Original article

Effect of a lipid-enriched diet on body composition and some regulatory hormones of food intake in growing rats

Effets d’un régime enrichi en lipides sur la composition corporelle et certaines hormones de régulation de la prise alimentaire chez le rat en croissance

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Résumé

Objectif. – L’objectif de cette étude était d’étudier les effets d’un régime hyperlipidique sur la composition corporelle, les principales hormones de régulation de la prise alimentaire (insuline, leptine, ghreline) et l’adiponectine.

Méthode. – Deux groupes de 16 rats chacun (âgés de 35 jours, et pesant 80 ± 6 g) ont été constitués. Au premier groupe (S) servant comme témoin, était administrée pendant dix semaines une alimentation standard. Un régime riche en lipides (G : 41,5, L : 38,5, P : 20 % calories) était donné au second groupe (L). Aliments et eau étaient administrés « ad libitum ».

Résultats. – La prise alimentaire totale, la masse corporelle, la surface du squelette et la masse maigre des rats L étaient moins élevées (6694 ± 178 vs 8160 ± 184 kcal, p = 0,01 ; 431 ± 38 vs 468 ± 25 g, p = 0,003 ; 72,19 ± 0,96 vs 76,07 ± 1,31 cm², p = 0,03 ; 369 ± 18 vs 409 ± 23 g, p = 0,0006), la différence de masse grasse n’était pas statistiquement significative (82,5 ± 17 vs 80 ± 17 g, p = 0,7). Les concentrations de ghreline et d’adiponectine étaient inférieures (1517 ± 224 vs 1915 ± 579 pg/ml, p = 0,03 ; 10 ± 3 vs 19 ± 3 μg/ml, p = 0,003) tandis que celles d’insuline et de leptine étaient inchangées (1,8 ± 1,5 vs 2,6 ± 1,4 ng/ml, p = 0,1 ; 16±11 vs 13 ± 10 ng/ml, p = 0,4).

Conclusion. – L’administration d’un régime riche en lipides à des rats en croissance aboutit à une hypophagie à l’origine d’un déficit de masse maigre. Les cinétiques différentes de certaines hormones de régulation de la prise alimentaire ne sont pas modifiées ; toutefois, la baisse de la ghreline notamment pourrait expliquer ladite hypophagie.

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Abstract

Objective. – The aim of the present study was to investigate the effects of a lipid-enriched diet on body composition and on main regulatory hormones of food intake (insulin, adiponectin, leptin, ghrelin).

Method. – Two groups of 16 rats, 35 days old, weighing 80 ± 6 g, were constituted. One group (S) was given a standard diet during 10 weeks and served as control. The second group (L) was given a lipidic-enriched diet (containing: G: 41.5, L: 38.5, P: 20% calorie). Food and water were given “ad libitum”.

Results. – Total food intake, body weight, skeletal area and lean body mass of rats eating lipid-enriched diet were lowered (6694 ± 178 vs 8160 ± 184 kcal, P = 0.01; 431 ± 38 vs 468 ± 25 g, P = 0.003; 72.19 ± 0.96 vs 76.07 ± 1.31 cm², P = 0.03; 369 ± 18 vs 409 ± 23 g, P = 0.0006), fat mass difference was not statistically significant (82.5 ± 17 vs 80 ± 17 g, P = 0.7). Blood ghrelin, adiponectin levels were lowered (1517 ± 224 vs 1915 ± 579 pg/ml, P = 0.03; 10 ± 3 vs 19 ± 3 μg/ml, P = 0.003) whereas insulin and leptin were unchanged (1.8 ± 1.5 vs 2.6 ± 1.4 ng/ml, P = 0.1; 16 ± 11 vs 13 ± 10 ng/ml, P = 0.4).

Conclusion. – A period of high fat diet in growing rats leads to a hypophagia, resulting in a lower lean body mass development. Some regulatory hormones of food intake did not change, while others significantly decreased, notably ghrelin being possible causal factor of the observed hypophagia linked to high fat diet.

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1. Introduction

Nutritional factors have a critical influence on the development of networks involved in metabolic disorders. High fat diet consumption is the main technique currently used to obtain obesity or overweight in adult rat. In rats submitted to high fat diets, the evolution of body composition during growth period depends on many factors: age, initial body weight, strain, fat source... etc. In rats of different ages, the oldest high fat diet rats showed a considerably higher fat proportion (compared with less old low fat diet rats) associated with a corresponding reduced body water content and an approximately identical body protein concentration [17]. Initial body weight is also an important factor of body composition evolution because, when comparing light and heavy rats submitted to a high fat diet, it was found that the heavy animals exhibited a highly significant increase in body fat content and a highly significant decrease in body water content [17]. Young Wistar rats fed with a high-fat diet became obese, with significant increases in the mass of visceral and subcutaneous fat depots [7]; an obese syndrome involving Long–Evans rats given high fat diet was also found [2,39]. Conversely, high fat fed Sprague–Dawley rats exhibited no significant increase of body fat [15], which permitted to conclude to a resistance to dietary obesity phenomenon in this strain of rats [28].

Generally, animals given high fat diets display a reduced lean body mass [15]. Concerning bones, a high fat diet is not indicated for its optimal development because it can alter mineralization process and mechanical properties [42]; rats fed on the long term with such a regimen exhibited a lower bone mineral content than that of the standard rats [38].

Energy homeostasis results from the balance between food intake and energy expenditure. Regulation of food intake is a complex mechanism involving peripheral signals addressing the central nervous system. Among these signals, ghrelin, insulin, leptin, adiponectin play a key role.

Ghrelin is an acylated 28 amino acid peptide [21] primarily secreted by the stomach and duodenum, although a minor portion of ghrelin synthesis occurs in other sites such as the hypothalamus, pituitary and lung. Ghrelin is a short-term signal which stimulates food intake [26] and reduces fat utilization in rodents [36].

Insulin is secreted in response to blood glucose concentrations increases. It also acts as long-term signal of food intake at peripheral level [29].

Leptin is a peptide released from fat cells into the circulation; it provides information on the availability of fat stores to the hypothalamus and acts as an afferent long-term satiety signal regulating appetite and energy expenditure in both rodents and humans [43].

Adiponectin, an adipocytokine produced by adipose tissue is not directly involved in food intake regulation process, but it acts by decreasing insulin resistance, reinforcing insulin action (by improving its sensitivity) [19]. Circulating levels of adiponectin are low in obesity [3] and increase after weight loss [41]. The serum levels do not correlate with fat mass per se but rather with insulin sensitivity [18].

Hormones influence energy homeostasis and nutrients in turn influence hormone levels. Therefore the aim of this study was to investigate the evolution of body composition and the behavior of hormones involved in food intake in response to a high fat diet in growing rats.

2. Materials and methods

2.1. Animals and treatments

This experiment was done in accordance with current legislation on animal experiments in France and was agreed by the local committee of ethics. Thirty-two Wistar male rats (35 days old, weighing 80 ± 6 g) were used. Groups of four rats were housed in 2.2 × 2.2 × 18 cm plastic cages at 22 ± 1 °C, with a 12:12 h light: dark cycle. Water and food were given “ad libitum” during 10 weeks.

Sixteen rats (L) were given a lipid enriched diet made of a mixture of 75 grams of laboratory chow (UAR A04, Villemoisson-sur-Orge, France) of known composition (72.2 % of carbohydrates, 7.7 % of fats and 20 % of proteins) to which was added 15 grams of commercial vegetable oil (fats 100 % comprising saturated 12 % of fatty acids, 41 % of monounsaturated fatty acids, 47 % of polyunsaturated fatty acids) and 10 grams of powdered skimmed milk (lactose, 51.7 %; fats, 1 %; proteins, 36 %); thus, the global composition and total energy value of this experimental diet were 41.5 % of carbohydrates, 38.5 % of fats, 20 % of protein and 425 Kcal/100 g. The other 16 rats (S) were given a standard diet (UAR, A04, Villemoisson sur Orge, France) for a 349 Kcal/100 g total energy.

2.2. Measurements

Animals were weighed daily for a permanent follow-up of body weight. Food intake was assessed by differential weighing daily for each group of four rats and totalized over the 10 weeks.

Fat mass (FM) and lean body mass (LBM) were assessed under chloral anesthesia by dual energy X-ray absorptiometry (DEXA) using a Hologic QDR 4500A (version 11.2.5) densitometer calibrated for small animals [6].

Rats were sacrificed 2 days after the end of the protocol between 08:00 and 10:00 h (post absorptive state), blood was collected following decapitation. Plasma was kept at –25 °C till the assay.
Hormones concentrations were analyzed in rat serum following manufacturer’s recommendations.

Adiponectin was measured by using a commercially available adiponectin ELISA kits (BioCat, Germany). The lowest limit of the sensitivity of the assay was 15.6 pg/ml; the intra-assay and inter-assay coefficients of variation were 1.9% and 7.5%, respectively.

Insulin and leptin were measured by ELISA kits (Linco Research, USA) with a minimum detection limit of 1 ng/ml; intra- and inter-assay variations were 1% and 7%, and 2% and 3%, respectively.

Plasma ghrelin concentration was measured according to manufacturer’s recommendations by homologous radioimmunoassay (Linco Research, USA). The minimum detectable amount for ghrelin was 10 pg per tube, intra- and inter-assay variations values were 3% and 16%, respectively.

2.3. Statistical analysis

All data are reported as the mean ± S.D. Comparisons between groups were made using Student’s t-test for non-paired series. Correlations were assessed by a simple regression analysis between variables; the level of significance was set at \( P < 0.05 \).

3. Results

Lipid-enriched diet rats had lower body weight (431 ± 38 vs. 468 ± 25 g, \( P = 0.003 \)), lean body mass (369 ± 18 vs. 409 ± 23 g, \( P = 0.0006 \)), skeleton area (72.19 ± 0.969 vs. 76.07 ± 1.317 cm\(^2\), \( P = 0.03 \)) (Table 1); body weight difference settled since the first week (Fig. 1).

Total food intake of L rats throughout the protocol was lower than that of S (6694±178 vs. 8160±184 kcal, –18%); their protein and carbohydrate intakes were less important (–293 and –3113 kcal) whereas lipid intake was higher (+1949 kcal) (Fig. 4).

We note that although the difference of food intake (g/day per rat) is evident from the first week, it became really significant only from the fourth week (Fig. 2).

When expressed as energy value (kcal/day per rat), L rats consumed more than S at the beginning of protocol; from the first to the fourth week the energy intakes were almost identical and presented a tendency to be lower in L than in S from the 4th to the 10th week, (Fig. 3).

Fat mass was not different between L and S rats (82.5 ± 17 vs. 80 ± 17 g, \( P > 0.05 \)), but lean body mass was lower (\( P = 0.0006 \)) for L than for S (Table 1).

Hormonal concentrations are presented in Table 2. Ghrelin and adiponectin concentrations were significantly lower in L than in S rats (1517 ± 224 vs. 1915 ± 579 pg/ml, \( P = 0.03 \) and 10 ± 3 vs. 19 ± 9 μg/ml, \( P = 0.003 \)).

Insulin and leptin remain unchanged (1.8 ± 1.5 vs. 2.6 ± 1.4 ng/ml, \( P > 0.05 \); 16 ± 11 vs. 13 ± 10 ng/ml, \( P > 0.05 \)).

There was a positive correlation between leptin and fat mass (\( R = 0.55, P = 0.007 \)) and a negative one between adiponectin and fat mass (\( R = 0.62, P = 0.02 \)).
The objective of this study was to investigate the body composition and responses of some regulatory hormones, insulin, adiponectin, ghrelin and leptin to a lipid-enriched diet in growing rats. First of all, we observed significant growth retardation in L versus S rats. The lipid-enriched diet induced hypoghrelinemia and hypoadiponectinemia insulin and leptin levels did not change.

There is of course a close relationship between final body weight and total food intake, but the deleterious effect of the lipid-enriched diet goes beyond this simple weight deficit since it impacts the body composition (Table 1): total fat mass although not significantly, is rather higher in L than S. On the opposite lean body mass is significantly lower in L; thus, the L rats growth is negatively affected by the hyperlipidic diet. This result is similar to that reported by some authors in adult rats [15,25,28]. The growth deficit observed in L rats may be explained by the reduced quantity of ingested food, especially the proteins, and also the carbohydrates (Fig. 4) which may limit the protein synthesis [1,40]. This growth retardation may also be highlighted by the skeletal dimensions: DEXA analysis of skeletons showed significantly lower area in L by comparison to S rats (Table 1).

The main question arising from these data concerns the reason why the lipid-enriched diet has an inhibiting effect on food intake, knowing that in such a study, rats given low-fat foods eat the greatest quantity of food but those fed high-fat foods have the highest energy intake [16]. Such an effect has already been reported and it is possibly related to digestibility and texture [12,31]. In our case, the reconstituted pellets had the same aspect, texture and dominant fish smell as the standard chow, since the milk powder and vegetable oil added had neutral effect on smell. The limiting effect was then independent of these parameters; it is reflected by the fact that at the beginning of experiment, L and S rats ate the same quantity of food. The reduction of food intake which appears progressively for L rats may be due to an adaptation faculty developed by animals; there is in L rats a rapid adaptation of food consumption with regard to energy value of the diet and not according to the quantity ingested in mass; this adaptation which aims to insure an adequate level of energy consumption [22,32] would be made by a decrease of food intake resulting from the satiety effect generated by the excess of fats and would be the consequence of a particular sensibility of rats L to the calorie properties of the hyperlipidic diet. Hypophagia found in this study is in agreement with that of the other authors having used a similar regimen. Reference [8] indicates that the total food intake is less higher after consumption of a diet rich in fat probably because of the inhibitor effect of these fats on the gastric emptying [30]. This phenomenon is also bound to the increased lipid oxidation during ingestion of hyperlipidic diet. This report is on the other hand in accordance with the idea that a strong increase of fats oxidation associated with a disturbance of carbohydrates metabolism is at the origin of hypophagia [23,31].

In an attempt to explain this hypophagia occurring after a long period of hyperlipidic diet, we assayed ghrelin, leptin and insulin, signals known to affect food intake and adiponectin. In our study, hyperlipidic diet was associated with ghrelin decreased levels; it is in agreement with other results [5,24]. Several parameters may explain this hypoghrelinemia. Ghrelin
secretion depends on food composition via metabolic signals related to fat and/or carbohydrate content of the diet [3,13] and hormonal interactions [27]. Ghrelin is negatively regulated related to fat and/or carbohydrate content of the diet [3,13] secretion depends on food composition via metabolic signals; those characteristics of diet lead L rats to consume a higher lipid and reversely a lower carbohydrate percentage. Knowing that high carbohydrates provoke an increase of ghrelin [33,37] and high fat a decrease [13], our result is not surprising; it is consistent with findings of authors mentioned above. The sensitivity of ghrelin secretion with regard to the diet composition is once again revealing in this study. Its down-regulation by fat ingestion might serve as a counter-regulatory mechanism to limit the development of dietary-induced adiposity.

An increase of insulin may lead to a drop in ghrelin level [8]. In this study, L rats insulinema was not different from that of S. As in a human study, this probably means that insulin in this case is not involved in ghrelin regulation [9,34].

As mentioned above, leptin levels were not different between the two groups, which excludes it for hypogrelinemia although such an effect of leptin was previously reported in [13].

Leptin levels were quite similar in the two groups, excluding any role of this hormone in the different food intakes. In fact, leptin levels presented as expected a close correlation with the fat mass ($R = 0.55, P = 0.007$). As fat masses were not significantly different between the two groups, the same was true for leptin.

Insulin showed a tendency only to be lower (not significant) in L than in S probably in relation to lower amounts of protein and carbohydrates intakes. Whatever, in this case, insulin did not influence food intake.

Adiponectin was negatively correlated with fat mass ($R = 0.62, P = 0.02$) and was lower in L than in S. A similar result was reported in high fat fed rats [11]. Hypoadiponectinemia has been suggested to be involved in the development of insulin resistance and hyperinsulinemia [4]. This is not the case in our study since insulin levels did not differ in the two groups. However, it was stated that low plasma adiponectin concentration precedes a decrease in insulin sensitivity [35].

The adiponectin/leptin ratio known as an index of insulin resistance [20] was effectively significantly lower in L than in S (Table 2). In conclusion, a period of high fat diet in growing rats leads to a hypophagia, resulting in a lower lean body mass development. This hypophagia might result from a response of the regulatory signals at the gastro-intestinal tract level. Such an effect could be related to the significant decrease of ghrelin.

References


