Increased gene expression of lipogenic enzymes and glucose transporter in white adipose tissue of suckling and weaned obese Zucker rats

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Previous experiments have shown that insulin-induced glucose utilization is increased in white adipose tissue of young obese Zucker rats. We have investigated the possible role of over-expression of the muscle/fat glucose transporter (Glut 4) and key lipogenic enzymes in this increased insulin-responsiveness. The amount or activity and the mRNA concentrations of Glut 4, fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) were measured before and after weaning in white adipose tissue of obese and lean Zucker rats. Comparison of the levels of Glut 4 and lipogenic-enzyme expression in 15-day-old suckling and 30-day-old weaned rats on a high-carbohydrate diet shows a marked increase in the latter group. The increase was, in lean and obese rats respectively, 6- and 7-fold for the amount of Glut 4 and 2- and 3-fold for its mRNA concentrations, 40- and 100-fold for the activity of lipogenic enzymes (FAS and ACC) and 30- and 10-fold for their mRNA concentrations. Furthermore, all these parameters, except the amount of Glut 4, were 2–5-fold higher in obese rats, both before and after weaning. Changes at weaning were largely blunted when rats were weaned on to a high-fat diet, although the differences between lean and obese rats persisted, and even became significant for the amount of Glut 4. Whatever the experimental conditions, plasma insulin levels were significantly higher in obese than in lean rats. These results indicate the existence of an enhanced expression of Glut 4, FAS and ACC in white adipose tissue of young obese *fa/fa* rats which could be related to the increased plasma insulin levels.

INTRODUCTION

The spontaneously obese Zucker *fa/fa* rat inherits its obesity as a homozygous recessive trait. They present, among other abnormalities, an increased insulin-responsiveness of glucose utilization in white adipose tissue just after weaning [1,2]. This increase could be mediated by an enhanced activity and/or concentration of intracellular effectors of insulin action. Among them, glucose transporters and lipogenic enzymes are likely candidates.

An enhancement of the glucose-transport capacity, of the number of glucose transporters and of their translocation to the plasma membranes has been described in 30-day-old obese Zucker rats [3]. Facilitated glucose transport is mediated by a family of tissue-specific proteins [4]. One of them, Glut 4, is expressed in tissues that exhibit insulin-regulatable glucose transport [5–8], and its expression varies in physiological or pathological conditions where changes in glucose transport activity have been demonstrated [9–13]. An increased expression of Glut 4 could thus be present in obese Zucker rats.

The activity and the mRNA concentrations of fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC), two major lipogenic enzymes, are low during suckling in normal rats and increase at weaning to a high-carbohydrate (HC) diet [14,15]. This could be brought about by changes in the nutritional and hormonal environment, since weaning to a high-fat (HF) diet precludes this increase [14,15]. Compared with lean rats, body-weight gain, fat deposition and white-adipose-tissue lipogenesis are enhanced in the obese *fa/fa* rats particularly after weaning to

a HC diet and, to a lesser extent, after weaning to a HF diet [16–19]. However, higher lipogenic rates and enzyme activities have been detected as early as the first week of life [16,18], although the mechanism (translational or pretranslational) remains unknown.

Insulin alone or in combination with other metabolic or hormonal factors could play a major role in the short- and long-term control of Glut 4 [9–13] and of lipogenic enzymes [20–23]. It has been clearly shown that basal plasma insulin is markedly increased after weaning in obese Zucker rats. In suckling *fa/fa* rats, post-absorptive plasma insulin concentrations have been found to be in the normal range, despite an enhanced insulin secretion in response to an acute glucose stimulation [19,24]. It has been suggested that the increased glucose transport and lipogenic capacities in white adipose tissue of the young *fa/fa* rats could be attributed to this hypersecretion of insulin [25].

The similarities between the young *fa/fa* rat and human obesity of recent onset have been underlined, especially in terms of over-utilization of glucose by the adipose tissue [26]. It is thus important to determine the mechanisms underlying the modified glucose metabolism in adipocytes during the development of obesity.

In the present study we have measured the activity and the mRNA levels of Glut 4, FAS and ACC in white adipose tissue of obese Zucker rats, early in the development of the syndrome, i.e. during the suckling period, or shortly after weaning, in relation to nutritional and hormonal changes occurring at these various developmental phases.

Abbreviations used: FAS, fatty acid synthase; ACC, acetyl-CoA carboxylase; HF, high-fat; HC, high-carbohydrate.

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METHODS

Animals

Animals were kept in a room at constant temperature ($23 \pm 1^\circ\text{C}$) and with a light-dark cycle (light on from 07:00h to 19:00h). They had free access to diet and water. Zucker rats were bred in our laboratory and were obtained by mating either (1) heterozygous (*Fa/fa*) lean females and obese (*fa/fa*) males or (2) homozygous (*Fa/Fa*) lean female and male rats. The studies were performed on 15- and 21-day-old suckling rats and on 22- and 30-day-old weaned rats. The rats were weaned at 21 days, either to a semi-synthetic high-carbohydrate (HC) diet [72% carbohydrate, 1% fat, 27% protein (by wt.)], or to a high-fat (HF) diet [$< 1\%$ carbohydrate, 72% fat, 28% protein (by wt.)]. Since suckling rats begin to nibble the food of the mother at 15 days of age, the mothers were fed with the HF diet from day 13 of lactation until weaning to avoid any consumption of carbohydrates by the pups between 15 and 21 days of age.

The genotype identification of the rats was made by either plotting inguinal fat-pad weight versus body weight for 15-day-old rats, or on the basis of their inguinal fat-pad weight at 21, 22 and 30 days of age [27]. The two genotypes *fa/fa* and *Fa/Fa* were studied.

All animals were killed by cervical dislocation at the onset of the light period, i.e. in the post-absorptive state. Blood was collected and plasma was kept frozen at -20°C for subsequent determinations of plasma glucose and insulin levels. Subcutaneous fat-pads of the inguinal region were immediately removed, frozen in liquid nitrogen and stored at -80°C for subsequent RNA extraction and enzyme-activity measurements.

Analytical techniques

Blood samples were immediately centrifuged, and plasma glucose levels were determined with a glucose analyser (Beckman 2). Plasma insulin levels were measured by radioimmunoassay (SB-INSI-5; ORIS, Gif/Yvette, France) with rat insulin as standard. Proteins were determined by the method of Bradford [28], with bovine serum albumin as standard. DNA was measured in tissue homogenates by a spectrofluorimetric assay [29]. The activity of FAS was determined by the spectrophotometric assay of Linn [30]. The maximal activity of ACC was determined as described by Majerus *et al.* [31] in the presence of 10 mM-citrate.

Quantification of glucose-transporter protein

Adipose tissue was homogenized with a Polytron in 10 vol. of 20 mM-Hepes/1 mM-EDTA/250 mM-sucrose, pH 7.4, and was centrifuged at 1000 *g* for 1 min at 4°C . Equal volumes of homogenates solubilized in Laemmli buffer were subjected to SDS/PAGE (10%-acrylamide gel). The proteins were electrically transferred to nitrocellulose membrane (BA 85; Schleicher & Schuell, Dassel, Germany). The molecular mass and the efficiency of the transfer were assessed by using rainbow protein markers (Amersham International, Amersham, Bucks., U.K.). The membranes were blocked with non-fat dry milk (Carnation Co., Los Angeles, CA, U.S.A.) in TBST [Tris-buffered saline (pH 7.6) with 0.1% Tween 20 (Sigma, St. Louis, MO, U.S.A.)] and incubated with an antibody against the Glut 4 protein, R820 [8,32], in the same solution, washed in TBST and reacted with 0.5 μCi of ^{125}I -Protein A (Amersham International)/ml for 1 h. The filters were again washed with TBST, exposed to Hyperfilm MP and quantified by scanning densitometry (Hoeffer GS 300, San Francisco, CA, U.S.A.).

RNA extraction and hybridization

Total RNA extraction was performed by the hot-phenol procedure of Scherrer & Darnell [33] modified as described by

Coupé *et al.* [14]. The concentration of RNA was determined by spectrophotometry (260 nm), and their integrity was systematically checked by electrophoresis in an 0.8%-agarose submarine minigel stained with ethidium bromide and observed under u.v. fluorescence.

Dot-blots were performed by denaturing increasing amounts (0.25–8 μg) of total RNA sample at 95°C for 2 min, which were then directly dotted on to a nylon membrane (Hybond N; Amersham International). Membranes were hybridized to the labelled probes as described previously [14,34]. Autoradiograms were obtained by exposure of radioactive nylon filters to Hyperfilm MP (Amersham International) at -80°C with intensifying screens and scanned by densitometric analysis.

The specificity of the various cDNA probes as well as the validity of the dot-blot procedure for mRNA quantification have been previously checked by Northern-blot analysis [14,34].

cDNA probes

The cDNA probes were kindly provided by Dr. A. G. Goodridge (pFAS 18 cDNA for rat liver FAS mRNA), Dr. K. H. Kim (P181-6 cDNA for rat mammary-gland ACC mRNA) and Dr. D. James (Glut 4) and were prepared by the multiprimer labelling-system kit (Amersham International).

Statistical analysis

Results are expressed as means $\pm$ S.E.M. Statistical analysis was performed by Student's *t* test for unpaired data.

RESULTS

Body weight was not significantly different in obese and lean rats until 30 days of age (Table 1). However, before that age, characteristics of white adipose tissue were modified, as reflected by the significantly lower concentrations of proteins, total RNA and DNA in obese compared with lean rats when expressed on a per-weight basis (Table 2). This is indicative of an increase in cell size in obese rats, the increase in cell number occurring later [35].

Glucose-transporter amount and lipogenic-enzyme activity

Since the cell size was different in lean and obese rats, we have assessed the glucose-transporter amount and the lipogenic-enzyme activities on a per-cell basis by normalizing to tissue DNA content (Table 3). The amount of Glut 4 was equally low during suckling in lean and pre-obese rats and increased to a similar extent at weaning on to a HC diet (Table 3). Weaning on to a HF diet prevented the increase in lean rats totally, but only

Table 1. Body weight and plasma glucose concentrations of lean (*Fa*) and obese (*fa*) Zucker rats during suckling (at 15 and 21 days of age) or after weaning on to either a HC (at 22 and 30 days of age) or a HF diet (at 30 days)

Results are means $\pm$ S.E.M. ($n = 5-9$). Lean versus obese Zucker: $\dagger P < 0.01$, $\ddagger P < 0.001$.

Genotype and age (days)	Body wt. (g)		Plasma [glucose] (mM)	
	<i>Fa</i>	<i>fa</i>	<i>Fa</i>	<i>fa</i>
15	22 $\pm$ 1	24 $\pm$ 1	6.3 $\pm$ 0.2	5.9 $\pm$ 0.2
21	30 $\pm$ 2	35 $\pm$ 1	5.9 $\pm$ 0.2	5.4 $\pm$ 0.3
22HC	29 $\pm$ 1	31 $\pm$ 1	6.0 $\pm$ 0.3	6.2 $\pm$ 0.3
30HC	48 $\pm$ 1	58 $\pm$ 2 $\dagger$	5.4 $\pm$ 0.1	5.7 $\pm$ 0.2
30HF	50 $\pm$ 2	55 $\pm$ 1 $\ddagger$	6.1 $\pm$ 0.3	8.2 $\pm$ 0.3 $\ddagger$

Table 2. Characteristics of white adipose tissue of lean (*Fa*) and obese (*fa*) Zucker rats during suckling (at 15 and 21 days of age) or after weaning on to either a HC (at 22 and 30 days of age) or a HF diet (at 30 days)Results are means $\pm$ S.E.M. ($n = 5-9$). Lean versus obese Zucker: * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$.

Genotype and age (days)	Protein (mg/g of tissue)		RNA (mg/g of tissue)		DNA (mg/g of tissue)	
	<i>Fa</i>	<i>fa</i>	<i>Fa</i>	<i>fa</i>	<i>Fa</i>	<i>fa</i>
15	13.9 $\pm$ 3.3	8.2 $\pm$ 0.8†	0.8 $\pm$ 0.1	0.58 $\pm$ 0.04*	1.34 $\pm$ 0.21	0.47 $\pm$ 0.07‡
21	9.0 $\pm$ 0.2	7.8 $\pm$ 0.3‡	0.56 $\pm$ 0.04	0.38 $\pm$ 0.03*	0.67 $\pm$ 0.03	0.45 $\pm$ 0.09
22HC	11.6 $\pm$ 0.8	7.7 $\pm$ 0.3‡	0.55 $\pm$ 0.04	0.37 $\pm$ 0.03*	0.81 $\pm$ 0.16	0.48 $\pm$ 0.10
30HC	16.2 $\pm$ 1.4	13.8 $\pm$ 0.9*	0.61 $\pm$ 0.04	0.41 $\pm$ 0.03*	0.87 $\pm$ 0.12	0.28 $\pm$ 0.06†
30HF	10.9 $\pm$ 1.1	6.5 $\pm$ 0.6†	0.36 $\pm$ 0.03	0.19 $\pm$ 0.03*	0.49 $\pm$ 0.14	0.14 $\pm$ 0.01†

Table 3. Enzyme activities and Glut 4 protein amount in lean (*Fa*) and obese (*fa*) Zucker rats during suckling (at 15 and 21 days of age) or after weaning on to either a HC (at 22 and 30 days of age) or a HF diet (at 30 days)FAS activity is expressed in nmol of NADPH oxidized/min per μ g of DNA; ACC activity is expressed in nmol of [14 C]bicarbonate fixed/min per μ g of DNA. Abbreviation: ND, not determined. Results are means $\pm$ S.E.M. ($n = 5-8$). Lean versus obese Zucker: * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$.

Genotype and age (days)	FAS		ACC		Glut 4 (arbitrary units/ μ g of DNA)	
	<i>Fa</i>	<i>fa</i>	<i>Fa</i>	<i>fa</i>	<i>Fa</i>	<i>fa</i>
15	22 $\pm$ 4	30 $\pm$ 2	6 $\pm$ 2	9 $\pm$ 2	21 $\pm$ 7	19 $\pm$ 2
21	32 $\pm$ 2	76 $\pm$ 9†	5 $\pm$ 1	35 $\pm$ 4†	ND	ND
22HC	110 $\pm$ 20	210 $\pm$ 20†	21 $\pm$ 3	41 $\pm$ 4†	ND	ND
30HC	1020 $\pm$ 80	2820 $\pm$ 280‡	252 $\pm$ 30	939 $\pm$ 110‡	112 $\pm$ 28	146 $\pm$ 26
30HF	250 $\pm$ 61	580 $\pm$ 72†	11 $\pm$ 2	273 $\pm$ 30‡	12 $\pm$ 0.2	60 $\pm$ 1‡

Fig. 1. FAS, ACC and glucose-transporter (Glut 4) mRNA concentrations in white adipose tissue and plasma insulin of obese (■) and lean (□) Zucker rats before (15 and 21 days) and after weaning on to a HC (22 and 30 days) or a HF diet (30 days)Data are means $\pm$ S.E.M. for 4-6 rats: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

partially in obese rats, leading to a 2-fold difference when expressed per μg of protein and a 6-fold difference when expressed on a per-cell basis (Table 3).

In both groups, the lipogenic-enzyme activities increased slightly between 15 and 21 days (Table 3). Weaning to a HC diet induced, at day 30, a 40- and a 100-fold increase in lipogenic-enzyme activity in lean and obese rats respectively as compared with 15-day-old rats (Table 3). In both genotypes, this increased enzyme activity was much decreased by weaning rats on to a HF diet. Except at the age of 15 days, lipogenic-enzyme activity was significantly higher in obese than in lean rats (Table 3).

Glucose-transporter and lipogenic-enzyme mRNA concentrations

The specific mRNA concentrations of Glut 4, FAS and ACC were quantified relative to total RNA concentration. In both obese and lean rats, the concentration of Glut 4 mRNA was low during suckling at 15 days of age and increased slightly from day 15 to 21 in obese rats (Fig. 1). At 1 day after weaning to a HC diet, the concentrations of Glut 4 mRNA were increased 6-fold in lean and 13-fold in obese Zucker rats. On day 30, there was a decrease to a concentration respectively 2- and 6-fold higher than in the corresponding 15-day-old suckling rats (Fig. 1). In rats weaned to a HF diet, the concentration of Glut 4 mRNA remained comparable with the concentration observed during suckling in lean rats, whereas it was still increased by 2-fold in obese *fa/fa* rats (Fig. 1). The important finding was that, except at 15 days of age, the relative concentration of Glut 4 mRNA was significantly higher in obese than in lean rats (Fig. 1). If the results are expressed on a per-cell basis (relative to DNA), the time-related pattern is not fundamentally different (results not shown). However, the differences in specific mRNA levels observed in obese rats are larger and significantly enhanced at 15 days of age (results not shown).

In lean rats, FAS and ACC mRNA concentrations did not change between 15 and 21 days (Fig. 1). By contrast, they increased markedly at weaning on to a HC diet, but not on to a HF diet. In obese rats the time-related changes were quite similar, except that there was a significant decrease in mRNA concentrations between 15 and 21 days (Fig. 1). Once again, the important finding was that, except at the age of 21 days, lipogenic-enzyme mRNA concentrations were significantly higher in obese than in lean rats (Fig. 1). If the results are expressed on a per-cell basis (relative to DNA), the differences in specific mRNA levels observed in obese rats are still larger (results not shown).

Plasma glucose and insulin concentrations

As depicted by Fig. 1, during suckling and after weaning, basal plasma insulin levels were significantly higher in the obese *fa/fa* rats than in lean *Fa/Fa* rats, despite no important changes in glycaemia (Table 1).

The consumption of a HC diet induced a 2- and 3-fold increase in basal plasma insulin levels in lean and obese rats respectively, without significant changes in those of glucose (Fig. 1 and Table 1). Weaning to a HF diet increased plasma insulin concentration 1.5-fold in both genotypes. However, plasma insulin concentration remained still higher and plasma glucose concentration was enhanced in the obese rats.

DISCUSSION

Obesity is a complex disorder in which the most obvious phenotype is an abnormal development of white adipose tissue. It has been shown in animal models of obesity that, very early in the time course of this pathogenesis, there was an increased insulin-responsiveness in white adipose tissue for both glucose transport and lipogenesis [1–3,36], which vanishes with age. A

similar trend for white-adipose-tissue metabolism has been suggested by comparing young and old obese humans [26]. In the genetically obese Zucker rats, increased insulin-responsiveness of white adipose tissue for glucose utilization is particularly pronounced after weaning, resulting in overt obesity. The present work was undertaken in the genetically obese *fa/fa* rats to study the factors which might be involved in this phenomenon.

The suckling–weaning transition in lean rats

In rats, the suckling period is characterized by the intake of a HF low-carbohydrate diet (milk [37,38]), whereas after weaning the adult diet is rich in carbohydrate and low in fat. Insulin remains at a low concentration during the suckling period but increases at weaning ([14,39]; the present study). During suckling, white adipose tissue is characterized by a low rate of basal and insulin-stimulated glucose utilization, which markedly increases at weaning on to a HC diet, but not at weaning on to a HF diet [40], which prevents, at least partially, the rise in plasma insulin levels ([14,39]; the present study).

It has been shown in the Wistar rat that glucose transport, the amount of insulin-regulatable glucose transporters and the lipogenic-enzyme activities were low during the suckling period and markedly increase after weaning to a HC diet, but not to a HF diet [14,15,34]. Parallel changes in the mRNAs for these effectors were observed during the suckling–weaning transition [14,34], suggesting a major role for a pre-translational mechanism. In the present study, we have found similar changes in the lean (*Fa/Fa*) Zucker rats.

The suckling–weaning transition in obese Zucker rats

Higher plasma insulin concentrations in obese *fa/fa* rats have been well described after weaning [1,2]. In the present study, we have shown that hyperinsulinaemia was present even before weaning. This is at variance with previous work, but could probably be explained by the genotype of the lean rats used (*Fa/Fa* against *Fa/fa*) and by the careful nutritional design. An increased insulin secretion during the suckling period in obese *fa/fa* rats is not surprising, since it has been shown that the parasympathetic and sympathetic activity, major regulators of insulin secretion [24], are respectively increased and decreased in obese pups even before weaning [24,41]. Moreover, it has been shown that glucose elicits an over-secretion of insulin in suckling pre-obese pups [24]. Weaning to the HC diet still re-enforces the hyperinsulinaemia of the obese *fa/fa* rats.

In white adipose tissue of obese *fa/fa* rats, Glut 4 expression was higher than in lean rats after weaning, although the trend was less marked for the protein than for the mRNA. This result is in agreement with a recent study [42] where an increase in Glut 4 protein and mRNA concentrations has been described in 30-day-old obese Zucker rats compared with their lean (*Fa/fa*) littermates. In that study, Hainault *et al.* [42] also reported a higher Glut 4 expression in 16-day-old obese rats. This last result is at variance with our results, and is probably due to the different diet conditions. Nevertheless, the increase in Glut 4 expression described in both studies can be related to the higher glucose transport observed in adipocytes of 30-day-old obese rats [3]. Once obesity is established, white adipose tissue becomes insulin-resistant [25], and in another model of genetic obesity, the *db/db* mouse, expression of Glut 4 in this tissue returns to normal [43]. In rats, parallel changes in Glut 4 amount, mRNA and insulin concentrations have been described in white adipose tissue in various situations such as starvation, diabetes and development [9–14,34]. Over-expression of Glut 4 in weaned obese rats could thus be related to the higher plasma insulin concentrations. It should be noted, however, that we did not find any differences in the expression of Glut 4 before weaning

between obese and lean rats, whereas plasma insulin concentrations were already higher in the former. Furthermore, Hainault *et al.* [42] found an increased Glut 4 expression in white adipose tissue of 16-day-old obese rats, with a similar plasma insulin concentration in the two genotypes. This indicates that insulin is certainly not the only factor involved in the enhanced expression of Glut 4 in white adipose tissue of obese Zucker rats.

It must be pointed out that the difference that we observe in the present study in terms of glucose-transporter concentration is much less marked than that previously described by using cytochalasin B measurements [3]. This could be related to the fact that, in contrast with these studies, the present weaning diet is fat-free. The obese Zucker rats are already hyperinsulinaemic, even when consuming a balanced diet [1,2]. Thus the increased insulin secretion linked to the absorption of an enhanced carbohydrate amount has probably a much smaller effect on the expression of Glut 4 in obese than in lean rats. This is supported by the fact that, when rats were fed with a HF diet, the difference is much more marked (Table 3). The rather modest difference in the expression of Glut 4 between lean and obese rats suggests that this effector of insulin action does not play a major role in the development of adipocyte hypertrophy.

In obese rats, concentrations of FAS and ACC mRNA were higher than in lean rats at 15 days of age, although FAS and ACC activities were not significantly different, suggesting a post-transcriptional regulation. It must be pointed out, however, that the activities measured at that age were very low. Later, the increased concentration of lipogenic-enzyme mRNA in obese *fa/fa* rats is in accordance with several studies reporting an early enhancement of lipogenesis and lipogenic capacities in white adipose tissue of this animal [16–19]. Weaning on to a HF diet decreases both the lipogenic-enzyme activities (the present study; [16]) and mRNA concentrations as compared with age-matched rats weaned on to a HC diet. A striking similarity is observed between the development, the over-expression of lipogenic enzymes and the plasma insulin levels, in keeping with the hypothesis that insulin could be a major regulator of FAS and ACC expression [21–24].

At 21 days of age, when the rats were still suckling, there was a slight decrease in lipogenic-enzyme mRNA concentrations, even when expressed on a per-cell basis. This could be due to the fact that pups start to eat the food of the mother around 15–16 days (when their eyes open), a diet richer in polyunsaturated fat than the milk [37,38]. Indeed, it has been shown that the increase in FAS mRNA concentrations induced by weaning to a HC diet was blunted when this HC diet was supplemented with 3% linoleic acid or linolenic acid [44].

The similarity in both Wistar and lean Zucker rats between the expression of effectors of insulin action and plasma insulin concentrations at weaning, as well as the over-expression of these effectors in hyperinsulinaemic obese rats, suggests that this hormone has an important role in the regulation of these effectors [14,34]. Hyperphagia, which has been described to occur in obese rats after weaning, could affect, either directly or by increasing plasma insulin, the Glut 4 and enzyme expression and activity [45]. This is not the case during suckling, since neither hyperphagia nor apparent change in milk composition related to the genotypic difference have been reported [37,46].

The increased plasma insulin levels of the pre-obese rats, which were present as early as 15 days of age in our dietary conditions and which increased further after weaning, could enhance, in white adipose tissue, on the one hand the efficiency of specific proteins (translocation of the glucose transporter to the plasma membrane [3], activation of ACC) and on the other hand their expression (the present paper). However, concerning the expression of Glut 4, it must be pointed out that insulin is

probably not the sole regulatory factor. Indeed, whereas plasma insulin increases in both lean and obese rats between 22- and 30-day-old HC-weaned rats, Glut 4 mRNA concentration decreases. Moreover, in HF-weaned rats, the marked decrease in plasma insulin concentration as compared with HC-weaned rats is concomitant with only a slight decrease in Glut 4 mRNA concentration.

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