MINIREVIEW

Genetic Obesity: Is the Defect in the Sympathetic Nervous System? A Review Through Developmental Studies in the Preobese Zucker Rat (43286C)

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Obesity of the Zucker rat is genetically transmitted as an autosomal recessive trait due to a mutation on a single gene that has been called "fa," for fatty (1). A single mutation theoretically implies that alteration of a single protein (or very few) should explain all the defects associated with the syndrome. The discovery of the etiology of this genetic obesity syndrome is a task of significance, since this animal model shares many similarities with obesity seen in humans who have insulin-resistance (2), or Type II diabetes (3). Recent reports which have demonstrated the strong genetic component in the transmittance of obesity in humans (4–6) also underscore the importance of the Zucker *fa/fa* rat as an appropriate model for studying obesity.

Over the past years, as new discoveries have been made and a better knowledge of the syndrome has evolved, several hypotheses have been proposed as primary causes for the development of this obesity (for reviews on Zucker rats, see Refs. 7–10). The main hypotheses are: an alteration of a single protein (lipoprotein lipase, fatty acid synthetase), the animal's hyperinsulinemia itself, defects in the control of the hypothalamic-pituitary-adrenal axis, and alterations in the central nervous system. However, it is noteworthy that the basis for most of the hypotheses for the etiology of this syndrome has been derived mainly from data obtained in adult rats. Both the problems associated with genotype identification before 16–18 days of age and the difficulty of establishing a Zucker rat colony (i.e., ≈80% of males and all females of the *fa/fa* genotype are sterile) have contributed to a relative dearth of studies in very young rats. Moreover, it has been shown recently that metabolic alterations present in rat pups (a slight hyperglycemia) can disappear (11, 12) or even display an opposite pattern after weaning (glucose utilization and thyroxine-5′-deiodinase activity in brown adipose tissue; 13, 14). Thus, it appears that, in search of etiologic factors, one should also take into consideration the emergence of anomalies in the very young preobese *fa/fa* rat. An improved knowledge of the different factors involved in the development of obesity in Zucker pups may also help a better understanding of the development of obesity in infants. Accordingly, the aim of this review is to present an up-to-date picture of our present knowledge of the young Zucker rat from birth until the age of 5 weeks.

In addition, tables are presented that gather the main data from studies performed in 0- to 5-week-old Zucker rats (Tables I–VIII).

As a preliminary remark, we would like to point out that in the literature, young Zucker *fa/fa* rats may be called either obese or preobese, provided that their genotype has been identified previously, although young *fa/fa* still look physically identical to their lean *Fa/fa* or *Fa/Fa* littermates. Actually, the obese phenotype of *fa/fa* rats begins to be visually detectable as the total body fat reaches ≈20% of the body weight, when these animals are approaching 4–5 weeks of age (15). Thus, young *fa/fa* rats should be considered preobese until the age of 3–4 weeks, i.e., until the beginning of the weaning period. However, before the

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0037-9727/91/1981-0528$3.00/0

effective detection of the phenotype by a mere visualization, genotypes can be determined around 16–18 days of age by the following methods: decreased oxygen consumption (16), core temperature (17), and weight of the inguinal fat pad versus the body weight (18). It is also possible to identify the genotype at 7 days of age based on the size and number of hypodermal adipocytes from skin biopsies (19), by sizing fat cells from inguinal fat pad biopsies (20) or by measuring the core temperature of rats pups on two consecutive days (Day 6 and Day 7; 21).

Since the transition from suckling to weaning is of paramount importance in the further development of obesity in Zucker rats, it should be noticed that in the rat, weaning naturally occurs between 3 and 4 weeks of age. As a consequence, in most laboratories, pups are generally separated from the dam between 21 and 30 days of age.

Obesity of the adult Zucker rat appears to result from defects in both components of energy balance, i.e., an increase in energy intake and storage, as well as a decrease in energy expenditure. However, when analyzing the temporal development of anomalies in the preobese rat, one can clarify the cause and effect relationship between some metabolic anomalies. Consequently, one can argue for the initial appearance of dysregulation in one component of energy balance compared with the other and, finally, discuss its etiology.

Table I. Whole Body[a]

	Day postpartum						
	0	2	7	14	21	28	35
Body wt		+	+	+	+	+	+
Food intake		0	0	+	+	+	+
Physical activity				0	–	–	–
Thermogenesis							
O_2 consumption		–	–	–	–	–	–
Diet-induced thermogenesis		0	–				
Cold-induced thermogenesis		–	–	–	–	–	
Core temperature	0	0	–	–	–	–	–
Water							
Total			0	0	0	0	
Turnover				0	0	+	
Protein							
Carcass			0	0	–	–	
Synthesis				–	0		
Lipid							
Carcass			+	+	+	+	+
Lipogenesis				0	+		
Glucose							
Turnover				–		+	
Tolerance				–	–	–	–

Note. The following tables are a compilation of the main data reported in 0- to 5-week-old Zucker rats. 0 = no difference between *fa/fa* and *Fa/Fa* or *Fa/fa* controls rats; – = a decrease in *fa/fa* rats compared with control rats; + = an increase in *fa/fa* rats compared with control rats. For clarity, data reported in *fa/fa* rats are rounded off to the closest week. E.g., a defect reported in 9-day-old *fa/fa* rats will be inserted in the Day 7 column. For each parameter, reference sources are listed. Abbreviations used in tables: FAS, fatty acid synthetase; ACoA case, acetyl-CoA carboxylase; CCE, citrate cleavage enzyme; ME, malic enzyme; G-6-PDH, glucose-6-phosphate dehydrogenase; 6-PGDH, 6-phosphogluconate dehydrogenase; GDP, guanosine 5′-diphosphate.

[a] Body weight: 15, 115, 146; food intake: 16, 20, 53, 116; physical activity: 152; thermogenesis: 17, 21, 29, 115, 116, 141; and water: 15, 28; protein: 15, 47; lipid: 15, 28, 153, 48, 139; glucose: 11–13, 72, 63, 65.

Table II. Plasma[a]

	Day postpartum						
	0	2	7	14	21	28	35
Hormones							
Corticosterone				0	–	0	+
Gastric inhibitory polypeptide					0	0	0
Glucagon				0			
Growth hormone							0
Insulin-like growth factor-1						+	0
Insulin	+	0	0	+	+	+	+
Prolactin							–
Testosterone						0	0
Triiodothyronine						–	–
Thyroxine						–	–
Thyroid-stimulating hormone							0
Lipids							
Triglycerides			0	+	+	+	+
Nonesterified fatty acids				0	0	+	+
Cholesterol			0	0	+	+	+
Phospholipids			+	+	+	+	+
Glucose				+	0	0	0

[a] For details, see *Note* to Table I. Hormones: 11, 60, 61, 64, 66, 79, 80, 81, 83, 84, 85, 153; lipids: 12, 40, 52, 53, 57; glucose: 11–13, 58, 154.

Table III. Central Nervous System[a]

	Day postpartum						
	0	2	7	14	21	28	35
Neurohormones							
Somatostatin							0
Neurotensin (binding sites)						–	
Glucose							
Uptake						0	
Protein							
Tyrosine quantity							–
Tyrosine aminotransferase							0
Hypothalamus							
Somatostatin							0
Glucose uptake						0	

[a] For details, see *Note* to Table I. Neurohormones: 61, 96; glucose: 13; protein: 60; Hypothalamus: 13, 61.

Increase in Energy Intake and Storage: Development of an Overactive Storage Component Independent of Hyperphagia or Hyperinsulinemia

Although hyperphagia clearly plays a major role in the development of this obesity, food restriction does

Table IV. Pancreas[a]

	Day postpartum						
	0	2	7	14	21	28	35
Weight						0	
Enzyme							
Amylase quantity						0	
and mRNA						0	
Hormones							
Insulin content	+		0	0	+	+	+
Somatostatin					0		0
Glucose							
Oxidation						0	

[a] For details, see *Note* to Table I. Weight: 155; enzyme: 155; glucose: 155; hormones: 11, 61.

Table V. Liver[a]

	Day postpartum						
	0	2	7	14	21	28	35
Weight			0	0	0	+	+
Lipid							
Triglyceride content			0	0	+	+	+
Lipogenesis			0	0	+	+	+
FAS			0	0	+	+	+
ACoA Case			0	0	0	+	
CCE			0	0	+	+	
ME			0	0	+	0	
G-6-PDH			0	0	+	+	+
6-PGDH			0	0	+	+	
Protein							
Synthesis				0	0		
Tyrosine aminotransferase					+	+	+
Tryptophan oxygenase							+
Serine dehydrogenase							+
Glucose							
Glucose production						0	
Glycogen content							+
Glyceraldehyde-3-phosphate dehydrogenase (mRNA)				0		+	

[a] For details, see *Note* to Table I. Weight: 40, 54; lipid: 17, 30, 40, 57, 59, 65; protein: 47, 83; and glucose; 33, 72, 139.

not prevent the development of the complete obese syndrome (22–25). In preobese *fa/fa* rats, hyperphagia starts to be detectable after the second week of life (15), in parallel with their new ability to eat *ad libitum* solid food due to the emergence of teeth (from 10 days of age; 26). Even by preventing the expression of hyperphagia by pair-feeding artificially fed rat pups from 10 to 20 days, *fa/fa* pups still deposit more fat than their lean counterparts, indicating an increased energy efficiency in these animals (27).

White Adipose Tissue. A greater increase in preobese pups carcass lipid and inguinal adipose tissue growth, the first adipose depot to develop, can be detected within the first postnatal week, i.e., at least 1 week before any detectable hyperphagia (20, 28, 29). From a metabolic point of view, activities from several

Table VI. Skeletal Muscle[a]

	Day postpartum						
	0	2	7	14	21	28	35
Protein							
Content				0	–	–	
Synthesis				–	–		
Glucose							
Uptake				0		0	0
Oxidation				0	0	0	0
Glycolysis				0	0		
Glycogen content							0
Lipid							
Oxidation			–				
Esterification			+				
Triglyceride content							+
LPL activity			0	–	0	0	0

[a] For details, see *Note* to Table I. Protein: 47; lipid: 51, 69, 156, 40; and glucose: 12, 13, 69, 72, 154.

Table VII. White Adipose Tissue[a]

	Day postpartum						
	0	2	7	14	21	28	35
Weight			+	+	+	+	+
Cellularity							
Size		0	+	+	+	+	+
Number			0	0	0	–	+
Lipid							
Triglyceride content		0	+	+	+	+	+
LPL			+	+	+	+	+
Lipogenesis				+	+	+	+
FAS			+	+	+	+	+
ACoA case					+	+	+
CCE			+	+	+	+	+
ME			+	+	+	+	+
G-6-PDH			0	+	+	+	+
6-PGDH			+	+	+	+	+
Glucose							
Uptake				+		+	
Oxidation				+		+	
GLUT1 protein				0		0	
GLUT1 mRNA				0		0	
GLUT4 protein				+		+	
GLUT4 mRNA				+		+	

[a] For details, see *Note* to Table I. Weight: 40; cellularity: 16, 29, 40, 157, 158; lipid: 2, 28, 29–31, 40, 65; glucose: 12, 13, 35, 36, 72, 159.

enzymes involved in lipogenesis (fatty acid synthetase, citrate cleavage enzyme, malic enzyme, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase; 30, 31), lipoprotein metabolism (lipoprotein lipase; 2, 20, 32), and glycolysis (glyceraldehyde-3-phosphate dehydrogenase; 33) are enhanced in inguinal white adipose tissue (WAT) from preobese pups before the emergence of hyperphagia. Thus, the presence of an overactive lipid storage in WAT is clearly anterior to and independent from the hyperphagia. Recent data obtained in 16-day-old pups, upon which a larger body

Table VIII. Brown Adipose Tissue[a]

	Day postpartum						
	0	2	7	14	21	28	35
Weight			+	+	+	+	+
Cellularity							
Size			0	+			
Lipid							
Lipid content		+	+	+	+	+	+
LPL			0	−	−	−	
Lipogenesis			+	+		+	+
FAS			+	+	+	+	+
ACoA case			0	+	+	+	+
CCE			0	+	+	+	+
ME			0	0	+	+	+
G-6-PDH			0	+	+	+	+
6-PGDH			+	+	+	+	+
Glucose							
Uptake				−		+	
Noradrenaline							
Content				−			
Turnover							−
Mitochondria							
Protein		0	−	−			−
Cytochrome oxidase		0	0	0	0	−	−
GDP binding		−	−	−	−	−	−
Uncoupling protein content				−	0		0
Uncoupling protein mRNA			−	−	−	−	−

[a] For details, see *Note* to Table I. Weight: 40; cellularity: 19; lipid: 37–40; glucose: 12, 13, 72; noradrenaline: 12, 140; mitochondria: 37, 119, 160.

of data is available due to the relative easiness of genotype detection, showed that the increased lipid storage capacities of WAT are regulated at transcriptional levels (34).

In 16-day-old Zucker rats, it has been shown that glucose uptake and metabolism and, accordingly, mRNA and protein levels of the major glucose transporter in inguinal WAT, GLUT4, are increased in *fa/fa*, while GLUT1 remains unchanged (12, 35). The glucose metabolized and directed toward the lipid synthesis and storage pathways is largely increased in fat cells of preobese rats (36). All the defects in lipid and glucose metabolism of WAT are intensified as preobese rats grow older, especially around the weaning period.

Brown Adipose Tissue. Brown adipose tissue (BAT) of *fa/fa* rats begins to accumulate a greater amount of triglycerides as early as at 2 days of age (37). *In vivo* lipogenesis and a subset of individual enzymes involved in fatty acid synthesis are accordingly enhanced in this tissue in the second week of life (38, 39). Hyperlipogenesis in BAT of *fa/fa* pups accounted for only 10–20% of its lipid content (39), suggesting that both decreased lipolysis and fatty acid oxidation contribute to the increased fat accretion. However, this has not been studied in BAT of *fa/fa* pups.

In BAT of 16-day-old *fa/fa* rats, in contrast to data obtained in WAT, glucose uptake is dramatically decreased (12) and GLUT1 protein abundance is diminished, while GLUT4 is unchanged (unpublished observations). Taking into account the high capacity for glucose disposal by BAT in normal rat pups (12), the large decrease in glucose utilization by BAT of preobese rats could explain both their diminished whole-body glucose turnover and their slight hyperglycemia, which both disappear at weaning (12, 13). The pattern of anomalies in BAT is opposite to WAT not only for glucose uptake, but also for lipoprotein lipase (LPL) activity, which is decreased from 14 days of age (40). This may be connected to the diminished sympathetic nervous system activity of preobese rats (see under Decreased Energy Expenditure, below), as both glucose transport and LPL are positively regulated by noradrenaline in BAT (41–45). After the emergence of hyperinsulinemia, both BAT and WAT demonstrated additional, but similar, defects in lipid synthesis, while glucose uptake is increased, but LPL activity remains lower in BAT of *fa/fa* rats as compared with controls rats (13, 40). This is in accordance with the fact that, in BAT, insulin can stimulate glucose transport (41, 42), but has no effect on LPL activity in fed animals (43, 46).

Skeletal Muscle. Skeletal muscle protein synthesis is decreased in 18-day-old rats (47), but there appears to be no differences between the protein mass of the two genotypes at this age (15). Cleary *et al.* (23) have suggested that the high rate of lipogenesis may lead to a removal of precursors for protein synthesis. Haggarty *et al.* (48), by showing that hyperlipogenesis of *fa/fa* rats is supported by carbon from the three basic nutrients, have invalidated this hypothesis. Martin *et al.* (10) argued that multiple alterations in muscle could not be caused by a single gene product change in the tissue, and suggested that the altered endocrine status (i.e., decreased growth hormone, somatomedin activity, and increased corticosterone) was a more likely explanation of abnormalities in skeletal muscle of *fa/fa* rats.

LPL activity is reduced in several muscular tissues of 2-week-old preobese rats, i.e., the heart, diaphragm, and thigh muscles (40). This can be explained by a decreased stimulation of these tissues by noradrenaline (49), where LPL is normally stimulated by catecholamines (50). In the diaphragm of 1-week-old *fa/fa* rats, there is both a decrease in β-oxidation and an increase in fatty acid esterification (51), which can be also explained by a diminished stimulation of this tissue by catecholamines. Theoretically, if LPL activity and fatty acid oxidation are reduced in the skeletal muscle mass, there should be more circulating triglycerides. Indeed, there are higher levels of circulating phospholipids and triacylglycerols by the end of the first and second week of life in preobese rats, respectively (40, 52).

Liver. The role of the liver in producing very low

density lipoprotein, and hence worsening the lipid serum levels, which is obvious in adult *fa/fa* rats (53–56), is of late appearance. It is only during the suckling to weaning period, but not before, that the liver of preobese animals begins to accumulate more triglycerides, is intensively lipogenetic (30, 40, 57–59), and is also a site for defects in protein metabolism (increased tyrosine aminotransferase, tryptophan oxygenase, and serine dehydrogenase activities [60]) and glucose metabolism (increased glycogen content, glyceraldehyde-3-phosphate dehydrogenase activity and mRNA levels, and lactate and pyruvate production [33, 61, 62]).

Pancreatic Hormones. These latter defects are apparently the result of a dysregulation in the endocrine function of pancreases of *fa/fa* rats that begins just prior to weaning. This is supported in part by the fact that hyperlipogenesis in the liver is abolished after streptozotocin treatment (58). The insulin and glucagon responses to a glucose or an arginine load are greater in 17-day-old preobese pups than in lean pups (63, 64), indicating an early onset of a glucose intolerance that is well-documented (11, 61, 63–66). Importantly, this defect can be prevented by the administration of acute atropine just before that of arginine, which suggests an increased parasympathetic stimulation (vague nerve) to the endocrine pancreas of *fa/fa* rats (63, 64). It is noteworthy that a decrease in plasma insulin levels, and an increase in pancreatic insulin content have been reported in the *fa/fa* fetus at 21 days, suggesting a very early alteration in the regulation of the β cell function (11). Indeed, in 5-day-old *fa/fa* pups, insulin release by perfused pancreas is increased following stimulation by acetylcholine, but not by glucose or arginine (N. Atef, C. Bulé, M.-T. Bihoreau, A. Ktorza, L. Picon, and L. Pénicaud, personal communication). This is in keeping with a possible increased sensitivity of the β cell of preobese rats to the parasympathetic nervous system activity. A recent measure made in our laboratory showed that hyperinsulinemia, previously reported to be undetectable before 17 days of age (30), is already present at 10 days of age (41.5 ± 3.29 vs 59.1 ± 4.89, $P < 0.01$, 20 *Fa/fa* vs 20 *fa/fa*). Regarding these recent data, interpretation of the increased lipogenesis in WAT of *fa/fa* pups, which starts to be detectable at 7 days of age and onward, should be re-estimated, and a role for insulin cannot now be excluded.

Insulin resistance concerning glucose metabolism, one of the main features of obesity (67, 68), has been well-studied in Zucker rats. The *fa/fa* rat develops hepatic and peripheral insulin resistance after weaning (69–74). This insulin resistance develops in muscle sooner than it does in white adipose tissue and even appears in some individual muscles (tibialis anterior, soleus) before it does in others (epitrochlearis, diaphragm; 13, 72). Differential insulin action on glucose utilization in muscle and WAT with time has been described in several other situations (75–78). This can potentiate anomalies already present before weaning (i.e., increase storage in WAT and decrease expenditure in BAT) by preferentially channelling glucose toward adipose tissue rather than toward the skeletal muscular mass (72).

Other Hormones. Circulating levels of hormones have been measured in Zucker rats between 4 and 6 weeks of age. Growth hormone and prolactin are decreased in *fa/fa* rats (79, 80) and insulin-like growth factor-1 is increased (81), while gastric inhibitory polypeptide, thyroid-stimulating hormone, *r*-triiodothyronine, and testosterone are unchanged (66, 80, 82, 83). Corticosterone has been observed as increased (61), decreased (60), or unchanged (84) in the fatty genotype. Triiodothyronine and thyroxine have been reported to be unchanged (61) or decreased in *fa/fa* rats (79, 85). While these hormone anomalies certainly contribute to the progression of the obese syndrome, their role in the onset of this obesity remains unclear due to the lack of data on preobese Zucker rats.

Several neurohormones (cholescystokinin, β-endorphin; 86–89) and neurotransmitters (noradrenaline, adrenaline, dopamine, and serotonin; 90–94) that are involved in the regulation of food intake, the pattern of intake, or diet selection exist at abnormal concentrations in the hypothalamus, pituitary, or several other brain regions of adult *fa/fa* rats. Neurotensin receptors, which are widely distributed in various brain regions in rats (95), are in young *fa/fa* rats, selectively decreased in only two brain areas (96) thought to be involved in thermoregulation (97). The importance of these abnormalities in central neurotransmitter and neuropeptide concentrations in the etiology of this obesity syndrome is unknown. These alterations might reflect a defect in the central nervous system (10). Alternatively, they might result from a new dynamic set point, i.e., a metabolic and hormonal re-equilibration, in response to the emergence of primary disorders and the following cascade development of secondary defects.

Adipsin. Adipsin is a serine protease homologue whose circulating levels and expression in WAT are severely depressed in several genetic and acquired obesities (98). It was thus considered a putative marker of obesities. Indeed, the mRNA level of adipsin is decreased in WAT of obese *fa/fa* rats (99). However, it is not altered in preobese *fa/fa* rats (99). This is also the case for the *db/db* preobese mice versus the obese mice (100).

"Pull" and "Push" Theories. Thus, the hyperactive fat accretions in white and brown adipose tissues are one of the primary defects detectable in preobese rat. Hypotheses relying on the defect of a single protein, in agreement with the single mutation present in the *fa/fa* rat, have been proposed in the context of a "substrate pulling theory" (fatty acids and glucose) for ex-

plaining the early excess fat storage of preobese pups. Both LPL (101) and fatty acid synthetase (30, 31), whose activities are increased in *fa/fa* pups at 7 days of age, were proposed as the primary genetic lesion leading to an increased fat storage, a decreased heat loss (hence, less need for thermogenesis), then, secondarily, to hyperphagia and hyperinsulinemia. Alternatively, a "substrate pushing theory" can be postulated. A diminution in peripheral oxidation of triglycerides by muscle due to a defect in LPL activity (40) and in glucose utilization by BAT (12), both explicable by a diminished sympathetic activity, would explain the elevated plasma concentration of these substrates, which in turn could lead to increased storage in WAT. In addition, an increase in glucose metabolism in white adipocytes could be involved, as hyperinsulinemia itself, in the high lipogenic activity of *fa/fa* pups (102, 103). One can also speculate that the increased plasma glucose concentration in preobese rats (11, 12) may trigger a higher pancreatic insulin response (104) that can be maintained with time. In effect, when β cells are stimulated for a short period of time (2–5 min) by cholinergic agonists in the presence of glucose, islets are sensitized to the level of glucose, and the resulting insulin secretory response is sustained for at least 45 min (105).

Decreased Energy Expenditure: A Role for a Diminished Sympathetic Activity

Thermogenesis. Thermoregulatory thermogenesis is usually partitioned into two components: shivering and nonshivering thermogenesis (NST; for a review, see Ref. 106). Whereas muscular shivering thermogenesis is evoked in response to exposure to cold, NST can be triggered by two stimuli, diet and cold exposure, i.e., diet- and cold-induced thermogenesis, respectively (106, 107). In rats, the shivering thermogenesis response to cold begins to be effective around 3 weeks after birth (108). Before this age, the heat production is only accounted for by NST, whose main effector is, in rats as in most mammals, brown adipose tissue (106, 109). The heat produced by BAT is of prime importance for the young rats' body thermoregulation (110), especially when pups are still hairless (hairs begin to grow around 10 days of age). It is now established that BAT thermogenesis plays a role not only in body temperature regulation, but also in energy balance too (107). Both are under the neural control of the sympathetic nervous system, whose main regulation center is located in the hypothalamus (106, 107, 111–114).

In Zucker *fa/fa* pups, a defect in thermoregulatory thermogenesis has been demonstrated using several different approaches. The first reported evidence was a decrease in core temperature in 16-day-old *fa/fa* pups, which was actually used by the authors as a method for genotype identification (17). Later on, this defect was shown to be detectable from Day 6 and onward, but not before (21, 28). Similar observations have been made comparing oxygen consumption, an index of metabolic rate or thermogenesis. Kaplan (16) reported lowered oxygen consumption in 18-day-old *fa/fa* rats and used this as a technique for genotype identification. It has since been shown to be one of the earliest defects of *fa/fa* pups, present as early as at 2 days of age (29, 115, 116). More specifically, by studying the two effectors of NST, Planche and Joliff (116) demonstrated that a diminished cold-induced thermogenesis was present in *fa/fa* rats at 2 days of age, 3 days before any defect in diet-induced thermogenesis. The same authors showed, in a study aiming at monitoring gas exchanges in parallel with measurements of white adipose tissue development, that the decreased thermogenesis of *fa/fa* rats preceded their white adipose tissue hyperdevelopment, thus suggesting the role of a primary energy deficit in the onset of this genetic obesity (29). Recently, Kaul *et al.* (117) have demonstrated that when artificially reared Zucker pups are maintained at thermoneutrality (in such a case, cold defense cannot be activated, 118), *fa/fa* rats do not deposit more body fat than their lean *Fa/-* littermates before 16 days of age. Thus, when shunting the need for cold-induced thermogenesis, obesity is not expressed before the third week of age. These data suggest that the role of a deficit in thermoregulatory thermogenesis is central to the development of this obesity.

Bazin *et al.* (37) have shown that BAT thermogenic capacity, as assessed by the binding of guanosine 5′-diphosphate to isolated mitochondrial membranes, is lower in *fa/fa* rats compared with their lean littermates at 2 days of age. Another study, performed in 10-day-old rats, has also shown a decrease in the uncoupling protein message levels in *fa/fa* pups (119). The reduced transcriptional levels of the gene encoding for the uncoupling protein can be stimulated to restore them to normal by either cold exposure or β-agonist administration (119) as early as the second week of life (120). These data agree with an alteration in the sympathetic stimulation of BAT in *fa/fa* rats. Such a defect was first indirectly demonstrated by a diminution of noradrenaline turnover (49). More recently, York *et al.* (121) showed a decreased rate of sympathetic firing in BAT of adult obese animals by direct measurement. A report of a lower noradrenaline level in BAT of *fa/fa* pups than in that of *Fa/fa* pups at 16 days of age (12) suggests that this decreased sympathetic activity is present early in life in preobese rats.

While the thermogenesis generated by activation of BAT needs adrenergic stimulation mediated through sympathetic innervation (for a review, see Ref. 113), a critical role for the thyroid hormone in the function of brown adipocytes has also been proposed (122–124). More recently, it has been shown that intra-adipocyte conversion of thyroxine to triiodothyronine, through

Type II thyroxine-5′-deiodinase activity, is required for the optimal thermogenic function of this tissue (125, 126; for a review, see Ref. 127). In 2-day-old pups, when heat production in BAT of *fa/fa* rats is already reduced, the activity of this enzyme is also decreased (128). Thyroxine-5′-deiodinase activity, known to be under adrenergic control (129), is totally restored in BAT of *fa/fa* pups after cold exposure or β-agonist drug injection (14). Furthermore, in BAT, the decreased LPL activity and mRNA levels of the *fa/fa* pups' BAT (40, 120) is also restored after an acute β-adrenergic stimulation (120).

Thus, it appears that in Zucker *fa/fa* rats, a defective thermogenesis (energy expenditure) is preceding the increased growth of white adipose tissue (29). The fact that the main metabolic defects of BAT from preobese rats are restored after cold stress or β-adrenergic administration (14, 119, 120) suggests that the muted protein(s) in Zucker *fa/fa* rats is partially active and that BAT receives inadequate stimulation by the sympathetic nervous system. Since the thermogenic response to noradrenaline and efferent sympathetic pathways from the hypothalamus are normal in *fa/fa* rats (130–133), the alteration probably resides in the hypothalamus or at a higher level of regulation, i.e., in the central nervous system.

Adrenalectomy. The thermogenic capacity of BAT in *fa/fa* rats can also be restored after adrenalectomy (134). Adrenalectomy reduces several anomalies that characterize the obesity of *fa/fa* rats, including increased weight gain, fat accretion, food intake, energy efficiency, hepatic lipogenesis, hepatic glycogen content, insulinemia, adipose tissue LPL activity, and plasma triglyceride levels, and decreased BAT noradrenaline turnover (84, 134–141). The administration of corticosterone reverses most of the effects of adrenalectomy (134–136, 138, 139, 141). These data have led to a hypothesis which proposes that glucocorticoids acting in the central nervous system through inhibition of hypothalamic corticotropin-releasing factor secretion might be the cause of the development of this genetic obesity (142–145). However, in marked contrast to what is observed in adult rats, if adrenalectomy is performed in 4-day-old pups, it does not prevent the emergence of obesity of *fa/fa* rats (146). It is possible that the high fat content of the suckling diet (147), the immature central nervous system (for a review, see Ref. 26), or the immature adrenal cortex function of rat pups (148, 149) may explain the absence of an effect of adrenalectomy in the development of obesity in *fa/fa* pups. In any case, increased fat accretion and decreased energy expenditure in adrenalectomized *fa/fa* pups, the main features of obesity to develop, remain present. Thus, it is unlikely that glucocorticoids play a role in the onset of this genetic obesity.

Conclusion

The chronological analysis of anomalies that emerge within the first postnatal week in *fa/fa* rats strongly suggests that increased lipid storage leading to obesity in Zucker rats is a consequence of a defect in energy expenditure. In the search for an unifying hypothesis that might explain the etiology of this genetic obesity, an alteration in the nervous system appears the most likely to agree with the monogenic transmittance of the syndrome. Such an alteration could cause a general defect in the peripheral organs, which are sympathetically innervated, and explain the decreased thermogenesis in BAT of *fa/fa* rats and in other organs like skeletal muscles (via decreased futile cycles activity; 150). It may also explain the decrease in the peripheral utilization of triglycerides and glucose, principally by skeletal muscles and BAT, which results in excess circulating levels of these substrates that could then be shunted for storage in WAT.

The hyperinsulinemia, which progressively develops in preobese rats from 10 days onward, could be explained either by an increased parasympathetic nervous system activity (8) or by a vagal sensitization of pancreatic insulin secretion to the increased levels of circulating glucose (105). This latter idea is consistent with a cause and effect dependency between a decreased sympathetic activity (possibly responsible for increasing plasma glucose levels) and the emergence of hyperinsulinemia.

Hyperphagia, the date of emergence of which cannot be separated from that of hyperinsulinemia, could be a consequence of the latter (78), and/or a defect in the hypothalamic control of food intake (151).

When hyperphagia and hyperinsulinemia develop, the anomalies in adipose tissue accretion get worse and a cascade development of new alterations develops in other organs (liver, muscles, brain, etc.). These alterations may give rise to a new dynamic metabolic and hormonal re-equilibration. As a result, anomalies present in older *fa/fa* rats may only be a consequence of earlier re-equilibrations and should be analyzed accordingly.

The etiology of the genetic obesity syndrome of Zucker rats appears to result from a disturbance in the regulation of the sympathetic nervous system, whose main and first expression is a decreased thermogenesis. The primary genetic defect(s) of *fa/fa* rats probably resides in discrete regions of the hypothalamus or at a higher level of regulation, i.e., in the central nervous system.

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