Effect of High Fat Diets on Energy Balance and Thermogenesis in Brown Adipose Tissue of Lean and Genetically Obese *ob/ob* Mice

STEWART W. MERCER[1,2] *AND PAUL TRAYHURN*[3]

Dunn Nutrition Laboratory, Medical Research Council and University of Cambridge, Cambridge CB4 1XJ, United Kingdom

ABSTRACT The effects on energy balance and brown adipose tissue thermogenesis of feeding high fat diets of differing fatty acid composition have been investigated in lean and genetically obese (*ob/ob*) mice. Groups of mice were fed either a low fat diet or a high fat diet based on corn oil or beef tallow for 2 wk. Energy intake and body weight gain were higher in both lean and obese animals fed the high fat diets than in respective mice fed the low fat diets. Carcass energy gain was greater for the obese than for the lean consuming each of the diets. Both lean and obese mice had a higher energy gain when fed the beef tallow diet than when fed the corn oil, despite isoenergetic intakes of the two diets. The thermogenic activity of brown adipose tissue, assessed from measurements of cytochrome oxidase activity and mitochondrial guanosine 5'-diphosphate (GDP) binding, were greater in both lean and obese mice fed the corn oil diet than in those fed the low fat diet. However, GDP binding and cytochrome oxidase activities in lean or obese mice fed the beef tallow diet were not different from those of mice of the same genotype fed the low fat diet. These results indicate that in both lean and obese (*ob/ob*) mice energy deposition and the stimulation of brown adipose tissue thermogenesis during the voluntary hyperphagia induced by feeding high fat diets are influenced by the fatty acid composition of the diet. A diet rich in polyunsaturated fatty acids appears to result in preferential stimulation of the thermogenic activity of brown adipose tissue, particularly in the *ob/ob* mouse. *J. Nutr.* 117: 2147–2153, 1987.

INDEXING KEY WORDS:

• *brown adipose tissue* • *diet-induced thermogenesis* • *obese mouse* • *dietary fat* • *polyunsaturated fatty acids*

There has been considerable interest in the extent to which the macronutrient composition of the diet may affect thermogenesis and whole-body energy flux, with some recent emphasis on the influence of dietary lipid (1–4). Studies on cold-acclimated rats (4, 5) and mice (6) have indicated that feeding high fat diets can specifically increase thermogenesis in brown adipose tissue, an organ now regarded as the major site of heat production by nonshivering mechanisms in small mammals (7, 8). This stimulatory effect of dietary lipid on thermogenesis in brown adipose tissue of cold-acclimated rodents involves an increase in the activity of the mitochondrial proton conductance pathway (5, 6), which is the major heat-generating mechanism in the tissue (9).

Recent research in obesity has stimulated a wider interest in brown adipose tissue because animal studies have suggested that the tissue is important in the regulation of energy balance (see 10–14). Normal rats and mice induced to overeat by feeding a "cafeteria" diet may resist the development of obesity by exhibiting a compensatory increase in energy expenditure. This phenomenon, generally termed diet-induced thermogenesis, is associated with a large increase in the thermogenic activity of brown adipose tissue (13–15). Conversely, brown adipose tissue thermogenesis is abnormally low in several different types of obese animal (see 10–12).

One of the most widely used animals in obesity research is the genetically obese (*ob/ob*) mouse. After 4 wk of age this mutant becomes hyperphagic (16), yet exhibits a low level of thermogenic activity in brown adipose tissue, the activity being unaffected by the in-

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[2]Present address: Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE, U.K.

[3]To whom correspondence should be directed at the present address: Nutrition and Metabolism Research Group, 536 Newton Research Building, University of Alberta, Edmonton, Alberta, Canada T6G 2C2.

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creased energy intake (17). This implies that the mutant has a defective capacity for diet-induced thermogenesis. Indeed, in some studies *ob/ob* mice fed a cafeteria diet have been reported to exhibit a reduced dietary stimulation of energy expenditure, together with a failure to show the full adaptive increases in brown adipose tissue thermogenesis that occur in lean mice (18). In other studies, however, a large activation of brown adipose tissue thermogenesis has been recorded in *ob/ob* mice fed a cafeteria diet (19).

Although most cafeteria diets are high in fat, a disadvantage of the cafeteria feeding regimen is the difficulty in accurately defining the nutrient composition of the diet, this being influenced both by the types of food items presented to the animals and by individual preferences for certain foods. Thus the influence of different dietary components, rather than hyperphagia per se, is difficult to assess, although attempts have been made to overcome this problem by feeding cafeteria diets of different gross nutrient composition (3, 20). In the present study we fed lean and obese (*ob/ob*) mice high fat diets of defined composition and compared the response by reference to a low fat/high carbohydrate diet. The aim of the study was to assess the influence of different dietary lipids on energy balance and brown adipose tissue thermogenesis in the two types of animal.

MATERIALS AND METHODS

Animals and diets. The animals used in this study were male lean (*ob/+* or *+/+*) and genetically obese (*ob/ob*) mice from a colony bred at the Dunn Nutrition Laboratory in which the *ob* gene is on the "Aston" background (21). Lean and obese littermates were taken at 40–47 d of age and caged individually in wire-mesh cages. They were fed either a low fat diet (3.4% wt/wt fat) or a high fat diet (20% wt/wt fat) containing corn oil or beef tallow. Food and water were available ad libitum, and the animals were maintained at a temperature of 22 ± 1°C with a 12 h light-dark cycle (lights on at 0700 h).

The low fat–high carbohydrate nonpurified diet (Spillers-Spratt Rodent Breeding Diet 1, Spratts Patent, Barking, Essex, U.K.) was identical to that used previously (6) and had the following gross composition (wt/wt: 21.3% protein (29.1% of total energy), 3.4% fat (10.6% of total energy), 41.8% starch and 2.8% sucrose (60.3% of total energy as carbohydrate). The high fat diets were also prepared as previously (6, 22): 200 g fat (corn oil or beef tallow), 100 g casein and 0.1 g vitamin E were mixed with 700 g of the low fat nonpurified diet. In the high fat diets, protein, fat and carbohydrate accounted for 23.4, 47.7 and 28.9% of total energy, respectively. Corn oil contains predominantly polyunsaturated fatty acids and has a linoleic (C18:2) acid content of approximately 60% (with 26% C18:1). Beef tallow is rich in saturated and monounsaturated fatty acids and has the following fatty acid composition: 41% C18:1, 19% C18:0 and 30% C16:0 + C16:1 (21).

Energy balance measurements. The mice were fed the experimental diets for 2 wk and food intake was measured over the entire period, corrections being made for spillage. Total fecal output was collected and samples taken for the determination of digestible energy intake (gross energy intake minus fecal energy). Metabolizable energy (ME) intake was calculated as 0.96 × digestible energy intake (23). At the end of the 2-wk experimental period the mice were killed by cervical dislocation and the carcasses (entire animal, minus the gut contents and the interscapular plus subscapular brown adipose tissue) softened by autoclaving. Following this they were homogenized and freeze-dried to constant weight without further processing.

The gross energy content of the dried carcasses, feces and diets was determined by bomb calorimetry, using a Gallenkamp adiabatic calorimeter (Gallenkamp, London, U.K.) calibrated with dry benzoic acid standards. The initial body energy was estimated from regression lines constructed from the carcass energy content of a group of eight pairs of male lean and obese littermates that were similar in age and weight range to the experimental animals at the start of the study.

Measurement of the thermogenic activity of brown adipose tissue. At the end of the 2-wk period of feeding the different diets, brown adipose tissue was removed from the interscapular and subscapular sites and cleared of adhering white adipose tissue. The tissue was weighed and homogenized and cytochrome oxidase activity was measured spectrophotometrically (24). The total protein content of the brown adipose tissue depots was determined by a modified Lowry procedure (25).

Mitochondria were isolated from brown adipose tissue (26), and guanosine 5'-diphosphate (GDP) binding was measured by incubating the mitochondria with 10 μM [^{3}H]GDP for 7 min at room temperature, as previously described (6, 26).

Statistical analysis. The statistical significance of differences between groups was assessed using Student's *t*-test.

RESULTS

Energy balance. In the lean mice, ME intake was higher in those fed the high fat diets than in those fed the low fat diet, and both body weight and carcass energy gain were significantly elevated on the high fat diets (**Tables 1 and 2**). However, carcass energy gain was higher in the lean mice fed the beef tallow diet than in lean animals fed the corn oil diet, despite the fact that the ME intake tended to be slightly lower in the former group than in the latter.

Carcass energy content was over threefold higher in the obese mice than in the lean animals at the start of

TABLE 1

***Body weight and food intake of lean and obese (ob/ob) mice fed a low fat diet or high fat diets*[1]**

Body weights and food intake	Low fat diet		Corn oil diet		Beef tallow diet	
	Lean (7)	Obese (7)	Lean (7)	Obese (6)	Lean (7)	Obese (7)
Initial body weight, *g*	32.4 ± 1.0	44.8 ± 1.7‡	32.0 ± 2.1	44.2 ± 1.5‡	32.6 ± 1.1	43.8 ± 1.1‡
Final body weight, *g*	35.5 ± 0.9	55.0 ± 2.1‡	36.9 ± 2.4	61.7 ± 3.4‡	39.4 ± 1.1[a]	65.1 ± 1.5‡[,b]
Body weight gain, *g*	3.1 ± 0.3	10.2 ± 1.7†	4.9 ± 0.5[a]	17.5 ± 2.2‡[,a]	6.8 ± 0.5[c,+]	21.4 ± 1.2‡[,c]
Food intake, *g/d*	5.4 ± 0.3	7.8 ± 0.5†	5.0 ± 0.3	8.0 ± 0.3‡	4.7 ± 0.2	8.2 ± 0.3‡

[1]Values are means ± SEM. $n = 7$ except for obese mice fed the corn oil diet. Lean and obese (*ob/ob*) mice were fed the low fat diet or one of the high fat diets for 2 wk at 22°C. Food intake was measured, with corrections for spillage, over the entire experimental period, and total fecal output was collected. $^{*}P < 0.05$, $^{\dagger}P < 0.01$, $^{\ddagger}P < 0.001$ compared with lean mice fed the same diet. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ compared with mice of the same genotype fed the low fat diet. $^{+}P < 0.05$ compared with mice of the same genotype fed the corn oil diet. For full experimental details see the text.

the experiment. Carcass energy gain and body weight gain over the experimental period were also considerably greater in the obese than in the lean (Tables 1 and 2). ME intake was higher in all the groups of *ob/ob* mice than in the lean littermates. The intake of obese mice fed the low fat diet was 146%, fed the corn oil diet was 165% and fed the beef tallow diet was 177% of that of lean mice.

Carcass energy gain was significantly higher in the *ob/ob* mice fed the high fat diets than in the obese animals fed the low fat diet and was higher in the group fed beef tallow than in the group fed the corn oil diet. The difference in carcass energy retention between the groups of *ob/ob* mice fed the high fat diets cannot be ascribed to differences in energy intake, because ME intake was virtually identical in these two diet groups (Table 2). Gross efficiency (kJ gain/kJ ME intake) was significantly higher in lean and obese mice fed the beef tallow diet than in those given the corn oil diet (Table 2).

From the measurements of energy intake and energy storage, the "apparent" energy expenditure of individual animals was calculated (Table 2). Apparent energy expenditure tended to be higher in the lean mice fed the corn oil diet than in the lean animals fed either the beef tallow diet or the low fat diet, although these differences were not statistically significant ($P > 0.05$). In the obese mice, the apparent energy expenditure was significantly higher in both the high fat dietary groups than in the low fat group. However, expenditure was higher in mice fed the corn oil diet than in those fed the beef tallow diet, despite the similar levels of energy intake in these two groups of obese mice.

The absolute increases in ME intake, carcass energy gain and apparent energy expenditure of the animals fed the high fat diets (above the levels of the same genotype fed the low fat diet) are shown in **Table 3**. Lean mice fed the corn oil diet had an ME intake 25% more than that of the lean animals fed the low fat diet, but only 18% of this extra intake was deposited. In contrast, lean mice fed the beef tallow diet deposited 77% of their "excess" energy intake. In the obese mutants, as in the lean animals, excess energy intake of mice fed the corn oil diet was very similar to that of

TABLE 2

***Energy balance in lean and obese (ob/ob) mice fed a low fat diet or high fat diets*[1]**

Energy	Low fat diet		Corn oil diet		Beef tallow diet	
	Lean (7)	Obese (7)	Lean (7)	Obese (6)	Lean (7)	Obese (7)
Me intake, *kJ*	1034 ± 66	1509 ± 94†	1296 ± 80[a]	2132 ± 90‡[,c]	1201 ± 58	2120 ± 73‡[,c]
Initial body energy, *kJ*	270.3 ± 16.0	818.7 ± 62.4‡	264.4 ± 32.1	806.3 ± 62.7‡	273.4 ± 17.9	781.7 ± 39.2‡
Final body energy, *kJ*	341.3 ± 17.7	1128.0 ± 51.3‡	383.4 ± 28.9	1307.7 ± 79.6‡	473.7 ± 19.9[c,+]	1436.7 ± 29.8‡[,c]
Gain in body energy, *kJ*	71.0 ± 10.4	309.2 ± 42.0‡	119.1 ± 17.1[a]	501.3 ± 43.6‡[,b]	200.3 ± 15.6[c,++]	653.7 ± 37.8‡[,c,+]
Gross efficiency, %	6.9 ± 1.2	20.5 ± 2.3†	9.2 ± 1.1	23.5 ± 1.2†	16.7 ± 1.4[++]	30.8 ± 1.1‡[,b,+++]
Apparent energy expenditure, *kJ*	963 ± 68	1200 ± 79[*]	1177 ± 84	1631 ± 48‡[,c]	1001 ± 59	1466 ± 49‡[,a,+]

[1]Lean and obese (*ob/ob*) mice were fed the diets for 2 wk at 23°C, as described in Table 1. Initial body energy was calculated from regression lines constructed from the energy content of lean and obese mice similar in initial age and weight range to the experimental animals. Final body energy was measured directly. Apparent energy expenditure was calculated from ME intake minus body energy gain. The results are given as mean values ± SEM, with the number of animals in parentheses. $^{*}P < 0.05$, $^{\dagger}P < 0.01$, $^{\ddagger}P < 0.001$ compared with lean mice fed the same diet. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ compared with mice of the same genotype fed the low fat diet. $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ compared with mice of the same genotype fed the corn oil diet.

TABLE 3

Diet-induced thermogenesis in lean and obese (ob/ob) mice fed different high fat diets

Measure	Corn oil diet		Beef tallow diet	
	Lean	Obese	Lean	Obese
Absolute extra ME[1] intake, *kJ*	262	623	167	611
Absolute extra body energy gain, *kJ*	48	192	129	345
Apparent extra energy expenditure, *kJ*	214	431	38	266
Proportion of extra intake deposited, %	18	31	77	56

[1]As compared with mice of the same genotype fed the low fat diet. Values shown are calculated from the data in Table 2.

those fed the tallow diet. However, in the *ob/ob* mice fed the corn oil diet only 31% of this extra intake was deposited, whereas 56% was deposited in the obese fed the beef tallow diet.

Thermogenesis in brown adipose tissue. The amount of brown adipose tissue (interscapular + subscapular) in the lean mice fed the corn oil diet was not different from that of mice fed the low fat diet, whereas mice fed the beef tallow diet had significantly more of the tissue (**Table 4**). This increase in tissue weight presumably reflects differences in lipid content since the total protein content was unaffected by dietary manipulation. In the obese mice, the amount of brown adipose tissue was significantly less in mice fed the high fat diets than in those fed the low fat diet, and this was more pronounced in mice fed the corn oil diet than in those fed beef tallow. Again, these changes are likely to reflect alterations in the fat content of the tissue since the total brown fat protein of mice fed the high fat diets was not lower than that of mice fed the low fat diet. Indeed, it was substantially higher in the *ob/ob* mice fed the corn oil diet, suggesting functional growth of the tissue in this group.

The total cytochrome oxidase activity in the tissue, an index of mitochondrial mass, is shown in Table 4. Cytochrome oxidase activity was lower in the *ob/ob* mice fed the low fat diet or the beef tallow diet than in lean littermates fed the same diet. However, the obese mice fed the corn oil diet exhibited a twofold increase in activity relative to the other groups of obese animals. Lean mice fed the corn oil diet also had significantly higher cytochrome oxidase activity than lean mice fed either of the other two diets. Thus, in both genotypes, feeding a corn oil diet resulted in a substantially greater mitochondrial mass and oxidative capacity of brown adipose tissue.

GDP-binding studies were performed on isolated brown adipose tissue mitochondria to assess the activity of the proton conductance pathway (Table 4). Specific GDP binding, expressed per milligram of mitochondrial protein, was not significantly influenced by dietary treatment in the lean mice, although on a whole-tissue basis the overall level of binding would be higher in the lean fed the corn oil diet than in the lean fed the other diets, as a consequence of the greater mitochondrial mass in these animals. In the obese mice, GDP binding was 186% higher in the corn oil group than in the obese group fed the low fat diet. Binding tended to be slightly higher in the obese fed the beef tallow diet than in the obese fed the low fat diet, but the difference was not statistically significant.

TABLE 4

Cytochrome oxidase activity and mitochondrial GDP binding in brown adipose tissue of lean and obese (ob/ob) mice fed different high fat diets[1]

Measure	Low fat diet		Corn oil diet		Beef tallow diet	
	Lean (6)	Obese (6)	Lean (6)	Obese (6)	Lean (6)	Obese (5)
Brown adipose tissue weight, *mg*	210.0 ± 11.5	737.0 ± 51.2‡	205.0 ± 9.2	546.0 ± 37.4‡^{,a}	278.3 ± 13.5$^{b,+}$	610.0 ± 15.3‡^{,a}
Brown adipose tissue protein content, *mg*	24.5 ± 2.0	18.3 ± 1.4*	26.8 ± 1.6	33.8 ± 2.5*^{,c}	26.6 ± 1.2	21.6 ± 1.5*$^{,+++}$
Total cytochrome oxidase activity, *μmol cytochrome c oxidized (min·tissue)*	38.7 ± 2.5	21.7 ± 1.7‡	51.3 ± 4.0^{a}	46.5 ± 4.5^{c}	39.7 ± 2.3^{+}	22.5 ± 2.2‡$^{,+++}$
Mitochondrial GDP binding, *pmol GDP/mg mitochondrial protein*	236.0 ± 23.1	164.0 ± 17.0*	257.8 ± 42.9	305.8 ± 39.6^{c}	258.4 ± 31.7	221.1 ± 54.5

[1]Lean and obese mice were fed the different diets for 2 wk at 23°C, as in Table 1. Brown adipose tissue was obtained from the interscapular and subscapular sites and pooled. Results are given as mean values ± SEM, with the number of animals in parentheses. *$P < 0.05$, ‡$P < 0.001$ compared with lean mice fed the same diet. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ compared with mice of the same genotype fed the low fat diet. $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ compared with mice of the same genotype fed the corn oil diet.

DISCUSSION

The present study has investigated the effects of two high fat diets of differing fatty acid composition on energy balance and indices of brown adipose tissue thermogenesis in lean and obese (*ob/ob*) mice. From the measurement of ME intake and carcass energy deposition, the results suggest that energy expenditure is stimulated more by a high fat diet based on corn oil, with its high level of polyunsaturated fatty acids, than by a high fat diet containing saturated and monounsaturated fatty acids (beef tallow). This differential response to dietary lipid composition was apparent in both lean and obese genotypes, although it was more pronounced in the lean animals. Only 18% of the *excess* energy intake of the lean mice fed the corn oil diet was retained in the carcass, compared with 77% in the lean fed the beef tallow diet, despite essentially isoenergetic intakes of the two diets. In the obese mice, less than a third of the extra energy intake was retained when the corn oil diet was fed compared with more than half when the beef tallow diet was fed. It should be emphasized that direct comparisons between the lean and obese animals in the present study are difficult because the obese were hyperphagic when fed each diet.

The differential response in both lean and obese animals to voluntary overfeeding when consuming high fat diets of differing composition suggests that some of the reported variability in the extent to which diet-induced thermogenesis is stimulated when cafeteria diets are fed (14, 23) may well be related to differences in the fatty acid composition of the particular diets selected. This factor would be in addition to other recognized variables, such as the strain and age of the animals (14, 27).

The energy cost of storage of the extra energy intake may account for some of the higher energy expenditure by animals fed the high fat diets, but this is unlikely to explain the differences in energy deposition between the corn oil and beef tallow groups. Even assuming that all of the extra energy deposited in the animals fed the high fat diets resulted from the de novo synthesis of fatty acids—which is unlikely since high fat diets suppress whole-body lipogenesis in rats and mice (21, 28)—this would account for only 8 and 16%, respectively, of the extra energy expenditure for lean and obese mice (consuming the corn oil diet), on the assumption that the energy cost of fat deposition is 0.36 kJ/kJ fat deposited (29). In the group receiving the beef tallow diet the cost of fat synthesis from carbohydrate could account for some 47% of the extra energy expended in the obese mice and all of the extra expenditure of the lean animals. These estimates are based on multiplying the "extra" energy deposited for each group by the energy cost of fat deposition from carbohydrate.

These calculations also assume that the excess energy deposited in mice fed the high fat diets was in the form of fat rather than protein. Although nitrogen balance was not measured in the present study, an indication of the composition of the extra energy retained can be seen from the energy density of the weight gained. The theoretical limits for energy gain are approximately 5 kJ/g for a gain consisting entirely of well-hydrated lean tissue and 33 kJ/g when the gain is essentially white adipose tissue (see 20). In the present study the average densities of weight gain were 21, 26 and 30 kJ/g wt gain for lean mice fed the low fat diet, corn oil diet and beef tallow diet, respectively. In the *ob/ob* mice the corresponding values were 33, 30 and 31 kJ/g wt gain. These values indicate that the predominant form of the extra energy stored was fat. In particular, the high and almost identical energy densities in the *ob/ob* animals fed the two high fat diets would discount differences in protein deposition as being of major significance in the large difference in energy retention observed between these two groups of obese mice.

Thus, even allowing for generous estimates of the cost of fat deposition, there is clear evidence of a stimulatory influence of the corn oil diet on energy expenditure in both lean and obese mice. In general terms, these diet-induced changes in whole-body energy expenditure were accompanied by similar changes in the thermogenic properties of brown adipose tissue. The total thermogenic activity of the tissue, which is a function of both the specific activity of the proton conductance pathway per milligram of mitochondrial protein and the total mitochondrial mass, was enhanced in the mice fed the corn oil diet with the obese mutants showing the largest increase. This group also showed the largest energy "gap" between intake and storage, consistent with a significantly higher energy expenditure when fed the corn oil diet. Lean mice fed the beef tallow diet showed no evidence of stimulation of diet-induced thermogenesis and exhibited no activation of brown adipose tissue. Obese animals fed the beef tallow diet showed a small augmentation in the activity of the proton conductance pathway, as indicated by the GDP binding values, but their cytochrome oxidase activity was not higher.

In relating these changes in brown adipose tissue to overall energy balance it should be emphasized that energy balance measurements assess the net change in energy deposition over the entire experimental period, whereas measurements on brown adipose tissue reflect the activity of the tissue at the termination of the experiment. Although total mitochondrial mass would not be expected to fluctuate greatly on a day-to-day basis, the activity of the proton conductance pathway does exhibit a distinct circadian rhythm (30). Thus the precise stoichiometry of the thermogenic activity of brown adipose tissue and whole-body energy flux cannot be clearly defined in the present study. It should also be emphasized that thermogenic mechanisms other than those associated with brown adipose tissue could be activated differentially in animals consuming the two high fat diets.

Previous work on *ob/ob* mice of the Aston strain with a cafeteria feeding regimen indicated a defective activation of diet-induced thermogenesis, associated with a failure to exhibit the full adaptive changes in the thermogenic activity of brown adipose tissue observed in lean animals (18). Interestingly, the cafeteria-fed *ob/ob* mice retained some 55% of their extra energy intake, a value that is almost identical to that of the mutants fed the beef tallow diet in the present work. The obese mice fed the corn oil diet in the present study did, however, show adaptive changes in the thermogenic activity of brown adipose tissue, and they retained only 31% of their extra energy intake. Thus differences in the level of polyunsaturated fatty acids in the diet may be of crucial importance in determining the extent to which voluntary hyperphagia activates thermogenesis in obese as well as in lean animals. Variations in the fatty acid composition of the diet may well explain the reported differences in the stimulation of brown adipose tissue thermogenesis in *ob/ob* mice fed cafeteria diets (18, 19).

It is noteworthy that the thermogenic activity of brown adipose tissue in obese mice fed the corn oil diet (the obese group with the highest activity) was at the same level as that of lean controls fed the low fat diet, despite the fact that energy intake was more than 100% higher in the former group. Thus, although altering the fatty acid composition of the diet appears to influence the extent to which diet-induced thermogenesis in brown adipose tissue is stimulated in *ob/ob* mice, the basic defective response to overfeeding still persists. Studies using different nonmutant strains of animals have clearly demonstrated that there are marked genetic differences in the extent to which obesity develops when feeding high fat diets (31) and the degree to which diet-induced thermogenesis is stimulated by overfeeding (27).

The mechanisms involved in the activation of brown adipose tissue by feeding a diet containing high levels of corn oil are unknown. In view of the importance of catecholamines in the activation and hypertrophy of the tissue (32), one explanation may be that dietary polyunsaturated fatty acids lead to stronger stimulation of the sympathetic nervous system than do saturated fatty acids. A second explanation is related to a possible direct influence of dietary polyunsaturated fatty acids on brown adipose tissue. Linoleic acid may be preferentially channeled toward brown adipose tissue (33), leading to increased uncoupling of respiration through the acute effects of fatty acids on activation of the proton conductance pathway (34, 35). Such a mechanism would, however, imply poor control of thermogenesis.

A third possible explanation of the present results involves the influence of dietary fats on membrane fatty acid composition (36). Changing the level of polyunsaturated fatty acids in the diet appears to modify membrane functions, such as receptor-enzyme coupling (37, 38) and hormone sensitivity (39). The *ob/ob* mice fed a low fat–high carbohydrate diet have defective coupling between the β-adrenoceptor and adenylate cyclase in the plasma membrane of the brown adipocyte (40), but chronic feeding of a diet rich in corn oil appears to correct this abnormality (41). That is, a high dietary intake of polyunsaturated fatty acids appears to have important effects on the "tightness of coupling" between these two membrane components, presumably via a change in plasma membrane lipid composition.

LITERATURE CITED

1. KASPER, H., THIEL, H. & EHL, M. (1973) Response of body weight to a low carbohydrate, high fat diet in normal and obese subjects. *Am. J. Clin. Nutr.* 26: 197–204.
2. ZED., C. A. & JAMES, W. P. T. (1982) Thermic response to fat feeding in lean and obese subjects. *Proc. Nutr. Soc.* 41: 32A (abs.).
3. ROTHWELL, N. J., STOCK, M. J. & WARWICK, B. P. (1983) The effect of high fat and high carbohydrate cafeteria diets on diet-induced thermogenesis in the rat. *Int. J. Obesity* 7: 263–270.
4. ROTHWELL, N. J. & STOCK, M. J. (1983) Acute effects of fat and carbohydrate on metabolic rate in normal, cold-acclimated and lean and obese (*fa/fa*) Zucker rats. *Metabolism* 32: 371–376.
5. MERCER, S. W. & TRAYHURN, P. (1984) A comparative study on the influence of high fat diets on thermogenesis in brown adipose tissue of cold-acclimated rodents. *Proc. Nutr. Soc.* 43: 146A (abs.).
6. MERCER, S. W. & TRAYHURN, P. (1984) Effect of high fat diets on the thermogenic activity of brown adipose tissue in cold-acclimated mice. *J. Nutr.* 114: 1151–1158.
7. FOSTER, D. O. & FRYDMAN, M. L. (1978) Non-shivering thermogenesis in the rat. II. Measurements of blood flow with microspheres point to brown adipose tissue as the dominant site of the calorigenesis induced by noradrenaline. *Can. J. Physiol. Pharmacol.* 56: 110–122.
8. FOSTER, D. O. & FRYDMAN, M. L. (1979) Tissue distribution of cold-induced thermogenesis in conscious warm- or cold-acclimated rats reevaluated from changes in tissue blood flow: the dominant role of brown adipose tissue in the replacement of shivering by nonshivering thermogenesis. *Can. J. Physiol. Pharmacol.* 57: 257–270.
9. NICHOLLS, D. G. & LOCKE, R. M. (1984) Thermogenic mechanisms in brown fat. *Physiol. Rev.* 64: 1–64.
10. TRAYHURN, P. (1984) The development of obesity in animals: the role of genetic susceptibility. *Clin. Endocrinol. Metab.* 13: 451–474.
11. TRAYHURN, P. (1986) Brown adipose tissue and energy balance. In: *Brown Adipose Tissue* (Trayhurn, P. & Nicholls, D. G., eds.), pp. 299–338, Edward Arnold, London.
12. HIMMS-HAGEN, J. (1985) Brown adipose tissue metabolism and thermogenesis. *Annu. Rev. Nutr.* 5: 69–94.
13. ROTHWELL, N. J. & STOCK, M. J. (1983) Diet-induced thermogenesis. In: *Mammalian Thermogenesis* (Girardier, L. & Stock, M. J., eds.), pp. 208–233, Chapman & Hall, London.
14. ROTHWELL, N. J. & STOCK, M. J. (1986) Brown adipose tissue and diet-induced thermogenesis. In: *Brown Adipose Tissue* (Trayhurn, P. & Nicholls, D. G., eds.), pp. 269–298, Edward Arnold, London.
15. ROTHWELL, N. J. & STOCK, M. J. (1979) A role for brown adipose tissue in diet-induced thermogenesis. *Nature (London)* 281: 31–35.
16. LIN, P.-Y., ROMSOS, D. R. & LEVEILLE, G. A. (1977) Food intake, body weight gain, and body composition of the young obese (*ob/ob*) mouse. *J. Nutr.* 107: 1715–1723.
17. MERCER, S. W. & TRAYHURN, P. (1984) The development of insulin resistance in brown adipose tissue may impair the acute cold-induced activation of thermogenesis in genetically obese (*ob/ob*) mice. *Biosci. Rep.* 4: 933–940.

18. TRAYHURN, P., JONES, P. M., MCGUCKIN, M. M. & GOODBODY, A. E. (1982) Effects of overfeeding on energy balance and brown fat thermogenesis in obese (*ob/ob*) mice. *Nature (London)* 295: 323–325.
19. HIMMS-HAGEN, J., HOGAN, S. & ZAROR-BEHRENS, G. (1986) Increased brown adipose tissue thermogenesis in obese (*ob/ob*) mice fed a palatable diet. *Am. J. Physiol.* 250: E274–E281.
20. ROTHWELL, N. J., STOCK, M. J. & TYZBIR, R. S. (1982) Energy balance and mitochondrial function in liver and brown fat of rats fed "cafeteria" diets of varying protein content. *J. Nutr.* 112: 1663–1672.
21. THURLBY, P. L. & TRAYHURN, P. (1979) The role of thermoregulatory thermogenesis in the development of obesity in genetically obese (*ob/ob*) mice pair-fed with lean siblings. *Br. J. Nutr.* 42: 377–385.
22. VAN DEN BRANDT, P. A. & TRAYHURN, P. (1981) Suppression of fatty acid synthesis in brown adipose tissue of mice fed diets rich in long chain fatty acids. *Biochim. Biophys. Acta* 665: 602–607.
23. BARR, H. G. & MCCRACKEN, K. J. (1984) High efficiency of energy utilization in "cafeteria"- and force-fed rats kept at 29°. *Br. J. Nutr.* 51: 379–387.
24. YONETANI, T. & RAY, G. S. (1965) Studies on cytochrome oxidase. VI. Kinetics of the aerobic oxidation of ferrocytochrome *c* by cytochrome oxidase. *J. Biol. Chem.* 240: 3392–3398.
25. SCHACTERLE, G. R. & POLLACK, R. L. (1973) A simplified method for the quantitative assay of small amounts of protein in biologic material. *Anal. Biochem.* 51: 654–655.
26. GOODBODY, A. E. & TRAYHURN, P. (1981) GDP binding to brown adipose tissue mitochondria of diabetic-obese (*db/db*) mice. *Biochem. J.* 194: 1019–1022.
27. ROTHWELL, N. J., SAVILLE, M. E. & STOCK, M. J. (1982) Effects of feeding a "cafeteria" diet on energy balance and diet-induced thermogenesis in four strains of rat. *J. Nutr.* 112: 1515–1524.
28. ROTHWELL, N. J., STOCK, M. J. & TRAYHURN, P. (1983) Reduced lipogenesis in cafeteria-fed rats exhibiting diet-induced thermogenesis. *Biosci. Rep.* 3: 217–224.
29. PULLAR., J. D. & WEBSTER, A. J. F. (1977) The energy costs of protein and fat deposition in the rat. *Br. J. Nutr.* 37: 355–363.
30. ROTHWELL, N. J., STOCK, M. J., WARWICK, B. P. & WINTER, P. D. O'B. (1983) Diurnal variations in circulating hormone levels and brown adipose tissue activity in 'cafeteria'-fed rats. *Comp. Biochem. Physiol. A Comp. Physiol.* 75: 461–465.
31. SCHEMMEL, R., MICKELSON, O. & GILL, J. L. (1970) Dietary obesity in rats: body weight and body fat accretion in seven strains of rats. *J. Nutr.* 100: 1041–1048.
32. MORY, G., BOUILLAUD, G., COMBES-GEORGE, M. & RICQUIER, D. (1984) Noradrenaline controls the concentration of the uncoupling protein in brown adipose tissue. *FEBS Lett.* 166: 393–396.
33. BECKER, W. (1984) Distribution of ^{14}C after oral administration of [1-^{14}C]linoleic acid in rats fed different levels of essential
34. LOCKE, R. M., RIAL, E., SCOTT, I. D. & NICHOLLS, D. G. (1982) Fatty acids as acute regulators of the proton conductance of hamster brown-fat mitochondria. *Eur. J. Biochem.* 129: 373–380.
35. LOCKE, R. M., RIAL, E. & NICHOLLS, D. G. (1982) The acute regulation of mitochondrial proton conductance in cells and mitochondria from the brown fat of cold-adapted and warm-adapted guinea pigs. *Eur. J. Biochem.* 129: 381–387.
36. WAHLE, K. W. J. (1983) Fatty acid modification and membrane lipids. *Proc. Nutr. Soc.* 42: 273–287.
37. HOUSLAY, M. D., HESKETH, T. R., SMITH, G. A., WARREN, G. B. & METCALFE, J. C. (1976) The lipid environment of the glucagon receptor regulates adenylate cyclase activity. *Biochim. Biophys. Acta* 436: 495–504.
38. HOUSLAY, M. D. (1985) Regulation of adenylate cyclase (EC 4.6.1.1.) activity by its lipid environment. *Proc. Nutr. Soc.* 44: 157–165.
39. GINSBURG, B. H., JABOUR, J. & SPECTOR, A. A. (1982) Effect of alterations in membrane lipid unsaturation on the properties of the insulin receptor of Ehrlich ascites cells. *Biochim. Biophys. Acta* 690: 157–164.
40. BEGIN-HEICK, N. & HEICK, H. M. C. (1982) Adenylate cyclase activity in brown adipose tissue of the genetically obese (*ob/ob*) mouse. *Can. J. Biochem.* 60: 910–916.
41. BEGIN-HEICK, N. (1982) Dietary modulation of adenylate cyclase activity in *ob/ob* mouse brown adipose tissue. *Clin. Invest. Med.* 5: 34B (abs.).