lesioned rats (for review, see Jeanrenaud *et al.*, 1985). However, further investigations are needed to provide direct evidence that an alteration in BAT stimulation by noradrenaline is present during the first days of life in *fa/fa* pups.

Conclusions

Our results show that whole-body glucose metabolism *in vivo* is decreased by 10% in 16-day-old conscious pre-obese pups compared with their lean littermates. Decreased glucose uptake in BAT of pre-obese pups was virtually the only factor responsible for the difference observed between the two genotypes. The present study adds evidence to the fact that BAT is a very early site of expression of the *fa* gene, and supports the hypothesis that a defect in the autonomic nervous system may be a primary cause of this genetic obesity.

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