Differential long-term dietary regulation of adipokines, ghrelin, or corticosterone: impact on adiposity

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Abstract

In the present experiment, we examined in Long-Evans rats the long-term effects of diets that differed in the energy provided by proteins (P) and fats (F) but provided a constant level of energy from carbohydrates (55%) on various hormones regulating feeding and metabolism. Sixty adult rats were fed for 2 months either a high-fat (protein-to-fat, PF 5/40), a control (PF 15/30), low-fat (PF 30/15), or high-protein (PF 40/5) diet *ad libitum*. Both the PF 30/15 and the PF 40/5 rats ate significantly less than their PF 5/40 and PF 15/30 counterparts throughout the experiment ($P < 0.001$). PF 40/5 rats weighed less than PF 15/30 rats ($PL = 0.04$). PF 40/5 and PF 30/15 rats had smaller epididymal and perirenal adipose tissue depots than PF 5/40 and PF 15/30 rats ($P < 0.05$ or less). Adiponectin (+25–47%) and leptin levels

in the PF 5/40 rats were higher than in the three other groups ($P < 0.0025$ or less). Ghrelin concentration in the PF 30/15 group was also higher than in the three other groups ($P < 0.001$ versus PF 5/40; $P < 0.05$ versus PF 15/30 and PF 40/5). Corticosterone level was 2- to 2.5-fold higher in PF 40/5 rats than in the three other groups ($P < 0.01$ or less). Immunoreactive insulin was not different between the four groups. Our current findings thus show that increases in the protein content resulted in a greater degree of leanness, but at sufficiently high levels, also activated the hypothalamo-pituitary axis. Ghrelin appeared to be down-regulated by increases in fat content and no obvious signs of insulin resistance were observed in any of the rats under study.

Journal of Endocrinology (2008) **196**, 171–179

Introduction

The macronutrient composition of the diet is a very important factor in the regulation of overall body weight and metabolism. Fats and carbohydrates are primarily considered to be sources of energy stores in either adipose tissue or liver, whereas proteins are considered to supply body-building elements. All three of these macronutrients can modulate feeding behavior in a manner that is mainly dependent upon their physical form, type, and quantity in the ingested diet (Sclafani 1987, Warwick & Schiffman 1992, Bray *et al.* 2004, Tome 2004). When combined, fats and carbohydrates augment diet palatability (Sclafani *et al.* 1996, Levine *et al.* 2003). High-fat (HF) sweet diets are also energy dense and are generally over consumed in the Western countries (Golay & Bobbioni 1997, Erlanson-Albertsson 2005). Indeed, long-term ingestion of HF/high-carbohydrate diets often leads to the development of overweight, and also of adiposity and metabolic abnormalities (Levin 2005). On the other hand, ingestion of high-protein (HP) diets leads to weight loss in association with decreased energy intake (Anderson & Moore 2004, Tome 2004). This is mainly due to the high satiating effects of proteins when compared with fats and carbohydrates (Jen *et al.* 1985, reviewed in Stubbs 1995,

Reid & Hetherington 1997). It is perhaps also due to a change in palatability. Accordingly, many types of unbalanced diets that show wide variation in their macronutrient composition have been proposed to decrease body weight in obese people (Golay *et al.* 1996, Astrup *et al.* 2004, Klein 2004, LaraCastro & Garvey 2004, SegallSaacson *et al.* 2004, Yancy *et al.* 2004, Dansinger *et al.* 2005, McAuley *et al.* 2005). Some diets have become extremely popular due to their gross effects upon body weight despite the lack of any clinical data showing their long-term impact on either cardiovascular risk or cancer development. Moreover, the classical ‘yo-yo’ phenomenon, i.e., a regain in body weight after discontinuing the diet is observed with many popular diets, indicating that there is often a low adherence and/or difficulties with maintaining them over a long period.

It is also known that the principal hormonal changes that are associated with each type of dietary macronutrient can have either favorable or adverse effects (Wolever 2003, Johnston *et al.* 2004, Stern *et al.* 2004). Development of insulin resistance, and more generally of the metabolic syndrome, have been well studied and the overconsumption of both fats and carbohydrates contributes to this development (Reaven 2005). Diet composition also affects adipose tissue hormones such as leptin (Stricker-Krongrad *et al.* 1998)

and adiponectin (Havel 2004, Kadowaki & Yamauchi 2005). It also modulates gastric hormones involved in feeding regulation like ghrelin (Beck *et al.* 2002, Erdmann *et al.* 2003, Greenman *et al.* 2004, Moesgaard *et al.* 2004, Sanchez *et al.* 2004, Lindqvist *et al.* 2005, Pavlatos *et al.* 2005).

Corticosterone (CORT), which is another important metabolic hormone that is linked to stress situations, is also sensitive to diet composition (Rabolli & Martin 1977, Devenport *et al.* 1991). Due to the highly variable diet compositions used in different studies, however, it is often quite difficult to compare the data of published reports. Hence, in our present long-term experiment in rats, we have established conditions in which the contribution of carbohydrates to energy balance is maintained at a constant (55% of total calories) level. We then examined the influence of ingestion of diets with proteins (P) and fats (F) providing different proportions of energy on body weight, feeding, and important metabolic hormones. The relationships between adiposity and these hormones (insulin, adipokines, ghrelin, and CORT) were analyzed.

Materials and Methods

Animals and protocol

Sixty male Long-Evans rats body weight (BW) 250–300 g; Centre d'Elevage R Janvier, Le Genest St Isle, France) were used in this experiment. The animals were placed in individual wire cages in an air-conditioned room with an automatic 12 h light/12 h darkness cycle (lights on at 0900 h). They were fed a standard laboratory chow (A 04, UAR-Villemoisson sur Orge, France) *ad libitum* and had tap water to drink for 1 week. During the habituation period to these conditions, the rats ingested about 63 kcal of chow per day.

Then, the rats were randomly distributed into four groups of 15 rats that were fed diets in which the PF energy ratio was symmetrically varied: a HF (PF 5/40), a 'control' (PF 15/30), a low-fat (PF 30/15), or a high-protein (PF 40/5) diet. Therefore, each diet provided 55% of its energy from carbohydrates in the form of two-thirds starch and one-third sucrose. The PF 15/30 diet was named 'control' by reference to Recommended Dietary Allowances for Humans. The exact composition of the four diets is shown in Table 1.

The rats were fed their respective diets for 2 months and both food intake corrected for occasional spillage and body weight were recorded twice weekly. At the end of the experimental period, the rats were killed by decapitation 3 h after the beginning of the light period. Food was withdrawn during this 3-h period in order that all animals would be in the same nutritional state. Trunk blood was sampled in tubes containing aprotinin (5000 IU/ml, Iniprol, Laboratoires Choay, Paris, France) and EDTA (1.2 mg/ml, Merck). In addition, the whole epididymal, perirenal, and abdominal subcutaneous fat depots were sampled and weighed.

Table 1 Composition of the four diets

	PF 5/40	PF 15/30	PF 30/15	PF 40/5
Ingredient (g/kg)				
Wheat starch	415	388	353	333
Sucrose	207	194	177	167
Margarine ^a	245	172	78	25
Casein	63	176	322	405
Salts ^b	40	40	40	40
Vitamins ^c	10	10	10	10
Cellulose	20	20	20	20
Energy (%)				
Carbohydrate	55	55	55	55
Fat	40	30	15	5
Protein	5	15	30	40
PF ratio	0.125	0.5	2	8
Energy density	4.52	4.23	3.85	3.64

All diets are supplemented with 2% methionine.

^aMargarine was a mixture of saturated and unsaturated fats provided by fish oil, soybean oil, colza oil, and palm oil.

^bSalt mixture (UAR 205b; Villemoisson sur Orge, France).

^cVitamin mixture (UAR 200b).

Hormone assays

Blood samples were centrifuged at 4 °C for 20 min and the plasma was distributed in aliquots for the determination of insulin, leptin, adiponectin, CORT, and ghrelin levels. These aliquots were kept at –20 °C until assayed.

Plasma glucose and triglycerides were measured by kits using enzymatic methods (BioMérieux, Marcy l'Etoile, France). The hormone levels were measured in duplicate in each case by specific radioimmunoassays using commercially available kits: insulin antibody-coated tubes for immuno-reactive insulin (IRI) measurements (Insulin-CT; Cis bio International, Saclay, France) with rat insulin as standard (NOVO, Copenhagen, Denmark), a rat ghrelin kit (RK-031-31; Phoenix Europe GmbH, Karlsruhe, Germany), a rat CORT kit (ICN Pharmaceuticals, Costa Mesa, CA, USA), and a rat leptin kit (RL-83 K; Linco, St Charles, MO, USA). Plasma samples were diluted for the ghrelin assay.

Calculation and statistics

Different indices were calculated: a food efficiency index was calculated as body weight gain during the experiment (in grams) divided by total food intake in grams or kcal; an adipogenicity index was calculated as weight of the three sampled fat depots divided by total food intake in grams or kcal; an insulin sensitivity index was calculated as plasma glucose (mmol/l) divided by plasma insulin (ng/ml). Results are given as the mean $\pm$ S.E.M. and were compared through variance analysis for repeated measures and/or covariance analysis followed by a *post hoc* protected least significant difference (PLSD) Fisher's test. Energy intake was the covariant when examining the effects of diet type on hormone profile, insulin sensitivity, and adiposity. Regression curves between hormones and either the adiposity or P/F

ratios were also calculated. A probability of 0.05 was considered statistically significant.

Results

Energy intake, body weight, and adiposity

There was no difference between the four groups in the chow intake during the habituation period. Energy intake variations during the experiment are shown in Fig. 1. Food intake in the four groups followed parallel variations during the experiment and there were significant effects of both diet and time ($P < 0.001$ for both). In the four groups, energy intake was elevated during week 1 and was stable from week 2 until the end of the experiment. During week 1, energy intake was 21–23% greater than the average intake during the following weeks in the PF 5/40, PF 15/30, and PF 30/15 groups ($P < 0.001$) and 17% greater in the PF 40/5 group ($P < 0.001$). However, there was a clear separation into two subgroups: one comprising the PF 5/40 and the PF 15/30 rats and the other consisting of the PF 30/15 and the PF 40/5 rats. Both PF 30/15 and PF 40/5 rats ingested significantly less energy than the two other groups (cf. Fig. 1) and over the course of the entire experiment, this reduction amounted to about 15% versus PF 15/30 ($P < 0.001$) and about 12% versus PF 5/40 ($P < 0.001$).

The measured body weight changes are shown in Fig. 2. During our 2-month experiment, all the rats regularly gained weight. There was a significant effect of diet type on body weight ($P < 0.001$) with the PF 15/30 rats having a higher body weight than the PF 40/5 rats ($P < 0.04$). Body weight gain over the experimental period was also lower in PF 40/5 rats than that in the three other groups (137.0 ± 4.3 g versus 154.5 ± 4.9 g (PF 5/40 rats; $P < 0.04$) versus 158.9 ± 6.3 g (PF 15/30 rats; $P < 0.01$) and versus 153.9 ± 6.4 g (PF 30/15 rats; $P < 0.04$)).

Adipose tissue weights are shown in Fig. 3. There was no difference between the adiposity levels of PF 5/40 and PF 15/30 rats regardless of the fat depot applied. PF 30/15 and

Figure 1 Energy intake (mean $\pm$ S.E.M.) in Long-Evans rats that had been given either a high-fat (PF 5/40), control (PF 15/30), low-fat (PF 30/15), or high-protein (PF 40/5) diet for 2 months ($n = 15$ per group). * $P < 0.01$ or less between on one hand PF 5/40 and PF 15/30 rats versus on the other hand PF 30/15 and PF 40/5 rats.

Figure 2 Body weight (mean $\pm$ S.E.M.) in Long-Evans rats that had been given either a high-fat (PF 5/40), control (PF 15/30), low-fat (PF 30/15), or high-protein (PF 40/5) diet for 2 months ($n = 15$ per group). * $P < 0.04$ between PF 15/30 and PF 40/5 rats.

PF 40/5 rats had smaller epididymal and perirenal adipose tissue depots than PF 5/40 and PF 15/30 rats ($P < 0.02$; cf. Fig. 3). The PF 40/5 rats had also a smaller epididymal fat depot than the PF 30/15 rats ($P < 0.05$). The PF 40/5 rats had a smaller abdominal subcutaneous adipose tissue than the PF 5/40 rats ($P < 0.005$) and than the PF 15/30 rats ($P < 0.003$).

There was a significant effect of diet type on the food efficiency index expressed per gram of ingested diet ($P < 0.001$). The PF 40/5 rats had a significantly lower food efficiency index than the three other groups (0.114 ± 0.004 vs 0.137 ± 0.004 (PF 5/40 rats; $P < 0.0001$), versus 0.129 ± 0.003 (PF 15/30 rats; $P < 0.01$) and versus 0.133 ± 0.004 (PF 30/15 rats; $P < 0.001$)). This effect totally disappeared when the index was expressed by kcal ingested ($P = 0.20$). A similar phenomenon was noted for the adipogenicity index, e.g., a significant effect of diet type when the index is expressed per gram of ingested diet ($P < 0.001$) with adipogenicity index of PF 40/5 rats lower than that in the three other groups ($P < 0.02$) but also a lower index for the PF 30/15 rats versus PF 5/40 ($P < 0.01$). Once again, the effect totally disappeared when the adipogenicity index was expressed per kcal ingested ($P = 0.38$).

Plasma parameters and hormone profiles

Plasma glucose and triacylglycerol (TG) levels are shown in Table 2. We found a significant effect of diet type on each parameter ($P < 0.005$ for plasma glucose and $P < 0.001$ for triglycerides). Plasma glucose levels were not different between PF 5/40 and PF 15/30 rats, but PF 30/15 and PF 40/5 rats had significantly lower plasma glucose concentrations than the PF 5/40 and PF 15/30 groups ($P < 0.05$; cf.

Figure 3 Different fat depot weight measurements (mean $\pm$ s.e.m.) in Long-Evans rats that had been given either a high-fat (PF 5/40), control (PF 15/30), low-fat (PF 30/15), or high-protein (PF 40/5) diet for 2 months ($n=15$ per group). AT, adipose tissue. ^a $P<0.05$ versus PF 5/40 rats; ^{aa} $P<0.01$ versus PF 5/40 rats; ^{aaa} $P<0.001$ versus PF 5/40 rats; ^b $P<0.05$ versus PF 15/30 rats; ^{bbb} $P<0.001$ versus PF 15/30 rats.

Fig. 4). PF 5/40 rats had significantly higher TG concentrations than the three other groups ($P<0.005$).

IRI levels as well as insulin sensitivity index were not significantly different between the four groups (Table 2).

Adipocytokines (leptin and adiponectin), CORT, and ghrelin concentrations are shown in Fig. 4. All four were also influenced by diet type ($P<0.0025$ for leptin, $P<0.01$ for ghrelin, $P<0.002$ for CORT, and $P<0.001$ for adiponectin). Leptin levels in PF 5/40 rats were significantly higher than that in the three other groups. Leptin production per unit total adipose tissue was significantly higher in PF 5/40 rats than that in the three other groups (0.77 ± 0.09 versus 0.60 ± 0.11 (PF 15/30), 0.64 ± 0.10 (PF 30/15), 0.66 ± 0.03 (PF 40/5); $P<0.025$). Ghrelin concentration in the PF 30/15 group was significantly higher than in the three other groups

($P<0.001$ versus PF 5/40; $P<0.05$ versus PF 15/30 and PF 40/5).

Adiponectin levels were significantly higher in PF 5/40 rats than that in either PF 15/30 (+25%; $P<0.001$), PF 30/15 (+40%; $P<0.001$), or PF 40/5 rats (+47%; $P<0.001$). In PF 15/30 rats, adiponectin level was higher than that in PF 40/5 rats (+16%; $P<0.04$). CORT concentrations were significantly higher (2- to 2.5-fold) in PF 40/5 rats than that in the three other groups ($P<0.001$ versus PF 5/40; $P<0.01$ versus PF 15/30 and $P<0.001$ versus PF 30/15).

Relationships between hormone levels, adiposity, and diet composition

Regression curves between the different hormones under study and the fat depot weights as an index of adiposity are

Table 2 Plasma parameters in rats after the ingestion of either a high-fat (protein-to-fat, PF 5/40), a control (PF 15/30), a low-fat (PF 30/15), or a high-protein (PF 40/5) diet for 2 months

	PF 5/40	PF 15/30	PF 30/15	PF 40/5
BG (mmol/l)	6.79±0.08	6.70±0.10	6.42±0.12*‡	6.31±0.09†,§
IRI (ng/ml)	9.65±1.07	8.50±0.74	9.60±0.94	8.28±0.83
IRI/BG	0.79±0.06	0.89±0.09	0.76±0.08	0.87±0.09
TG (mmol/l)	4.04±0.36	2.87±0.32†	2.45±0.22†	2.25±0.18†

BG, blood glucose; IRI, immunoreactive insulin; TG, triglycerides. * $P<0.01$ versus PF 5/40; † $P<0.001$ versus PF 5/40; ‡ $P<0.05$ versus PF 15/30; § $P<0.01$ versus PF 15/30.

Figure 4 Plasma leptin, ghrelin, adiponectin, and corticosterone (CORT) concentrations (mean $\pm$ S.E.M.) in Long-Evans rats on either a high-fat (PF 5/40), control (PF 15/30), low-fat (PF 30/15), or high-protein (PF 40/5) diet for 2 months ($n=15$ per group). ^{aa} $P<0.01$ versus PF 5/40 rats; ^{aaa} $P<0.001$ versus PF 5/40 rats; ^b $P<0.05$ versus PF 15/30 rats; ^c $P<0.05$ versus PF 30/15 rats; ^{ccc} $P<0.001$ versus PF 30/15 rats; ^{dd} $P<0.01$ versus PF 40/5 rats; ^{ddd} $P<0.001$ versus PF 40/5 rats.

shown in Fig. 5. There was a strong correlation found between leptin levels and both adiposity ($r=0.79$; $P<0.0001$) and final body weight ($r=0.69$; $P<0.0001$) as well as between leptin production per unit deep abdominal adipose tissue (epididymal+perirenal) and final body weight ($r=0.28$; $P=0.03$). A significant correlation was also observed between adiposity and on the one hand IRI ($r=0.54$; $P<0.0001$) and on the other hand adiponectin ($r=0.47$; $P<0.001$). In the case of ghrelin,

there was a tendency for an inverse correlation ($P<0.08$) with adiposity when calculated for the four groups. This correlation became significant when calculated for the PF 5/40, PF 15/30, and PF 30/15 rats only ($r=-0.39$; $P<0.01$).

There was a negative correlation found between the P/F ratio and either leptin ($r=-0.35$; $P<0.01$), adiponectin ($r=-0.44$; $P<0.001$), or total adipose tissue weight ($r=-0.42$; $P<0.001$).

Figure 5 Correlations between total fat depots weight and either plasma leptin, immunoreactive insulin (IRI), adiponectin, or ghrelin concentrations in Long-Evans rats on either a high-fat (PF 5/40), control (PF 15/30), low-fat (PF 30/15), or high-protein (PF 40/5) diet for 2 months. $n=15$ per group.

Discussion

Obesity is becoming a worldwide epidemic with the prevalence of obese people increasing rapidly particularly in Western developed countries (James 2004). During the last decade, numerous types of diet that are either low in fat or low in carbohydrates have been recommended in order to induce weight loss in obese people (Bravata *et al.* 2003, Astrup *et al.* 2004, LaraCastro & Garvey 2004, McAuley *et al.* 2005). More recently, there has been an emphasis on protein-rich diets (Tome 2004, Astrup 2005) but all these diets are associated with energy restriction in order to be more efficient. The goal of our present experiment was to study the metabolic adaptation of normal rats to the long-term ingestion of diets that differed in their protein and fat energy content but provided the same percentage of energy from carbohydrates. The hormonal and metabolic effects obtained at the end of this experiment without food restriction was analyzed in relation with body weight and energy intake variations.

The increased palatability of the four diets versus chow, possibly due at least to the presence of sucrose, likely explains the intake of the four groups during week 1 of the experiment. Nonetheless, the rats ingesting the two protein-rich diets ate less than the two other groups. From week 2, there was a metabolic adaptation to these new diets but the clear dichotomy in energy intake between the PF 40/5 and PF 30/15 groups on one hand and the PF 15/30 and PF 5/40 groups on the other hand remained. The two former groups with the highest level of protein went on to consistently eat less than either the PF 15/30 or PF 5/40 rats. A similar reduction in energy intake has been observed in human when the protein content of the diet is increased (Skov *et al.* 1999, Due *et al.* 2004, Weigle *et al.* 2005). The change of diet palatability due to the higher fat content and to the slightly higher sweetness of the PF 15/30 and PF 5/40 diets could also explain the difference in energy intake with the PF 30/15 and the PF 40/5 rats. All four groups of animals in this study regularly gained weight during the course of the experiment but the PF 40/5 rats gained less weight than the three other groups. Food efficiency in the PF 40/5 rats expressed per calorie ingested was not significantly different and the effect on body weight can therefore be attributed to the lower intake of this group. Changes in intake had much more clear-cut impact upon adiposity levels as a marked reduction in deep abdominal adipose tissue mass was observed in PF 30/15 rats and PF 40/5 rats. A lower effect was also observed for the subcutaneous abdominal adipose tissue. These results confirm previous observations of the effects of high-protein diets on visceral adiposity in human or rats (Due *et al.* 2004, Lacroix *et al.* 2004). The differences between PF 30/15 or PF 40/5 rats and the two other groups are also likely to be due to some changes in energy expenditure as thermogenesis is increased by a high-protein diet (Johnston *et al.* 2002, Halton & Hu 2004).

The data from our PF 5/40 rats show that the 10% increase in diet fat content to the detriment of the protein level did not induce any significant change in energy intake when compared with the control rats. This indicates that under conditions of normal carbohydrate availability, rats can rapidly adapt their intake to such relevant changes in the diet composition and control their body weight in a satisfactory manner.

A specific hormonal profile was attached to the phenotype of each dietary group. PF 40/5 and PF 5/40 rats were particularly representative of this phenomenon. In our experiment, PF 40/5 rats had the lowest leptin levels. This is consistent with their lowest adiposity. They differed from the other groups in two respects, their ghrelin and CORT levels. When ghrelin is chronically injected either in the brain or in the periphery, it stimulates food intake and decreases energy expenditure leading to overweight development (Tschöp *et al.* 2000). This hormone is also sensitive to the fat and carbohydrate content of the diet (Beck *et al.* 2002). Accordingly, we found an inverse correlation between the fat content of the diet and the plasma ghrelin concentrations in the PF 5/40, PF 15/30, and PF 30/15 groups that confirmed some of our previous observations (Beck *et al.* 2002). The correlation between ghrelin levels and the dietary fat content was no longer evident when the PF 40/5 rats were included in the calculations, thus suggesting that the regulation by ghrelin is either no more functional or less operational when a very low-fat/high-protein content is present in the diet. The threshold is for a P/F ratio > 2 . This is in agreement with the persistence of ghrelin at levels that are lower than the baseline for a longer duration following a high-protein (45%) meal (AlAwar *et al.* 2005).

In the PF 40/5 rats, the absence of a further augmentation in ghrelin concentrations is likely to be partly due to their high CORT levels. Ghrelin concentration is indeed lower after a prednisolone treatment or exhibits a strong inverse temporal association with serum cortisol in normal subjects (Otto *et al.* 2004, Espelund *et al.* 2005). The CORT increase might also contribute cooperatively with ghrelin to some replenishment of energy stores through their combined action in adipose tissue (Tung *et al.* 2004). So, the PF 40/5 rats might be more susceptible to weight regain after a dietary change by stimulating fat intake through their high CORT status (Bligh *et al.* 1990, 1993, Prasad *et al.* 1995). Additionally, CORT increase is also a sign of activation of the hypothalamo-pituitary axis (HPA). The HPA activation is partly dependent on the macronutrient composition of the diet (Rabolli & Martin 1977, Devenport *et al.* 1991). For basal CORT, we did not measure any difference between the PF 5/40 and either PF 15/30 or PF 30/15 rats. These data agree with previous experiments showing the absence of variations in basal CORT levels after exposure to a HF diet (Rabolli & Martin 1977, Kamara *et al.* 1998, Legendre & Harris 2006). However, we cannot exclude a change in sensitivity to stress in the PF 5/40 rats as HF diets augment HPA responsivity to restraint stress with an impact that may vary with the duration

of exposure to a HF content (Tannenbaum *et al.* 1997, Kamara *et al.* 1998, Legendre & Harris 2006). On the other hand, the presence of sucrose in the diets could have counteracting effects as sucrose or palatable diet ingestion induces a decrease in CORT response to restraint (Strack *et al.* 1997, Pecoraro *et al.* 2004) but as HF content, does not alter basal CORT. The high basal CORT measured in the PF 40/5 rats was rather unexpected even if a CORT increase has been observed after a high-protein meal (Gibson *et al.* 1999). This large increase constitutes a sign of alteration of the HPA axis in the PF 40/5 rats. It is therefore possible that these rats will react differently than the other groups to environmental cues that are either related to nutrition or to other behaviors (e.g., fear, aggressiveness, etc). This is supported, for example, by a greater activity/reactivity of the PF 40/5 rats when opening the cage door for feeding them (personal and non-quantified observations). Ghrelin might contribute to this situation through its anxiogenic effects (Carlini *et al.* 2002). However, only the measurement of other components of the HPA axis such as central corticotropin-releasing factor in association with different behavioral tests will allow us to confirm this hypothesis in the future.

Our PF 5/40 rats showed the highest levels of leptin that may have served to regulate their food intake near control levels. Their leptin production by unit adipose tissue was also higher and positively associated with final body weight. This confirms several previous findings showing that the leptin system is very sensitive to the fat content of the diet (Ahren *et al.* 1997, Cooling *et al.* 1998, Stricker-Krongrad *et al.* 1998) but not those of Ainslie and colleagues who have shown a negative association between leptin production and body weight in rats fed a moderately HF diet (Ainslie *et al.* 2000). Diet type and/or rat strain might explain these differences.

The adiponectin levels were paradoxically elevated in the PF 5/40 rats as in rodents; HF diet is associated with insulin resistance and decreased adiponectin (Yamauchi *et al.* 2001). Our data, however, agree with previous findings in humans showing a positive association between adiponectin and total fat intake (Pischoon *et al.* 2005) and similar variations of adiponectin with the PF ratio have been noted in a recent paper (Morens *et al.* 2005). This indicates therefore an adaptation to maintain a normal insulin action. This is confirmed by the absence of difference in the insulin sensitivity index that we calculated but the absence of insulin resistance needs, however, to be confirmed by glucose tolerance tests. Maintenance of adequate adiponectin concentration might be related to the low ghrelin levels in PF 5/40 rats. Adiponectin gene expression in adipocytes, which is correlated with plasma levels (Degawa-Yamauchi *et al.* 2005), is indeed impaired by chronic exposure to ghrelin (Ott *et al.* 2002). With a PF ratio of <2, ghrelin appeared to be metabolically active and its lower levels might also help to limit energy intake in PF 5/40 rats. The only adverse dietary effect that we noted in PF 5/40 rats concerned the high concentration of plasma triglycerides that may augment the risk of cardiovascular diseases.

Between the two groups with extreme P/F ratios, variations in ghrelin concentration in the PF 15/30 and PF 30/15 rats indicate that regulatory mechanisms are functioning in a proper manner in these animals to control fat deposits in PF 15/30 rats and stimulate fat storage in PF 30/15 rats at least through ghrelin. A similar increase in ghrelin secretion has been observed in human and rats when fats were substituted for protein in a way similar to our PF 30/15 rats (Vallejo-Cremades *et al.* 2004, Weigle *et al.* 2005). Ghrelin levels, however, are unchanged under dietary conditions in which carbohydrates are substituted for fat and when the protein content is unchanged, e.g., when the diet is switched from 35% fat/45% carbohydrates/20% proteins to 15% fat/65% carbohydrates/20% proteins (Weigle *et al.* 2003). This is also the case when the switch is between protein and fat at a lower (37%) level of carbohydrates (Moran *et al.* 2005). All these observations indicate that each macronutrient plays an important role for ghrelin regulation.

In conclusion, we show from our current experiments that the PF 40/5 diet comprising a normal supply of energy from carbohydrates was the less obesogenic diet of the four diets that were tested in this study. It was, however, the sole diet that we found could be associated with some signs of HPA activation which might have negative effects in the long term. Quite similar positive effects on adiposity were obtained with the PF 30/15 diet which is less extreme in its macronutrient composition. It is interesting to note that in the conditions we used no obvious signs of insulin resistance were observed. Hence, it will now be interesting to combine these diets with some energy restriction in order to assess whether the same type of regulation occurs and whether differences in body weight loss can be obtained in selected obese subjects.

Acknowledgements

The authors thank Dr C Villaume for his help in bibliographic search, Mrs F Bergerot for her excellent technical assistance, and Mr A Fillion for his help in animal care. These data have been presented under abstract form at the 14th European Congress on Obesity in Athens (Greece). This study was supported by a Research Prize of Institut Benjamin Delessert (Paris). The authors had no conflict of interest to declare.

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Received in final form 12 October 2007

Accepted 16 October 2007

Made available online as an Accepted Preprint
16 October 2007