Endocrine, *vol. 30, no. 1, 69–74, August 2006* 0969–711X/06/30:69–74/$30.00
ENDO (Online) ISSN 1559-0100

Effects of High-Fat Diets on Body Composition, Hypothalamus NPY, and Plasma Leptin and Corticosterone Levels in Rats

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We investigated the adipogenic effects of high-fat diets with different fat sources, as well as their influence on hypothalamus NPY content (NPY) and plasma corticosterone (Cs) and leptin (Lep) concentrations. In a 6-wk experiment performed in 28 male Wistar rats, high-fat diets (approx 60% of energy as fat) containing sunflower oil (S), rapeseed oil (R), palm oil (P), or lard (L) as the fat source were applied. Carcass composition was analyzed by standard methods; NPY, Cs, and Lep were determined by RIA method. Gas chromatography was applied to measure fatty acid composition of dietary fats. S group had the highest body fat content and Lep and the lowest Cs and NPY, while L rats had high Lep and the highest Cs and NPY. We conclude that the fatty acid composition of dietary fat is of high importance where effects of high fat diets on adiposity, as well as on plasma levels of both hormones and hypothalamus NPY content are concerned.

Key Words: Dietary fat; leptin; corticosterone; NPY; adiposity.

Introduction

Energy homeostasis is monitored by the central nervous system (CNS), notably the hypothalamus that integrates peripheral neuronal and hormonal signals indicative of energy status. The signals are then transmitted to higher centers of brain that produce behavioral responses affecting food intake and energy expenditure, and to the periphery, where they regulate the output of nutrients from endogenous sources *(1,2)*. Fundamental to energy balance is the "adiposity negative feedback" whereby hormonal signals generated in proportion to fat mass and recent energy balance (leptin, insulin) act at the level of hypothalamus nuclei to reduce appetite and stimulate thermogenesis *(3,4)*.

Received March 21, 2006; Revised June 7, 2006; Accepted June 21, 2006.
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In addition, a negative feedback system operates between adipocytes (leptin) and the adrenal cortex (glucocorticoids), because glucocorticoids stimulate the synthesis and release of leptin; leptin, in turn, blunts both basic and corticotropin-stimulated production of adrenal hormones *(5,6)*.

Both glucocorticoids and leptin exert their influence on energy balance via hypothalamic orexigenic neuropeptide Y (NPY). On the one hand, glucocorticoids stimulate the secretion of NPY, which in turn augments the synthesis and secretion of glucocorticoids. Leptin, on the other hand, has an inhibitory effect on the synthesis and secretion of NPY. Thus, NPY, leptin, and glucocorticoids may be described as the neurohormonal system regulating energy metabolism of an organism *(4)*.

Bearing the crucial regulatory functions of hormones in mind, it appears that any imbalance in this system can lead to metabolic changes in peripheral tissues and may be one of the reasons for the development of many non-communicable diseases such as arteriosclerosis, hypertension, and non-insulin dependent diabetes mellitus. Several lines of evidence suggest that it is obesity that lies at the very heart of the abovementioned diseases *(7)* and the link between obesity and diet is well known. Excess of energy brings about a positive energy balance, and, if long lasting, leads to deposition of adipose tissue and overweight or obesity. However, the adipogenic effects of diets seem to be related, both quantitatively and qualitatively, to the composition of diets, in particular to the amount of certain fatty acids (FAs) and to their modulatory effect on hormonal and neurohormonal systems *(8,9)*.

As demonstrated by Widmaier *(10)*, Stricker-Krongrand et al. *(11)*, Wang et al. *(12)*, and Lin et al. *(13)*, dietary fat exerts both a direct and an indirect impact on the synthesis and secretion of glucocorticoids and leptin, as well as on hypothalamus NPY expression. However, little is known about the effect of dietary fat composition on peripheral signaling molecules (i.e., leptin and glucocorticoids) and the key neuronal molecule, NPY, involved in the regulation of energy balance. Hence, we sought to explore the relationship between fatty acid composition of dietary fats and their adipogenic effects, as well as hypothalamus NPY, and plasma corticosterone and leptin concentrations in rats reared on high-fat diets (approx 60% energy as fat) containing different types of fat.

Table 1
Body Weight Gain, Intake of Food, Protein, Fat, C16:0, C18:0, C18:1, and C18:2 Fatty Acids and Gross Energy in Rats Fed on High-Fat Diets (57.7–61.0% Energy as Fat) Containing Different Sources of Fat During 6 wk of Experiment[1]

		Dietary fat[2]			
	Unit	S	R	P	L
Body weight gain	g/d	2.92 ± 0.19[a]	2.11 ± 0.20[c]	2.72 ± 0.14[a,b]	2.86 ± 0.15[a]
Food intake	g/d	15.73 ± 0.21[b]	14.21 ± 0.41[c]	16.95 ± 0.35[a]	14.56 ± 0.27[c]
Protein intake	g/d	3.08 ± 0.04[b]	2.91 ± 0.08[b,c]	3.47 ± 0.07[a]	2.84 ± 0.05[c]
Fat intake	g/d	6.12 ± 0.08[a]	5.14 ± 0.15[b]	6.37 ± 0.13[a]	5.14 ± 0.09[b]
C16:0 intake	g/d	0.35 ± 0.005[c]	0.19 ± 0.006[d]	3.23 ± 0.070[a]	1.40 ± 0.03[b]
C18:0 intake	g/d	0.141 ± 0.002[b]	0.042 ± 0.001[c]	0.16 ± 0.003[b]	0.77 ± 0.01[a]
C18:1 intake	g/d	1.46 ± 0.02[c]	3.52 ± 0.10[a]	2.59 ± 0.05[b]	2.46 ± 0.05[b]
C18:2 intake	g/d	4.16 ± 0.05[a]	1.04 ± 0.03[b]	0.34 ± 0.01[c]	0.35 ± 0.01[c]
Energy intake	kJ/d	399.6 ± 5.3[b]	351.0 ± 10.2[c]	425.4 ± 8.7[a]	354.0 ± 6.6[c]

[1]Values are mean ± standard error of mean ($n = 7$). Means in a row with different letters differ, $p \leq 0.05$.
[2]S, sunflower oil; R, rapeseed oil; P, palm oil; L, lard.

Table 2
Body Composition of Rats after 6 wk of Feeding of High-Fat Diets (57.7–61.0% Energy as Fat) Containing Different Sources of Fat (g/100 g Body Weight)[1]

	Dietary fat[2]			
	S	R	P	L
Fat	24.16 ± 1.31[a]	19.48 ± 1.64[b]	19.15 ± 0.97[b]	19.80 ± 1.24[b]
Protein	19.65 ± 0.34[a]	20.80 ± 0.35[b]	20.80 ± 0.16[b]	20.61 ± 0.28[b]
Ash	3.12 ± 0.05	3.26 ± 0.08	3.09 ± 0.05	3.20 ± 0.05

[1]Values are means ± standard error ($n = 7$). Means in a row with different letters differ, $p \leq 0.05$.
[2]S, sunflower oil; R, rapeseed oil; P, palm oil; L, lard.

Results

Food Intake

Consumption of experimental diets was significantly different among all groups; namely, the type of dietary fat did affect food, energy, and main nutrient intakes (ANOVA, $p < 0.0001$). Both food and energy intakes were found to be the highest in rats fed on P diet and the lowest in rats reared on R and L diets ($p < 0.001$ for both parameters). Rats fed on S and P diets consumed more fat than animals fed on R and L diets ($p < 0.001$). In addition, animals fed on P diet consumed more protein than other groups ($p < 0.002$) and S rats consumed more protein than L rats ($p < 0.001$) (Table 1).

Fatty acid intake was dependent on the fat source and FAs content in the experimental diets (ANOVA, $p < 0.0001$). The highest intake of palmitic acid was found in rats fed on the P diet, followed by a lower level of intake in L rats. Feeding lard resulted in the highest stearic fatty acid intake in L group. The lowest intake of both of the aforementioned FAs was observed in rats fed on the R diet ($p < 0.001$ for both parameters). For oleic acid, animals fed on R diet had the highest C18:1 intake, whereas it was the lowest in S rats ($p < 0.001$). With respect to linoleic acid, the intake was the lowest in P and L rats and the highest in S rats ($p < 0.001$) (Table 1).

Body Weight and Body Composition

Feeding different dietary fats brought about significant variation in body weight gain ($p < 0.0001$). Rats maintained on R diet gained less weight than animals consuming S, P, and L diets (Table 1).

With respect to body composition, dissection observations showed the fatty deposits in internal organs (including liver, adrenals, heart, and aorta), subcutaneous tissue, as well as muscles to be most prominent in S rats. Dietary fat type affected significantly both body fat and protein contents (ANOVA, $p < 0.04$), while the source of fat had no statistically significant effect on carcass ash content (ANOVA, NS). Among groups, S rats were found to have the highest content of body fat and the lowest body protein content as compared to other dietary groups (Table 2).

Hypothalamus NPY Content, Plasma Leptin, and Corticosterone Concentration

Fat type significantly influenced hypothalamus NPY content and plasma corticosterone concentrations (ANOVA, $p < 0.0001$ for both parameters) but did not affect plasma leptin levels (ANOVA, NS).

Hypothalamus homogenate NPY of rats fed on S and R diets was lower than in rats consuming P or L diets ($p < 0.001$), whereas the highest plasma level of corticosterone was observed in L rats (Fig. 1A). Moreover, the plasma corticosterone was higher in rats fed on the R diet as compared with rats fed on S diet ($p < 0.001$) (Fig. 1B). Rats fed on S diet tended to have slightly higher plasma leptin than other groups (Fig. 1C).

Discussion

Diet is one of the most significant determinants of body weight and composition, and of synthesis and secretion of hormones and neuropeptides involved in the regulation of metabolism and energy homeostasis. There is a general agreement that laboratory animals reared on high-fat diets have higher body weight gain *(14,15)*, higher body fat content *(16)*, and larger fat depots *(17,18)* compared to animals fed on low fat diets. Furthermore, energy deposition in adipose tissue may depend on the type and composition of the dietary fat consumed.

Of particular importance in the regulation of body fat reserves is the influence of dietary fat and certain fatty acids on the synthesis and plasma level of leptin and glucocorticoids. Conceivably, fatty acids reaching the hypothalamus via bloodstream could also directly influence the synthesis of NPY, and, in consequence, affect food intake, energy expenditure, and adipose tissue deposition *(8,13,16,19–24)*.

The present study was thus undertaken to examine the effect of the type of dietary fat on body weight and body composition (especially fat content), as well as on plasma concentrations of leptin and corticosterone, and hypothalamus NPY content in rats. In particular, we sought to address the issue of whether the intake of certain fats, independently of food intake, predisposes to obesity and what mechanisms are behind this putative effect. In all diets used in our experiment, approx 60% of energy was supplied by fat. Moreover, the diets were differentiated in terms of fat source and, thereby, in terms of different fatty acid content.

A striking difference in body fatness was discovered among the groups. The highest body weight gain and body fat content was found in animals reared on the diet containing fat provided by sunflower oil, although they consumed less food than palm oil group, whose body weight gain was similar. In addition, body organs of S animals were much fattier (dissection observation) than that of rats fed other diets. In contrast, body fatness of rats that consumed the R diet rich in rapeseed oil (a source of C18:3 FA being the precursor for the synthesis of C20:5 and C22:6 FAs) was

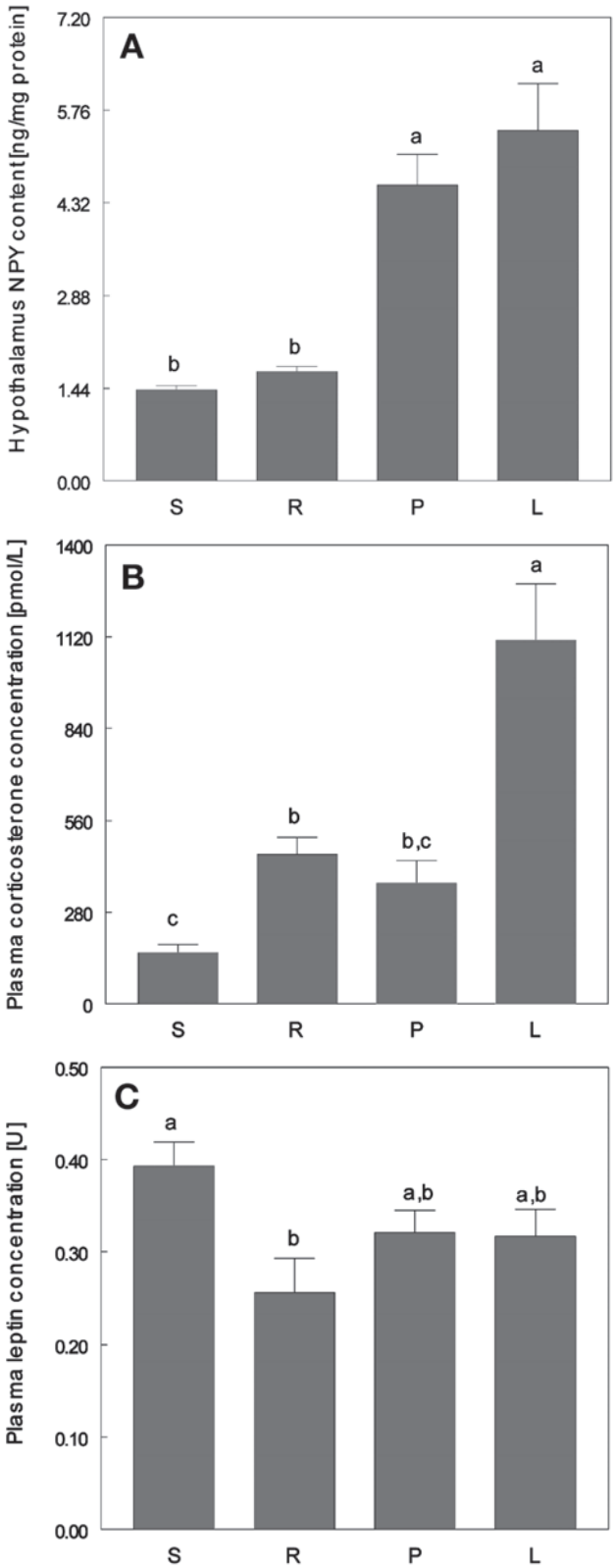

Fig. 1. Hypothalamus neuropeptide Y content (ng/mg protein) (**A**) plasma corticosterone concentration (pmol/L) (**B**) and plasma leptin concentration (ng/mL/g body fat) (**C**) in rats fed on high fat diets (57.7–61.0% energy as fat) containing sunflower oil (S), rapeseed oil (R), palm oil (P), or lard (L) after 6 wk of experiment (mean values with their standard errors for seven animals); a,b,c, different letters indicate significant differences between groups ($p \leq 0.05$).

similar to that of rats maintained on the diets containing high amounts of SFAs, i.e., palm oil or lard. Food intake of R and L rats was, however, lower than that of P rats.

The above findings are quite intriguing, taking into account the results by Raclot et al. *(8)*, Rustan et al. *(25)*, and Benhizia et al. *(26)*, who showed that the high intake of dietary *n* – 3 PUFAs, mainly C20:5 and C22:6 FAs, in a high-fat diet prevents the accumulation of fat in the body of experimental animals. As opposed to SFAs, *n* – 3 PUFAs are esterified to triglycerides to a lower proportion and downregulate genes involved in lipogenesis, while they upregulate genes controlling β-oxidation of FAs and thermogenesis in brown adipose tissue (BAT) through, as shown by Matsuo et al. *(27)* and Takeuchi et al. *(28)*, increased sympathetic activity of BAT.

The reasons for the observed discrepancies are not clear. A strong possibility may be the difference in food intake. Rats fed on R diet consumed the lowest amounts of diet and therefore had the lowest body weight gain. However, food intake of L rats was similar to that of R rats, but their body weight gain was significantly higher. This difference points to another mechanism(s) contributing to the observed discrepancies. We speculate that PUFA-induced thermogenesis *(27,28)* and, in consequence, total energy expenditure, could have protected the R animals from the deposition of extra energy in the adipose tissue. Another possible explanation could be the difference in food consumption and diet composition, namely fat level, between our experiment and previous studies. Diets applied in the present study supplied approx 60% energy as fat, so the huge surplus of energy could have blunted the positive, effects of PUFAs when consumed in moderate amounts, and could have resulted in the unfavorable effects with respect to body weight and composition in S group.

As body weight and fat content can be seen as a result of the balance between energy intake and energy expenditure, both processes being highly dependent on hormonal and/or neurohormonal factors, we measured plasma concentrations of corticosterone and leptin, as well as hypothalamus NPY content. Research to date has shown that glucocorticoids and leptin act antagonistically in the regulation of energy metabolism *(29)*. Generally, adrenal hormones have anabolic effects: they stimulate NPY synthesis in the CNS and promote food intake, but decrease energy expenditure. Peripherally, glucocorticoids promote lipogenesis, while leptin decreases NPY synthesis and activates lipolysis and β-oxidation of fatty acids *(29–32)*.

In the present study, we demonstrated that rats that consumed high amounts of *n*–6 PUFAs (C18:2 FA present in sunflower oil diet) tended to have higher plasma leptin levels than other dietary groups. Apart from the effect on the development of adipose tissue, the high fat intake has been shown to stimulate directly the expression of ob gene and thereby to contribute to a rise in the plasma leptin levels *(33,34)*. Hence, it cannot be excluded that hyperleptinemia observed in S rats reflected the direct effect of fat consumed by the animals. Our results would thus endorse the positive correlation between PUFAs intake and plasma leptin concentration shown by Stachoń et al. *(21)* and the hyperleptinemic effects of PUFAs found in rats by Cha and Jones *(22)*. We found high plasma leptin levels also in rats reared on P and L diets. These observations are in harmony with the results by Wang et al. *(24)*, who showed that 7-wk feeding of mice on a diet supplying 58% energy as fat provided by a mixture of safflower oil (rich in *n* – 6 PUFA) and beef tallow (with high amounts of SFA and MUFA) resulted in higher plasma leptin levels than in mice fed on a diet with either safflower oil or fish oil (a rich source of *n* – 3 PUFA).

We also showed a positive effect of sunflower oil on plasma corticosterone levels as compared to other dietary fats, especially rapeseed oil and lard. The lowest corticosterone concentration observed in S rats was in harmony with our previous observation that rats fed on sunflower oil diet had the lowest deoxycorticosterone 11β-hydroxylase activity *(21)*. Conversely, it was significantly higher in rats fed high fat diets with lard or cod oil as fat sources.

Because SFAs stimulate adrenal cortex and induce hypercorticosteronemia *(36)*, we suggest that a two- up to threefold difference in corticosterone concentration between lard group and other feeding regimens could be ascribed to a high intake of stearic fatty acid and its stimulatory effect on deoxycorticosterone 11β-hydroxylase activity.

Dietary fat may affect the synthesis and content of numerous hypothalamic neuropeptides (including NPY) that are involved in the central regulation of energy metabolism. However, it has not been shown unequivocally whether diet per se modulates the level of NPY synthesis and/or release, or whether only peripheral factors are involved. Leptin and glucocorticoids are among these peripheral factors as their receptors were demonstrated in hypothalamus NPY-ergic neurons *(35,36)*.

Our observations indicate that hypothalamus NPY content varies with the FA composition of the dietary fat and that this effect may depend on the influence of particular fatty acids on the secretion of leptin and corticosterone. Rats consuming sunflower or rapeseed oil (rich in PUFAs) had significantly lower hypothalamus NPY contents than those fed on diets containing mainly SFAs (e.g., palm oil or lard). The inverse relationship between leptin and hypothalamus NPY, as well as between leptin and plasma corticosterone in group S, support the effective negative feedback operating between periphery and high brain centers. In other dietary groups, especially in groups P and L, the negative feedback between these hormones is impaired, as indicated by high plasma leptin levels and concomitant high NPY hypothalamus contents.

There are two apparent explanations for our findings. One reason for the high hypothalamus NPY content, at least in L group, could be upregulation of NPY synthesis

accounted for by high plasma corticosterone concentration. Second, the high hypothalamus NPY contents in P and L rats were paralleled by high plasma leptin levels indicative of leptin-resistance of the hypothalamus and the loss of leptin's inhibitory effect on NPY expression. In this respect, rapid induction of leptin resistance after voluntary overfeeding is a well-documented phenomenon *(2)*.

In conclusion, the present study has shown that type of dietary fat, i.e., its fatty acid composition, affects plasma concentrations of leptin and corticosterone, as well as the hypothalamic content of NPY. Even high intake of sunflower oil conserves the feedback mechanism between peripheral hormones regulating energy metabolism and hypothalamus, whereas high SFAs intake disturbs these key physiological mechanisms.

Materials and Methods

Animals, Diets, and Experimental Protocol

The study protocol was reviewed and approved by the Third Local Animal Care and Use Committee in Warsaw (Poland). Male Wistar rats (n = 28, with an initial body weight of 240–260 g) were obtained from the Medical Research Center of Polish Academy of Sciences (Warsaw, Poland). Rats were individually housed in stable environmental conditions [temperature 22°C, air humidity 50–60% and 12 h (06.00–18.00) light:12 h (18.00–06.00) dark cycle]. The animals were given free access to food and water throughout the study.

After a 2-wk adaptation period, the animals were randomly assigned to experimental groups (n = 7) fed on separate high-fat (40% by weight) diets differing in the fat source: sunflower oil (S), rapeseed oil (R), palm oil (P), and lard (L), for 6 wk. Gross energy of diets was within the range of 24.1 (L diet) and 25.2 MJ/kg (S diet), with 57.7 (L)–61.0% (S), 23.0 (S)–24.5 % (L), and 16.1 (S)–17.8% (L), of energy supplied by fat, carbohydrates, and protein, respectively. Table 3 reports data on the most abundant fatty acids in the experimental dietary fats. Throughout the experiment, all rats were weighed every third day and consumption of the experimental diets was controlled daily taking spillage into account.

Table 3
Content of the Most Abundant Fatty Acids and Total Saturated Fatty Acids (SFAs), Monounsaturated Fatty Acids (MUFAs) and Polyunsaturated Fatty Acids (PUFAs) in Dietary Fats (g/1 kg Fat)

	Dietary fat[1]			
Fatty acid(s)[2]	S	R	P	L
C14:0	0.0	0.0	6.2	10.4
C16:0	57.3	37.2	507.3	272.9
C18:0	23.1	8.2	25.2	149.5
∑SFAs	80.4	45.4	539.8	434.2
C16:1 $n-7$	0.0	0.0	0.0	14.2
C18:1 $n-9$	239.1	683.8	406.0	478.6
∑MUFAs	239.1	695.6	406.0	492.8
C18:2 $n-6$	680.5	201.9	54.2	68.8
C18:3 $n-3$	0.0	57.0	0.0	0.0
∑PUFAs	680.5	258.9	54.2	68.8

[1]S, sunflower oil; R, rapeseed oil; P, palm oil; L, lard.
[2]C14:0, capric acid; C16:0, palmitic acid; C18:0, stearic acid; C16:1 $n-7$, palmitoleic acid; C18:1 $n-9$, oleic acid; C18:2 $n-6$, linoleic acid; C18:3 $n-3$, alpha-linolenic acid.

Sample Collection and Storage

At the end of the experiment, the rats were subjected to a 12-h food deprivation, anesthetized intraperitoneally with thiopental (120 mg/kg body weight; Biochimie GmbH, Austria), and sacrificed via exsanguination by cardiac puncture.

Body organs and cavities were visually evaluated for the assessment of adipose tissue deposits. Thereafter, the hypothalamus was dissected from each animal, weighed, immediately frozen in liquid nitrogen, and stored at –80°C for NPY analysis. Plasma was stored at –23°C for fatty acid and hormone determination. The remaining carcasses were weighed and stored at –23°C for subsequent body composition analyses. Sample collection was performed on three consecutive days within the first 2 h of the light phase of the diurnal cycle.

Body Composition

After autoclaving and homogenization, ash, protein and fat were determined in carcasses by standard analytical methods *(37)*.

Hypothalamus NPY Content

NPY content was determined in 0.5 *M* acetic acid extracts of hypothalamus homogenates by radioimmunoassay using a Neuropeptide Y (Human, Rat) RIA Kit (Phoenix Pharmaceuticals, Inc. Belmont, CA, USA). Assay sensitivity was 3–15 pg/sample, with cross-reactions: rat, human, and porcine NPY 100%, human PYY 1.99%, rat and porcine PYY 0.01%. NPY content was expressed in ng/mg protein of hypothalamus homogenate as determined by the Lowry method *(38)*.

Plasma Corticosterone

Plasma corticosterone was measured using a Rat Corticosterone RIA Kit (Diagnostic Systems Laboratories, Inc., Webster, TX, USA). The intra- and interassay coefficients of variation were 3.4% and 7.3%, respectively. Assay sensitivity was 2.7 ng/mL.

Plasma Leptin

Plasma leptin was measured using a Rat Leptin RIA Kit (Linco Research, Inc., St. Charles, Missouri, USA). Inter- and intraassay coefficients of variation were 4.8% and 3.3%, respectively. Method sensitivity was 0.5 ng/mL, with cross-

reactions: rat and murine leptin 100%, human leptin < 2%, canine leptin 0.5%. To adjust plasma leptin levels for differences in body fat content in rats of particular dietary groups, leptin concentration was expressed per gram of body fat [ng/mL/g].

Fatty Acids

The fatty acid profile of dietary fats (C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C16:1, C18:1, C20:1, C22:1, C18:2, C18:3, C20:4, C20:5, C22:6) was determined by gas chromatography (Hewlett Packard, USA).

Statistical Analysis

Data are presented as means ± SEM. Data were analyzed by analysis of variance (ANOVA) with type as the discriminating factor, and by Fisher's least significant difference post hoc test. Differences with $p \leq 0.05$ were considered to be significant. All statistical analyses were performed using the computer program STATGRAPHICS® Plus 4.0 (Manugistics, Rockville, MD, USA).

Acknowledgments

This work was supported in part by Grant 5P06G02517 from the KBN (State Committee for Scientific Research) and by the Ministry of Education in Poland.

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