Effects of dietary fat types on body fatness, leptin, and ARC leptin receptor, NPY, and AgRP mRNA expression

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Wang, Hongqin, Len H. Storlien, and Xu-Feng Huang. Effects of dietary fat types on body fatness, leptin, and ARC leptin receptor, NPY, and AgRP mRNA expression. Am J Physiol Endocrinol Metab 282: E1352–E1359, 2002.—Some, but not all, fats are obesogenic. The aim of the present studies was to investigate the effects of changing type and amount of dietary fats on energy balance, fat deposition, leptin, and leptin-related neuropeptides: leptin receptor, neuropeptide Y (NPY), agouti-related peptide (AgRP), and proopiomelanocortin (POMC), in C57Bl/6J mice. One week of feeding with a highly saturated fat diet resulted in a 50% and 20% reduction in hypothalamic arcuate NPY and AgRP mRNA levels, respectively, compared with a low-fat or an n-3 or n-6 polyunsaturated high-fat (PUFA) diet without change in energy intake, fat mass, plasma leptin levels, and leptin receptor or POMC mRNA. Similar neuropeptide results were seen at 7 wk, but by then epididymal fat mass and plasma leptin levels were significantly elevated in the saturated fat group compared with low-fat controls. In contrast, fat and leptin levels were reduced in the n-3 PUFA group compared with all other groups. At 7 wk, changing the saturated fat group to n-3 PUFA for 4 wk completely reversed the hyperleptinemia and increased adiposity and neuropeptide changes induced by saturated fat. Changing to a low-fat diet was much less effective. In summary, a highly saturated fat diet induces obesity without hyperphagia. A regulatory reduction in NPY and AgRP mRNA levels is unable to effectively counteract this obesogenic drive. Equally high fat diets emphasizing PUFAs may even protect against obesity.

saturated fat; n-3 polyunsaturated fat; n-6 polyunsaturated fat; hypothalamus; leptin

NEUROPEPTIDE Y (NPY) is a potent, centrally acting orexigenic peptide with a high concentration in the hypothalamic arcuate nucleus (Arc). There is considerable evidence of a role for NPY in regulation of food intake and energy balance. This has recently been reviewed (1). A number of studies (5, 6, 9, 17), although not all (13), have demonstrated that a high-fat diet can induce decreased Arc NPY mRNA expression in rodents. The level of NPY in Arc is regulated by leptin (4). Leptin is a signal protein produced by adipocytes and is positively correlated to the size of body mass (27). Intracerebroventricular injection of leptin significantly re-

duce the level of Arc NPY mRNA expression in mouse Arc (26). Conversely, overexpression of Arc NPY mRNA has been reported in mice that lack leptin (ob/ob) and in leptin receptor-deficient db/db mice (14, 18). Therefore, it is possible that any reduction in NPY message level induced by high-fat feeding is due to either a direct effect or an indirect effect via increased circulating leptin levels subsequent to the fat-induced positive energy balance and white adipose accumulation.

In a further complexity, it has been reported that the majority of Arc NPY neurons produce agouti-related peptide (AgRP) (7). Functionally, AgRP has a similar effect to that of NPY in promoting energy intake and decreasing energy expenditure, albeit via a different mechanism. AgRP is an antagonist of α-melanocortin-stimulating hormone (α-MSH) acting at the level of melanocortin receptor subunit 4 (MCR4) (11). In mice, lack of functional leptin (ob/ob) produces a fivefold increase in Arc AgRP mRNA expression, which can be reversed by leptin treatment (20). These studies show that leptin plays an inhibitory role in controlling the production of AgRP. Nutritional status is also important in the regulation of AgRP production, as it has been demonstrated that food deprivation can dramatically increase AgRP mRNA expression in mice (16).

The aim of this study is to investigate the effects of altering both the level and type of dietary fat on the level of leptin and leptin-related hypothalamic neuropeptide mRNA expression, including NPY, AgRP, α-MSH, and the leptin receptor.

MATERIALS AND METHODS

Animals. Three-week-old C57Bl/6J male mice were obtained from the Animal Resource Centre (Perth, Australia; the mouse genomic background was tested by allozyme electrophoresis and random amplified polymorphic DNA-PCR analysis). Mice were housed individually in a temperature-controlled room (20 ± 2°C) with a 12:12-h light-dark cycle (lights on at 0700) and were given ad libitum access to tap water throughout the study.

Diets and experimental procedure. Mice were fed standard laboratory chow for the 1st wk to allow them to adjust to the new environment. Mice were then randomly assigned to a low-fat (10% of calories as fat) or one of the high-fat diets

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In the present study, three groups of mice were fed the same concentration (58% kcal) of fat, but different fat types (Table 1). In mice fed a highly saturated fat diet, edible tallow (52°C melting point; Unilever, Australia) contributed equally to fat calories. In mice fed high n-3 polyunsaturated fatty oil (Meadow Lea Foods Australia) contributed all 58% of fat, with a high n-6-to-n-3 (n-6/n-3) PUFA ratio. The n-3 fatty oil contributed all 58% of fat calories. The saturated fat diet was thus high in saturated fat, with a high n-6-to-n-3 (n-6/n-3) PUFA ratio. The n-3 PUFA diet was also almost equally high in saturated fat, but with a high level of n-3 PUFAs and a very low n-6/n-3 PUFA ratio. The n-6 PUFA diet was the most unsaturated, but with a very high n-6/n-3 PUFA ratio. Detailed compositions of the respective diets are as detailed previously (24). Diets were freshly made every week and stored at 4°C. Mice were given fresh food at 1600 each day. Food consumption was measured daily, and body weight was measured weekly.

After being fed with a high-fat or a low-fat diet for 1, 7, or 11 wk, mice were killed by an overdose of pentobarbitone sodium anesthesia (120 mg/kg ip) between 0700 and 1100. The total carcass was weighed, and adipose tissue (epididymal, perirenal, and inguinal) was dissected free and also weighed. Blood was taken by heart puncture, and plasma was stored at −20°C for later analysis of leptin levels. The brains were removed quickly, frozen immediately in liquid nitrogen, and stored at −70°C. The brains were cryostat sectioned (12-μm-thick coronal sections) at −15°C and mounted on slides. To gain consistency of tissue sections to be hybridized, sections were washed in 1× SSC buffer at room temperature. Finally, sections were dipped sequentially in Milli-Q water, 70% ethanol, and 95% ethanol before air-drying and exposure to Hyperfilm (Amersham). After exposure for 2 wk, X-ray films were developed using standard procedures.

Quantification and data analysis. All films were analyzed by using a computer-assisted image analysis system, Multi-Analysys, connected to a GS-690 Imaging Densitometer (Bio-Rad). Quantification of mRNA expression levels in various brain regions was obtained by measuring the average density of hybridization spots on X-ray film.
of each region in five adjacent brain sections, and then the values were compared against a 14C-labeled autoradiographic standard (Amersham UK). All data are shown as means ± SE for groups based on a minimum of five mice in each group. Analyses by ANOVA, followed where appropriate by Dunnett’s test, were performed using the JMP statistical package (SAS Institute, Cary, NC).

RESULTS

Food intake. After 1 and 7 wk of the diets, no significant differences were found in a total cumulative calorie intake in mice fed saturated, n-6 PUFA, and n-3 PUFA diets (Fig. 2, A and B). However, from Fig. 3A, it can be seen that among high-fat groups from weeks 5–7, the saturated fat diet group showed a lower intake than those of the PUFA groups \( [F(2, 21) = 3.76, P = 0.04] \). In contrast, Fig. 3B showed that mice on a saturated fat diet always had a higher body weight gain throughout all time points examined. In addition, the low fat-fed group did show a significantly lower cumulative energy intake compared with all high-fat diet groups after 7 wk of diet. Four weeks of dietary reversal show that a change in the diet from saturated to an n-3 PUFA or a low-fat diet significantly decreased the total cumulative energy intake compared with mice that continued the saturated fat diet \( [528 \pm 19, 448 \pm 13, \text{and} 593 \pm 11 \text{kJ•wk}^{-1} \text{mouse}^{-1}, \text{respectively}; \ F(2,14) = 85.26, P < 0.001, \text{Fig. 2C}] \). Compared with the last week on the previous diet, there were significant reductions in the highly saturated to low-fat (HS-LF, −12%) and HS to n-3 PUFA (HS-n3, −16%) groups compared with the group continuing on the HS diet (Fig. 2D). Compared with the group continuing on the HS diet, the HS-n3 group had a small but significant reduction in caloric intake in weeks 8, 9, 10, and 11 \( (−21, −10, −6, \text{and} −8\%, \text{respectively}) \). A more profound reduction was seen in the group switched to LF diet in weeks 8, 9, 10, or 11 \( (−18, −25, −28, \text{and} −27\%, \text{respectively}) \). During the reversal phase, the rate of body weight gain was reduced in the HS-n3 group but was not statistically different between the HS and HS-LF groups (Fig. 3B).

Body fatness. Relative body fatness was presented as body fat index, estimated as the amount of epididymal, perirenal, and inguinal fat depots per 100 g of body weight (Fig. 4). After 1 wk of diet, no significant differ-

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**Fig. 2.** Total cumulative energy intake over 1 (A) and 7 (B) wk on the diets and for the 4 subsequent wk of dietary reversal (C). Changes of energy intake (D) were compared after 1 wk of dietary reversal to the last week on previous diet. Diets were, as described in MATERIALS AND METHODS, highly saturated (HS), n-6 PUFA (n-6), high n-3 PUFA (n-3), or low-fat (LF). \( P \leq 0.05 \) vs. LF diet; \( P \leq 0.01 \) vs. HS fat diet; \( P \leq 0.05 \) vs. HS-LF.

**Fig. 3.** Food intake (A) and body wt (B) in mice fed an HS, n-3, n-6, or LF diet; or mice fed a high n-3 PUFA (HS-n3) or an LF (HS-LF) diet for 4 wk after 7 wk of an HS diet.
ferences were found in the body fat index between groups fed a highly saturated, n-6 PUFA, or low-fat diet (2.13 ± 0.08, 1.77 ± 0.22, 1.84 ± 0.10, respectively). However, the n-3 PUFA group had a significantly lower body fat index compared with each of the three other groups (1.28 ± 0.06, F(3,35) = 7.17, P < 0.001, Fig. 4A). After 7 wk of diet, the mice fed a highly saturated fat diet had the highest relative body fat index (4.75 ± 0.29), significantly higher (P < 0.01) than n-3 PUFA and low-fat diet groups, where the latter two groups differed [n-3 PUFA: 2.37 ± 0.21, LF: 3.32 ± 0.35, F(3,35) = 8.65, P < 0.01, Fig. 4B]. Again, no difference was found in the body fat index between the n-6 PUFA and low-fat diets. Four-week dietary reversal showed that using a low-fat diet to replace the saturated fat diet did not significantly reduce the body fatness (group changed to low-fat diet: 5.11 ± 0.41; group continuing the saturated fat diet: 6.11 ± 0.55; Fig. 4C); however, changing to the n-3 PUFA diet did reduce it (2.16 ± 0.25, −64%, F(2,33) = 38.20, P < 0.01).

Plasma leptin. After 1 wk of diet, no statistical differences were found in the levels of plasma leptin among groups (Fig. 5A). After 7 wk of diet, a significant increase in plasma leptin was found in mice fed the saturated fat diet [4.8 ± 0.5 ng/ml, F(3,35) = 9.53, P < 0.001] compared with the mice fed the n-3 PUFA, n-6 PUFA, or low-fat diet (2.3 ± 0.1, 3.9 ± 0.1, 1.6 ± 0.2 ng/ml, respectively, Fig. 5B). Four weeks of dietary reversal showed that changing from a saturated to an n-3 PUFA fat diet resulted in a significant decrease in plasma leptin to a level comparable to that of the low-fat diet group [Fig. 3C, F(2,26) = 4.07, P = 0.029]. However, the group switched to low fat were intermediate and not different from the saturated fat group. Furthermore, plasma leptin levels correlated well with the body fat index after 7 wk of diets (r = 0.42, P = 0.007) and 4 wk of diet reversal (r = 0.34, P = 0.042), but not after 1 wk of diet (r = 0.12, P = 0.49).

Leptin receptor mRNA expression. After 1 wk of diet, no significant difference was found in the level of leptin receptor mRNA expression in the choroid plexus, Arc, or ventromedial hypothalamic nucleus (VMH) across diet groups (Fig. 6, A, B, and C). After 7 wk of diet, the saturated fat diet group showed a significant increase (+26, +21, and +30%) in the levels of leptin receptor mRNA expression in the choroid plexus, Arc, and VMH compared with the low-fat diet group (Fig. 6, A, B, and C). However, all of these increases in the saturated fat diet group disappeared after 11 wk of diet (not shown). In the VMH, leptin receptor mRNA expression was significantly lower in mice fed an n-6 and an n-3 PUFA diet than in mice fed the saturated fat diet (Fig. 6C). Changing the diet to a saturated to an n-3 PUFA or a low-fat diet for 4 wk had no significant effects on the leptin receptor mRNA expression in choroid plexus, Arc, or VMH (Fig. 6D).

NPY mRNA expression. After 1 wk of diet, the saturated fat diet group had a significantly reduced level of Arc NPY mRNA expression compared with the n-6 PUFA, n-3 PUFA, and low-fat diet groups (−47, −50, and −54%, respectively, Fig. 7A). No significant differences were found in Arc NPY mRNA expression among

![Fig. 4. Body fat index was measured after 1 (A) and 7 (B) wk of diet and 4 wk of dietary reversal (C). Mice were fed HS, n-6, n-3, or LF diets. *P ≤ 0.05 vs. LF diet; **P ≤ 0.05 vs. HS diet; ***P ≤ 0.001 vs. HS-LF.](image-url)

![Fig. 5. Levels of plasma leptin (ng/ml) were measured after 1 (A) and 7 (B) wk of diets and 4 wk of dietary intervention (C). Mice were fed HS, n-6, n-3, or LF diets. *P ≤ 0.05 vs. LF diet; **P ≤ 0.05 vs. HS diet; ***P ≤ 0.05 vs. HS-LF.](image-url)
The latter three groups. After 7 wk of diet, the highly saturated fat diet group showed even further reduced Arc NPY mRNA expression compared with high n-3 PUFA, n-6 PUFA, and low-fat diet groups (to \(50\), \(60\), and \(71\%\), respectively, Fig. 7B). Again, no significant differences were seen among the latter three groups. Changing the diet from the saturated to an n-3 PUFA or a low-fat diet significantly reversed Arc NPY mRNA expression (by \(441\) and \(346\%) compared with keeping mice on the saturated fat diet (Fig. 7C).

AgRP mRNA expression. After 1 wk of diet, a significant reduction of Arc AgRP mRNA was found in mice fed the saturated fat diet (\(-21\), \(-20\), and \(-27\%\), Fig. 8A) compared with mice fed the n-6 PUFA, n-3 PUFA, or low-fat diet. No significant differences were found in the levels of Arc AgRP mRNA expression among the latter three groups. After 7 wk of diet, the saturated fat diet group still showed a reduced Arc AgRP mRNA expression (\(-23\), \(-13\), and \(-20\%, \text{Fig. 8B}\) compared with n-3 PUFA, n-6 PUFA, and low-fat diet groups. Again, no significant differences were found in the levels of Arc AgRP mRNA expression among the latter three groups. Four weeks of diet reversal showed that substitution of the saturated with the n-3 PUFA or low-fat diet had profound effects on Arc AgRP mRNA expression. Changing the diet from the saturated to a low-fat or a high n-3 PUFA fat diet significantly reversed Arc AgRP mRNA expression, by \(+181\) and \(+237\%\), respectively, compared with mice staying on the saturated fat diet. Continued feeding of mice with the saturated fat diet led to an even more pronounced reduction, to \(<25\%\) of Arc AgRP mRNA expression when compared with the low-fat diet group. The reduction in NPY mRNA expression was fairly specific to the Arc. We have also quantified the levels of NPY mRNA expression in the primary motor cortex, primary sensory cortex, and amygdala. No dietary effects were observed in these areas at any time point (data not shown here).

POMC mRNA expression. We have also measured Arc POMC mRNA expression in mice fed different types of fat and low-fat diets. We found no significant
either the n-3 or n-6 families. The main rated fat with a high n-6/n-3 PUFA ratio or PUFAs of Discussion

The present study has examined hypothalamic NPY, AgRP, POMC, and leptin receptor mRNA expression in response to high-fat diets emphasizing either saturated fat with a high n-6/n-3 PUFA ratio or PUFAs of either the n-3 or n-6 families. The main findings of this study are that a highly saturated fat diet potently decreases Arc NPY and AgRP mRNA expression and that this decrease occurs before significant elevations of circulating leptin levels can be detected. In contrast, diets equally high in total fat, but in which that fat is more predominantly PUFA, do not alter Arc NPY or AgRP mRNA expression levels compared with a low-fat diet. The decreased Arc NPY and AgRP mRNA expression levels induced by a highly saturated fat diet can be reversed by substitution of the diet with either high n-3 PUFAs or low fat. POMC mRNA expression is not affected by dietary fat level or type, at least within the time frame studied.

A good deal of the research investigating the influence of diet on hypothalamic NPY mRNA expression and protein levels has focused on the balance between carbohydrate and fat. However, there are intertwined issues here beyond just macronutrient exchange. These include altered energy density, increased total caloric intake, and changes in body weight and fatness. The current study allows an instructive dissection of these issues.

The reduction in Arc NPY mRNA with obesogenic high-fat feeding found here has been reported from a number of laboratories, including our own (5, 6, 9, 23, 25). Arc NPY protein levels follow mRNA levels down with longer feeding periods. As well, NPY protein in the paraventricular hypothalamic nucleus falls with long-term feeding and development of obesity (23). The argument has then been made about the carbohydrate-to-fat ratio as a controller of hypothalamic NPY. Compared with a high n-3 fat diet, the current study shows that a high-fat diet with a higher proportion of saturated fat perturbs the NPY (and AgRP) system. This study suggests that it is not the level of dietary fat per se that influences NPY expression, but that it is some obesogenic property or properties of saturated fats, which are detected either peripherally or centrally. The NPY/AgRP systems then could be seen to be reacting in a homeostatic manner. In the case of the PUFA diets, there is no evidence for increased body fatness (and even less fatness in the case of the n-3 PUFA diet; see Fig. 4), and the lack of change in Arc NPY mRNA (or even slight rise by week 7 in the n-3 group) is appropriate. The issue is clearly not dietary fat vs. carbohydrate.

The homeostatic response (inhibition) of Arc NPY and AgRP mRNA expression in the highly saturated fat group should then, at least as one important factor, have been associated with hypophagia and have worked to normalize weight (and fat) gain. That is clearly only partially the case. If we first look at the high saturated fat vs. low fat comparison, then we see the effects of both fat type and increased dietary energy density. At 1 and 7 wk of feeding, Arc NPY and AgRP mRNA expressions are reduced in the highly saturated fat group by some 47 and 50% and 21 and 23%, respectively, compared with the low-fat group. However, despite these reductions, food intake was increased by 17 and 14%, and rate of body weight gain was also significantly higher in the highly saturated fat diet group compared with the low-fat group.

Comparisons among the high-fat diet groups allow us to separate out the effect of diets of higher caloric density. Here it can be seen that, because the low-fat and PUFAs diets were similar in terms of NPY mRNA expression, there is again a substantial reduction (∼50%) in the highly saturated fat group compared with the PUFA groups. However, in this situation, there is very small, but significant, reduction in food intake of some (e.g., ∼15% over weeks 4–7). This may indicate that, when caloric density is controlled, indeed downregulation of the NPY and/or AgRP systems does act to reduce intake. However, the effects are slow to take effect, extremely weak in relation to the size of the downregulation, and are more a small speed-bump than a roadblock on the highway to obesity.

These data then would seem to show that, indeed, the NPY and AgRP systems are reacting to some signal indexing positive energy balance (driven by the highly

![Graph showing levels of agouti-related peptide mRNA expression](https://example.com/graph.png)
saturated fat diet) and are reacting appropriately. What is equally apparent is that the reduced Arc NPY and AgRP are insufficient to maintain energy homeostasis. Importantly, it is not high-fat diets per se that have this effect. High-fat diets emphasizing PUFAs do not act to impair energy balance, and the Arc NPY or AgRP systems are appropriately not responding. This strongly argues for a dysregulation in other parts of the energy balance system induced specifically by saturated fat, or at least by the fatty acid profile of that diet. Leptin is a potent regulator of the NPY system and one such candidate. However, at week 1, when Arc NPY mRNA levels were reduced by one-half, no increase in circulating leptin and no change in leptin receptor mRNA expression could be detected. If altered leptin dynamics were to account for the change in NPY, then a fairly subtle alteration in the 24-h profile would have to be implicated. This is possible but unlikely. Furthermore, if leptin had caused downregulation of Arc NPY, it should elevate Arc POMC mRNA as well (15, 19). However, this is not the case in this study, as there were no differences in POMC mRNA expression after 1 wk of diet. The conclusion that leptin is not responsible for the decline in NPY with fat feeding supports earlier suggestions (23). Other regulatory mechanisms must therefore exist in the regulation of Arc NPY and AgRP mRNA expression in the mice fed a highly saturated fat diet.

One of the possible explanations for the effects of the different high-fat diets is specific fatty acid modulation of gene expression. Clarke and coworkers (2, 3) showed very early that fatty acid synthase (FAS) gene expression was increased by diets high in saturated and monounsaturated fats, but that when the predominant fat was n-6 PUFAs, FAS activity was lower, and when it was n-3 PUFAs, FAS activity was returned almost to control level. Because endogenous lipogenesis creates saturated fats, an increased FAS activity will result in a “saturating down” of the whole body fatty acid pool, adding to the overall effect of saturated fats. This major increase in the body’s saturated fatty acid pool may then directly result in obesity via decreases in metabolic rate (e.g., reduced ion “leakiness of membrane and reduced adrenergic binding”; see Refs. 12 and 22). Equally, the effects may be indirect. Genes implicated in the mature phase of adipocyte proliferation are highly expressed in rats fed saturated and monounsaturated fats (21). In contrast, expression was suppressed to some extent by n-6 PUFAs and profoundly by n-3 PUFAs. There are numerous secreted proteins from adipocytes besides leptin, and one of these, which is primarily expressed in the transition phase from pre- to mature adipocyte, might potently regulate NPY and associated neuropeptides. Of course, although the effects of different dietary fatty acid profile might be on gene expression in adipose and other peripheral tissues, a direct effect on brain is also possible. This exciting possibility might be addressed in future work with brain slice preparations.

An important aspect of the current studies is the effect seen with the dietary reversal phase. Both change in dietary fat profile (n-3 diet) and alteration in fat/carbohydrate balance (i.e., low-fat diet) had beneficial effects on body fatness. However, it is striking that these effects are very modest with the low-fat diet, compared with major effects with change only in dietary fat profile. This is equally reflected in the leptin levels. In contrast, both dietary reversal interventions resulted in “normalization” (to the chronic low-fat control) of NPY and AgRP mRNA expression. This again suggests the possibility of direct effects peripherally on gene expression (in adipose tissue) of individual fatty acids, which tune metabolism away from storage and toward mobilization of triglycerides. An analysis of adipocyte gene expression (e.g., lipoprotein lipase, perilipin, hormone-sensitive lipase, and the like) and of fatty acid efflux in the early period after dietary reversal intervention would be informative.

The lack of effect of highly saturated fat diet on POMC expression over the current dietary time frame is consistent with our earlier work (10) and that of others (8). The current results extend this observation by showing that neither fat/carbohydrate balance nor dietary fatty acid profile has effects. Whatever the factor or factors are that alter NPY/AgRP expression, they are not effective on the POMC system. Finally, a limitation of the current study is that the observed changes in NPY gene expression may not precisely reflect NPY release. It is possible that dietary fat profile might somehow differentially affect cell membrane phospholipid composition and protein secretion and interaction. This possibility should be tested in future studies.

In summary, the current studies demonstrate that changing the fatty acid profile of the diet alone can profoundly alter the expression of major hypothalamic neuropeptides of energy balance. The effects would appear to relate to whether a particular dietary fatty acid profile is obesogenic or not, but dysregulation appears independent of leptin as a mediator. The next step is to understand the elements of the body energy balance homeostatic network, which are dysregulated by saturated fats.

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