Developmental and nutritional changes of *ob* and PPARγ2 gene expression in rat white adipose tissue

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The *ob* gene encodes leptin, a hormone which induces satiety and increases energy expenditure. The peroxisome proliferator-activated receptor γ2 isoform (PPARγ2) gene encodes a transcription factor which controls adipocyte differentiation and expression of fat-specific genes. We have studied the regulation of these two genes in white adipose tissue (WAT) during the suckling–weaning transition. Suckling rats ingest a high-fat diet (milk). Fat-pad weight barely varied during the last week of suckling. *ob* mRNA levels, which were very low in 15-day-old rats, rose ∼ 6-fold until weaning at 21 days. When the rats were weaned on to a standard (high-carbohydrate) laboratory chow, epididymal WAT enlarged ∼ 7-fold, and *ob* mRNA kept increasing progressively and doubled between 21 and 30 days. This evolution contrasted with that of fatty acid synthase (FAS) mRNA, which increased sharply, but only after weaning. To distinguish between the influence of developmental and nutritional factors on *ob* expression, a group of rats was weaned on to a high-fat diet. This prevented the rise in glycaemia and insulinaemia and the decrease in plasma non-esterified fatty acids

which otherwise occurred at weaning. This also resulted in a slight (10–15 %) decrease in food intake and body weight gain. Under this high-fat diet, the rise of *ob* mRNA in WAT was augmented (3.7-fold in 30- versus 21-day-old pups), whereas the normal rise in FAS mRNA levels was attenuated. Fat-pad weights and adipocyte cell size and number were roughly similar in high-carbohydrate- and high-fat-weaned pups. mRNA levels of PPARγ2, like those of *ob*, were low in the WAT of 15-day-old suckling pups, doubled at 21 days, and reached a maximum as soon as 23 days. This evolution further differed from that of *ob* mRNA in not being influenced by diet composition. In conclusion, *ob* expression markedly increases during the suckling–weaning transition, and this effect is accentuated by a high-fat diet. Qualitative nutritional changes in *ob* mRNA were correlated with neither acute changes in adipose-tissue mass, nor cell size/number, nor variations in insulinaemia. PPARγ2 also increased during suckling, but rapidly reached a plateau after weaning and no longer changed thereafter. Unlike *ob*, PPARγ2 was not influenced by the diet composition.

INTRODUCTION

Adipocytes may function as endocrine cells and play an active role in the regulation of energy homoeostasis. The *ob* gene is selectively expressed in fat-cells. It encodes leptin, a newly identified hormone which induces satiety and increases energy expenditure, thereby regulating the size of body fat (lipostat model) (reviewed in [1]). Morbidly obese mice, which are homozygous for the *ob* mutation, fail to produce leptin, but lose weight when injected with the hormone (reviewed in [1]). One target tissue of leptin is the hypothalamus, where specific receptors have been identified [2,3]. The evidence that adipocytes can communicate directly with the brain has aroused considerable interest in the origin and fate of fat-cells and, thence, in a member of the peroxisome proliferator-activated receptor subfamily of nuclear hormone receptor, PPARγ$_2$. It is the first adipocyte-specific transcription factor identified so far, and, *in vitro* at least, it appears to control the expression of several other fat-specific genes and adipocyte differentiation itself (i.e. adipose-cell number). Forced expression of this gene in fibroblasts initiates a transcription cascade that leads to conversion in cells of the adipose lineage [4,5].

The suckling–weaning transition is a physiological situation during which dramatic nutritional changes occur spontaneously. At weaning, suckling rats, which had been ingesting a high-fat (HF) diet (milk), are switched to a standard (high-carbohydrate, HC) laboratory chow. These nutritional changes, and the ensuing

hormonal and metabolic modifications, underlie the marked increase in size and lipid content that normally occurs in white adipose tissue (WAT) at weaning [6]. They also contribute to the development of overt obesity in susceptible animals [6,7].

In the present work, we have studied the regulation of *ob* and PPARγ2 genes in WAT during the suckling–weaning transition. To distinguish between the influence of ontogenic and nutritional factors, a group of rats was weaned on to an HF diet. The expression of genes coding for fatty acid synthase (FAS) and phosphoenolpyruvate carboxykinase (PEPCK), two enzymes which are mainly regulated by variations of the nutritional state [6,8], was also measured for comparison.

MATERIALS AND METHODS

Animals and research design

Wistar rats, bred in the Faculty animal house, were used. On the day of delivery, the litter size was standardized to eight pups per mother. The studies were performed on male rats only, at the ages of 15 and 21 days (suckling rats) and 23, 25 and 30 days (weaned rats). At 21 days, one group of rats was weaned to a semi-synthetic HC diet composed of (% of total gross energy): 62 carbohydrate (cornstarch/dextrose, 1:3), 12 fat (corn oil/colza oil/lard, 1:1:3), 26 protein (casein). The other group was weaned to an HF diet composed of (% of total gross energy): < 1 carbohydrate, 72 fat (corn oil/colza oil/lard, 1:1:3), 26 protein

(casein) (U.A.R., Villemoisson, France). Because suckling rats begin to nibble the food of their mothers around 15 days of age [8], the mothers were fed an HF diet from day 14 of lactation until weaning to avoid any consumption of carbohydrates by the pups during the last week of lactation [8]. At weaning, the young rats were housed individually. Pups destined to be killed at 30 days were housed in metabolism cages permitting accurate measurements of food and gross energy intake. All rats were maintained at a constant temperature (22 °C) with a 12 h/12 h light (07.00–19.00 h)/dark cycle.

The animals were killed by decapitation in the morning (08.30–10.30 h). Blood was collected, and plasma kept at -20 °C until glucose, non-esterified fatty acid (NEFA) and insulin measurements. Pairs of epididymal and inguinal fat-pads were immediately removed, weighed, frozen in liquid nitrogen, and stored at -70 °C for subsequent RNA extraction or morphometric determinations.

RNA extraction and Northern-blot analysis

Total RNA was isolated from the fat tissue of each individual rat (30 days) or from pooled tissue of two or three rats (younger animals), with an acid guanidinium thiocyanate/phenol/chloroform mixture as previously described [9]. The concentration of RNA was determined by absorbance at 260 nm. For Northern-blot analysis, 30 μg of total RNA was denatured in a solution containing 2.2 mM formaldehyde and 50 % (v/v) formamide by heating at 95 °C for 2 min. RNA was then size-fractionated by 1 %-agarose-gel electrophoresis, transferred to a Hybond-N membrane (Amersham International, Amersham, Bucks., U.K.) and cross-linked by UV irradiation. The integrity and relative amounts of RNA were assessed by Methylene Blue staining of the blot.

The *ob* cDNA probe was prepared as described in [9]. The PPARγ_2 [5] and β_3-adrenoceptor (β_3-AR) [10] cDNA probes were prepared as follows. Products of 391 bp and 434 bp were obtained respectively for PPARγ_2 and β_3-AR after reverse-transcription PCR on total RNA from rat adipose tissue [sense primers: 5′-TATGGAGCCTAAGTTTGAGT-3′ (PPARγ2), 5′-TGGGACTCCTCGTAATGCCACCAC-3′ (β_3-AR); antisense primers: 5′-GCAATCAATAGAAGGAACAC-3′ (PPARγ2), 5′-CACTCGAGCATAGACGAAGAGCATTAC-3′ (β_3-AR)]. The FAS and PEPCK cDNA probes were kindly supplied by Dr. A. G. Goodridge [11] and Dr. R. W. Hanson [12] respectively. Hybridizations with the different radiolabelled probes and subsequent washings of the membranes were performed as reported in [9]. The filters were then exposed to Kodak X-OMAT AR films for 4–72 h at -70 °C with intensifying screens. The same filters were successively hybridized with the different probes. The intensities of the RNA bands on the blots and of 18 S RNA on membrane were quantified by scanning densitometry (Sharp Scanner JX 325 combined with Image Master Software; Pharmacia) and expressed as absorbance (A). Ribosomal 18 S RNA levels were similar in all groups.

Microscopic determination of fat-cell size and estimation of fat-cell number

In 15- and 21-day-old pups, microscopic measurements could not be done on the same samples as mRNAs, because of the small size of the fat-pads. Two groups of pups matched for body weight and fat-pad weight were therefore used. In older rats the fat-pad size was sufficient to allow both measurements.

Epididymal adipose tissue was allowed to thaw for 3–4 min at room temperature, fixed in Bouin's solution, embedded in paraffin and stained with Hemalun/Eosin. Diameter profiles of

fat-cells were measured by planimetry with a semi-automatic image analyser (Videoplan, Kontron Bildanalyse, Eching, Germany) and treated by the Wicksell transformation, to obtain the actual size of the cells [13]. Accurate determination of cell size required analysis of $\sim$ 100 cells per sample, as shown by the lack of change in the coefficient of variation when n was further increased. Fat-cell number was calculated by dividing the adipose-tissue mass by the mean cell volume and the density of fat tissue (0.94 g/ml at 37 °C).

Analytical procedures

Plasma glucose, insulin and NEFA were measured as previously described [9].

Presentation of the results

Results are means $\pm$ S.E.M. for the indicated number of rats. Comparisons between groups were carried out by an unpaired Student's t test or analysis of variance followed by the Newman–Keuls test for multiple comparisons, when appropriate. Differences were considered statistically significant at $P < 0.05$.

RESULTS

The body-weight gain of rats weaned on to an HF diet was slightly lower ($\sim$ 14 % at day 30; $P < 0.01$) than that of control rats weaned on to a more standard (HC) laboratory chow (Figure 1). This slight decrease can probably be ascribed to a 10 % reduction in the daily food consumption: over the last 7 days of the study, i.e. when rats were fully adapted to solid food and individual housing in metabolism cages, the average calorie intake (based on gross energy intake) was 144.8 ± 4.2 kJ (34.6 ± 1.0 kcal)/day for HF versus 161.1 ± 4.2 kJ (38.5 ± 1.0 kcal)
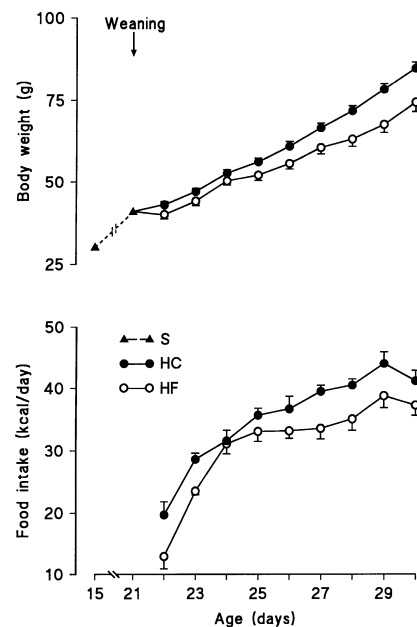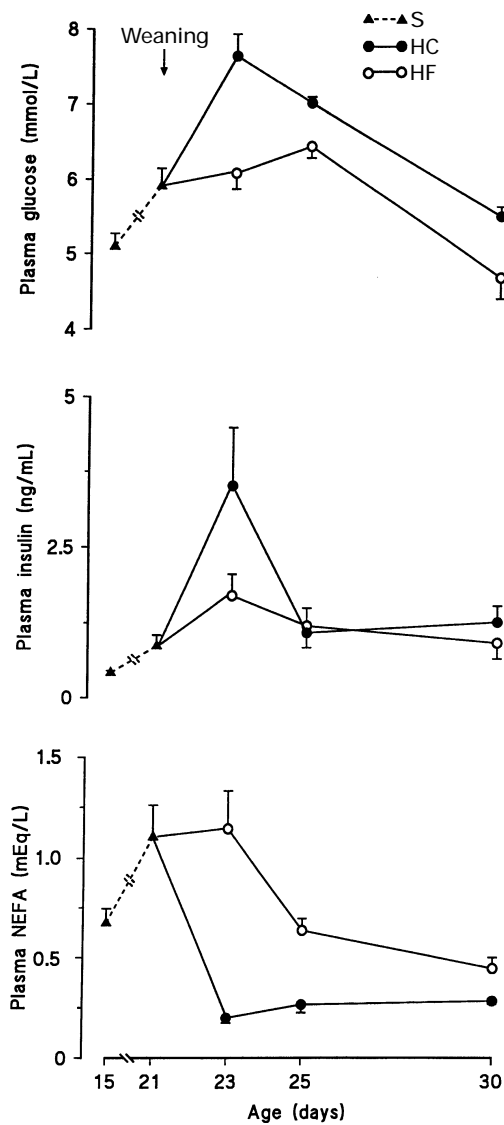

Figure 1   Changes in body weight and food intake during the suckling–weaning transition

Suckling (S) rats were weaned to a high-carbohydrate (HC) or high-fat (HF) diet at 21 days of age. The animals were housed in individual metabolism cages from weaning onward. Values are means $\pm$ S.E.M. for ten rats in each group. Note: 1 kCal $\equiv$ 4.184 kJ (the S.I. unit).

Table 1   Fat-pad weight, adipocyte volume and number during the suckling–weaning transition

(a) Weight of pairs of epididymal and inguinal fat-pads in rats weaned at 21 days of age to an HC or HF diet. (b) Adipocyte volume was measured microscopically by planimetry: $\sim$ 100 cells were analysed per rat. (c) Adipocyte number was calculated on the basis of the average values of the two aforementioned parameters. Values are means $\pm$ S.E.M. from seven (15 days), seven (21 days) or ten (30 days, HC) and ten (30 days, HF) rats. [a]$P < 0.001$ versus 15 days; [b]$P < 0.001$ versus 15 and 21 days.

	Epididymal				Inguinal	
Age (days) ...	15	21	30		30	
Diet ...			HC	HF	HC	HF
(a) Fat-pad weight (mg)	18.1 ± 0.8	$63.1^a \pm 6.4$	$396^b \pm 29$	$365^b \pm 17$	1427 ± 84	1304 ± 118
(b) $10^3 \times$ Adipocyte volume (μm^3)	8.54 ± 0.67	$26.3^a \pm 1.4$	$69.1^b \pm 3.4$	$71.3^b \pm 3.4$		
(c) $10^6 \times$ Adipocyte number/depot	2.35 ± 0.24	2.54 ± 0.20	$6.12^b \pm 0.34$	$5.51^b \pm 0.27$		

Figure 2   Changes in plasma glucose, insulin and NEFA levels during the suckling–weaning transition

Suckling (S) rats were weaned to a high-carbohydrate (HC) or high-fat (HF) diet at 21 days of age. Measurements were made on the blood obtained when the animals were killed. Values are means $\pm$ S.E.M. for ten individual rats in each group. mEq/L = mequiv./litre.
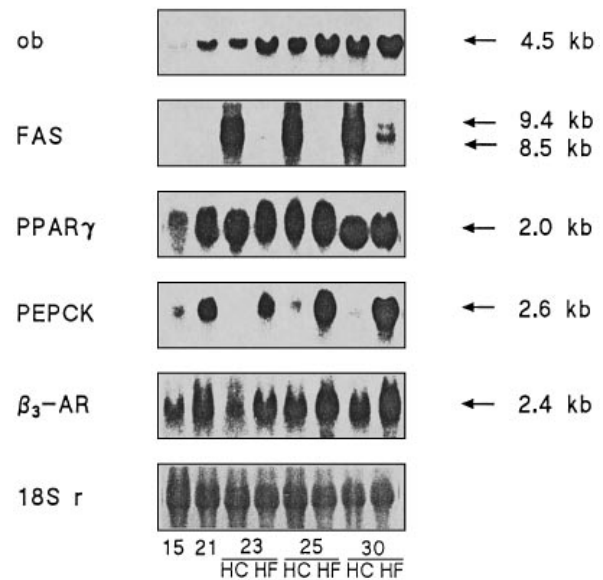
Figure 3   Northern-blot analysis of *ob*, FAS, PPARγ2 ('PPARγ'), PEPCK and β₃-AR mRNA in epididymal WAT during the suckling–weaning transition

All lanes were loaded with 30 μg of total RNA. The filters were then successively hybridized with the different radiolabelled cDNA probes. This figure is representative of four (15, 21, 23 and 25 days) or ten (30 days) determinations. Spots of 18 S rRNA ('18S r') obtained after Methylene Blue staining of the membranes are shown to indicate similar loading of RNA.

/day for HC rats ($n = 10$; $P < 0.02$). Fat-pad weight and fat-cell size increased $\sim$ 3-fold during the last week of suckling, while cell number did not change (Table 1). After weaning, fat tissue markedly enlarged ($\sim$ 6-fold for epididymal fat between 21 and 30 days of age) due to a 2–3-fold increase in adipocyte size and number. This development was not significantly affected by the type of diet (HC or HF) administered to weaned pups (Table 1).

The glycaemia of pups increased by about 1 mmol/l during the last week of the suckling period (Figure 2). In rats weaned on to an HC diet, a substantial further rise in glycaemia occurred within the first 2 days post-weaning. This was accompanied by a marked increase in insulinaemia and a decrease in plasma NEFA ($P < 0.01$ or less for these three parameters in 23- versus 21-day-old pups). Weaning on to an HF diet largely prevented these
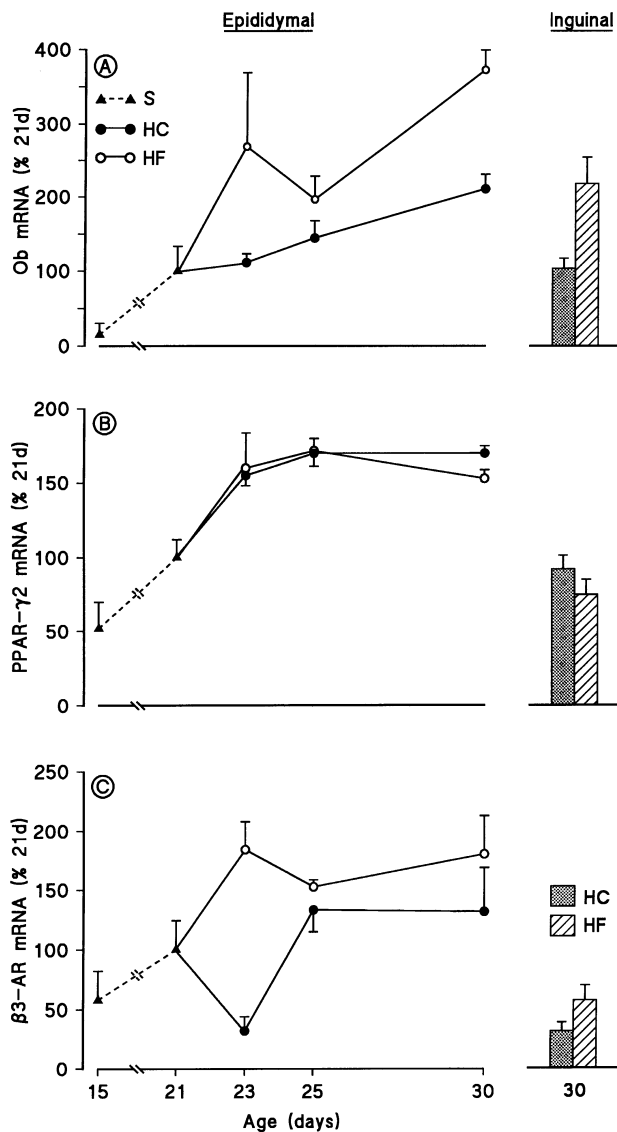
Figure 4   Expression of different genes in epididymal and inguinal fat during the suckling–weaning transition

Suckling (S) rats were weaned to a high-carbohydrate (HC) or high-fat (HF) diet at 21 days ('21 d') of age. mRNA levels of (**A**) *ob*, (**B**) PPAR)γ2 and (**C**) β_3-AR in epididymal and inguinal adipose tissue are shown. Values are means $\pm$ S.E.M. for four determinations in each group, except for 30 day-old groups ($n = 10$). Total RNA was isolated from each individual rat (30 days) or from pooled tissue of two or three rats (younger animals). mRNA levels were quantified by scanning densitometry of autoradiographic signals obtained from Northern blots (30 μg of total RNA on each lane). Results are expressed as percentages of the values obtained from epididymal adipose tissue of 21-day-old rats.

changes. There was no pronounced rise in insulinaemia, and plasma glucose and NEFA levels of HF rats remained respectively lower or higher ($P < 0.05$) than those of HC rats up to 30 days of age (Figure 2).

ob mRNA levels were low in epididymal WAT of 15-day-old suckling pups and rose $\sim$ 6-fold until weaning at 21 days (Figures 3 and 4A). Then *ob* mRNA kept increasing progressively and doubled between 21 and 30 days under an HC diet. The rise in *ob* mRNA was larger in epididymal WAT of HF-weaned rats (3.7-fold between 21 and 30 days). Thus, at day 30, *ob* mRNA levels were 77 % higher in epididymal WAT of HF than of HC rats ($P < 0.01$). A similar difference ($+111\%$; $P < 0.01$) was

observed in inguinal WAT of HF- versus HC-fed rats. However, average *ob* expression was lower in inguinal than in epididymal tissue (Figure 4A), confirming that it may be characterized by an adipose region-specificity [9,14]. This observation can be extended to the other genes studied (cf. Figure 4).

Because *ob* expression may be influenced by variations in body weight and/or quantitative changes in food intake [9,15–18], two subgroups of the HC and HF rats killed at 30 days were analysed to ascertain the direct impact of dietary fat. These subgroups (each comprising seven out of ten rats from the initial groups) were matched for daily food intake [average over the last 7 days of the study: HC, 153.6 $\pm$ 1.68 (36.7 $\pm$ 0.4); HF, 149.8 $\pm$ 5.02 (35.8 $\pm$ 1.2) kJ (kcal)/day] and for body weight (at 21 days: HC, 40 $\pm$ 1 g; HF, 41 $\pm$ 1 g and at 30 days: HC, 81 $\pm$ 1 g; HF, 77 $\pm$ 3 g). When these subgroups were compared, *ob* gene expression remained higher ($P < 0.03$ or less) in HF than in HC rats (by 104 % in epididymal and 138 % in inguinal fat at day 30). This indicates that HF diet specifically enhances *ob* gene expression.

mRNA concentrations of PPARγ_2, like those of ob, increased (2-fold; $P < 0.01$) during the last week of suckling. After weaning, they increased further (by 55 %; $P < 0.01$) reaching a maximum by as soon as 23 days. This evolution also differed from that of *ob* mRNA in being not influenced by the diet composition (Figures 3 and 4B).

Unlike *ob* and PPARγ_2, FAS mRNA was almost undetectable during suckling. Yet, as previously described [8], FAS expression increased sharply after weaning to a HC diet, and this rise was strongly attenuated in HF rats (Figure 3).

In line with previous data [6], PEPCK mRNA did not significantly rise during the last week of the suckling period. After weaning to a HC diet, PEPCK expression fell to undetectable levels in 23 day-old pups (concomitantly with the rise in insulinaemia) and re-increased slightly thereafter to preweaning values. By contrast, PEPCK expression was stimulated by weaning to a HF diet (3.5-fold in 30- versus 21-day-old pups), and the levels reached at each time point of this period were higher than the corresponding values in HC rats ($P < 0.05$ or less in epididymal or inguinal WAT) (Figure 3).

β_3-AR mRNA increased during the suckling-weaning transition. A transient decrease of β_3-AR expression occurred after weaning to an HC diet ($P < 0.05$; Figures 3 and 4C), but there was no significant difference in β_3-AR mRNA concentrations between HC and HF groups from day 25 onwards (Figures 3 and 4C).

DISCUSSION

The present study shows that *ob* mRNA levels markedly increase during the suckling–weaning transition, and that this effect is accentuated by an HF diet. *ob* gene expression is thus submitted to both developmental and nutritional regulatory mechanisms.

In control rats, a large increase in *ob* gene expression was observed during the last week of suckling. *ob* expression kept increasing after weaning, but at a lower rate. Later in life, *ob* mRNA has been reported to increase slowly with the size of epididymal WAT [15,19]. These observations apparently fit with the lipostat theory which suggests that leptin serves as a signal reflecting the state of energy stores and is produced in proportion to total body-fat mass, cell size or number (reviewed in [20]). *ob* mRNA increased 6.7-fold during the last week of suckling, whereas adipocyte number changed little and cell size enlarged 3-fold. This supports the hypothesis that *ob* expression is stimulated in response to hypertrophy rather than hyperplasia [21]. However, after weaning to a standard diet, adipocyte size similarly increased (2.6-fold) between 21 and 30 days, and *ob* mRNA only

doubled. Yet, as previously suggested [21], filling of small adipocytes could have a greater influence on *ob* expression than filling of larger adipocytes. Changes in fat-cell size and/or number could thus be involved in developmental changes in *ob* expression. On the other hand, *ob* expression was higher in HF than in HC rats, although there were no differences in epididymal and inguinal fat-pad weights, and in fat cell size and number. Thus, in agreement with our and other previous findings [9,22,23], the present results indicate that there is no tight relationship between *ob* expression and adipocyte size and number during certain acute phases of metabolic changes.

Our observations also show that *ob* expression is regulated not only by quantitative (i.e. fasting/refeeding) [9,16,17] but also by qualitative alterations in diet. The greater *ob* expression under an HF diet is consonant with that observed in FBV mice fed a so-called 'western diet' [24]. This previous study did not clearly establish, however, the exact contribution of increased dietary fat on *ob* expression because of several confounding factors [24,25]. First, the western diet was enriched not only with fat (41 % of calories) but also with sucrose (30 % of calories), and it was not possible to determine which component was responsible for *ob* overexpression. Second, obesity develops in FBV mice chronically offered the western diet (as it may occur in some susceptible inbred strains of rodents), and overt obesity itself (genetic or acquired forms) often results in increased *ob* mRNA and protein levels [22–24]. Our study unambiguously shows that an HF diet may specifically and rapidly upregulate *ob* gene expression, independently of any increase in body weight.

The mechanisms underlying the nutritional and developmental changes of *ob* expression during the suckling–weaning transition remain unknown. The possible role of changes in adipose-tissue mass or cell size/number has already been discussed. Several other factors may be involved. First, glucocorticoids increase *ob* gene expression *in vivo* [26] and in most [27,28], though not all [29], *in vitro* studies. The increase in free plasma corticosterone between days 12–14 and day 19 [6] is likely to stimulate *ob* expression during the last week of suckling. Whether the subtle increase in corticosterone levels or responsiveness reported under an HF diet [30,31] suffices to explain the post-weaning difference between the two diets remains unsettled. Secondly, several lines of evidence suggest that insulin stimulates *ob* gene expression *in vivo*. *ob* mRNA and protein levels were decreased by fasting and re-induced by refeeding, concomitantly with the changes in insulinaema [9,16,17,22]. Moreover, high insulin levels during euglycaemic–hyperinsulinaemic clamps up-regulated *ob* gene expression [14,17,32]. In the present study, the rise in insulin occurring within the first 2 days of weaning to an HC diet was accompanied by a sharp increase of FAS mRNA levels and a decrease of PEPCK mRNA levels, as expected from their known regulation and/or potentiation by insulin [33,34]. The concomitant increase in *ob* expression was only marginal (11 %) and smaller than in HF-weaned rats, whose diet prevented the rise in insulinaema. Thus the observed changes in *ob* gene expression are probably not mediated by insulin. Thirdly, besides hormones, nutrients themselves could be involved in *ob* gene regulation. Hyperglycaemic-clamp studies have shown that glucose does not, by itself, modulate *ob* expression [17]. However, NEFA are stimulatory *in vitro* (B. Reul and S. M. Brichard, unpublished work). It is thus possible that, together with glucocorticoids, they contribute to the higher *ob* expression in HF-weaned rats and to the rise in *ob* mRNA during the last week of suckling.

Recent studies have shown that leptin secretion is regulated at pre-translational levels [22,23]. The changes in plasma leptin should thus vary in parallel with those in *ob* mRNA. If this assumption is correct, plasma leptin levels were probably higher in HF- than in HC-weaned rats. One may therefore wonder whether, besides lesser palatability and rewarding effect of the diet, high leptin levels could contribute to the slight decrease in food intake and body-weight gain of these HF rats. However, when two subgroups of HC- and HF-weaned rats ($n = 7$ for each) were matched for daily food intake and body weight, *ob* gene expression remained elevated in the latter. Thus, in a substantial subgroup of HF rats, food intake was not decreased in spite of presumably high leptin levels. This would suggest, at variance with the hypothesis expressed above, that sensitivity to leptin may actually be reduced in some HF pups, as reported for mice fed the western diet and for obese rodents or humans in general [15,23,24]. This would also imply that fat-induced resistance to leptin may precede overt obesity or occur independently of it. Since insulin resistance is a common characteristic of obese subjects [35] and HF pups [36], it is possible that resistance to leptin and insulin develop concomitantly.

In vitro studies with adipocyte-cell lines indicate that PPAR*γ*2 may play a key role in the development of the adipose lineage, and that its transcriptional activation may be potentiated by a variety of lipids or lipid-like compounds [4], including naturally occurring polyunsaturated fatty acids. The developmental and nutritional regulation of the PPAR*γ*2 gene has not yet been characterized *in vivo*. Our study clearly shows that PPAR*γ*2 mRNA increased during suckling, but rapidly reached a plateau after weaning and no longer changed thereafter. In particular, PPAR*γ*2 expression was not influenced by the diet composition. The receptor, therefore, appears to be submitted to developmental, but not to qualitative nutritional regulatory mechanisms.

PPAR*γ*2 is an adipocyte-specific, nuclear transcription factor that is activated by endogenous ligands, among which are arachidonate metabolites of the prostaglandin J2 group [37]. It has been proposed to regulate the expression of the *ob* gene [38]. However, we show here that the expression of PPAR*γ*2 differed from that of *ob* in reaching a maximum by as soon as 2 days after weaning. Moreover, it remains to be determined how the ligands of PPAR*γ*2 change during the suckling–weaning transition.

Several lines of evidence indicate that the β_3-AR may play a pivotal role in obesity. β_3-specific agonists have potent anti-obesity and anti-diabetic effects in both animals and humans; expression of the receptor is impaired in rodent models of obesity, and a missense mutation in the gene may lead to visceral obesity in man (reviewed in [39,40]). Moreover, recent *in vivo* and *in vitro* studies have proposed that the sympathetic system and β_3-agonists may modulate *ob* expression [28,41], though this point is still controversial [18]. Our study demonstrates a developmental rise in β_3-AR mRNA during the suckling–weaning transition. The most striking nutritional effect was produced by the HC diet and consisted in a very transient (day 23) decrease of β_3-AR mRNA. It is possible that this inhibition results from the concomitant rise in insulinaemia, since insulin rapidly lowered β_3-AR mRNA and protein levels in 3T3-F442A adipocytes [41].

In conclusion, we have shown that *ob* expression markedly increases during the suckling–weaning transition, and that this effect is accentuated by an HF diet. PPAR*γ*2 also increased during suckling, but rapidly reached a plateau after weaning and no longer changed thereafter. Unlike ob, PPAR*γ*2 was not influenced by the diet composition.

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