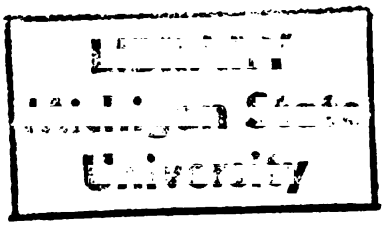

This is to certify that the

dissertation entitled

REGULATION OF ENERGY BALANCE
AND BODY COMPOSITION IN THE
OBESE (OB/OB) MOUSE

presented by

COLLEEN KAY SMITH

has been accepted towards fulfillment
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Major professor

Dale R. Romsos

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Regulation of energy balance and body composition in the obese (ob/ob) mouse.

by

Colleen Kay Smith

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ABSTRACT

REGULATION OF ENERGY BALANCE AND BODY COMPOSITION IN THE OBESE (ob/ob) MOUSE

By

Colleen Kay Smith

The purpose of these studies was to describe the effects of acclimation to mild cold on energy balance and body composition of ob/ob mice, and to determine the importance of the adrenal gland in the development of obesity. Cold-acclimation (4 or 8 weeks housing at 14C) normalized energy intake, body weights and efficiency of energy retention of young obese mice, but hindlimb muscle gain was suppressed to 40% that of lean mice. Plasma corticosterone concentration of control obese mice was 2-4 times that of leans and cold-acclimation resulted in a further increase in corticosterone in obese mice, with no change in leans. To determine the importance of this high corticosterone concentration in the development of obesity, obese and lean mice were adrenalectomized or sham-operated at 3 or 6 weeks of age. Adrenalectomy normalized body weights, energy intake and efficiency of energy retention of obese mice fed stock diet. Adrenalectomy also improved hindlimb muscle gains 50-100% in obese fed stock diet, and plasma insulin concentrations of these obese mice were almost normalized. These effects of adrenalectomy were probably

due to a combination of the direct lack of corticosterone and the restoration of insulin sensitivity to the tissues of obese mice. Previous studies had shown that consumption of semipurified diets by obese animals after adrenalectomy prevented the effects of adrenalectomy. Consistent with this, adrenalectomy of obese mice fed a high-fat diet had no effect on energy balance, muscle gains or plasma insulin concentration. The same results were observed in obese mice fed a semipurified high-carbohydrate (glucose) diet after adrenalectomy. Insulin sensitivity was not normalized by adrenalectomy in obese mice fed either semipurified diet. The restoration of insulin sensitivity appears to be necessary for the effects of adrenalectomy to be expressed in obese mice. Consumption of stock diet allowed insulin sensitivity to be restored, and adrenalectomy prevented the complete development of obesity. When insulin resistance was maintained in adrenalectomized obese mice by their consumption of a semipurified high-carbohydrate or high-fat diet, adrenalectomy did not prevent obesity. Thus, the effects of adrenal secretions on the development of obesity in ob/ob mice appear to be dependent on the insulin status of the animal.

DEDICATION

To my parents

ACKNOWLEDGEMENTS

I would first like to thank Dr. Dale Romsos, without whose patience and guidance this project would not have been possible. I could not have asked for a better advisor. I would also like to thank Dr. Jerry Vander Tuig, whose helpfulness in the lab was a model for me. Finally, I must thank my parents, and Dennis, whose moral (and financial) support was invaluable.

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Chapter 1. General Introduction

Under most conditions, the ob/ob mouse is more efficient at retaining dietary energy in its carcass than its lean littermates. This is not due simply to the increased energy intake of obese mice, since energy efficiency of obese mice pair-fed to leans is still almost 2 1/2 times that of leans (79,89). Thus, the increased efficiency of energy retention in obese mice must be due to reduced energy expenditure. There is considerable evidence indicating that a reduction in thermoregulatory thermogenesis in obese mice plays a major in this reduced energy expenditure (12,78,79,80,82). At thermoneutral environmental temperature (33C), the resting metabolic rate of obese and lean mice is the same (80), although obesity still develops in the ob/ob mice at 33C. Body temperature and oxygen consumption of obese mice declines repidly upon exposure to a cold (3C) environment (12). The average duration of survival of obese at 3C was 2.2 hours, while lean mice survived for more than a week at this temperature (50). This failure to resist cold stress was attributed to a failure to increase thermogenesis rather than to a failure to prevent heat loss; this conclusion was confirmed by succeeding studies (80,82). The increase in oxygen consumption in obese mice after norepinephrine administration was only half that observed in leans, supporting the hypothesis of impaired non-shivering thermogenesis in obese mice (80). Since brown adipose tissue (BAT) is the major organ responsible for thermoregulatory thermogenesis in rodents (24), it seems likely that there is a defect in heat production by BAT of obese mice at environmental temperatures

below thermoneutrality.

The mechanism for heat production by BAT involves the uncoupling of oxidative phosphorylation, generating relatively large quantities of heat without the production of ATP. The sequence of events leading to heat production in BAT begins with a stimulus, such as cold stress, which activates the sympathetic nervous system. BAT is extensively innervated by sympathetic nerves. Norepinephrine, released by the sympathetic nerve endings, binds to B-adrenergic receptors on BAT cells and activates adenyl cyclase, leading to increased cAMP production. The increased cAMP activates a lipase, and accelerates lipolysis in the BAT cells. The free fatty acids which are released are used as fuel for increased mitochondrial oxidation. BAT mitochondria contain a unique proton conductance pathway which makes the inner mitochondrial membrane more permeable to the inward movement of protons. This pathway is regulated by a 32,000 D polypeptide which is on the outer surface of the inner mitochondrial membrane. The polypeptide is inhibited by purine nucleotides; removal of the inhibition allows operation of the proton conductance pathway. Fatty acids released during lipolysis stimulate this proton conductance pathway.

This sequence of events in BAT provides two means of assessing the function of the pathway. Measurement of norepinephrine turnover in BAT provides information about the activity of the sympathetic nervous system in the tissue (41). The ability of the 32,000 D polypeptide to bind purine nucleotides, such as GDP, provides a means of assessing the capacity of BAT for heat production (16). At 25-28C, both norepinephrine turnover and GDP binding to BAT mitochondria

are about 60% lower than in BAT of lean mice (33,41). This confirms the presence of a defect in heat production by BAT. This reduced heat production allows obese mice to expend less energy on thermoregulation and so to be more efficient in retaining consumed energy in their carcasses.

Even though obese mice die relatively rapidly during acute exposure to severe cold, they can survive exposure to less severe cold (14C) (33,41), as well as step-wise acclimation to severe cold (80). Exposure to 14C for several weeks increased both norepinephrine turnover and GDP binding in BAT of obese mice 2 - 2 1/2 times as much in obese mice as in leans, though neither norepinephrine turnover nor GDP binding reached the same level in obese mice as in leans (33,41). The effect of this mild cold exposure on overall energy balance is not known. It seems likely that since BAT of obese mice was stimulated more by cold exposure than that of lean mice, efficiency of energy retention in the obese would be reduced to be more like that of lean mice. However, calculation of efficiency of energy retention from data in the one report available on energy efficiency of obese mice housed at 17C or 23C for 10 days showed that the relative differences in energy efficiency between obese and lean mice were even greater at 17C than at 23C (79). This was a relatively short term exposure to a slightly higher temperature compared to the length of time and temperature used in the studies on BAT, so it is possible that thermogenic mechanisms were not fully activated in the mice in this 10 day study. Studies on energy balance after longer term cold exposure could help to clarify this.

Not only is energy retained with a higher efficiency in obese mice than in leans, it is also partitioned quite differently in the two phenotypes. This results in a markedly different body composition in obese mice as compared to leans. At 7-8 weeks of age, ad libitum fed obese mice weigh up to 1 1/2 times as much as their lean littermates (83). This difference in body weights gradually increases, so that by 37 weeks of age, obese mice weigh twice as much as leans. The percentage body fat in obese mice is almost twice that of leans at 7 weeks of age, and increases to almost 4 times that of leans by 37 weeks (83). The fat-free carcass of these obese mice averages only 17% of their body weight, compared to 32% for lean mice (83). Fat-free hindlimbs, representative of skeletal muscle, weigh approximately 20% less in ad libitum fed obese mice than in leans between 7 and 37 weeks of age (83). As with the elevated efficiency of energy retention, this abnormal body composition is not simply a result of excess energy intake by obese mice (8,17,35,79,86). Obese mice pair-fed to leans still gain more body weight and body energy than lean mice, but less carcass protein (8,79). The percentage body fat of obese mice pair-gained to lean mice was still 3 1/2 times that of leans, and their percentage lean body mass was only half that of the lean mice (17). These data indicate that obese mice have a higher percentage of body fat and a smaller muscle mass than lean mice, even when their food intake and body weight equals that of lean mice.

Skeletal muscle growth may be linked to bone growth (36), so it seems likely that if muscle growth is reduced in obese mice, bone growth may also be reduced. No data are available to confirm this,

although there is one report in which tail length (an indicator of linear body growth) of obese mice was significantly less than that of leans (45). Further evidence supporting this link between skeletal muscle and bone growth comes from studies in the Zucker rat. In 15 week old obese rats, skeletal muscle weighed 10% less, and the tibia and femur were 10-15% shorter than in their lean counterparts (60). If the bones of obese mice are shorter than those of leans, they could exert less of a trophic effect on muscle and so contribute to the reduced muscle mass of obese mice.

Environmental temperature may influence the body composition of obese mice as well as their efficiency of energy retention. At 20 - 25C, hindlimb muscle of obese mice weighs about 20% less than that of leans; there is a preliminary observation that hindlimb muscle of obese mice housed at 14C weighs less than half as much as muscle of lean mice, suggesting that cold-acclimation may further reduce muscle growth in obese mice (46). The effects of long term cold-acclimation on body composition of obese mice have not been described, however.

Several hormonal imbalances are present in obese mice which may contribute to their abnormal energy retention and body composition. The plasma insulin concentration of obese mice is 3-5 times that of lean mice from 6-60 weeks of age (26,29). Even when obese mice were pair-gained to leans, plasma insulin of the obese mice was 2-3 times that of leans from 5-17 weeks of age (17). However, plasma glucose of obese mice is 10-40% higher than that of leans during most of this time (26), indicating impaired sensitivity to insulin in obese mice. Insulin has an important role in the regulation of both energy balance and body composition. Several studies have shown a

requirement for insulin in the normal functioning of brown adipose tissue. Both GDP binding and the content of the 32,000 D protein were reduced in diabetic rats compared to normo-insulinemic controls, and conversely, were increased in hyperinsulinemic rats compared to controls (71). Interscapular BAT of streptozotocin-diabetic rats was atrophied compared to controls, and basal and norepinephrine-stimulated heat production were both reduced in diabetic rats (70). Severely diabetic rats could not sustain the increased metabolic rate needed for survival in a cold environment (58). However, moderately diabetic rats retained sufficient insulin activity to survive at cold temperatures for up to 3 months (51). The hypertrophy of BAT which occurred when rats were fed a cafeteria diet was not observed in lactating rats fed the cafeteria diet (1). Plasma insulin was low in these lactating rats; insulin administration enabled the hypertrophy of their BAT stimulated by the cafeteria diet. Rothwell and Stock also found a requirement for insulin in thermogenesis induced by diet (63). These studies indicate a requirement for insulin in both non-shivering and diet-induced thermogenesis; the primary site of both of these processes is brown adipose tissue (24,62). The presence of insulin resistance in obese mice could prevent these actions of insulin on the BAT, resulting in their reduced energy expenditure and high efficiency of energy retention.

The anabolic effects of insulin on skeletal muscle are blunted due to the development of insulin resistance in muscle. From 6 weeks of age on, insulin binding by skeletal muscle of obese mice is lower than in lean mice (29). Defects in glucose transport and utilization

occur even earlier in obese mice - by 4 weeks of age - indicating that these intracellular defects may have a more important role in the development of insulin resistance in muscle than the defects in binding, which occur when the resistance is more fully expressed (48). As mentioned earlier, plasma insulin of 3 week old obese mice is already considerably higher than that of leans, and blood glucose is also elevated in the obese mice, indicating that the insulin resistance may already be present in these 3 week old mice (18). Muscle weights of obese mice are already significantly lower than that of lean mice by 5 weeks of age and remain lower than leans through at least 37 weeks of age (83). The development of insulin resistance in the skeletal muscle of obese mice is thus consistent with their reduced muscle mass.

Corticosterone is another hormone which probably has an important role in the abnormal energy balance and body composition of obese mice. Corticosterone is the primary glucocorticoid in rodents (87) and plasma corticosterone concentrations in obese mice range from 2-5 times that of lean mice (19,26,31). Functional receptors for glucocorticoids have been identified in BAT, indicating that this thermogenic tissue is a glucocorticoid target organ (21). Small amounts of glucocorticoids (below physiological concentrations) are necessary for the increase in fatty acid mobilization and thermogenesis potentiated by cold and norepinephrine (13). However, this is probably a permissive role of glucocorticoids, since chronic administration of corticosterone to intact mice, resulting in elevated plasma concentrations of the hormone, reduces GDP binding in BAT, indicating a reduction in the capacity for heat production in

these mice (25). Body weight gain, food intake and metabolic efficiency were all higher in the corticosterone treated mice compared to untreated mice. None of these studies could clarify whether the effects of glucocorticoids on energy balance were a direct effect of the hormone on BAT or an indirect effect, working via the sympathetic nervous system. It does seem clear, however, that glucocorticoids suppress BAT activity and reduce energy expenditure. This is consistent with the findings of elevated plasma corticosterone, reduced energy expenditure and increased efficiency of energy retention in ob/ob mice compared to their lean counterparts.

The high concentration of plasma corticosterone in obese mice probably also contributes to their abnormal body composition. High doses of glucocorticoids reduce rates of muscle protein synthesis (60,61) and increase rates of muscle protein breakdown (66). Muscle protein breakdown has been shown to be higher in obese mice than in leans (83). When rats were given doses of corticosterone which tripled their normal plasma corticosterone concentration for 8 days, there was a 20% reduction in the weight of their gastrocnemius muscle when expressed as a percentage of their body weight (67). Thus, the effects of corticosterone on muscle protein synthesis and breakdown appear to have a net effect in decreasing muscle weight. High concentrations of corticosterone also promote insulin resistance through decreases in insulin binding (38,55,57). This provides an indirect mechanism by which high concentrations of corticosterone can suppress muscle growth.

The key role of adrenal secretions in the abnormal energy

balance and body composition of obese rodents can be demonstrated by adrenalectomizing these animals and following their development. Adrenalectomy reduced the food intake of several obese animals, including the Zucker obese rat, the VMH lesioned rat, the GTG-induced obese mouse and the ob/ob mouse (7,14,49,65), so that it was equal to or less than that of their lean counterparts. Adrenalectomy of obese Zucker rats also reduced their efficiency of energy retention by 50% so that it equaled that of leans (49). This reduction in efficiency could not have been due to the reduced food intake alone - pair-feeding sham-operated obese rats to leans did not result in a reduction in efficiency of energy retention (6). Thus, the decrease in efficiency of energy retention in adrenalectomized obese rats must have been due to increased energy expenditure. Consistent with this, GDP binding to mitochondria of BAT of adrenalectomized obese rats was increased more than 4 fold compared to sham-operated obese, indicating an increased capacity for heat production in BAT of adrenalectomized obese (49). Adrenalectomy also increased norepinephrine turnover of BAT of ob/ob mice almost 3 fold, so that it almost equaled that of lean mice (84). The effect of adrenalectomy on overall energy balance of obese mice is not known, however. The specific absence of corticosterone appears to be responsible for these effects of adrenalectomy. Administration of corticosterone, but not desoxycorticosterone, (a mineralocorticoid), prevented the effects of adrenalectomy on body weight gain and food intake in GTG-induced obese mice (14). Corticosterone administration also reversed the increase in GDP binding in adrenalectomized obese rats (34). Thus, adrenal secretions, and specifically

corticosterone, play an important role in controlling energy balance in obese rodents.

Adrenalectomy of obese rodents also reduces their body weight so that it is equal to or only slightly greater than that of lean mice (7,14,49,65). Body composition of obese rodents must also be altered by adrenalectomy, because body energy density of adrenalectomized obese rats and ob/ob mice was 25-30% less than that of sham-operated obese, indicating a lower proportion of body fat in the adrenalectomized obese (49,84). The excess fat accumulation seen in GTG-induced obese mice was prevented by adrenalectomy (14), and in ob/ob mice, adrenalectomy reduced the weight of white adipose tissue by over 50% (65). Muscle growth of obese animals may also be improved by adrenalectomy, since the gastrocnemius muscle of adrenalectomized ob/ob mice weighed 20% more than that of sham-operated obese (65). This increase in muscle mass was probably facilitated by the return of insulin sensitivity to skeletal muscle of adrenalectomized ob/ob mice (56). As with the effects of adrenalectomy on energy balance, these effects of adrenalectomy on body composition also seem to be due to the absence of corticosterone. Administration of cortisone to adrenalectomized obese mice prevented the reduction in growth rate and adipose tissue weight as well as the increase in tail length and muscle weight observed in untreated adrenalectomized obese mice (65).

The composition of the diet consumed by obese animals may also affect energy balance and body composition. Obese mice fed a high-fat diet consumed more energy and gained more body weight than those fed a high-carbohydrate diet (20). Lean mice fed a high-fat

diet from 3-6 weeks of age gained only 30% more energy than leans fed a high-carbohydrate stock diet, but obese mice fed the high-fat diet gained more than twice as much energy as high-carbohydrate fed obese (45). All of this excess body energy gain was made up of fat energy with no change in the gain in lean energy. This indicates a substantial shift in the body composition of obese mice fed high-fat diet to an even higher proportion of fat than is found in obese mice fed high-carbohydrate diet. These effects of the high-fat diet on body composition may have been mediated by insulin - in rats fed high-fat diets, muscle and adipose tissue were resistant to the actions of insulin (43,75,91). The diet consumed by animals may also affect the outcome of adrenalectomy. When VMH-lesioned rats were ovariectomized and adrenalectomized and then fed a pelleted stock diet, thay did not demonstrate the hyperghagia and excess body weight gain normally observed in VMH-lesioned control rats (52). However, when ovariectomized-adrenalectomized rats were fed a liquid high-carbohydrate or semi-purified high-fat diet, they consumed as much food and gained almost as much body weight as VMH-lesioned controls. The effect of diet on the outcome of adrenalectomy in ob/ob mice is not known, since the studies reported so far have all utilized a pelleted stock diet.

Thus, the overall aim of this research was to describe the effect of acclimation to mild cold on energy balance and body composition, and to determine the importance of the adrenal gland in the development of obesity. A further aim was to determine a possible mechanism by which the changes in energy balance and body composition caused by cold-acclimation and adrenalectomy occur. The

following objectives were established to meet these aims:

1. To provide further data on the effect of mild cold-acclimation on energy balance, body composition and plasma corticosterone and insulin concentrations.

2. To determine the effect of adrenalectomy on the development of the obesity syndrome in ob/ob mice.

3. To examine the impact of diet on the outcome of adrenalectomy.

4. To examine the interaction of adrenalectomy and diet composition as they affect insulin responsiveness.

Chapter 2. Cold-acclimation of obese (ob/ob) mice: Effects on energy balance.

Obesity in the genetically obese (ob/ob) mouse is only partially a result of hyperphagia, since pair feeding these animals with their lean counterparts does not prevent the development of obesity (8,44). Thus, these obese mice must utilize dietary energy more efficiently than their lean littermates.

Reduced energy expenditure for thermoregulatory thermogenesis may partially explain this increased energy efficiency (body energy gain/energy intake) of obese mice (80). When housed at a temperature near thermoneutrality (33C), body temperature is the same in lean and obese mice and obese mice are less than 1 1/2 times as efficient as leans (46,85). However, when obese mice are housed at temperatures approximating those of many vivariums (20 - 25C) their body temperature is 1 1/2 - 2 1/2C lower than that of lean mice and they are 2 1/2 times as efficient at retaining dietary energy than lean mice (39,44,80).

These differences in body temperature and energy efficiency of obese mice at the different environmental temperatures may be caused by low heat production by brown adipose tissue (BAT) of obese mice at 23C (33,80). BAT is the major organ responsible for thermoregulatory heat production in rodents (24). The observation that obese mice die of hypothermia within only a few hours after abrupt exposure to 4C, probably because they are unable to sufficiently increase heat production in their BAT, supports this hypothesis (50).

Obese mice can, however, survive exposure to less severe cold (14C), as well as stepwise acclimation to a lower temperature (4C) (2,33,41). Although body temperature is still lower in obese mice than in leans after 10-20 days at 14C, BAT has been shown to be activated in these mice (33,41,46). At 20-25C, both norepinephrine turnover, an estimator of sympathetic nervous system stimulation of BAT, and GDP binding, an estimator of the capacity for heat production, are about 60% lower in BAT of obese mice than in lean mice (33,41). Exposure to 14C for several weeks increased both parameters 2 - 2 1/2 times as much in obese mice as in lean mice, though neither quite reached the same level in the obese mice as in leans (33,41). Exposure to 4C for 4 weeks increased GDP binding in obese mice by almost 3 times compared to values for obese mice at 23C, resulting in no difference in GDP binding between obese and lean mice at 4C (2).

Based on these observations, one might expect that differences in energy efficiency between obese and lean mice housed at 14C would be less than those observed at 20 - 25C, due to the greater activation of BAT in obese mice housed at 14C rather than at 20 - 25C relative to the responses of lean mice housed at the same two temperatures. But calculation of efficiency of energy retention from data in the one report on energy efficiency of obese mice at temperatures below normal animal room temperature showed a greater relative decrease in energy efficiency in lean mice (from 13.0% at 23C to 8.8% at 17C) than in obese mice (from 29.6% at 23C to 24.9% at 17C) after 10 days at 17C (79). Thus, the relative differences in energy efficiency between obese and lean mice were even greater at 17C than at 23C. At

17C, obese mice were 2.8 times as efficient as lean mice, whereas at 23C, obese mice were only 2.2 times as efficient as lean mice (79). It is possible, however, that thermogenic mechanisms were not sufficiently activated in obese mice during this short term study (10 days) to produce the lower efficiency of energy retention predicted from results of longer-term studies on BAT metabolism in cold-acclimated obese mice (2,33,41),

The present study was conducted to assess effects of mild cold (14C) exposure for 4 - 8 weeks on energy balance in young and near-adult obese (ob/ob) and lean mice. In addition, after 4 weeks of cold exposure, mice were returned to 23C to assess changes in energy balance during a reacclimation period of 4 weeks.

Materials and Methods

Animals

Obese (ob/ob) and lean (ob/+ or +/+) male mice were obtained from our breeding colony of C57BL/6J-ob/+ mice. The breeding colony was housed at 23C and the mother was removed from the pups at 3 weeks of age. At 3 1/2 weeks of age, obese and lean pairs were separated from their littermates and were housed individually in solid-bottom, plastic cages with wood shavings as bedding. At 4 weeks of age, mice were assigned to one of two environmental temperatures (23C or 14C). During the first week of exposure to 14C, paper was placed in each cage as extra nesting material. All mice had food (Wayne Lab-Blox, Allied Mills, Chicago, IL) and water available ad libitum. Food intake and body weights were recorded weekly. Room lights were on from 0700- 1900 hours daily.

Experimental Design

Experiment 1 was designed to determine effects of chronic cold exposure on body weight and energy balance of young obese and lean mice. Both obese and lean mice at 4 weeks of age were divided into 5 groups of 9-10 mice each. Group 1 was killed at 4 weeks of age to serve as an initial group. Groups 2 and 3 were housed at 23C for 4 and 8 weeks, respectively, and groups 4 and 5 were housed at 14C for 4 and 8 weeks, respectively. In addition, one group of obese mice housed at 23C for 4 weeks was pair-fed to lean mice.

The purpose of Experiment 2 was to determine effects of chronic cold exposure on near adult obese and lean mice, as well as to determine responses of mice to reacclimation to 23C after 4 weeks of cold exposure. Because Experiments 1 and 2 were conducted concurrently, mice housed at 23C from 4-12 weeks of age in Experiment 1 (group 3) served as controls in Experiment 2 (Figure 1). The cold-acclimated group was housed at 23C from 4 through 8 weeks of age, and then at 14C from 9 through 12 weeks of age. The reacclimated group was housed at 14C from 4 through 8 weeks of age and then at 23C from 9 through 12 weeks of age.

Analyses

At the end of each experimental period, mice were killed by cervical dislocation and the intact carcasses were frozen until analysis. Body energy was measured by direct calorimetry. Carcasses were first softened by heating in an autoclave at 100C, then homogenized (Brinkmann Polytron, Brinkmann Instruments, Westbury, NY) in 2-3 times their weight of water. An aliquot of homogenate was dried at 50C and used for determination of energy with an adiabatic

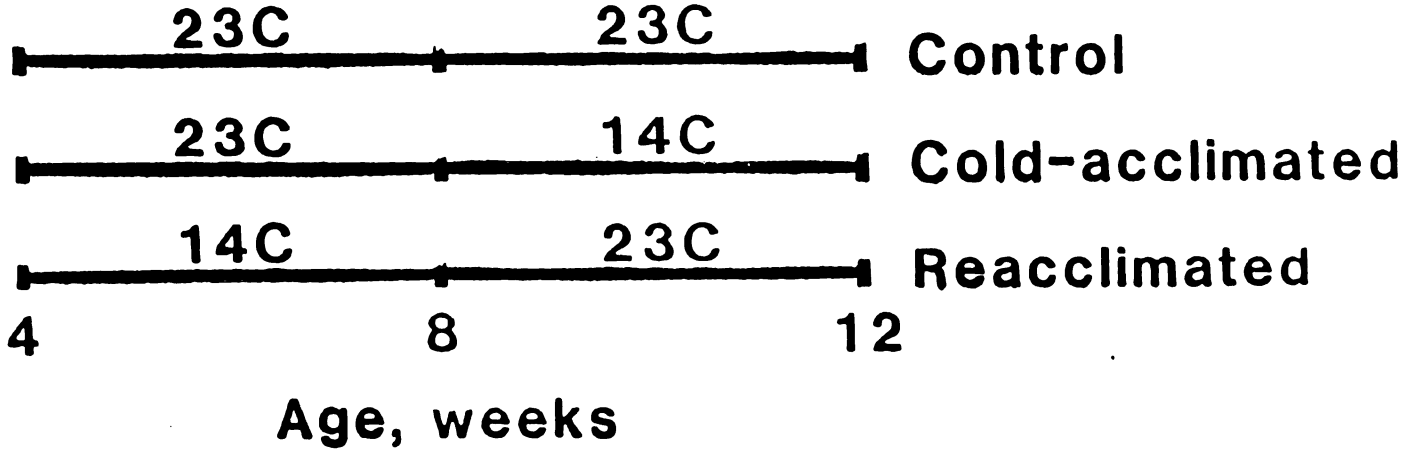

Figure 1. Design of Experiment 2. Numbers above horizontal lines indicate the temperature at which the mice were housed during the time period. Group names are at the end of each line.

calorimeter (Parr Instrument Co., Moline, IL). The diet was also analyzed for energy content and was found to contain 3.81 kcal/g.

Energy efficiency was calculated as body energy gained divided by energy consumed during a 4 week period. Body energy gain was calculated as body energy content at the end of the experimental period minus the predicted body energy content at the beginning of the experiment. Body energy at the beginning of each experimental period was predicted from a linear regression equation based on body weights and body energy contents of appropriate groups (ie,groups 1,2 and 4) from Experiment 1. Body weights of mice at the beginning of each experimental period were then used to predict initial body energy for each mouse. Heat production was calculated as the difference between estimated metabolizable energy intake and body energy gain. Metabolizable energy intake was calculated as gross energy intake minus fecal energy with a correction for the energy associated with urinary nitrogen and was found to be 74% of gross energy intake.

Data are presented as means + SEM. Data were analyzed by analysis of variance. Treatment differences were determined by Duncan's multiple-range test ($P < 0.05$) (74).

Results

Experiment 1

When housed at 23C, obese mice consumed 30% more energy per day than leans, as expected (Table 1). Lean mice at 14C consumed 50% more than leans at 23C, but cold acclimated obese mice consumed only 8% more energy than obese controls. As a result, energy intakes of obese and lean mice housed at 14C were the same.

Table 1. Energy Intake of Mice from 4-12 Weeks of Age

	Environmental Temperature	
	23C	14C
	(kcal/day)	
Lean	15.5 ± 0.3	23.7 ± 0.3+
Obese	21.2 ± 0.5*	22.9 ± 0.7+

Mean ± SEM (n=9-11)

*Significant (P< .05) difference between lean and obese at the same temperature.

+Significantly (P< .05) different from the 23C group within phenotype.

At 23C, obese mice weighed more than leans at both 8 and 12 weeks of age (Figure 2A). Obese mice at 14C gained approximately 60% less weight than obese mice at 23C. Weight gain of lean mice at 14C, however, was depressed only slightly. As a result, obese mice at 14C weighed the same as leans.

Obese mice at 23C gained over 200 kcal energy between 4 and 12 weeks of age (Figure 2B). Lean mice gained approximately 1/4 this much energy during this period. Energy gain of obese mice was markedly depressed by cold; they gained less than 100 kcal between 4 and 12 weeks of age. Thus the carcasses of obese mice housed at 14C for 8 weeks contained less than 1/2 as much energy as obese mice at 23C. Body energy in lean mice was depressed only after 8 weeks of cold exposure (Figure 2B).

From 4-8 weeks of age, obese mice housed at 23C retained 22.0 + 0.4% of the gross energy consumed; they were 3 1/2 times as efficient at retaining body energy as leans (Figure 3). Obese mice housed at 23C from 4-8 weeks of age and pair-fed to lean mice retained 19.8 + 0.7% of gross energy consumed. Energy efficiency of both obese and leans declined significantly with age, though efficiency of leans decreased more. Consequently, between 9 and 12 weeks of age, obese mice at 23C were 7 times as efficient as leans (Figure 3).

Cold exposure, as expected, led to a decrease in energy efficiency. Energy efficiency of obese mice, however, was depressed more than that of leans so that between 4 and 8 weeks of age, obese mice at 14C were only twice as efficient as leans (Figure 3). Between 9 and 12 weeks of age, differences between obese and lean mice at 14C were abolished; neither group retained a significant

Figure 2

Body weights and body energy of mice from 4-12 weeks of age. Environmental temperature noted by numbers on the right of each data line. Each point is the mean + SEM of 9-11 mice. += Significant (P<Ø.Ø5) difference due to temperature within phenotype. Obese mice weighed significantly more than leans at 8 and 12 weeks of age at 23C. Body energy was significantly higher in obese mice than in leans at all ages and both temperatures.

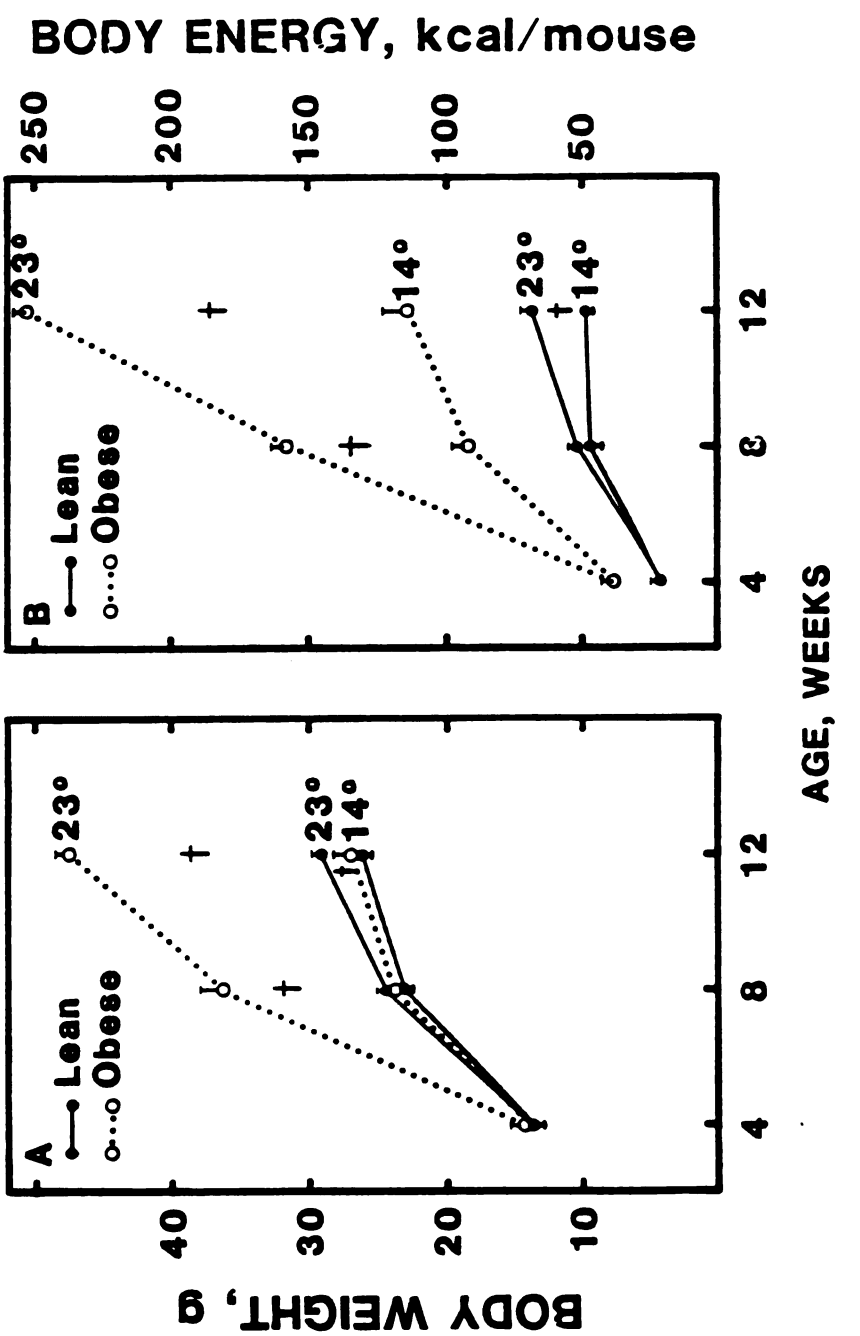

Figure 2. Body weights and body energy of mice from 4-12 weeks of age.

Figure 3

Energy efficiency of mice from 4-12 weeks of age. Each bar is the mean + SEM of 9-11 mice. *= Significant (P<Ø.Ø5) difference between lean and obese at the same temperature and age. += Significant (P>Ø.Ø5) difference due to temperature within age group and phenotype.

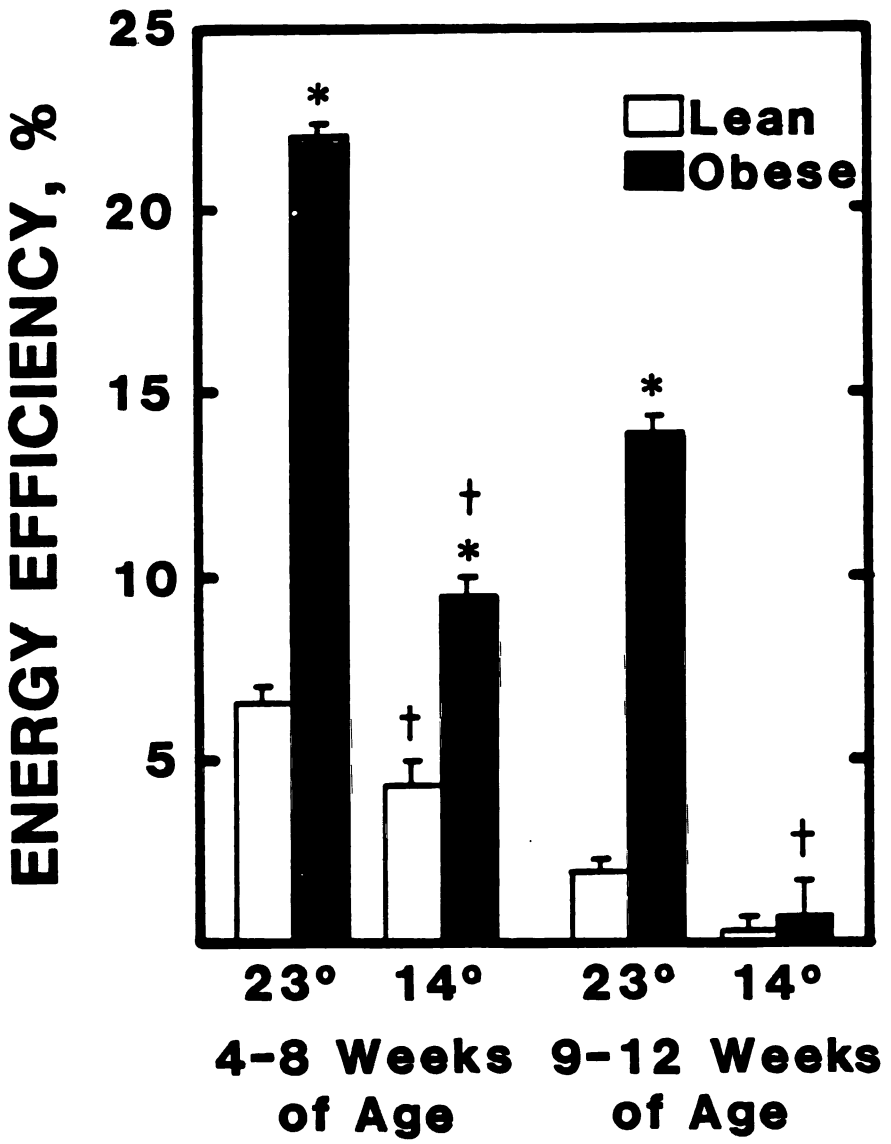

Figure 3. Energy efficiency of mice from 4 - 12 weeks of age.

amount of the energy consumed. This represents, to our knowledge, the first demonstration of an equalized efficiency of energy retention in intact young adult obese and lean mice fed ad libitum.

When housed at 23C from 4-8 weeks of age, calculated heat production was 267 and 294 kcal/mouse in obese and lean mice, respectively. In obese and lean mice housed at 14C from 4-8 weeks of age, heat production was 40% higher (364 and 422 kcal/mouse, respectively).

Experiment 2

When switched from 23C to 14C at 9 weeks of age, both obese and lean mice increased their energy intake by approximately 50% (Table 2). Weight gain of these mice was reduced to approximately 1/3 that of respective controls (Figure 4A and 4B). Leans in this group did not gain body energy, whereas obese mice gained 32 kcal, which is approximately 1/3 of the amount of energy gained by obese controls (Figure 4C and 4D). Both obese and lean mice retained energy with a lower efficiency than did their counterparts maintained at 23C, but obese mice still maintained a higher efficiency than leans (Figure 5).

When obese mice that had been housed at 14C for 4 weeks were reacclimated to 23C, their energy intake decreased to equal that of their obese control counterparts maintained at 23C throughout the experiment (Table 2). Energy intake of lean mice during this period of reacclimation to 23C remained 12% higher than lean controls. Both obese and lean mice gained body weight and body energy at approximately the same rate as controls (Figure 4A and 4B).

Figure 4

Body weights and body energy of mice from 9-12 weeks of age. Mice were housed at 23C through 12 weeks of age, or at 23C through 8 weeks of age and at 14C from 9-12 weeks of age (23C to 14C) or at 14C from 4-8 weeks of age and at 23 C from 9-12 weeks of age (14C to 23C). Each point is the mean + SEM of 6-11 mice. *= Significantly ($P<0.05$) different from 23C group within phenotype.

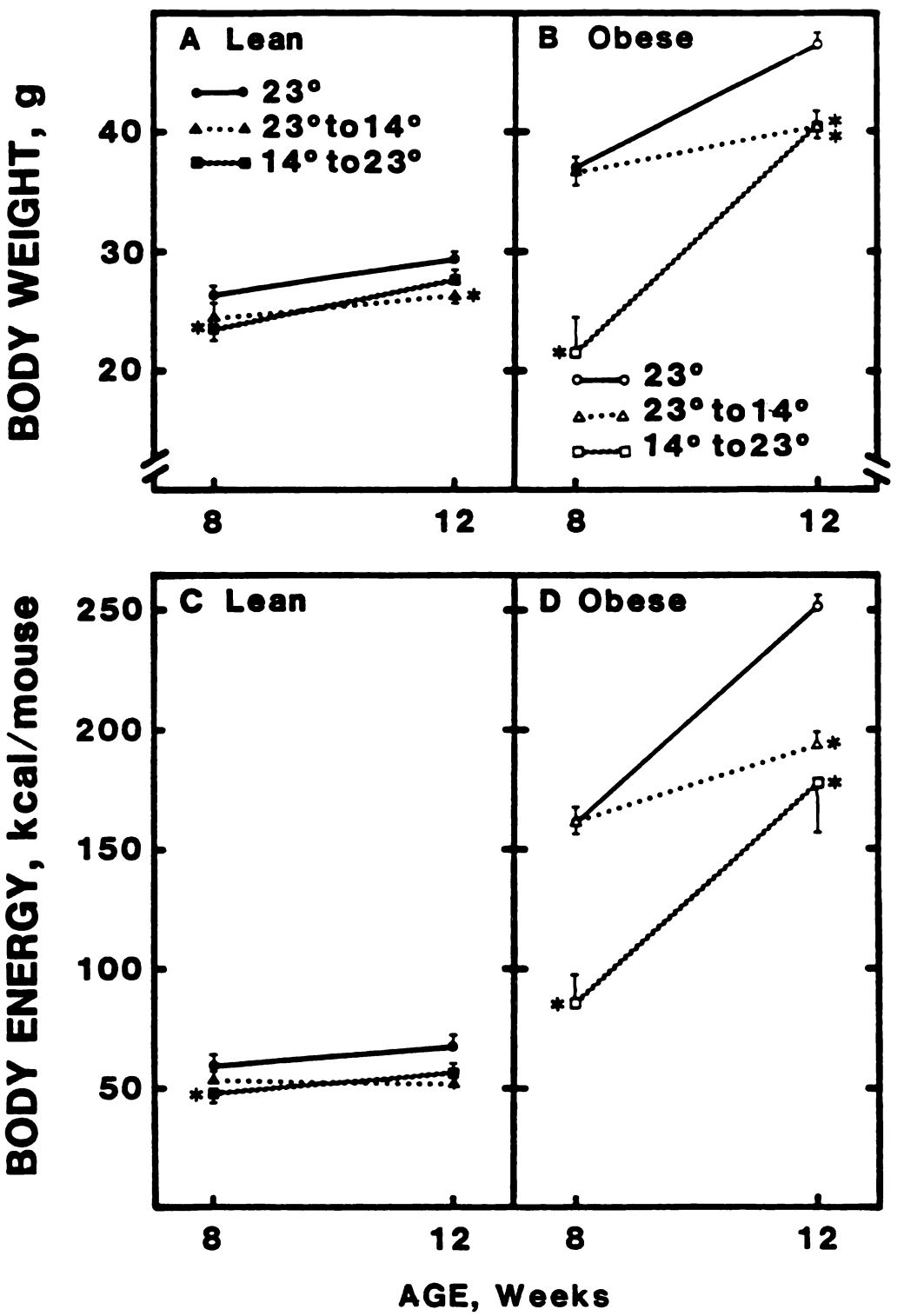

Figure 4. Body weights and body energy of mice from 9-12 weeks of age.

Table 2. Energy Intake of Mice from 9-12 Weeks of Age

	Environmental Temperature		
	23C	23C to 14C	14C to 23C
		(kcal/day)	
Lean	15.5 ± 0.2	23.6 ± 0.4^{+}	17.4 ± 0.4^{+}
Obese	21.8 ± 0.8*	31.0 ± 0.5^{*+}	22.4 ± 0.6*

Mean ± SEM (n=9-11)

*Significant (P <.05) difference between lean and obese in the same treatment group.

$^{+}$Significantly (P <.05) different from the 23C group within phenotype.

Figure 5

Energy efficiency of mice from 9-12 weeks of age. Each bar is the mean + SEM of 6-11 mice. Mice were housed at 23C through 12 weeks of age (23), or at 23C through 8 weeks of age and at 14C from 9-12 weeks of age (23C to 14C), or at 14C from 4-8 weeks of age and at 23C from 9-12 weeks of age (14C to 23C). *= Significant (P<Ø.Ø5) difference between lean and obese at the same temperature. += Significantly (P<Ø.Ø5) different from 23C group within phenotype.

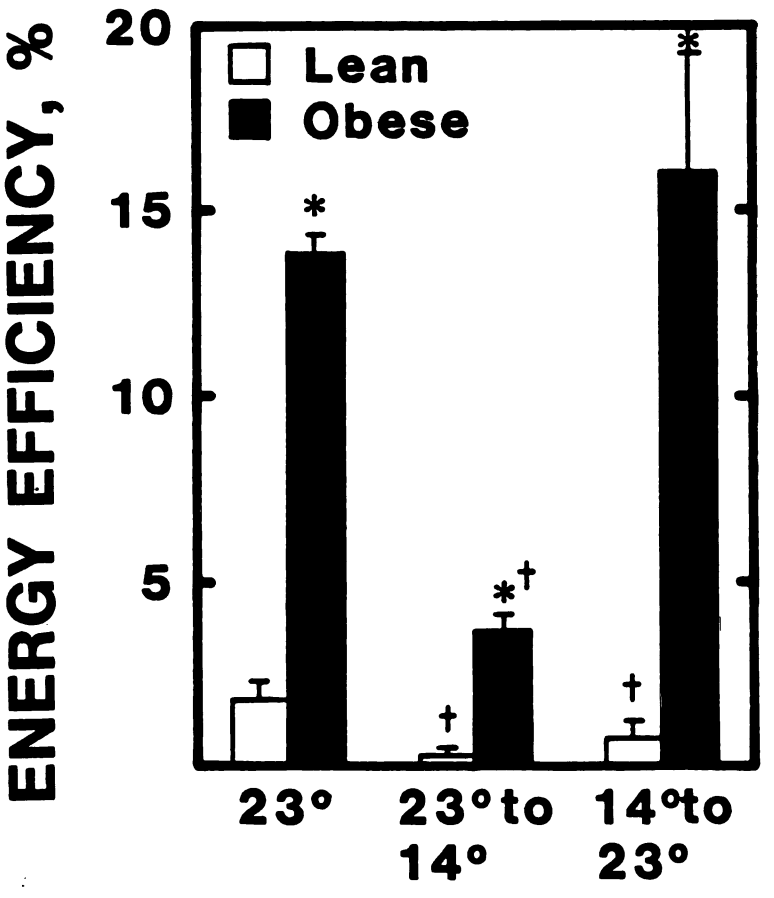

Figure 5. Energy efficiency of mice from 9 - 12 weeks of age.

Efficiency of energy retention in reacclimated obese mice was the same as that of obese mice maintained at 23C throughout the experiment (Figure 5), but efficiency of energy retention in reacclimated lean mice was only 1/2 that of lean controls.

Discussion

These results confirm those of previous studies where both obese (ob/ob) and lean mice were shown to survive prolonged exposure to mild cold (14 - 17C) (2,33,41,46,80). Lean mice increased their energy intake to compensate for increased energy needs at 14C, so that even though energy efficiency decreased in these mice, weight gain was maintained. Young obese mice, on the other hand, increased energy intake only slightly when housed at 14C and gained less than half as much weight as obese controls. Although energy intake and body weights of lean and obese mice were the same after 8 weeks of cold exposure, body energy content of obese mice was still 2.3 times that of leans. The cold-acclimated obese mice must therefore have retained more fat than their lean counterparts. This was confirmed in a companion study - although body fat content was reduced in cold acclimated obese mice, the percentage body fat was maintained close to that of obese controls (Chapter 3). Muscle weights were lower in obese control mice than in lean controls; cold acclimation led to an even further reduction in muscle weights in obese mice, but did not influence muscle weights in lean mice.

Efficiency of energy retention of obese mice was lowered more by cold acclimation than that of leans (Figures 3 and 5). Thus, after 4 weeks at 14C, the difference in efficiency between obese and lean mice was less than that at 23C (whether obese mice at 23C were fed ad

libitium or were pair-fed to lean mice) and after 8 weeks at 14C, there was no difference in energy efficiency between phenotypes (Figure 3). In contrast, Thurlby and Trayhurn found that the difference in energy gain between pair-fed obese and lean mice, a reflection of energy efficiency, was greater in mice housed at 17C than in those housed at 23C (79). They suggested that at the lower temperature (17C), obese mice utilized less energy for thermoregulation than did lean mice. This would allow obese mice to be even more efficient in retaining energy that lean mice at the lower temperature. Because their study lasted only 10 days, it is likely that adaptive thermoregulatory processes in BAT were not fully activated in their obese mice.

Calculation of heat production in our study confirmed that both young obese and lean mice increased heat production by approximately 40% when chronically exposed to mild cold. Part of this increase in heat production in lean mice would have been caused by increased energy expenditure associated with digestion, absorption and metabolism of the extra food consumed and part could have been attributed to increased non-shivering thermogenesis, probably originating in BAT (24). Because energy intake was only slightly higher in young cold-exposed obese mice than in obese controls, their increase in non-shivering thermogenesis must have been greater than that observed in lean mice. As discussed in the Introduction, results from several previous studies support this suggestion of a greater responsiveness of heat production by BAT of obese mice to cold acclimation (2,33,42).

There is one other treatment which normalizes efficiency of energy

retention in obese rodents. Adrenalectomy of obese Zucker rats reduced their energy efficiency by half so that it equaled that of lean rats (50). The reduction in energy efficiency was most likely due to increased brown adipose activity; mitochondrial protein content and GDP binding were both increased in the adrenalectomized obese rats, indicating an increased capacity for heat production. Adrenalectomy also increased sympathetic nervous system stimulation of brown adipose tissue of ob/ob mice - norepinephrine turnover in brown adipose of adrenalectomized obese mice was more than 1 1/2 times that of sham-operated obese, almost equal to that of leans (84). It seems likely that this increased brown adipose activity in adrenalectomized obese mice would result in reduced efficiency of energy retention, but overall measures of energy balance in obese mice after adrenalectomy have not been reported. Adrenal secretions thus appear to have an important role in the regulation of brown adipose tissue activity in obese rodents. High concentrations of glucocorticoids (as are found in ob/ob mice) have been shown to suppress brown adipose activity (25); adrenalectomy would remove this inhibition, allowing greater energy expenditure and a lower efficiency of energy retention. It is not clear whether this effect of adrenal secretions on brown adipose is a direct effect on the tissue itself, or an indirect effect, mediated by another agent which is influenced by the adrenal secretions.

Efficiency of energy retention in reacclimated obese mice increased from 10% after 4 weeks at 14C (Figure 3) up to 16% after 4 weeks of reacclimation (Figure 5). However, reacclimated obese mice remained hyperphagic relative to lean mice. In normal mice this

might be expected to activate diet-induced thermogenesis (62). Rothwell and Stock have suggested that diet-induced thermogenesis has the same metabolic origin as non-shivering thermogenesis (BAT) and that, given a change in environmental conditions, heat production in BAT induced by cold can "switch over" to heat production induced by overeating, or vice versa (62). According to this hypothesis it might have been expected that the increased heat production in BAT of obese mice induced by cold acclimation would have "switched over" to heat production induced by overeating during reacclimation to 23C, resulting in a continued low efficiency of energy retention. This did not occur; energy efficiency of reacclimated obese mice returned to the same level as in control obese mice rather than remaining at the lower level found during cold acclimation. Therefore, the signal mechanism to switch on diet-induced thermogenesis in obese mice may be defective. This suggestion is supported by findings of Trayhurn et al. that obese mice induced to overeat by being offered a varied, palatable diet (cafeteria feeding) demonstrated less diet-induced thermogenesis than cafeteria-fed lean mice (80). Efficiency of energy retention in reacclimating lean mice in our study was even lower than that of the lean mice after 4 weeks of cold exposure (Figure 3 and 5). This decrease in efficiency during reacclimation agrees with a recent report in which the lower efficiency of cold-adapted (5C) male rats persisted for almost 3 months after being returned to 25C (11). It is also consistent with Rothwell and Stock's hypothesis that heat production in BAT induced by cold acclimation may "switch over" to heat production induced by overeating during reacclimation (62).

In conclusion, although obese mice are relatively unresponsive to a change in environmental temperature from 33C to 25C (46,85), they can activate thermoregulatory processes to almost the same level seen in lean mice when a greater stimulus is provided by decreasing the temperature to 14C. This eventually leads to equalized energy efficiency between obese and lean mice, as well as to equalization of body weight, though not of body composition (Chapter 3). Restoration of energy efficiency to the same level as in control obese mice occurred within 4 weeks in obese mice reacclimated to 23C, indicating that hyperphagia in obese mice was an insufficient stimulus to maintain the elevated rates of heat production attained during previous cold-acclimation.

Chapter 3. Cold-acclimation of obese (ob/ob) mice: Effects on skeletal muscle and bone.

Young adult obese (ob/ob) mice weigh approximately twice as much as lean mice but their fat-free carcass and hindlimbs (indices of skeletal muscle growth) weigh 20 - 40% less than those of lean mice (3,74). When obese mice were housed at 14C for 8 weeks, their body weight gain was reduced so that their body weight at the end of this period was equal to that of lean mice (Chapter 2). However, body energy content of these cold-acclimated obese mice was more than twice that of the leans, indicating that their body composition was still different than that of lean mice, despite their equal body weights. A preliminary observation that hindlimb muscle weight of obese mice housed at 14C was less than half that of lean mice housed at this temperature lends support to this hypothesis (46).

The present study was conducted to quantitate changes in growth of skeletal muscle and bone in obese (ob/ob) mice which occur during acclimation to mild cold (14C). In another genetically obese rodent (obese Zucker rats) both skeletal muscle weights and bone lengths are reduced compared to lean counterparts (69). Body fat was quantitated to determine the extent to which obese mice maintain their high percentage body fat when weight gain is reduced by cold acclimation (Chapter 2). Because the concentration of corticosterone in plasma of obese mice is 2-6 times higher than in lean mice (31,54) and high concentrations of glucocorticoids have been shown to result in muscle wasting and altered fat deposition (77), plasma corticosterone was measured. Finally, concentrations of insulin in plasma were

measured to determine the relationship of this anabolic hormone to the catabolic glucocorticoid during cold acclimation.

Materials and Methods

Animals

At 4 weeks of age, obese (ob/ob) and lean (ob/+ or +/+) male mice were assigned to one of two environmental temperatures (23C or 14C). All mice had food (Wayne Lab-Blox, Continental Grain Co, Chicago, IL) and water available ad libitum. Room lights were on from 0700 to 1900 hours daily.

Experimental Design

Experiment 1 was designed to determine changes in body composition (selected muscle weights, bone length and total body fat) resulting from chronic cold exposure of young obese and lean mice. Obese and lean mice at 4 weeks of age were divided into 5 groups of 9-10 mice each. Group 1 was killed at 4 weeks of age to serve as an initial group. Groups 2 and 3 were housed at 23C for 4 and 8 weeks, respectively, and groups 4 and 5 were housed at 14C for 4 and 8 weeks, respectively.

The purpose of Experiment 2 was to determine effects of cold acclimation on body composition of near adult obese and lean mice as well as to determine if body composition was restored to equal that of controls in mice returned to 23C after 4 weeks of cold exposure. Experiments 1 and 2 were run concurrently; therefore mice housed at 23C from 4-12 weeks of age in Experiment 1 (group 3) were used as controls in Experiment 2 (Figure 6). The 2 experimental groups

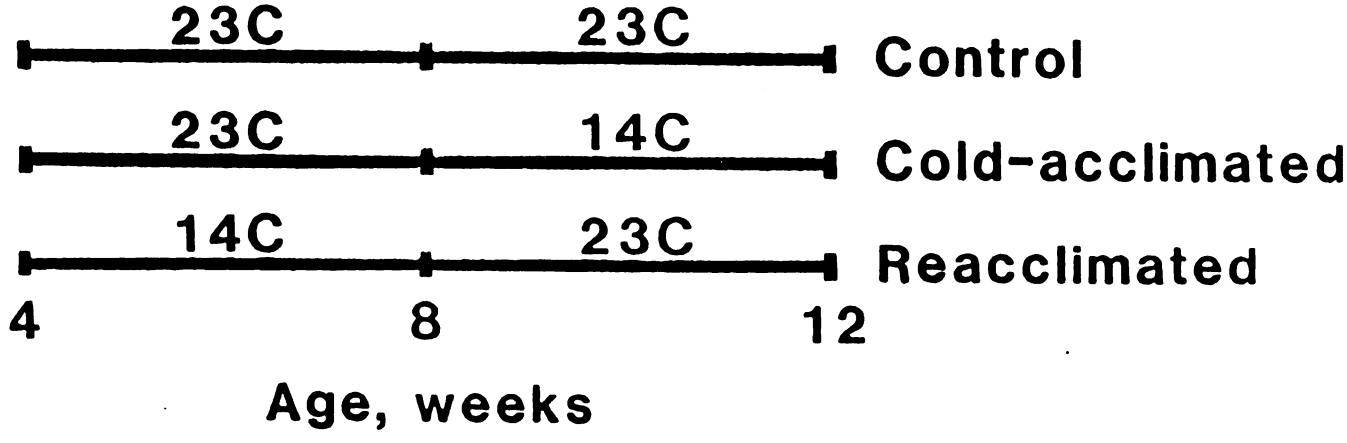

Figure 6. Design of Experiment 2. Numbers above horizontal lines indicate the temperature at which the mice were housed during the time period. Group names are at the end of each line.

consisted of 10 obese and lean pairs - one group housed at 23C until until 9 weeks of age, then at 14C from 9-12 weeks of age, and another group housed at 14C from 4-8 weeks of age, then at 23C from 9-12 weeks of age. Experiments 1 and 2 here correspond to Experiments 1 and 2 in Chapter 2.

Effects of cold acclimation of young obese and lean mice on plasma corticosterone, the major circulating glucocorticoid in mice (27), and plasma insulin were determined in Experiment 3. Obese and lean mice at 4 weeks of age were housed at 23C or 14C for 4 weeks.

Analyses

At the end of each experimental period in Experiments 1 and 2, mice were killed by cervical dislocation and the intact carcasses were frozen. When the carcasses were thawed, the soleus, extensor digitorum longus (EDL) and psoas muscles were each isolated and weighed. These muscles were chosen on the basis of fiber type and function. The soleus contains red and intermediate type fibers, and functions in locomotion, whereas the EDL is composed mostly of white fibers, and functions in support (28). Both of these are hindlimb muscles. The psoas, located along the spinal column, is composed of mixed fiber types and also serves in support. Weight of the total hindlimb musculature was recorded after stripping all of the remaining muscles from the bones and removing all visible connective tissue and fat. Lengths of each tibia and femur were also recorded. Averages of the weights of muscles or lengths of bones from both hindlimbs or sides were used to calculate means.

One of the psoas muscles from mice in Groups 2 and 4 (Experiment 1) was utilized to determine RNA and DNA content by the method of

Schmidt and Thannheuser as modified by Munro and Fleck (53). Briefly, RNA was hydrolyzed in base, then solubilized in acid and determined colorimetrically at OD 260. DNA was quantitated by the indole method. The contralateral muscle was used to determine protein. A sample of muscle was digested in 0.3M NaOH for 18 hours at 37C and protein of an aliquot was determined by the method of Lowry et al. (47).

Body fat was determined on an aliquot of the entire carcass, including all tissues removed for weighing. Carcasses were first softened in an autoclave at 100C, then were homogenized (Brinkmann Polytron) in 2-3 times their weight of water. Duplicate aliquots of the homogenate were removed and fat was determined gravimetrically after chloroform:methanol extraction.

At the end of Experiment 3, mice were lightly anesthetized with ether (for 15 seconds) and blood samples were collected from 9-10 pairs of obese and lean mice maintained at each temperature (23C or 14C) at 0800 and from another 9-10 pairs from both temperatures at 1600 hours. These times correspond to approximately the lowest and highest concentrations respectively, of plasma corticosterone during its circadian rhythm (30). All blood samples were collected from the orbital sinus and were completed within 2 minutes of the time the mouse was removed from its cage, including the ether exposure. This was a short enough period of time to prevent increased plasma corticosterone due to stress (10). Plasma was stored at -40C. Corticosterone was determined by radioimmunoassay after ethanol extraction using corticosterone antiserum B3-163 (Endocrine Sciences, Tarzana, CA). Insulin was determined by radioimmunoassay using a kit

from Micromedic Systems Inc. (Horsham, PA).

Data are presented as mean + SEM. Data was analyzed by analysis of variance. Treatment differences were determined by Duncan's multiple range test ($P<0.05$) (74).

Results

Experiment 1

At 4 weeks of age, weights of all muscles examined, except soleus, were significantly lower in obese mice than in lean mice (Figure 7). In general, muscles of obese mice housed at 23C from 4 to 12 weeks of age gained weight only half as rapidly as those of lean mice (Figure 7). These results support earlier observations of depressed skeletal muscle accumulation in obese mice at 23-25C (3,87).

Cold acclimation markedly depressed muscle growth in obese mice, but affected growth of muscles in lean mice only minimally (Figure 7). Soleus and EDL of obese mice gained less than 1 mg during 8 weeks of cold exposure. Weights of psoas and total hindlimb muscles of obese mice did not increase during the first 4 weeks at 14C, but during the next 4 weeks, these muscles gained 25 and 90 mg, respectively. As a result, after 8 weeks of cold exposure, muscles of obese mice weighed only 35-45% as much as those of lean mice. Body weights of 12 week old lean mice were 29 + 0.5 and 26 + 0.6 g at 23C and 14C respectively, and 48 + 0.6 and 27 + 1.9 g in obese mice at 23C and 14C respectively. Thus, the lower skeletal muscle weights in cold-acclimated obese mice as compared with lean mice are not due to a lower body weight of the obese mice. Hearts of obese mice

Figure 7

Muscle weights of mice from 4-12 weeks of age. Total hindlimb muscle refers to all muscle stripped from the hindlimb except the soleus and EDL. Environmental temperature noted by numbers at the right end of each data line. Each point is the mean + SEM of 9-11 mice. +=Significant ($P<0.05$) difference due to temperature within phenotype. Within temperature treatment, muscle weights of obese mice are significantly lower than those of lean mice at all ages except for the soleus at 4 weeks of age.

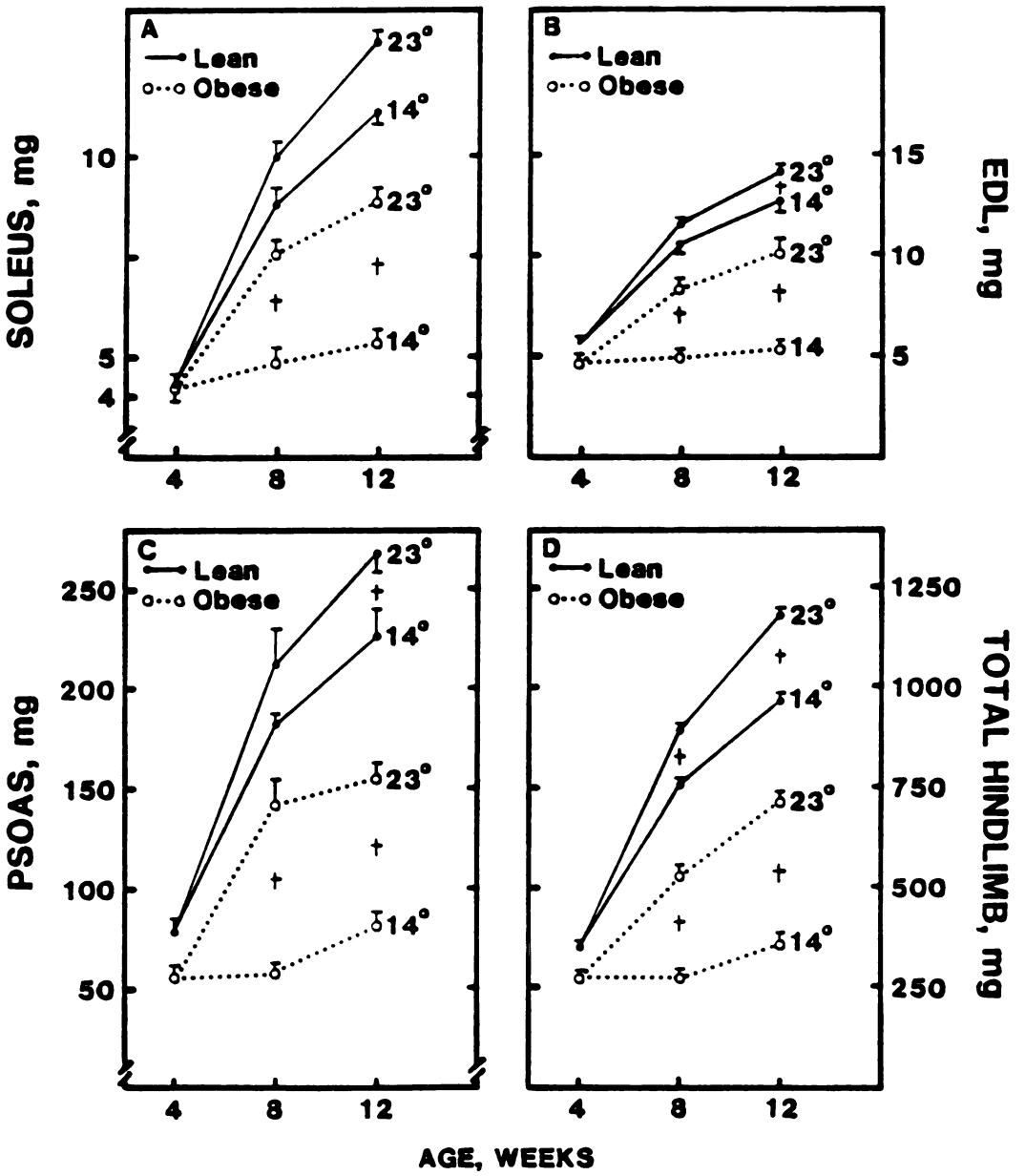

Figure 7. Muscle weights of mice from 4-12 weeks of age.

housed at 23C and 14C for 8 weeks weighed 139 + 6 mg and 209 + 6 mg respectively, while those of lean mice housed at 23C and 14C weighed 152 + 5 mg and 208 + 13 mg respectively, indicating that heart muscle of obese mice, unlike skeletal muscle, was not adversely affected by cold exposure.

Neither phenotype nor cold acclimation affected protein, RNA and DNA concentration in the psoas of 8 week old mice (Table 3). Because the psoas of obese mice weighed only 69% and 36 % as much as psoas of leans at 23C and 14C, respectively, the total muscle content of these constituents was, however, correspondingly lower in obese mice than leans (data not shown). These findings are in agreement with those of Shapira et al., who found no difference in these parameters between obese and lean Zucker rats, despite differences in muscle weights (69).

The tibia and femur demonstrated the same pattern of growth as the muscles (Figure 8). At 23C, the bones of obese mice were 90-95% as long as those of leans. Cold acclimation only slightly depressed the rate of gain in length of the tibia and femur in lean mice, but caused a marked depression in gain in the tibia and femur in obese mice. In cold acclimated obese mice, the bones lenghtened by approximately 15% from 4-12 weeks of age as compared with 29% in leans.

Lean mice housed at 23C deposited about 1.8 g body fat whereas obese mice gained almost 15 g body fat between 4 and 12 weeks of age (Figure 9). Cold-acclimated obese mice gained approximately 5 g fat from 4 to 8 weeks of age compared to about a 1 g gain in lean mice. Neither obese nor lean mice gained body fat during the second 4 weeks

Table 3. Composition of Psoas Muscle

Phenotype	Protein	RNA	DNA
	ug/mg muscle		
	Temperature-23C		
Lean	164±3	2.94±0.28	0.77±0.08
Obese	158±4	2.76±0.21	0.61±0.05
	Temperature-14C		
Lean	154±5	2.78±0.32	0.76±0.05
Obese	144±6*	2.47±0.36	0.72±0.05

Mean ± SEM (n=9-11)

*Significantly ($P < 0.05$) different from obese at 23°C.

Figure 8

Bone lengths in mice from 4-12 weeks of age. Environmental temperature noted by numbers at the right end of each data line. Each point is the mean + SEM of 9-11 mice. += Significant ($P<0.05$) difference due to temperature within phenotype. Within temperature treatment, both bones are significantly shorter in obese mice than in lean mice at all ages examined at both temperatures.

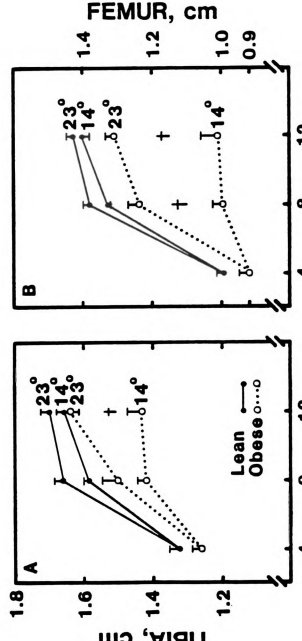

Figure 8. Bone lengths in mice from 4-12 weeks of age.

Figure 9

Total body fat and percentage body fat in mice from 4-12 weeks of age. Environmental temperature noted by numbers at the right end of each data line. Each point is the mean + SEM of 9-11 mice. += Significant (P<Ø.Ø5) difference due to temperature within phenotype. Within temperature treatment, total body fat and percentage body fat are significantly higher in obese mice than in lean mice at all ages examined.

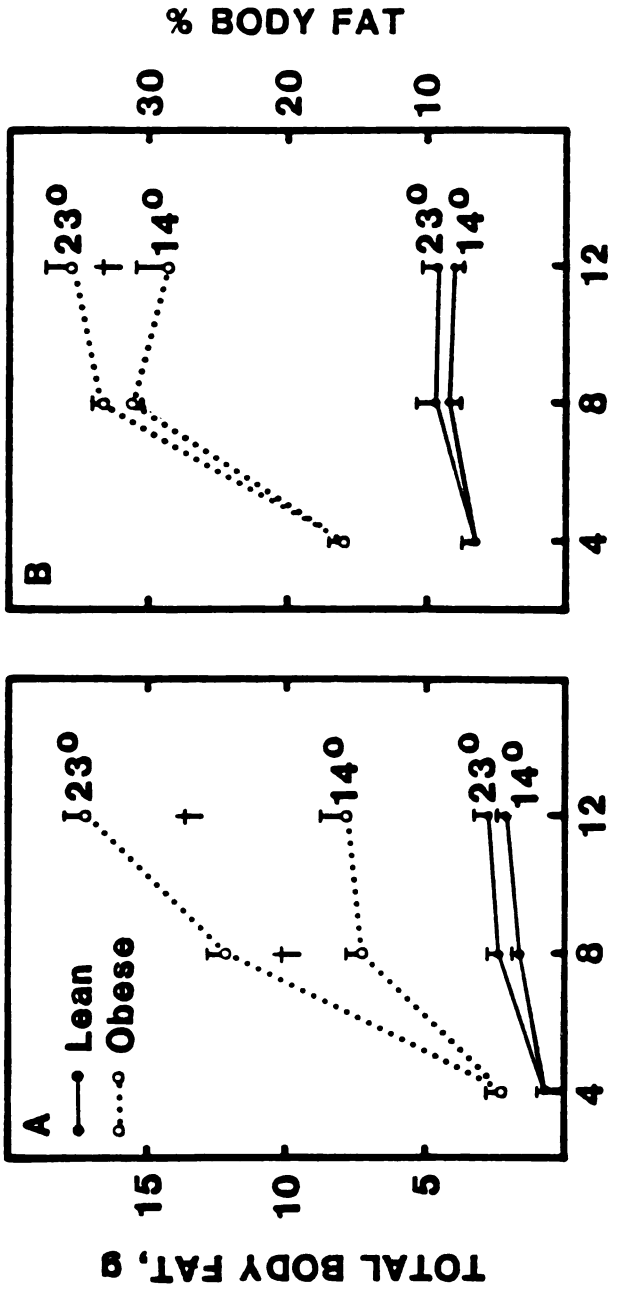

Figure 9. Total body fat and percentage body fat in mice from 4-12 weeks of age.

at 14C.

Cold acclimation had no effect on percentage body fat in lean mice (Figure 9B). Obese mice at 14C maintained the same percentage body fat as obese controls during the first 4 weeks at 14C, but from 9-12 weeks of age, cold-acclimated obese mice showed a slight decrease in percentage body fat. This occurred because total body fat remained constant during this period while body weight increased by 3.1 g.

Experiment 2

Cold acclimation of near-adult mice slowed muscle growth as it had in younger mice and the effects were more pronounced in obese mice than lean mice (23 to 14;Figure 10A). Because the responses of the soleus, EDL and psoas muscles to treatment paralleled that of the total hindlimb muscle only the latter is presented. Weight of the total hindlimb muscle did not increase in obese mice housed at 14C from 9-12 weeks of age, whereas in lean mice, muscle weight increased by 20%. Cold exposure from 9-12 weeks of age depressed the rate of increase in bone length in both obese and lean mice so that at 12 weeks of age the tibia of cold-acclimated obese lean mice was 15-20% shorter than that of appropriate controls (Figure 10B). Cold-acclimated lean mice maintained body fat, as did lean controls (Figure 10C). Cold-acclimated obese mice gained less than 1 g fat while controls gained approximately 5 g fat.

Total hindlimb muscle of lean mice reacclimated to 23C after 4 weeks of cold exposure weighed the same as this muscle from lean controls, but total hindlimb muscle of reacclimated obese mice still weighed only 85% as much as that of obese controls (14 to 23; Figure

Figure 10

Total hindlimb muscle weights, bone lengths and total body fat of mice from 9-12 weeks of age. Total hindlimb muscle refers to all muscle stripped from the hindlimb except the soleus and EDL. Each point is the mean + SEM of 9-11 mice. Mice were housed at 23C through 12 weeks of age (23), at 23C through 8 weeks of age and at 14C from 9-12 weeks of age (23 to 14), or at 14C from 4-8 weeks of age and at 23C from 9-12 weeks of age (14 to 23). *= Significantly ($P<0.05$) different from the 23C group within phenotype.

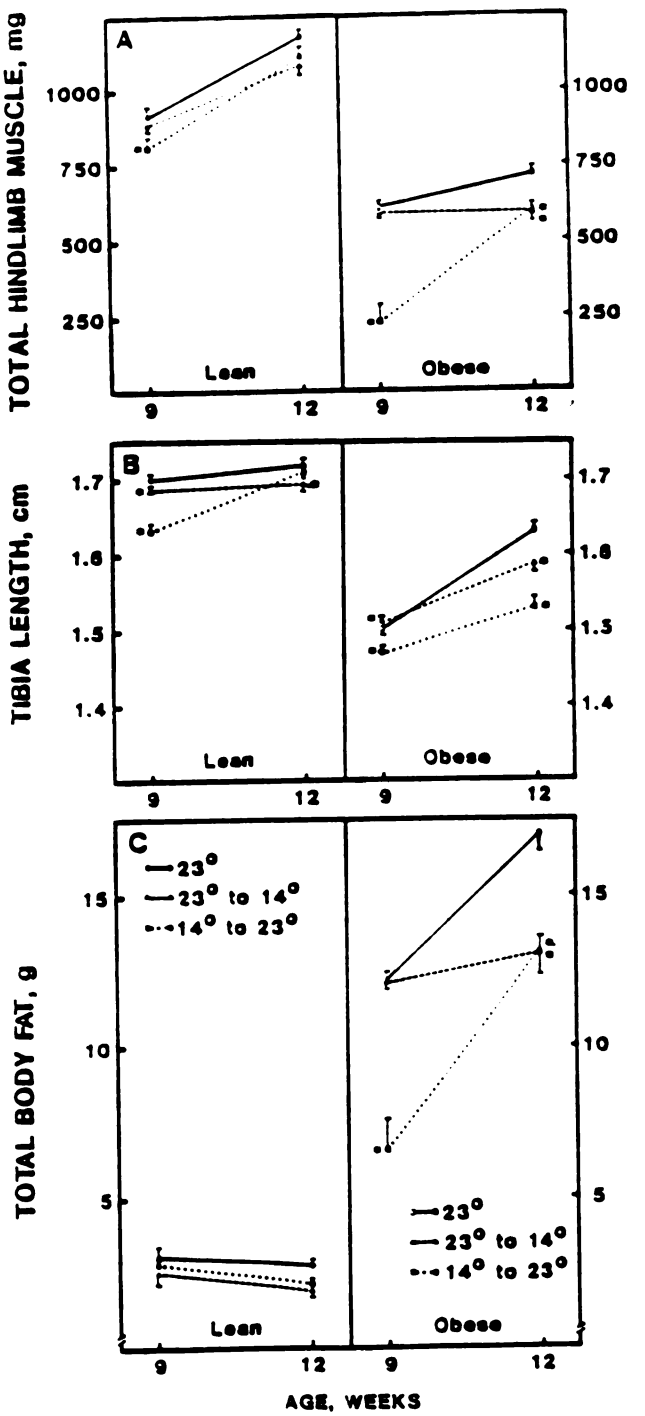

Figure 10. Total hindlimb muscle weights, bone lengths and total body fat of mice from 9-12 weeks of age.

10A). The tibia of reacclimated obese mice lengthened at only half the rate of obese controls so that at 12 weeks of age, it remained shorter than the tibia from obese controls (Figure 10B). The tibia of reacclimated lean mice lengthened by more than two-fold that of lean controls, so that at 12 weeks of age it was the same length as the control.

Lean mice reacclimated to 23C did not gain body fat, whereas reacclimated obese mice gained approximately the same amount of body fat os obese controls (Figure 10C). Reacclimated obese mice still had significantly less body fat than obese controls, however.

Experiment 3

Concentration of corticosterone in plasma of obese mice housed at 23C was higher than that of lean mice, though the difference between obese and lean was only significant at 1600 hours (Figure 11A and B). This higher concentration of corticosterone in obese mice is in agreement with results of Herberg and Kley (31) and Naeser (54). Cold acclimation increased the concentration of corticosterone in plasma of obese mice compared to obese mice at 23C at both times of the day, though this was statistically significant only at 0800 hours. In lean mice, plasma corticosterone concentrations were unaffected by cold acclimation. The concentration of corticosterone in plasma of cold acclimated obese mice was thus 5-10 times that of cold-acclimated lean mice.

Plasma insulin concentration of obese mice housed at 23C was 20 times that of lean mice at this temperature (Figure 11C). Cold exposure led to a dramatic reduction in insulin of obese mice, from 411 to 137 uUnits/ml. Despite this reduction in insulin

Figure 11

Plasma corticosterone and insulin concentrations in mice at 8 weeks of age. They had been housed at the indicated temperatures for 4 weeks. Each point is the mean + SEM for 9-10 mice. Top panel, plasma corticosterone at 0800 hours. Middle panel, plasma corticosterone at 1600 hours. Bottom panel, plasma insulin. *= Significant ($P<0.05$) difference between obese and lean at the same time and temperature. += Significant ($P<0.05$) difference due to temperature within phenotype.

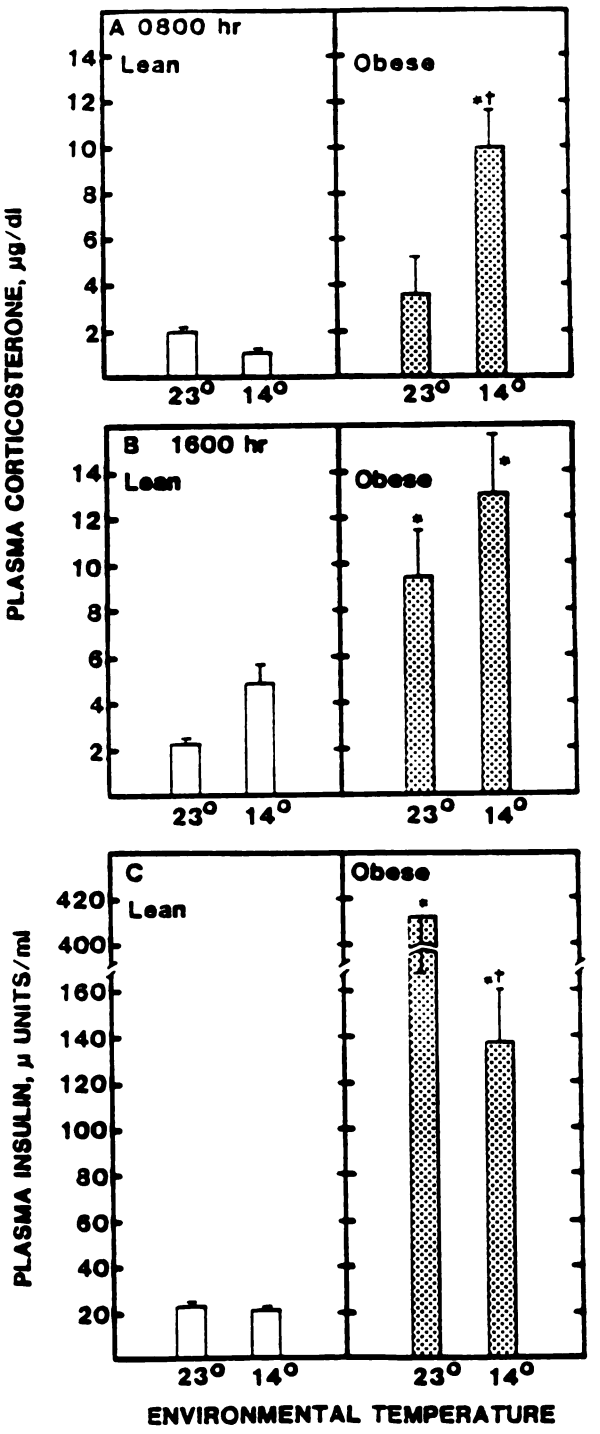

Figure 11. Plasma corticosterone and insulin concentrations in mice at 8 weeks of age.

concentration in obese mice at 14C, cold exposed obese mice were still hyperinsulinemic relative to leans. The concentration of insulin in plasma of lean mice was unaffected by cold acclimation.

Discussion

Skeletal muscle of obese mice housed at 23C weighed only about 65% as much as much as muscle of lean mice. This effect was not seen only in hindlimb muscle - the psoas, located in the trunk, followed the same pattern of response as the other muscles examined. This is consistent with previous reports showing that skeletal muscle of obese mice, based on the weight of the fat-free carcass or hindlimb, weighed only about 80% as much as that of leans (3,83). Chronic mild cold exposure resulted in even more dramatic differences in muscle weights between obese and lean mice. After 8 weeks at 14C, skeletal muscles of obese mice weighed only 35-45% as much as muscles of lean mice, even though their body weights were the same.

As mentioned earlier, skeletal growth is a stimulus for skeletal muscle growth (36). Shapira and coworkers found that the tibia and femur of obese Zucker rats were approximately 10% shorter than those of lean rats, and suggested that these shorter bones exerted less of a trophic effect on the muscle, contributing to the reduced skeletal muscle mass of obese rats (69). In our study, hindlimb bones of obese mice were significantly shorter than those of lean mice and cold acclimation reduced bone growth even more in obese mice than in lean. These changes in bone growth parallelled the changes in muscle growth. It is possible then, that the slower growth of the skeleton

of obese mice compared to leans, contributed to their reduced skeletal muscle mass. It is unlikely however, that the 10-15% reduction in bone length of cold acclimated obese mice was entirely responsible for their dramatic reduction in muscle accumulation.

Corticosterone reduces skeletal muscle growth through several mechanisns. High doses of glucocorticoids lead to decreased rates of muscle protein synthesis (60,61) and increased rates of muscle protein breakdown (66). The 5-10 fold elevation in concentration of corticosterone observed in the cold-acclimated obese mice might contribute to their accelerated rates of muscle breakdown and reduced muscle accumulation (83). A 3-fold increase in concentration of plasma corticosterone for 8 days caused a 20% reduction in weights of gastrocnemius muscle, as a percentage of body weight, of rats (67). Elevated concentrations of plasma corticosterone also promote insulin resistance, which could reduce muscle growth (55). Further support for the involvement of glucocorticoids in reduced muscle accumulation in obese mice comes from Ohshima et al.; they showed that adrenalectomy increased muscle mass of obese mice (65). Thus, there is some evidence supporting a role for elevated plasma corticosterone in the reduced muscle growth of obese mice, though the causal relationship between these must be further clarified.

Although the concentration of insulin in plasma of obese mice was lowered by cold acclimation they were still hyperinsulinemic relative to lean mice (Figure 10C). Considering the high concentrations of corticosterone in plasma of cold-acclimated obese mice it is likely that their muscles remained insulin resistant (29,48). These factors together with low concentrations of growth hormone (42) and

testosterone (76) in plasma of obese mice probably contributed to the restriction of skeletal muscle growth in cold-acclimated obese mice even though their intake of nutrients equaled that of lean mice.

Retained energy was partitioned into body fat and muscle in a pattern that enabled cold-acclimated obese mice to maintain a similar percentage fat (Figure 8) and skeletal muscle (data not shown) as age-matched obese controls. Because of the limited rate of body weight gain and concomitant increase in percentage body fat, skeletal muscle gain was severely limited in the cold-acclimated obese mice. Pair-fed obese mice (data not shown) and pair-fed obese Zucker rats (9) also maintain a body composition (percentage body fat and skeletal muscle) similar to that of ad libitum fed obese counterparts. However, their reduction in muscle gain is not as severe as that of cold-acclimated animals because the pair-fed obese animals gain more body weight than their ad libitum-fed lean counterparts. These genetically obese rodents thus tenaciously maintain an elevated percentage body fat even during periods of restricted growth.

Reacclimation of the obese mice to 23C reduced their energy expenditure more than energy intake, consequently their rate of body energy gain was again accelerated (Chapter 2).Concomitantly, the rate of skeletal muscle gain increased in the obese mice (Figure 1Ø). It is likely that muscle weights of the reacclimated obese mice would have been restored to the same level as obese controls if the study had been extended beyond 4 weeks of reacclimation.

In conclusion, though body weights of obese and lean mice are equalized by chronic cold exposure, obese mice maintain their obese

body composition (increased percentage body fat and decreased percentage skeletal muscle) by limiting skeletal muscle accumulation. Elevated concentrations of plasma corticosterone in combination with reduced concentrations of several anabolic hormones may have a key role in maintaining this obese body composition. Finally, obese mice demonstrate the ability to accelerate their rate of muscle accumulation when reacclimated to 23C after 4 weeks of cold exposure.

Chapter 4. Effects of adrenalectomy on energy balance of obese (ob/ob) mice are diet-dependent.

Adrenalectomy reduces food intake by 35-60% and body weight gain by 50-90% in several obese animal models, so that intake and gain are similar to that of their lean counterparts (7,14,49,65,91). This normalization of energy intake and weight gain does not necessarily indicate that these adrenalectomized obese animals normalized their body fat content, however, since obese animals pair-fed or pair-gained to lean animals still deposit body fat at a faster rate than lean counterparts (5). Based on currently available evidence it appears that adrenalectomy may reduce body fat accumulation of obese animals to a greater extent than can be explained by the reduction in energy intake. The high efficiency of energy retention usually observed in obese Zucker rats was reduced by adrenalectomy to equal that of lean rats (49). This reduction in efficiency was probably not due to reduced energy intake, since in control obese rats, a reduction in energy intake failed to reduce their efficiency of energy retention (5). The reduction in energy efficiency in adrenalectomized obese rats was more likely caused by increased energy expenditure, possibly resulting from increased activity of brown adipose tissue. This suggestion of increased brown adipose activity is supported by the findings that mitochondrial proteincontent and GDP binding to mitochondria of brown adipose, a measure of the capacity of the tissue for heat production, were increased in adrenalectomized obese rats so that they equaled that of leans (49). Adrenalectomy also increased norepinephrine turnover in

brown adipose tissue of ob/ob mice to nearly the same level as in lean mice, indicating increased brown adipose tissue metabolism and perhaps a reduced efficiency of energy retention (91). Energy balance of adrenalectomized ob/ob mice has not been directly examined, however.

Consistent with the reduction in efficiency of energy retention in adrenalectomized obese Zucker rats, their body composition is also altered by adrenalectomy. Adrenalectomy of obese Zucker rats prevented the 25% increase in body energy density observed in sham-operated obese rats, indicating that the adrenalectomized obese rats had a lower proportion of body fat than sham-operated obese (49). Adrenalectomy affects body composition of other obese animals as well. In GTG-treated mice, adrenalectomy prevented the accumulation of excess carcass lipid seen in sham-operated GTG-treated mice (14). In ob/ob mice, the weight of the gastrocnemius muscle was increased after adrenalectomy so that it was similar to that of lean mice (65), and presumably fat accumulation was decreased.

All of the above-mentioned studies examining the effects of adrenalectomy on obese animals were conducted with animals fed a pelleted stock diet. Several lines of evidence indicate that the diet fed to obese animals may be important in influencing the outcome of adrenalectomy. In VMH-lesioned rats, combined ovariectomy-adrenalectomy prevented the hyperphagia and excess body weight gain usually seen in VMH-lesioned rats if they were fed a pelleted stock diet. However, when a semi-purified, high-fat or a liquid, high-carbohydrate diet was fed, the

ovariectomized-adrenalectomized rats overate to almost the same extent as intact VMH-lesioned rats (52). Treatments other than adrenalectomy which alter the development of obesity are also affected by diet. When hypophysectomized obese rats were fed a pelleted stock diet, their body weights steadily declined and they eventually died (59). However, when the hypophysectomized obese rats were fed a softer, more palatable diet, they maintained their body weights and the obesity attained prior to hypophysectomy. Vagotomy blocked hyperphagia and the development of obesity in VMH knife-cut rats fed a pelleted stock diet, but when these rats were fed several palatable foods in addition to stock diet, they gained more weight than either non-vagotimized VMH lesioned or sham-operated rats (68). Finally, the metabolism of brown adipose tissue, which has been shown to be altered by adrenalectomy of obese animals (34,49,91), is also influenced by diet. Heroux et al. found that the metabolic potential of brown adipose tissue was higher in rats fed a semipurified diet than in rats fed a pelleted stock diet (32). These studies demonstrated the important role of diet in determining the outcome of several treatments of obese animals. Therefore, it was of interest to examine the influence of diet on the outcome of adrenalectomy in ob/ob mice.

The present study was designed to determine the effects of adrenalectomy on energy balance and body composition of obese (ob/ob) and lean mice fed either a high-carbohydrate, stock diet or a high-fat, semipurified diet. The pelleted stock diet was chosen to facilitate comparisons of this study with those from previous studies on adrenalectomy of obese animals. The high-fat

diet was chosen to compare to the stock diet because ob/ob mice demonstrate a marked degree of hyperphagia and weight gain on this diet (44) so that any effects of adrenalectomy on these parameters should be obvious. Finally, the extent to which obesity has developed in animals appears to influence the effects of adrenalectomy. Adrenalectomy of GTG-treated mice which were not yet obese resulted in the maintenance of food intake and body weight at the same levels as in lean (non-GTG-treated) controls (14). But, when GTG-treated mice were adrenalectomized after obesity had developed, there was a rapid decline in food intake and body weight to levels considerably below those of untreated lean control mice (15). Therefore, this study was designed to examine the effects of adrenalectomy before obesity in ob/ob mice was markedly obvious (3 weeks of age) and after obesity was more fully developed (6 weeks of age).

Materials and Methods

Animals

Obese (ob/ob) and lean (ob/+ or +/+) male mice were obtained from our breeding colony of C57BL/6J ob/+ mice. The breeding colony is housed at 23C with room lights on from 0700 to 1900 hours daily. Pups are weaned at 3 weeks of age. At 3 or 6 weeks of age, obese and lean pairs were separated from their littermates and housed individually in solid-bottom plastic cages with wood shavings as bedding. After 1-2 days of adaptation to single housing, mice were bilaterally adrenalectomized (Adx) or sham-operated (Sh)

through dorsal incisions while under ether anesthesia. The adrenal glands were gently lifted to the opening of the incisions and curved scissors were used to remove the glands along with a small amount of surrounding adipose tissue, taking care not to touch the adrenals with the dissecting instruments. Sham-operation consisted of locating the adrenal glands and exposing them as for adrenalectomy, but without excising them. Incisions were closed with stainless steel wound clips. Mice were exposed to ether for approximately 4 minutes and had recovered from the anesthesia within the following 5 minutes. After surgery, drinking water of Adx mice was replaced with physiological saline (0.9% NaCl). All mice had food (see below for diets) and water (or physiological saline) available ad libitum. Food intake and body weights were monitored 3 times weekly.

Experimental Design

Experiment 1 was designed to examine the effects of adrenalectomy on energy balance and body composition of obese and lean mice fed a stock diet (Wayne Lab-Blox, Wayne Pet Food Div., Continental Grain Co., Chicago, IL). Obese and lean mice were adrenalectomized or sham-operated as described above at either 3 or 6 weeks of age and were fed stock diet for 3 weeks. Initial groups of 3 or 6 week old obese and lean mice were killed for baseline data.

Experiment 2 was designed to assess how consumption of a high-fat diet affected the outcome of adrenalectomy in obese and lean mice. The same procedures as followed in Experiment 1 were followed here, except the mice were fed a high-fat, semipurified diet containing (g/100g) mineral mix, 4.83 (1); vitamin mix, 1.38 (1); cellulose, 5.52; methionine, 0.41; choline chloride, 0.28; casein, 27.61;

cerelose 27,31; corn oil, 16.33; and tallow, 16.33 for 3 weeks after adrenalectomy or sham-operation. This diet provided 60% of metabolizable energy as fat, 20% as protein and 20% as carbohydrate.

Experiment 3 was designed to examine the effects of restricted intake of the high-fat diet on the outcome of adrenalectomy. Obese and lean mice were adrenalectomized or sham-operated at 6 weeks of age. They were fed an amount of high-fat diet to provide them with the same gross energy intake as 6-9 week old sham-operated lean mice in Experiment 1. The diet was given to the mice each day at 1000-1200 hours. Some of this food was still in the food cups at 2000 hours, indicating that the mice did not consume all of their food during the light cycle. Mice were killed at 9 weeks of age for further analyses.

Analyses

At the end of the experimental periods, two blood samples were taken from the orbital sinus of each mouse at 2000 hours, the approximate time of peak corticosterone concentration in mice (64). The first sample was taken within 2 minutes of the time the mouse was removed from its cage, including 15 seconds of ether exposure, and served as a nonstressed baseline sample. The second blood sample was taken 10 minutes later and served as a stressed sample. Plasma was assayed for corticosterone concentration by radioimmunoassay (Endocrine Sciences, Tarzana, CA). Plasma insulin concentration was also determined by radioimmunoassay (Radioassay Systems Laboratories, Inc., Carson, CA) on baseline plasma samples pooled from 2-3 mice in the same treatment group. Only those adrenalectomized mice whose baseline corticosterone was less than half that of sham-operated

mice and whose stressed corticosterone was not increased by more than 20% above the baseline concentration were included in the analyses. Mice were killed by cervical dislocation after the second blood sample was taken, and carcasses were frozen until analyzed. After thawing, the soleus, gastrocnemius and psoas muscles were isolated and weighed. These muscles were chosen to provide a variety of fiber types, functions and locations. The soleus, in the hindlimb, contains both red and intermediate type fibers and functions in locomotion. The gastrocnemius, also in the hindlimb, contains red and white fibers and funtions in locomotion. The psoas, located along the spinal column, contains mixed fiber types and functions in support. The remaining hindlimb muscle was also stripped from the bones and weighed. The sum of the weights of all the hindlimb muscles (soleus + gastrocnemius + remaining hindlimb muscle) is reported as total hindlimb muscle. The length of the tibia was also recorded. Averages of the weights of muscles or length of bones from both hindlimbs or sides were used to calculate means.

Body energy of the carcass (including all tissues removed for weighing) was measured by direct calorimetry. Carcasses were softened in an autoclave at 100C, then homogenized (Brinkmann Polytron, Brinkmann Instruments, Westbury, NY) in 2-3 times their weight of water. An aliquot of homogenate was dried at 50C and used for the determination of energy with an adiabatic calorimeter (Parr Instrument Co., Moline, IL). Body energy density was calculated as kcal/g body weight. The diets were also analyzed for gross energy content (stock diet = 3.9 kcal/g; high-fat diet = 5.3 kcal/g). Energy efficiency was calculated as body energy gained divided by

gross energy consumed during the 3 week experimental period. Body energy gain was calculated as body energy content at the end of the experimental period minus the predicted body energy content at the beginning of the experiment. Body energy at the beginning of each experimental period was predicted from a linear regression equation based on body weights and body energy contents of the initial groups. Body weights of the mice at the beginning of each experimental period were used to predict initial body energy for each mouse. This linear regression method was also used to calculate gain in muscle weight and tibia length. This method of predicting initial body energy, muscle weights and bone length was validated by comparing the actual and predicted values for these parameters in a separate group of mice. By this method, actual values for initial body energy, muscle weight and bone length were within 5% of predicted values.

Data are presented as means + SEM. Data were analyzed as a 2 x 2 factorial design by analysis of variance. Where interaction between treatment effects (phenotype x surgery) was significant ($P<0.05$), Bonferroni t-tests were used to determine specific treatment effects (80).

Results

Experiment 1 (Stock diet).

Basal concentrations of plasma corticosterone of sham-operated obese mice were almost 3 times those of lean mice (Figure 12). Plasma corticosterone concentrations of sham-operated mice increased

by 3-10 ug/dl after stress. Successful adrenalectomies were confirmed in 85% of the mice that underwent adrenalectomy. These adrenalectomized mice did maintain a low concentration of plasma corticosterone, but there was little or no increase in corticosterone after stress (Figure 12).

Body weights of all 4 groups of mice were similar from 3-5 weeks of age (Figure 13A). Between 5 and 6 weeks of age, sham-operated obese mice gained considerably more weight than adrenalectomized obese and both groups of lean mice. Adrenalectomy of obese mice at 3 weeks of age prevented this excess body weight gain, so that their final body weight equaled that of lean mice.

Between 6 and 9 weeks of age, sham-operated obese mice gained more than 5 times as much body weight as sham-operated leans (Figure 13B). Adrenalectomy of 6 week old obese mice reduced body weight gain to less than half that of sham-operated obese mice (Figure 13B). By 8 weeks of age, body weights of adrenalectomized obese were significantly less than those of sham obese, and this difference widened by 9 weeks of age.

Energy consumption of sham-operated obese mice from 3-6 weeks of age was the same as that of lean mice, contrary to what is usually seen at this age (15,28) (Figure 14A). This occurred because the sham-operated obese mice consumed very little food for the first several days after surgery, and their food intake only slowly increased. Adrenalectomy reduced energy intake of obese mice by 17%, though this was not statistically significant. From 6-9 weeks of age, energy consumption of sham-operated obese mice followed a more normal pattern; sham obese mice consumed 50% more energy than sham

Figure 12

Plasma corticosterone concentrations of mice fed stock diet from 3-6 weeks of age (A) or 6-9 weeks of age (B). Cross-hatched portion of bars represents non-stressed concentrations. Open portion of bars represent stressed concentrations, obtained 10 minutes after initial exposure to ether. Sh = sham-operated and Adx = adrenalectomized. Vertical line on top of each bar is the SEM of 9-14 mice. Non-stressed and stressed plasma corticosterone of sham obese was significantly ($P<0.05$) higher than that of sham leans, except for stressed values at 6-9 weeks of age. Adrenalectomy reduced corticosterone to equally low concentrations in obese and leans at body ages ($P<0.05$).

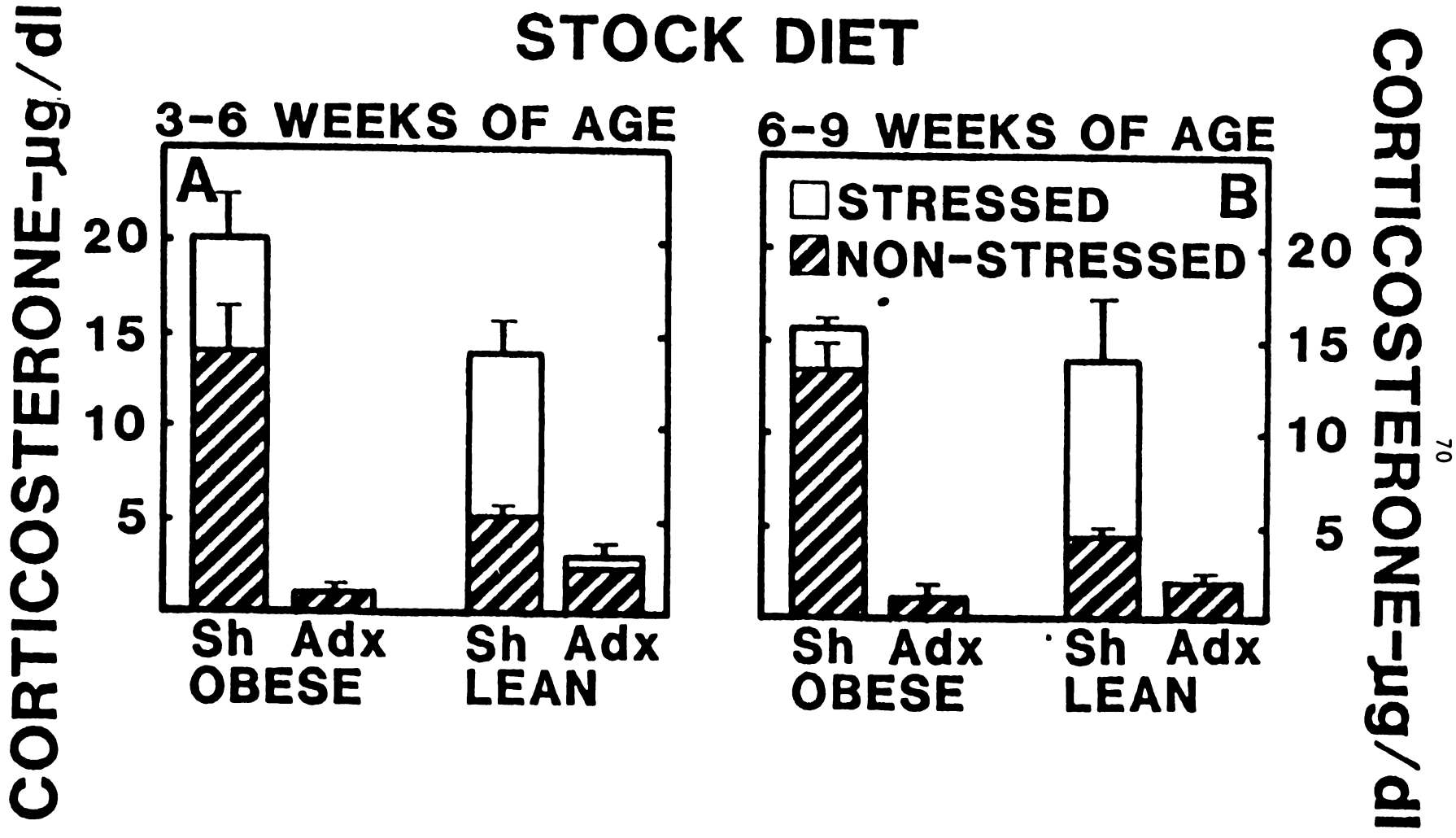

Figure 12. Plasma corticosterone concentrations of mice fed stock diet from 3-6 weeks of age or 6-9 weeks of age.

Figure 13

Body weights of mice fed stock diet from 3-6 weeks of age (A) and 6-9 weeks of age (B). Surgical treatment noted at the end of each line; Sh = sham-operated and Adx = adrenalectomized. Different letters indicate a significant difference ($P<0.05$) between groups at the same age. There were no significant differences between body weights of any of the groups at 3,4, or 5 weeks of age (panel A).

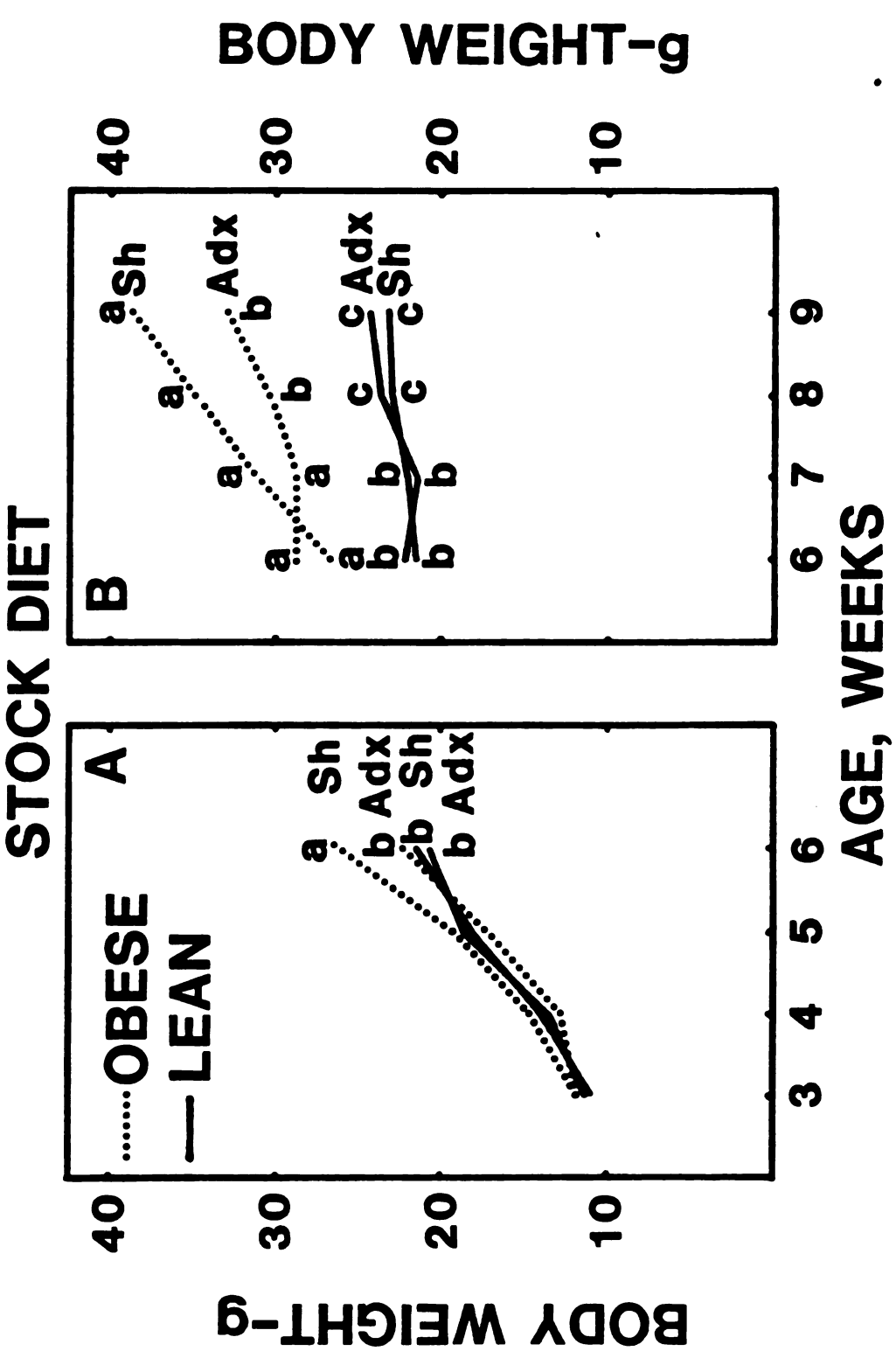

Figure 13. Body weights of mice fed stock diet from 3-6 weeks of age and 6-9 weeks of age.

Figure 14.

Energy consumption, gain and efficiency of mice fed stock diet from 3-6 weeks of age (A,C,E) and 6-9 weeks of age (B,D,F). Vertical line at the top of each bar is the SEM of 9-14 mice. Different letters above the bars indicate a significant difference ($P<0.05$) between groups of the same age.

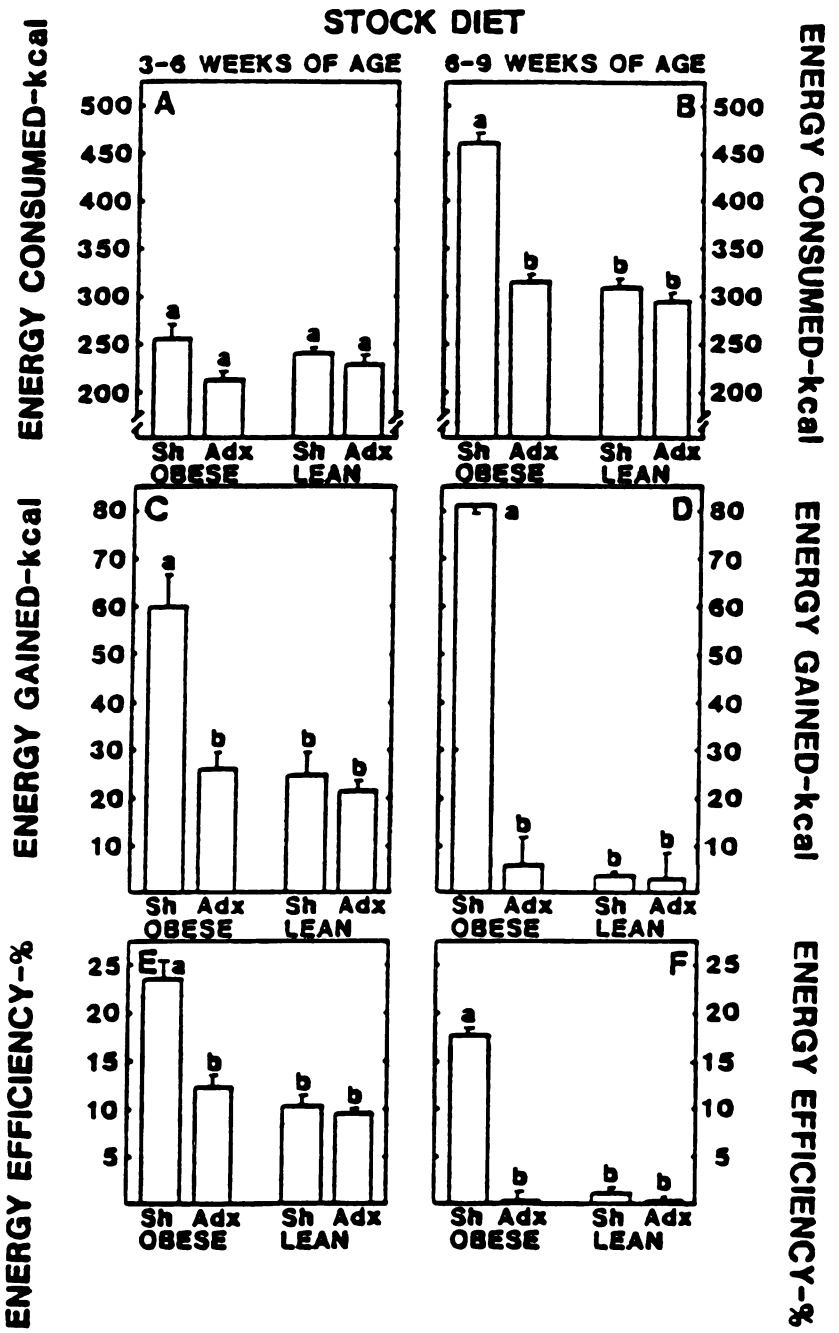

Figure 14. Energy consumption, gain and efficiency of mice fed stock diet from 3-6 weeks of age and 6-9 weeks of age.

leans (Figure 14B). Recovery from sham-surgery was more rapid in these older obese mice. Adrenalectomy of 6 week old obese mice reduced their energy intake to equal that of leans.

Sham-operated obese mice gained almost 2 1/2 times as much body energy between 3 and 6 weeks of age as sham-operated leans (Figure 14C). Adrenalectomy of 3 week old obese mice fed stock diet prevented this excess energy gain. The difference in energy gain between sham obese and lean mice was even more pronounced from 6-9 weeks of age, when sham obese mice gained 15 times as much body energy as sham leans (Figure 14D). Adrenalectomy of 6 week old obese mice prevented the large energy gain observed in sham obese mice.

The high body energy gain of 3-6 week old sham obese mice coupled with their only slightly elevated energy intake resulted in an efficiency of energy retention more than twice that of sham leans (Figure 14E). Adrenalectomy of 3 week old obese mice normalized their energy efficiency to equal that of lean mice. Between 6 and 9 weeks of age, sham-operated obese mice continued to retain body energy with a higher efficiency than sham leans (Figure 14F). As in the younger mice, adrenalectomy of 6 week old obese mice normalized their efficiency of energy retention to the level of lean mice. Adrenalectomy of lean mice did not affect any of these parameters of energy balance as compared with sham leans.

Energy density of sham-operated obese mice increased by 40% between 3 and 6 weeks of age, compared to a 20% increase in sham-operated leans (Figure 15A). The higher energy density of sham-operated obese mice indicates that they had a higher proportion of body fat than lean mice. Adrenalectomy of obese mice fed stock

Figure 15

Energy density, muscle weights and bone lengths of mice fed stock diet from 3-6 weeks of age (A,C,E,G) and 6-9 weeks of age (B,D,F,H). Cross-hatched portion of bars represents means of predicted values for mice at the beginning of the experiment. All predicted values for obese mice at the beginning of the experiment were significantly different ($P<0.05$) than initial leans. Open portion of bars represents gain during the experimental period. Sh = sham-operated, Adx = adrenalectomized. Vertical lines on each bar are the SEM of 9-14 mice. Different letters above the bars indicate significant ($P<0.05$) differences between groups at the end of the experimental period.

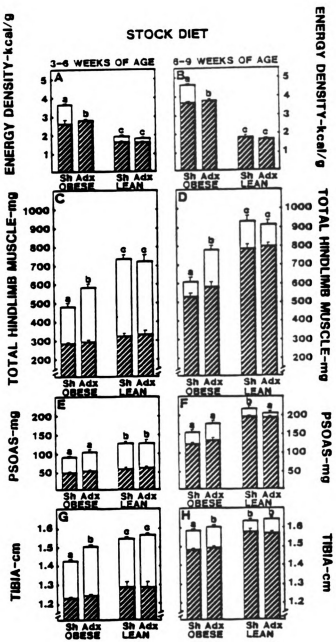

Figure 15. Energy density, muscle weights and bone lengths of mice fed stock diet from 3-6 weeks of age and 6-9 weeks of age.

diet prevented the increase in body energy density of 3-6 week old mice, though it remained significantly higher than that of lean mice. Energy density of 6-9 week old sham-operated obese mice increased by 25%, and, as in the 3-6 week old mice, this increase was prevented by adrenalectomy (Figure 15B)

The lower energy density of adrenalectomized obese mice compared to sham-operated obese indicates that adrenalectomy increased the proportion of lean body mass in obese mice. This was confirmed by the muscle weight data. The soleus and gastrocnemius muscles showed the same pattern of changes as the total hindlimb muscle, so they are not presented. Hindlimb muscles of 3 week old obese mice already weighed 17% less than muscles of lean mice, and sham-operated obese mice gained only 48% as much hindlimb muscle as lean mice from 3-6 weeks of age (Figure 15C). Adrenalectomy improved muscle gain of obese mice by almost 50%, so that their final hindlimb muscle weight was 80% of leans. Hindlimb muscle of unoperated 6 week old obese mice weighed 30% less than that of lean mice (Figure 15D). Sham-operated obese mice gained only 80 mg hindlimb muscle during the 3 week experimental period, so that muscle weight at 9 weeks of age was only 65% of leans. Adrenalectomy of 6 week old obese mice significantly increased their hindlimb muscle gain so that by 9 weeks of age, the muscle of adrenalectomized obese mice weighed 87% as much as leans. Results for the psoas muscle indicate that these effects on muscle were not restricted to the (Figure 15E). Adrenalectomy improved gain in psoas weights of 3 and 6 week old obese mice by 40%, but psoas weights of adrenalectomized obese were still less than those of lean controls because muscles of obese mice weighed less

than muscles of lean mice at the time of surgery.

The tibia of 3 week old obese mice was 95% as long as the tibia of lean mice, and between 3 and 6 weeks of age the tibia of sham-operated obese mice lengthened by less than that of leans (Figure 15G). Adrenalectomy of obese mice improved gain in tibia length by 30%, but final tibia length was still only 96% that of leans. Adrenalectomy of 6 week old obese mice did not significantly improve gain in tibia length; the tibia of 9 week old adrenalectomized obese mice was 98% as long as that of lean mice (Figure 15H).

Plasma insulin concentration of 6 week old sham-operated obese mice was 4 1/2 times that of sham leans (Figure 16A). Adrenalectomy of 3 week old obese mice fed stock diet prevented this hyperinsulinemia from developing, and at 6 weeks of age plasma insulin concentration of adrenalectomized obese equaled that of leans. By 9 weeks of age, plasma insulin concentration of sham-operated obese mice was 20 times that of sham leans (Figure 16B). Adrenalectomy of 6 week old obese mice reduced plasma insulin concentration so that plasma insulin of 9 week old adrenalectomized obese mice was only 4 times that of lean mice.

Experiment 2 (High-fat diet)

Basal concentrations of plasma corticosterone of 3-6 week old and 6-9 week old sham-operated obese mice were 2 - 2 1/2 times that of sham leans (Figure 17A and 17B). Plasma corticosterone was doubled or tripled after stress in all groups of sham-operated mice. Successful adrenalectomies were confirmed in 65% of the mice that underwent adrenalectomy. As in Experiment 1, the adrenalectomized

Figure 16

Plasma insulin concentrations of mice fed stock diet from 3-6 weeks of age (A) and 6-9 weeks of age (B). Sh = sham-operated and Adx = adrenalectomized. Vertical line on top of each bar is the SEM of 3-5 samples of plasma pooled from 2-3 mice each. Different letters above the bars indicate a significant ($P<0,05$) difference between groups of the same age.

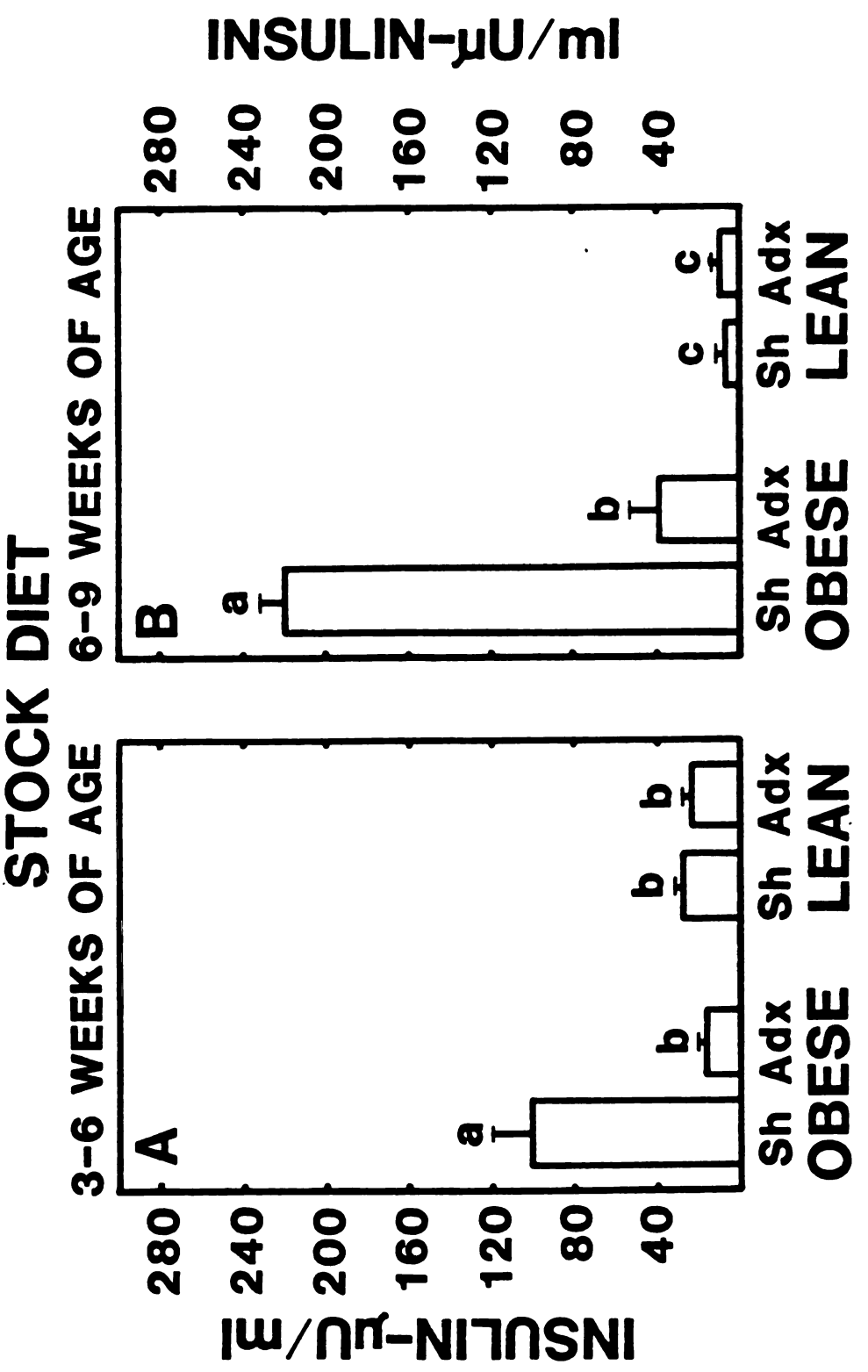

Figure 16. Plasma insulin concentrations of mice fed stock diet from 3-6 weeks of age and 6-9 weeks of age.

Figure 17

Plasma corticosterone concentrations of mice fed high-fat diet from 3-6 weeks of age (A) and 6-9 weeks of age (B). Cross-hatched portion of bars represents non-stressed concentrations. Open portion of bars represents stressed concentrations, obtained 10 minutes after initial ether exposure. Sh = sham-operated and Adx = adrenalectomized. Vertical line on top of each bar is the SEM of 9-14 mice. Non-stressed and stressed plasma corticosterone of sham obese was significantly higher ($P<0.05$) than sham leans at both ages. Adrenalectomy reduced corticosterone to equally low concentrations in obese and leans at both ages ($P<0.05$).

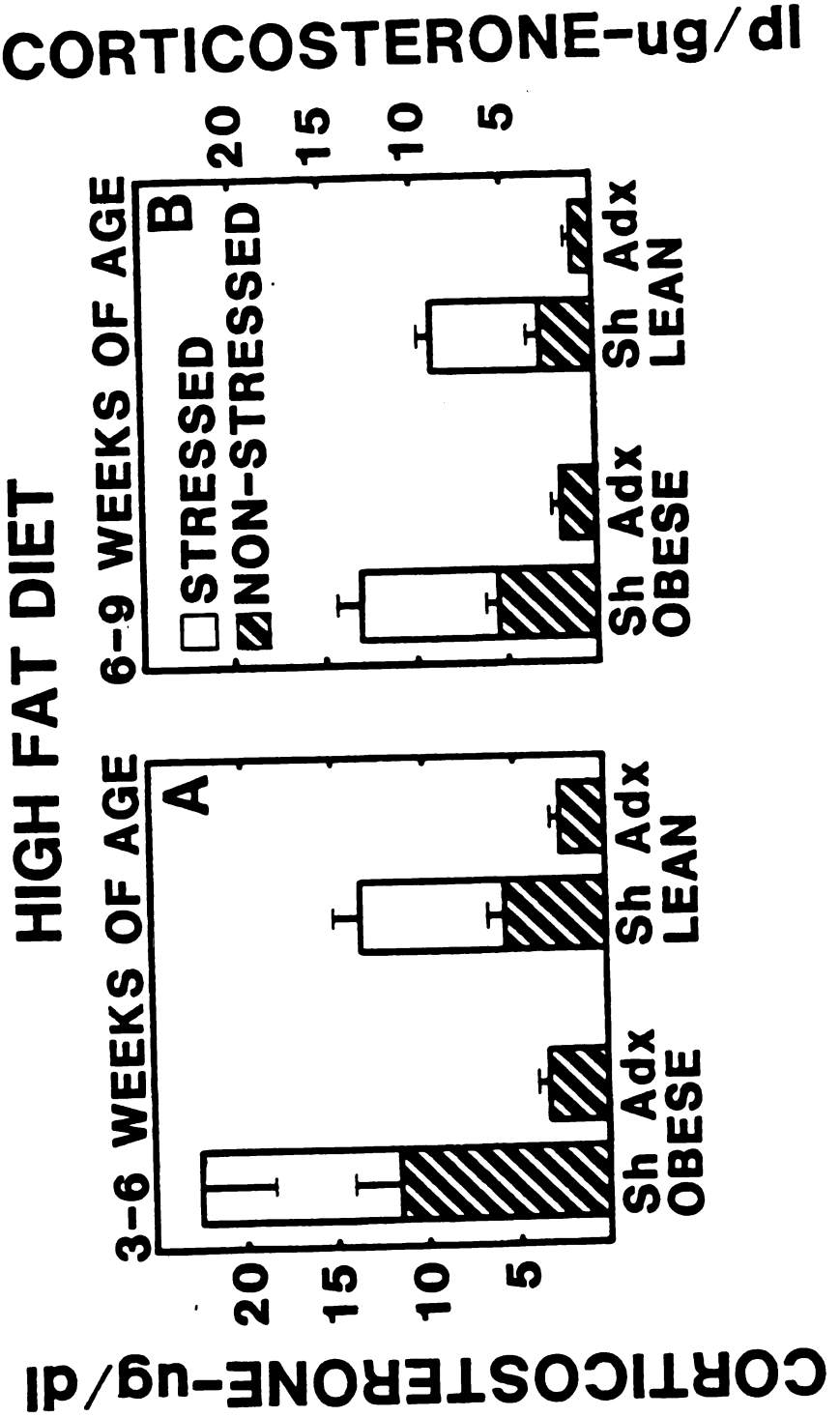

Figure 17. Plasma corticosterone concentrations of mice fed high-fat diet from 3-6 weeks of age and 6-9 weeks of age.

mice maintained a low concentration of circulating corticosterone, but the response to stress was absent.

Sham-operated obese mice fed the high-fat diet gained twice as much body weight as sham leans between 3 and 6 weeks of age, and by 6 weeks of age, sham obese mice weighed 55% more than leans (Figure 18A). Adrenalectomy of 3 week old obese mice fed high-fat diet did result in a 25% decrease in weight gain, but their weight gain was still 60% higher than leans. At 6 weeks of age, adrenalectomized obese mice weighed 33% more than leans. This contrasts with the normalization of body weight of 3-6 week old adrenalectomized mice fed stock diet (Figure 13A). From 6-9 weeks of age, sham-operated obese mice gained 3 times as much body weight as sham leans (Figure 18B). Adrenalectomy of 6 week old obese mice fed high-fat diet only reduced weight gain by 18%, so that body weight of 9 week old adrenalectomized obese was still 50% greater than that of leans.

As in Experiment 1, 3-6 week old sham-operated obese mice did not consume significantly more energy than sham-operated leans. (Figure 19A). However, adrenalectomy reduced energy consumption in both obese and lean mice. From 6-9 weeks of age, sham-operated obese mice fed the high-fat diet consumed 18% more energy than sham leans, and adrenalectomy did not reduce energy intake in either obese or lean mice.

Sham-operated obese mice fed high-fat diet gained 4 1/2 times as much body energy as sham leans from 3-6 weeks of age (Figure 19C). Adrenalectomy resulted in a significant 30% decrease in energy gain by obese mice, but this gain was still more than 3 times that of lean mice. Sham-operated obese mice fed high-fat diet continued to

Figure 18

Body weights of mice fed high-fat diet from 3-6 weeks of age (A) and 6-9 weeks of age (B). Surgical treatment noted at the end of each line. Sh = sham-operated and Adx = adrenalectomized. Different letters indicate a significant difference ($P<0.05$) between groups at the same age.

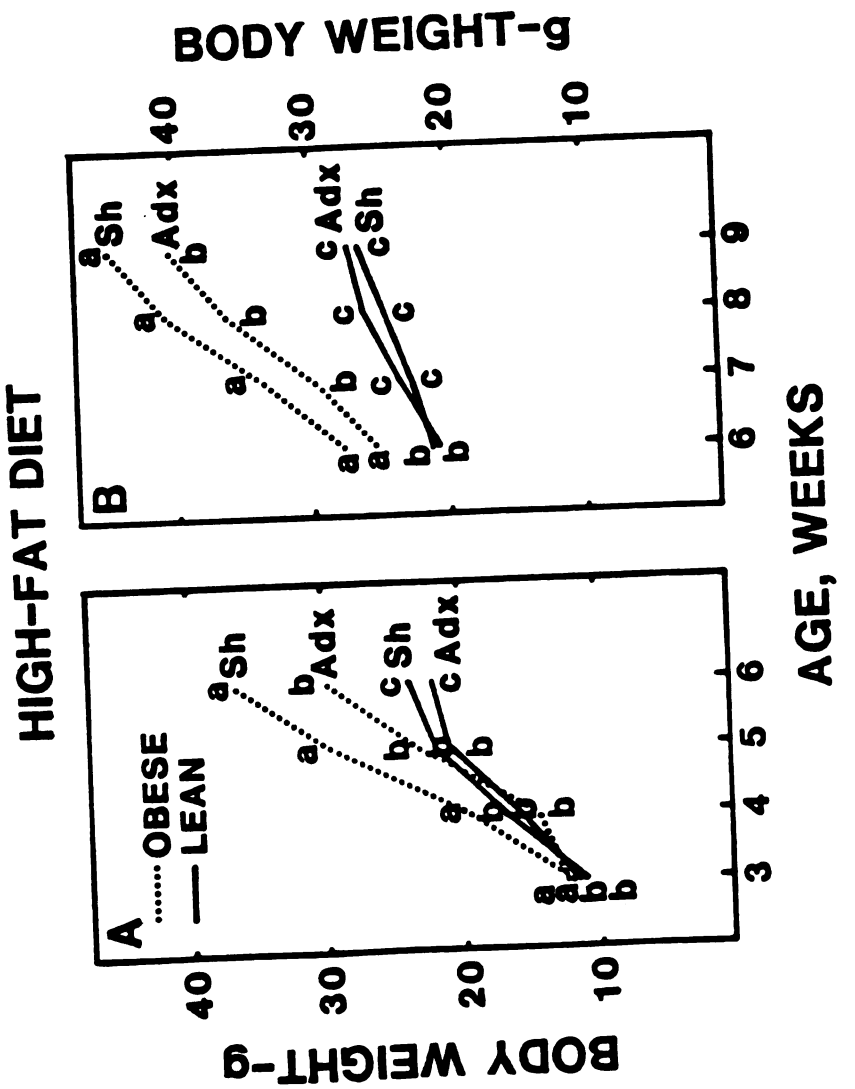

Figure 18. Body weights of mice fed high-fat diet from 3-6 weeks of age and 6-9 weeks of age.

Figure 19

Energy consumption, gain and efficiency of mice fed high-fat diet from 3-6 weeks of age (A,C,E) and 6-9 weeks of age (B,D,F). Vertical line at the top of each bar is the SEM of 9-14 mice. Different letters above the bars indicate a significant difference ($P<0.05$) between groups of the same age.

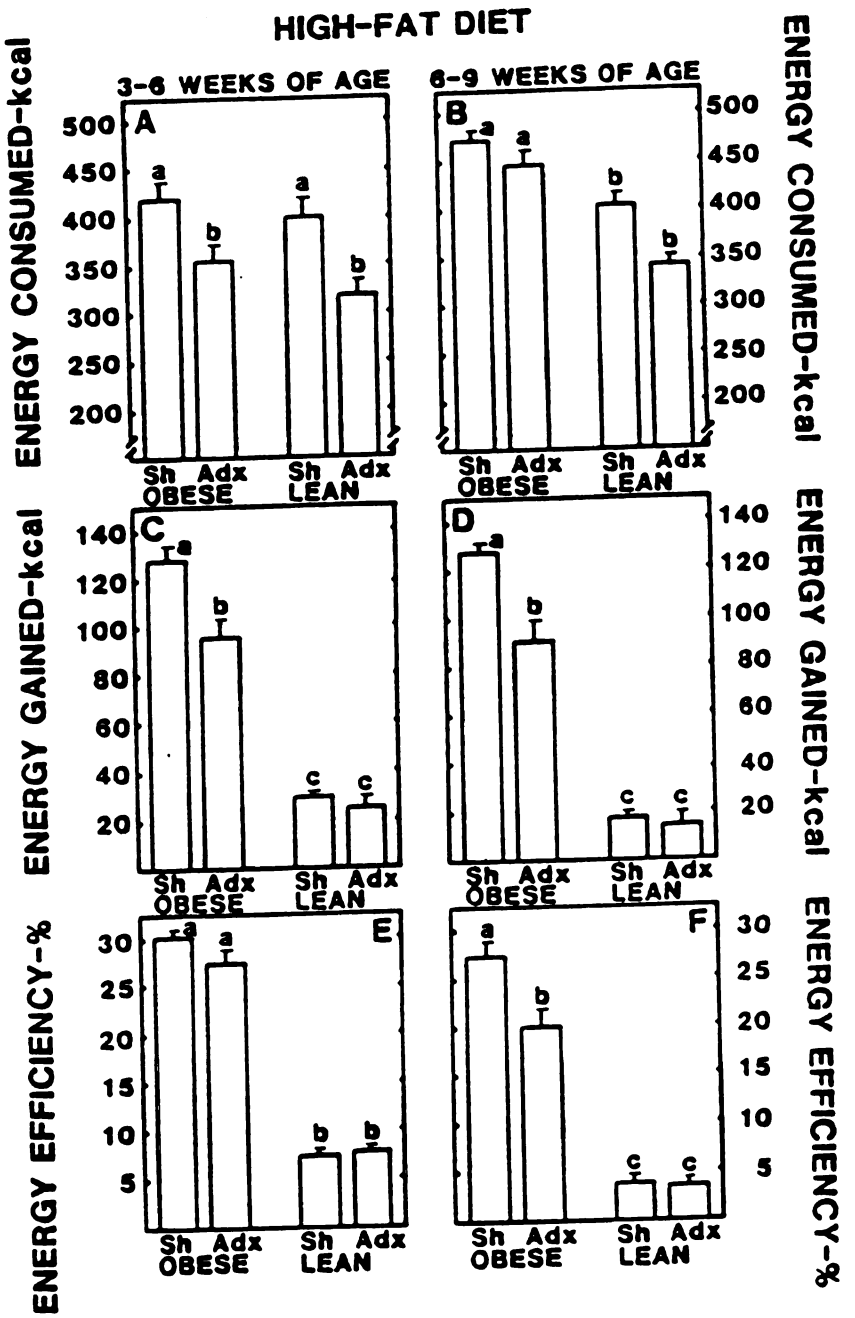

Figure 19. Energy consumption, gain and efficiency of mice fed high fat diet from 3-6 weeks of age and 6-9 weeks of age.

gain significantly more energy than sham leans from 6-9 weeks of age (Figure 19D). Adrenalectomized obese mice gained 30% less energy than sham obese from 6-9 weeks of age, but this was still 4 1/2 times the energy gain of lean mice. Thus, the normalization in efficiency of energy retention seen in adrenalectomized obese mice fed stock diet was not seen when adrenalectomized obese mice were fed the high-fat diet (Figure 19E and 19F). Efficiency of energy retention of 3-6 week old adrenalectomized obese mice was only 10% lower than that of sham-operated obese mice and was 3 1/2 times the efficiency of lean mice. From 6-9 weeks of age, sham-operated obese mice retained energy with 6 times the efficiency of sham leans (Figure 19F). Efficiency of energy retention of adrenalectomized obese mice was slightly (25%) less than that of sham-operated obese, but was still 4 1/2 times the efficiency of lean mice.

Consumption of the high-fat diet also blocked the effects of adrenalectomy on changes in body composition in obese mice. Energy density of sham-operated obese mice fed high-fat diet increased by 67% between 3 and 6 weeks of age, so that at 6 weeks of age, energy density of obese mice was more than twice that of leans (Figure 20A). Adrenalectomy of 3 week old obese mice did not prevent this increase in energy density as it had in obese mice fed stock diet. Energy density of sham-operated obese mice increased by 38% between 6 and 9 weeks of age (Figure 20B). Adrenalectomy of 6 week old obese mice did not prevent the increase in energy density from 6-9 weeks of age, and body energy density of adrenalectomized obese mice remained more than twice that of lean mice. These results indicate that consumption of the high-fat diet prevented the improvement

Figure 20

Energy density, muscle weights and bone lengths of mice fed high-fat diet from 3-6 weeks of age (A,C,E,G) and 6-9 weeks of age. Striped portion of bars represents means of predicted values for mice at the beginning of the experiment. All predicted values for obese mice at the beginning of the experiment were significantly different ($P<0.05$) than initial leans. Open portion of bars represents gain during the experimental period. Sh = sham-operated and Adx = adrenalectomized. Vertical lines on each bar are the SEM of 9-14 mice. Different letters above the bars indicate a significant ($P<0.05$) difference between groups at the end of the experimental period.

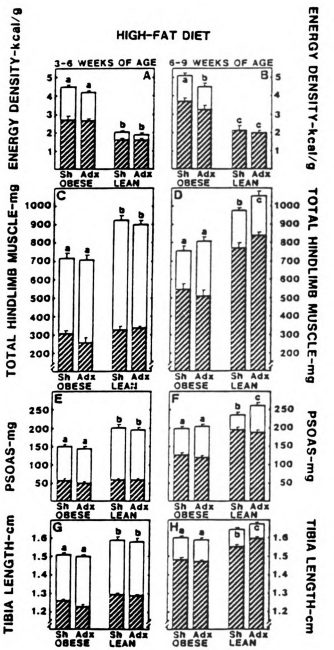

Figure 20. Energy density, muscle weights and bone lengths of mice fed high-fat diet from 3-6 weeks of age and 6-9 weeks of age.

in lean body mass seen when obese mice fed stock diet were adrenalectomized. The results for muscle weights confirm this (Figures 20C, 20D, 20E, 20F). Adrenalectomy of 3 or 6 week old obese mice fed high-fat diet did not increase muscle gain in these obese mice - final hindlimb muscle weight remained at 75% that of leans (Figure 20C, 20D). The results for the psoas muscle confirm this lack of effect of adrenalectomy on muscle mass in obese mice fed the high-fat diet (Figure 20E, 20F). Likewise, adrenalectomy failed to increase tibia length in obese mice fed the high-fat diet (Figure 20G, 20H), in contrast to the increases in tibia length observed after obese mice fed the stock diet were adrenalectomized.

Plasma insulin concentration of 6 week old obese mice was 9 times that of sham leans (Figure 21A). Adrenalectomy of obese mice fed the high-fat diet did not prevent this hyperinsulinemia - plasma insulin of 6 week old adrenalectomized obese was the same as that of sham obese. By 9 weeks of age, plasma insulin concentration of sham-operated obese mice was almost 25 times that of leans (Figure 21B). Adrenalectomy partially prevented this hyperinsulinemia in 6-9 week old obese mice, but their plasma insulin concentration was still 9-10 times that of leans.

Experiment 3 (High-fat diet - restricted).

Basal concentration of plasma corticosterone of sham-operated obese mice fed high-fat diet in restricted amounts was 16.0 ug/dl, as compared to 6.7 ug/dl in sham leans. Stressed concentrations of corticosterone in obese and lean mice were 30.8 and 14.6 ug/dl, respectively. Successful adrenalectomies were confirmed in 70% of the mice that underwent adrenalectomy. The mean basal corticosterone

Figure 21

Plasma insulin concentrations of mice fed high-fat diet from 3-6 weeks of age (A) and 6-9 weeks of age (B). Sh = sham-operated and Adx = adrenalectomized. Vertical line on top of each bar is the SEM of 3-5 samples of plasma pooled from 2-3 mice each. Different letters above the bars indicate a significant ($P<0.05$) difference between groups of the same age.

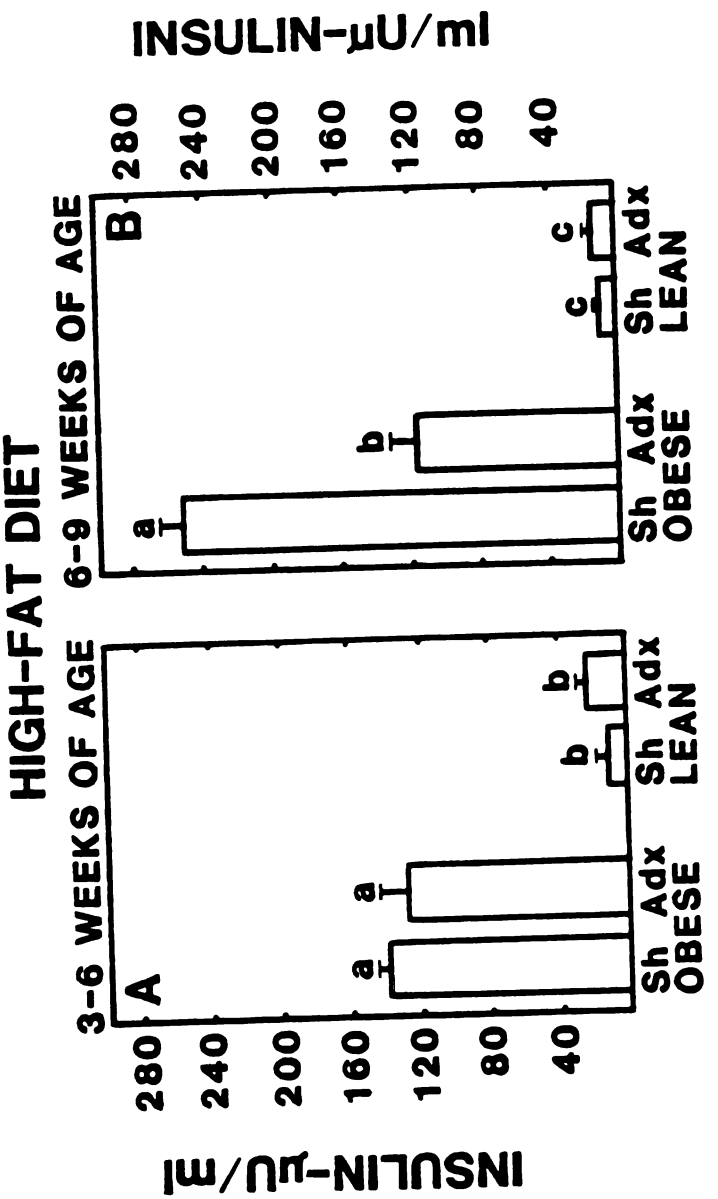

Figure 21. Plasma insulin concentrations of mice fed high-fat diet from 3-6 weeks of age and 6-9 weeks of age.

concentrations in adrenalectomized obese and lean mice were 4.4 and 2.5 ug/dl, respectively, with no increase after stress.

All groups of restricted-fed mice consumed approximately 300 kcal between 6 and 9 weeks of age (Table 4). Compared to ad libitum fed mice consuming the high-fat diet, this represented a restriction of 50% and 25% for obese and lean mice, respectively. Sham-operated obese mice gained more than 6 times as much body energy as sham leans (Table 4). Adrenalectomy reduced energy gain, but it was still almost 5 times that of lean mice. Sham-operated obese mice retained energy 7 times more efficiently than sham-operated leans (Table 4). Adrenalectomy of the restricted-fed obese mice reduced the efficiency of energy retention by 50%, but this was still 5 times the efficiency of leans. Energy density of these sham-operated obese mice was the same as that of sham obese fed the high-fat diet ad libitum (Table 4 and Figure 20B). Adrenalectomy of food-restricted obese mice only reduced their body energy density by 10%, indicating that their lean body mass was not increased by adrenalectomy. Adrenalectomized obese mice gained only slightly more hindlimb muscle than sham obese; final hindlimb muscle weight in the adrenalectomized obese was still only 70% that of adrenalectomized leans (Table 5). Thus, restricting the energy intake of mice fed the high-fat diet to the same as that of mice fed stock diet did not change the outcome of adrenalectomy in the mice fed high-fat diet.

Table 4. Energy balance of energy restricted mice [1]

	Energy consumed, kcal		Pooled SEM[2]
	Sham	Adx[3]	
Obese	303[a,4]	289[b]	2.84
Lean	299[a]	295[a,b]	
	Energy gained, kcal		
Obese	65[a]	26[b]	2.61
Lean	10[b,c]	6[c]	
	Energy efficiency, %		
Obese	22[a]	11[b]	0.82
Lean	3[b,c]	2[c]	
	Energy density, kcal/g		
Obese	5.1[a]	4.6[b]	0.09
Lean	2.0[b,c]	1.9[c]	

[1]Six obese and lean mice were fed high-fat diet for 3 weeks to match energy intake of 6-9 week old lean mice in Experiment 1.

[2]Pooled SEM = pooled standard error of the mean.

[3]Data presented as the mean of 9-11 mice, Sham = sham-operated and Adx = adrenalectomized.

[4]Different superscripts indicate a significant difference ($p < 0.05$) between groups.

Table 5. Muscle weights of restricted mice[1]

	Sham	Adx
	Total hindlimb muscle, mg	
Obese	660±21[2,3]	788±27
Lean	1020±17	998±44
	Psoas, mg	
Obese	157±6	163±7
Lean	234±4	221±4

1. Six obese and lean mice were fed high-fat diet for 3 weeks to match energy intake of 6-9 week old lean mice in Experiment 1.

2. Data presented as the mean ± SEM of 9-11 mice, Sham=sham-operated and Adx=adrenalectomized.

3. Different superscripts indicate a significant difference ($p<0.05$).

Discussion

These results demonstrate that adrenalectomy of obese mice fed stock diet normalized their efficiency of energy retention. Removal of the adrenals is one of only two manipulations, the other being prolonged cold exposure (Chapter 2), that has been shown to normalize efficiency of energy retention in ad libitum-fed obese mice. The effects of adrenalectomy on energy balance of obese mice fed stock diet cannot be attributed to their reduced energy intake, since pair-feeding intact obese mice to leans did not decrease their efficiency of energy retention (Chapter 2). Thus, adrenalectomy must reduce the efficiency of energy retention in obese mice by increasing their energy expenditure. The relatively low energy expenditure in intact obese mice is thought to result in large part from low heat production by brown adipose tissue (87). Heat production by brown adipose tissue is stimulated by the sympathetic nervous system, and turnover of norepinephrine in the tissue is an indicator of sympathetic stimulation. The low rates of norepinephrine turnover observed in brown adipose tissue of intact obese mice (41) (about half the rates observed in lean mice) were increased to nearly equal the rates in brown adipose of lean mice when obese mice were adrenalectomized (91). This increased sympathetic stimulation of brown adipose tissue would be expected to lead to a greater heat production by this tissue, and consequently, a lower efficiency of energy retention, consistent with the results of the present study. Adrenalectomy of obese Zucker rats also reduced their efficiency of

energy retention to the same as that of lean mice (49). Evidence was presented to implicate increased thermogenesis by brown adipose tissue in this lowering of efficiency of energy retention in adrenalectomized obese rats (49).

Although adrenalectomy normalized efficiency of energy retention in obese mice fed stock diet, it did not reverse the obesity which had developed in these animals prior to surgery. Body energy density of sham-operated obese mice increased by approximately 30% during each of the 3 week experimental periods, indicating a shift to a higher proportion of body fat as the animals aged. Adrenalectomy of obese mice fed stock diet prevented this increase in energy density, though their energy density was still higher than that of lean mice. These findings are the same as those with adrenalectomized Zucker obese rats - body energy density of adrenalectomized obese rats remained similar to that of a group killed at the start of the experiment (49).

For body energy density to have remained stable in adrenalectomized obese mice as compared to the increase in sham-operated obese, the proportions of lean tissue gain must have been greater in adrenalectomized than in sham obese. Consistent with this, muscle gain of adrenalectomized obese mice fed stock diet was significantly higher than that of sham-operated obese. This improvement was seen in the muscle from the trunk as well as in hindlimb muscle, though the increase was statistically significant only in the hindlimb muscle. In agreement with our results, Saito and Bray found that gastrocnemius muscle of adrenalectomized obese mice was significantly heavier than that of sham-operated obese

(65). Bone length was also improved by adrenalectomy of obese mice in the present study, especially in the 3-6 week old group. This increased growth of the bone may have contributed to the increased muscle growth (36).

Adrenalectomy had little or no effect on energy balance and body composition of lean mice. These results are similar to those from other studies in which adrenalectomy resulted in changes in energy balance and body composition in obese animals, but had little effect on leans (49,65). Thus, it appears that the adrenal gland is necessary for the development of obesity in stock-fed obese animals, but not for the normal development of weanling lean animals.

The effects of adrenalectomy on energy balance and body composition of obese mice did not differ markedly between 3 week old mice in which obesity was not yet obvious and 6 week old mice in which obesity was more fully developed. At both ages, adrenalectomy reduced energy gain and efficiency and improved muscle gain. This contrasts with the effects of adrenalectomy in GTG-treated mice. In GTG-treated mice which were not yet obese, adrenalectomy resulted in the maintenance of food intake and body weight at the same level as in lean (non GTG-treated) mice (14). In obese GTG-treated mice, adrenalectomy resulted in a rapid decline in both food intake and body weight gain and the maintenance of these low levels (15). These obese GTG-treated mice were considerably older (4 1/2 months of age) and heavier (40-55 g) than our 6-9 week old obese mice (average initial weight = 28 g) when adrenalectomy was performed. This may have contributed to the more drastic effects of adrenalectomy on food intake and weight gain in the obese GTG-treated mice compared to our

6-9 week old obese mice.

Although our results demonstrate a clear effect of adrenalectomy on energy balance and body composition of obese mice, they do not distinguish which of the adrenal secretions is responsible for the development of obesity. Several lines of evidence point to the glucocorticoid, corticosterone (the primary form of glucocorticoid in rodents (87)), as the effector. Plasma corticosterone of sham-operated obese mice is 2-5 times that of lean mice (Figures 1 and 6). This high concentration of corticosterone may contribute to the high efficiency of energy retention in obese mice, through the suppression of brown adipose tissue activity (34). High concentrations of corticosterone may also contribute to reduced muscle mass in obese mice, through decreased muscle protein synthesis and increased protein breakdown (60,66). Additional evidence for a role for glucocorticoids includes the observations that administration of glucorticoids to adrenalectomized obese animals prevents the effects of adrenalectomy (7,34,65). By contrast, administration of desoxycorticosterone, a mineralocorticoid, to adrenalectomized GTG-treated mice decreased food intake and body weight to levels below that of either adrenalectomized GTG-treated or untreated mice (14).

The question remains as to whether the adrenal secretions (probably corticosterone) affect energy balance and body composition directly or indirectly. As mentioned earlier, prolonged cold exposure is another treatment which normalizes efficiency of energy retention (though not muscle mass (Chapter 3)) in obese mice.

However, plasma corticosterone concentrations were even higher in cold-acclimated obese mice than in obese mice housed at normal room temperature. Thus, the two treatments which result in normalized efficiency of energy retention in obese mice (namely, adrenalectomy and prolonged cold exposure), have opposite effects on plasma corticosterone. This argues against a direct role of corticosterone in altering energy balance. Also, permissive amounts of glucocorticoids are required for the normal response of brown adipose to norepinephrine and cold (22). This would support the argument against a direct action of glucocorticoids in suppressing brown adipose activity. A third argument against a direct role of corticosterone in altering energy balance and body composition comes from the data obtained when the high-fat diet was fed to adrenalectomized obese mice.

Consumption of the high-fat diet prevented most of the effects of adrenalectomy in obese mice. Body weight gain was somewhat reduced in these adrenalectomized obese mice, but was still 1 1/2 - 3 times that of lean mice. This contrasts with the results in stock-fed obese mice, where adrenalectomy reduced body weight gain so that it equaled that of lean mice. Energy intake of adrenalectomized obese mice fed the high-fat diet was 10-30% higher than that of adrenalectomized leans, and efficiency of energy retention was 3 1/2 - 4 times that of leans. Energy density of adrenalectomized obese mice increased by 25-55% during the 3 week experimental periods, unlike in adrenalectomized obese mice fed stock diet. Muscle and bone gain of obese mice fed the high-fat diet were also not improved by adrenalectomy. Thus, unlike in stock-fed ob/ob mice, obesity in

adrenalectomized ob/ob mice fed the high-fat diet continued to develop to almost the same degree as in sham-operated ob/ob mice. Restricting the energy intake of high-fat diet to the same level as that of leans fed stock diet did not appreciably alter the outcome of adrenalectomy in obese mice fed high-fat diet.

Thus, the diet fed to obese mice had a dramatic effect on the outcome of adrenalectomy. The two diets used here differed in both form (pelleted stock diet vs. powdered semipurified diet) and composition (high-carbohydrate stock diet vs. high-fat semipurified diet). It is not clear from this study which of these factors was most critical. However, other studies have also shown differences due to the form or composition of the diet. The effects of combined ovariectomy-adrenalectomy of VMH-lesioned rats fed a pelleted stock diet were prevented when either a semipurified high-fat or a liquid high-carbohydrate diet was fed, suggesting the importance of the form of the diet (soft or liquid semipurified diets vs. hard pelleted diet) in determining the response to treatment (52). The composition of the diet may also play an important role in determining metabolic effects of treatments. Several studies have shown that the consumption of a high-fat diet by rats can result in insulin resistance in muscle and adipose tissue, even though energy intake and plasma insulin concentrations were the same as those of rats fed high-carbohydrate diets (37,84,91). Thus, both the form and composition of the diets may have contributed to the results of adrenalectomy in ob/ob mice. The absence of any major effects of adrenalectomy on energy balance and body composition of obese mice fed high-fat diet indicates that adrenal secretions per se are not

responsible for the development of obesity in ob/ob mice fed a high-fat diet.

The effects of adrenalectomy on obese mice must therefore be mediated by some other factor which can be influenced by diet. One possibility for this mediator is tissue responsiveness to insulin. In sham-operated obese mice fed stock diet, plasma insulin concentration was 4-30 times that of lean mice, and plasma corticosterone was 2-5 times that of leans. This hyperinsulinemia coupled with the high plasma corticosterone concentration would contribute to and maintain the insulin resistance observed in skeletal muscle and adipose tissue of obese mice (29,48,55). Adrenalectomy of obese mice fed stock diet not only reduced their plasma corticosterone, it also resulted in normal to near-normal concentrations of plasma insulin. The lower concentration of both corticosterone and insulin should enable muscle and adipose tissue to regain insulin sensitivity. A recent study by Ohshima et al. (56) confirmed that adrenalectomy of obese mice fed stock diet did restore insulin sensitivity to muscle of ob/ob mice. This would facilitate the improved growth of muscle seen in adrenalectomized obese mice.

The reduction in efficiency of energy retention observed in adrenalectomized obese mice may also have involved tissue responsiveness to insulin as a mediator. As mentioned earlier, increased activity of brown adipose tissue of adrenalectomized obese animals may be largely responsible for this reduced efficiency. Both norepinephrine turnover (84) and GDP binding (49) have been shown to be restored to the levels found in lean animals following adrenalectomy of stock-fed obese animals, indicating normalized

metabolic capacity of brown adipose tissue. Insulin has been demonstrated to be required for thermogenesis in brown adipose (63). However, it is not clear whether insulin acts directly on the brown adipose tissue or indirectly via central activation of thermogenesis. Thus, the decreased capacity for thermogenesis observed in intact obese mice (33,41) could be due to resistance at a central site of activation, or the brown adipose tissue itself may be resistant to stimulation of thermogenesis by insulin. Adrenalectomy of ob/ob mice might restore the sensitivity of either or both of these sites to insulin, resulting in more normal stimulation of the sympathetic nervous system, the return of insulin-stimulated increases in heat production, and a decrease in the efficiency of energy retention.

Insulin sensitivity may also be the key to the mechanism whereby consumption of the high-fat diet prevented the effects of adrenalectomy in obese mice. Several studies have shown that adipose tissue and skeletal muscle of rats fed high-fat diets are resistant to insulin's actions on glucose metabolism, even though their energy intake and plasma insulin concentrations were similar to those of rats fed high-carbohydrate diets (37,43,75,91). Consumption of the high-fat diet by our adrenalectomized obese mice may have resulted in the production of insulin resistance, masking the effect of adrenalectomy in restoring insulin sensitivity. Thus, thermogenesis in brown adipose tissue of adrenalectomized obese mice fed the high-fat diet may have remained similar to that of sham-operated obese mice, allowing the efficiency of energy retention to remain elevated. The improvements in muscle gain in adrenalectomized obese

mice may also have been blocked by insulin resistance induced by the high-fat diet. Because lean mice did not become grossly obese or exhibit reduced muscle accumulation when fed the high-fat diet, one would have to speculate that ob/ob mice have a greater susceptibility to the development of insulin resistance than lean mice, if in fact tissue sensitivity to insulin contributes to the development of obesity in ob/ob mice.

Chapter 5. Effects of adrenalectomy on energy balance, body composition, and insulin sensitivity of obese mice mice fed semipurified diets.

When obese mice fed stock diet are adrenalectomized, their efficiency of energy retention is reduced to equal that of lean mice and skeletal muscle weights are improved by 20-30% (Chapter 4). However, when obese mice are fed a semipurified high-fat diet, these effects of adrenalectomy are not observed. Thus, the effects of adrenalectomy on energy balance in obese mice are dependent on the diet consumed by the mice.

The reduced efficiency of energy retention in adrenalectomized obese mice fed stock diet is probably due to increased energy expenditure, resulting from increased heat production by brown adipose tissue. Adrenalectomy of obese rodents has been demonstrated to increase norepinephrine turnover and GDP binding in brown adipose tissue to almost the same level as in leans (49,84). The mechanism for this increased brown adipose activity, as well as for the increased muscle mass of adrenalectomized obese mice, may involve changes in tissue responsiveness to insulin. Both adipose tissue and skeletal muscle of intact obese mice are resistant to insulin's actions (29,48). Insulin is required for the normal thermogenic function of brown adipose tissue (63); resistance to insulin's actions on brown adipose could contribute to reduced energy expenditure in obese mice, resulting in their high efficiency of energy retention. Insulin resistance could also contribute to the reduction in muscle mass observed in intact obese mice (Chapter 3).

Adrenalectomy of obese mice fed stock diet restores insulin sensitivity to muscle tissue (56). This is probably an important factor in the improvement in muscle mass of adrenalectomized obese mice fed stock diet. Restoration of insulin sensitivity to brown adipose tissue or to sites regulating brown adipose would allow insulin-stimulated thermogenesis in adrenalectomized obese mice fed stock diet. This could contribute to their reduced efficiency of energy retention. Thus, the primary effects of adrenalectomy on energy balance and body composition of obese mice fed stock diet may have been due in large part to the restoration of insulin sensitivity to their tissues. Consumption of high-fat diet by the obese mice may have induced insulin reisistance in muscle and adipose, as it has been shown to do in rats (37,43). This insulin resistance induced by the high-fat diet may have overridden the effect of adrenalectomy in restoring insulin sensitivity.

This experiment was designed for two purposes. The first purpose was to compare the effects of adrenalectomy on energy balance and body composition in mice fed either a semipurified high-carbohydrate diet or a semipurified high-fat diet. This was done to test the hypothesis that it was the high proportion of fat in the semipurified high-fat diet that prevented the effects of adrenalectomy on energy balance and body composition in obese mice fed this diet. Thus, two semipurified diets which differ only in the proportion of fat and corbohydrate were used, rather than comparing the stock diet and a semipurified diet, which differ in several ways in addition to the fat content.

The second purpose of this experiment was to determine if adrenalectomy restores insulin sensitivity in obese mice and whether the diet consumed by the adrenalectomized obese would affect insulin sensitivity. This was done to test the hypothesis that changes in energy balance and body composition induced by adrenalectomy can be correlated with improved insulin sensitivity. A glucose challenge was used as an indicator of insulin sensitivity in the mice. Clearance of a glucose load in intact obese mice is abnormal relative to lean mice (72,73,88). This method measures a biological response (glucose removal from the blood) to endogenous insulin and includes the assumption that the defect in the action of insulin is in the tissues' response to insulin, and is not due to a structural abnormality in the insulin molecule nor to abnormal release from the pancreas. This has been shown to be the case for ob/ob mice (23,27). A drawback of this method is that several hormones besides insulin also act to influence glucose metabolism. A primary example is corticosterone, which acts to raise blood glucose concentrations. Plasma corticosterone concentrations were measured at several time points during the glucose tolerance test.

Mice were adrenalectomized at 4 weeks of age, since recovery from both adrenalectomy and sham surgery was found to be slow in younger mice in previous experiments. Body weights of 4 week old obese and lean mice are still similar, so that effects of adrenalectomy on the development of obesity should be obvious. The diets were fed for 3 weeks, which has been shown to be sufficient to observe alterations in energy balance and body composition due to the development of obesity or due to adrenalectomy.

Materials and Methods

Animals

Obese (ob/ob) and lean (ob/+ or +/+) male mice were obtained from our breeding colony of C57BL/6J-ob/+ mice. The breeding colony is housed at 23C with lights on from 0700 to 1900 hours daily. Pups are weaned at 3 weeks of age. At 3 1/2 weeks of age, obese and lean pairs were separated from their littermates and housed individually in solid-bottom plastic cages with wood shavings as bedding. After 3-4 days of adaptation to single housing, mice were bilaterally adrenalectomized (Adx) or sham-operated (Sh) through dorsal incisions while under ether anesthesia. After surgery, drinking water of Adx mice was replaced with physiological saline (0.9% NaCl). An initial group of 4 week old mice was killed for baseline data. All mice had food and water (or saline) available ad libitum. Food intake and body weights were monitored 2-3 times weekly.

Mice were fed either a high-carbohydrate or high-fat semipurified diet for 3 weeks. The high-carbohydrate diet contained (g/100g) mineral mix (4), 3.5; vitamin mix (4),1.0; cellulose, 5.0; methionine, 0.3; choline chloride, 0.2; casein, 20.0; cerelose, 62.0; and corn oil, 8.5, providing 60% of the metabolizable energy as carbohydrate, 20% as protein, and 20% as fat. The high-fat diet had the same composition with the following exceptions (g/100 g):mineral mix, 4.8; vitamin mix, 1.4; casein, 27.6; cerelose, 27.3; corn oil, 16.3; and tallow, 16.3, providing 60% of the metabolizable energy as fat, 20% as protein, and 20% as carbohydrate.

Analyses

At the end of the experimental period, the glucose challenge was performed. Mice were fasted for 12 hours (from 2100 hr - 0900 hr the next morning) before the test. A baseline (0 minute) blood sample of approximately 100 ul was taken from the orbital sinus of each unanesthetized mouse, within 1 minute of removing the mouse from its cage. An intraperitoneal injection of 25 mg glucose (in a volume of 0.25 ml) was given immediately after this baseline blood sample, and subsequent blood samples of approximately 40 ul each were taken from the orbital sinus at 5, 10, 20, 40, 60 and 90 minutes after the glucose injection. Mice were killed by cervical dislocation immediately after the 90 minute blood sample was taken. Plasma from each time point was assayed for glucose (Boehringer-Mannheim, Indianapolis, IN). Plasma from the 0, 10, 40, and 60 minute time points was assayed for corticosterone by radioimmunoassay (Endocrine Sciences, Tarzana, CA). Only those adrenalectomized mice whose plasma corticosterone at 0 minutes was less than half that of sham-operated mice and whose corticosterone concentration at 10 minutes was not increased by more than 20% above the baseline (0 minute) concentration were included in the analyses.

The hindlimb muscles were stripped from the bones and weighed, and the length of the tibia was recorded. Body energy of the carcass (including the tissues removed for weighing) was measured by direct calorimetry. Carcasses were softened in an autoclave at 100C, then homogenized (Brinkmann Polytron, Brinkmann Instruments, Westbury, NY) in 2-3 times their weight of water. An aliquot of homogenate was dried at 50C and used to determine energy content with an adiabatic

calorimeter (Parr Instrument Co., Moline, IL). Body energy density was calculated as kcal/g body weight. The diets were also analyzed for gross energy content (high-carbohydrate diet= 4.4 kcal/g; high-fat diet= 5.6 kcal/g). Energy efficiency was calculated as body energy gained divided by gross energy consumed during the 3 week experimental period. Body energy gain was calculated as body energy content at the end of the experimental period minus the predicted body energy content at the beginning of the experiment. Body energy content at the beginning of the experiment was predicted from a linear regression equation based on body weights and body energy content of the initial group. Body weight of each mouse at the beginning of the experiment was then used to predict its initial body energy content. This linear regression method was also used to predict initial hindlimb muscle weights and tibia lengths for the calculation of muscle and bone gains.

Data are presented as mean + SEM. Data were analyzed by 2-way analysis of variance. Specific treatment effects were determined by Bonferroni t-test (74).

Results

Energy consumption of sham-operated obese mice fed the high-carbohydrate and high-fat diets was 17% and 10% higher, respectively, than that of sham leans (Table 6). Adrenalectomy reduced energy consumption of obese mice more than that of lean mice so that intake of adrenalectomized obese equaled that of leans.

Sham-operated obese mice fed either the high-carbohydrate or

Table 6. Energy consumption and body weights of obese and lean mice[1]

	High-carbohydrate diet			High-fat diet		
	Sham[2]	Adx	Pooled SEM[3]	Sham	Adx	Pooled SEM
	Energy consumed, kcal					
Obese	426[a,4]	322[b]	16.6	440[a]	319[b]	22.3
Lean	365[b]	328[b]		405[a]	326[b]	
	Weight gain, g					
Obese	16[a]	12[b]	0.5	20[a]	12[b]	0.8
Lean	9[c]	8[c]		9[c]	8[c]	
	Final body weight, g					
Obese	30[a]	26[b]	0.8	35[a]	27[b]	1.0
Lean	24[c]	23[c]		24[c]	23[c]	

1. Obese and lean mice were sham-operated or adrenalectomized at 4 weeks of age and fed the appropriate diet for 3 weeks.

2. Sham=sham-operated, Adx=adrenalectomized.

3. Pooled SEM=Pooled standard error of the mean.

4.Data presented as the mean of 9-10 mice. Different superscripts indicate a significant difference ($P<0.05$) between groups within diet.

high-fat diets gained almost twice as much body weight as sham-operated leans (Table 6). Adrenalectomy reduced weight gain of obese mice by 25-35%, but final body weight of adrenalectomized obese was still 16% higher than leans.

Sham-operated obese mice fed the high-carbohydrate and high-fat diets gained 4 - 4 1/2 times as much body energy as sham leans between 4 and 7 weeks of age (Table 7). Adrenalectomy reduced energy gain of obese mice by 40-50%, but this was still almost 3 times the gain of leans.

Sham-operated obese mice fed either the high-carbohydrate or high-fat diet were 4 - 4 1/2 times as efficient at retaining dietary energy as sham leans (Table 7). Adrenalectomy reduced energy efficiency of obese mice by almost 30%, but it was still almost 3 times that of leans. Adrenalectomy did not affect the energy gain or efficiency of energy retention of lean mice fed either the high-carbohydrate or high-fat diet (Table 7).

Energy density of 4 week old obese and lean mice was 2.8+0.05 and 1.5+0.02 kcal/g, ntion in obese mice were all reduced by approximately the same extent after adrenalectomy, but remained significantly higher than in lean mice, regardless of which diet they consumed. Hindlimb muscle gain in obese mice fed the high-carbohydrate diet was improved more by adrenalectomy than that of obese mice fed the high-fat diet, though hindlimb muscle of adrenalectomized obese on both diets obese indicates that adrenalectomy improved the proportion of lean body mass of obese mice. The muscle data for obese mice fed high-carbohydrate diet confirmed this. Hindlimb muscle of 4 week old obese and lean mice

Table 7. Energy gain and efficiency of energy retention of obese and lean mice[1].

	High-carbohydrate diet[2]			High-fat diet		
	Sham	Adx	Pooled SEM[3]	Sham	Adx	Pooled SEM
	Energy gain, kcal					
Obese	98[a,4]	59[b]	3.8	121[a]	64[b]	6.0
Lean	23[c]	21[c]		27[c]	22[c]	
	Energy efficiency, %					
Obese	23[a]	18[b]	0.8	28[a]	19[b]	1.6
Lean	6[c]	6[c]		6[c]	7[c]	

1. Obese and lean mice were sham-operated or adrenalectomized at 4 weeks of age and fed the appropriate diet for 3 weeks.

2. Sham=sham-operated, Adx=adrenalectomized.

3. Pooled SEM=Pooled standard error of the mean.

4. Data presented as mean of 9-10 mice. Different superscripts indicate significant difference ($P<0.05$) between groups within diet.

weighed 310 and 420 mg, respectively. Sham-operated obese mice fed the high-carbohydrate diet gained only 200 mg hindlimb muscle, which is less than half the gain of sham leans. As a result, final hindlimb muscle weight of sham-operated obese mice was only 60% that of leans (Table 8). Adrenalectomy improved hindlimb muscle gain of obese mice by 53%, so that final muscle weight of adrenalectomized obese mice fed high-carbohydrate diet was almost 75% that of adrenalectomized leans.

Improvements in muscle gain were not as marked in obese mice fed the high-fat diet. Sham-operated obese mice fed the high-fat diet gained 295 mg hindlimb muscle, which was about 60% as much as sham leans. As a result, final hindlimb muscle weight of sham-operated obese mice was only 66% that of sham leans (Table 8). Adrenalectomy improved muscle gain of obese mice fed high-fat diet to only 317 mg, so that final muscle weight remained at about 70% that of leans. Thus, adrenalectomy of obese mice fed a high-carbohydrate diet did significantly improve muscle gain, whereas in obese mice fed high-fat diet, adrenalectomy did not improve muscle gain. The tibia of sham-operated obese mice fed either high-carbohydrate or high-fat diet increased in length slightly more than that of sham leans, but because the initial bone length in sham obese was only 90% that of initial leans, final tibia length of sham obese was only 92% that of sham leans (Table 8). Adrenalectomy did not improve the gain in tibia length in obese mice fed either diet. Adrenalectomy had no effect on energy density, muscle weight or bone length of lean mice.

The plasma glucose concentration of sham-operated obese mice fed the high-carbohydrate diet increased to more than 4 1/2 times the

Table 8. Energy density, muscle weights and bone lengths of obese and lean mice[1].

	High-carbohydrate diet[2]			High-fat diet		
	Sham	Adx	Pooled SEM[3]	Sham	Adx	Pooled SEM
	Energy density, kcal/g					
Obese	4.5[a,4]	3.6[b]	0.09	4.6[a]	3.8[b]	0.11
Lean	1.9[c]	1.9[c]		2.0[c]	1.9[c]	
	Total hindlimb muscle, mg					
Obese	508[a]	617[b]	27.5	596[a]	641[a]	31.5
Lean	850[c]	852[c]		904[b]	882[b]	
	Tibia, cm					
Obese	1.5[a]	1.5[a]	0.05	1.5[a]	1.5[a]	0.01
Lean	1.6[b]	1.6[b]		1.6[b]	1.6[b]	

1. Obese and lean mice were sham-operated or adrenalectomized at 4 weeks of age and fed the appropriate diet for 3 weeks.

2. Sham=sham-operated, Adx=adrenalectomized.

3. Pooled SEM=Pooled standard error of the mean.

4. Data presented as mean of 9-10 mice. Different

superscripts indicate significant difference ($P<0.05$) between groups within diet.

fasting level by 20 minutes after a glucose load, and remained elevated during the remainder of the glucose challenge (Figure 22A). In lean mice, plasma glucose concentration increased to a peak of approximately 3 times the fasting level by 20 minutes, then stayed at approximately 500 mg/dl until 60 minutes, and finally decreased by 90 minutes. Glucose concentrations of sham-operated obese mice were 1 1/2 - 2 times that of sham-operated leans from 10-. Adrenalectomy resulted in lower plasma glucose concentrations from 10 minutes on in both obese and lean mice (Figure 22A). The glucose challenge curves for adrenalectomized obese and lean mice basically paralleled those of their sham-operated counterparts, though plasma glucose concentrations in adrenalectomized mice were only 65-80% of shams.

The results for the glucose challenge in mice fed the high-fat diet were similar to those from mice fed the high-carbohydrate diet. Plasma glucose concentration of sham-operated obese mice fed high-fat diet averaged 1 1/2 -2 times that of sham-operated leans from 10-90 minutes (Figure 22B). Unlike in high-carbohydrate fed obese mice, however, plasma glucose of sham obese fed the high-fat diet peaked at 60 minutes and then decreased. The glucose challenge curve for sham-operated lean mice fed the high-fat diet was flatter than that of high-carbohydrate fed leans from 10-90 minutes, and the peak glucose concentration in leans fed the high-fat diet was slightly lower than that of leans fed the high-carbohydrate diet. Adrenalectomy reduced plasma glucose at all time points (except for 0 minutes in leans) by 20-40% in obese and 25-35% in lean mice fed the high-fat diet (Figure 22B).

Plasma corticosterone concentrations of sham-operated obese mice

Figure 22

Glucose challenge curves for obese and lean mice fed a high-carbohydrate diet (A) or a high-fat diet (B) from 4-7 weeks of age. Mice were given a dose of 25 mg glucose in .25 ml and blood was sampled from the orbital sinus at the indicated times. Sh=sham-operated, Adx=adrenalectomized. Obese mice are designated by the dotted line, lean mice are designated by the solid line. +=Significant difference due to adrenalectomy within diet and phenotype. Each point is the mean of 9-11 mice.

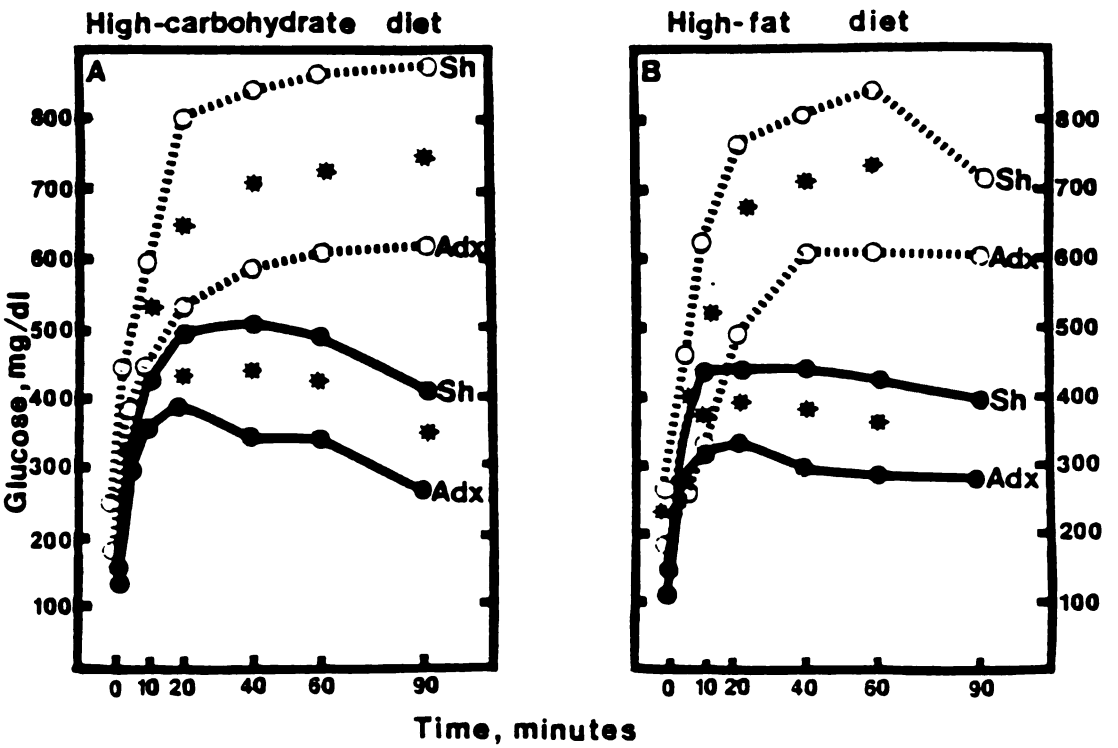

Figure 22. Glucose challenge curves for mice fed high-carbohydrate or high-fat diet from 4-7 weeks of age.

fed the high-carbohydrate diet were more than 4 times that of sham-operated leans at the 0 minute time point (Table 9). Between 0 and 60 minutes, plasma corticosterone concentration of sham-operated obese mice doubled, and increased almost 7 1/2 fold in sham leans. Due to this relatively large increase in corticosterone of lean mice compared to obese, there was no difference in plasma corticosterone concentrations of obese and lean mice at 40 and 60 minutes. Successful adrenalectomies were confirmed in 69% of the mice fed the high-carbohydrate diet. These adrenalectomized mice maintained a low concentration of plasma corticosterone which rose only slightly during the glucose challenge (Table 9). The plasma corticosterone concentration of sham-operated obese mice fed the high-fat diet was twice that of sham-operated leans at 0 minutes (Table 10). The plasma corticosterone concentration of sham obese remained significantly higher than that of leans at the 10 minute time point, but due to large variations within the groups, there were no differences between sham obese and lean mice at 40 or 60 minutes. Successful adrenalectomies were confirmed in 82% of the mice that underwent surgery. As in the mice fed the high-carbohydrate diet, these adrenalectomized mice maintained a low concentration of plasma corticosterone with only a slight increase during the glucose challenge (Table 10).

Discussion

Adrenalectomy of obese mice had similar effects on their energy balance and body composition whether they were fed the

Table 9. Plasma corticosterone concentrations in obese and lean mice fed high-carbohydrate diet[1].

	Plasma corticosterone, ug/dl[2]			
	Time, minutes			
	0	10	40	60
Sham				
Obese	16.0[a,3]	20.7[a]	31.5[a]	30.9[a]
Lean	3.8[b]	10.4[b]	26.2[a]	27.0[a]
Adx				
Obese	2.0[c]	2.3[c]	2.8[b]	2.9[b]
Lean	1.8[c]	2.0[c]	4.1[b]	4.1[b]
Pooled SEM[4]	0.75	0.95	2.42	2.10

1. Obese and lean mice were sham-operated or adrenalectomized at 4 weeks of age and fed a high-carbohydrate diet for 3 weeks.
2. Plasma corticosterone was measured on plasma from blood taken from the orbital sinus at 0,10,40,and 60 minutes after a glucose load (25 mg/mouse).
3. Data presented as the mean of 9-10 mice. Different superscripts indicate a significant difference ($P<0.05$) between groups fed the same diet and at the same time.
4. Pooled SEM=Pooled standard error of the mean.

Table 10. Plasma corticosterone concentrations in obese and lean mice fed high-fat diet[1].

	Plasma corticosterone, ug/dl[2] Time, minutes			
	0	10	40	60
Sham				
Obese	10.5[a,3]	16.6[a]	27.2[a]	31.4[a]
Lean	4.8[b]	10.8[b]	21.8[a]	24.1[a]
Adx				
Obese	2.5[c]	2.3[c]	3.0[b]	2.7[b]
Lean	1.4[c]	1.5[c]	2.3[b]	2.5[b]
Pooled SEM[4]	0.64	0.82	1.80	2.21

1. Obese and lean mice were sham-operated or adrenalectomized at 4 weeks of age and fed a high-fat diet for 3 weeks.

2. Plasma corticosterone was measured on plasma from blood taken from the orbital sinus at 0,10,40, and 60 minutes after a glucose load (25 mg/mouse).

3. Data presented as the mean of 9-10 mice. Different superscripts indicate a significant difference ($P<0.05$) between groups fed the same diet and at the same time.

4. Pooled SEM=Pooled standard error of the mean.

high-carbohydrate or high-fat diet. Body weight, body energy gain, and efficiency of energy retention in obese mice were all reduced by approximately the same extent after adrenalectomy, but remained significantly higher than in lean mice, regardless of which diet they consumed. Hindlimb muscle gain in obese mice fed the high-carbohydrate diet was improved more by adrenalectomy than that of obese mice fed the high-fat diet, though hindlimb muscle of adrenalectomized obese on both diets remained lower than that of leans. These results contrast with those from obese mice fed stock diet, where adrenalectomy normalized body weight, energy gain and efficiency of energy retention and markedly improved muscle gains. Thus, the effects of adrenalectomy in obese mice fed a high-carbohydrate semipurified diet were not the same as those observed in obese mice fed a high-carbohydrate stock diet.

The results of the glucose challenge were also similar in obese mice fed either the semipurified high-carbohydrate or high-fat diet. Adrenalectomy did not normalize the response to a glucose load in obese mice fed either diet. The consistently high concentrations of corticosterone in sham-operated obese mice throughout the glucose challenge may have resulted in even higher plasma glucose concentrations than are usually observed in non-stressed obese mice. These high concentrations of corticosterone may also have suppressed insulin release, which would have slowed the rate of glucose utilization by insulin sensitive tissues. The reduced plasma glucose concentrations after a glucose load indicate that adrenalectomized obese mice were more sensitive to insulin's action in lowering blood glucose than sham-operated obese mice. However, these results do not

indicate the normalization of insulin sensitivity in adrenalectomized obese mice fed either diet. In contrast, a recent study showed that adrenalectomy of obese mice fed stock diet reduced their fasting blood glucose so that it equaled that of sham-operated leans (88). In addition, the glucose tolerance curves of adrenalectomized obese mice were the same as those of lean mice, and significantly lower than those of sham-operated obese mice at every time point. Unlike in mice fed semipurified diets then, adrenalectomy of obese mice fed stock diet does appear to normalize sensitivity to the actions of insulin. This is supported by Ohshima et al.'s finding that adrenalectomy abolished the insulin resistance in the muscle of obese mice (56). The results from the glucose challenge in the present study as well as these other tests of insulin sensitivity are consistent with the changes in energy balance and body composition observed in adrenalectomized obese mice fed the various diets and with the hypothesis that the diet consumed by obese mice influences the outcome of adrenalectomy through changes in insulin sensitivity. When adrenalectomized obese mice were fed stock diet, insulin sensitivity was restored (56,88), possibly contributing to the reduction in efficiency of energy retention and the improvement in muscle mass. However, when a semipurified high-carbohydrate or high-fat diet was consumed by adrenalectomized obese mice, the return of insulin sensitivity was blocked,and this may have resulted in the maintenance of high efficiency of energy retention and the reduced muscle mass.

Thus, the diet consumed by obese mice was important in influencing the outcome of adrenalectomy, but not simply because of differences in the proportion of fat in the diet, as was hypothesized

for this experiment. The semipurified diets differ in several ways from the stock diet. The source of the nutrients as well as their proportions are different in the two types of diets. The form of the diets also differs - the stock diet is a hard pelleted diet and the semipurified diet is a soft, powdered diet. Although this experiment did not completely separate these differences in diet composition and form as they affect adrenalectomy, the observation that specific metabolic effects of adrenalectomy, such as efficiency of energy retention and muscle growth, were altered by the diet points towards diet composition as the more important factor. Diet composition has been shown to have specific effects on brown adipose tissue metabolism as well as on insulin sensitivity of several tissues (32,37,43). Studies by Mook et al. (52) first showed the importance of diet in influencing the outcome of adrenalectomy. When fed a pelleted stock diet, VMH-lesioned rats which underwent combined ovariectomy-adrenalectomy did not demonstrate the excess food intake and body weight gain seen in control VMH-lesioned rats. However, when ovariectomized-adrenalectomized rats were fed either a semipurified high-fat or a liquid fortified eggnog diet after VMH lesioning, they consumed almost as much energy and gaines much body weight as VMH-lesioned controls. Although the form of the diets (pellets vs. powdered or liquid) may have affected the hyperphagia of these rats, no evidence was presented to show that the form of the diet would have any effects beyond those on food intake.

Adrenalectomy also lowered the glucose challenge curves of lean mice by 20-30% compared to sham-operated leans. In another study, fasting blood glucose of lean mice was reduced by almost 30% after

adrenalectomy (88). Thus, adrenalectomy may also improve responsiveness to insulin in lean mice, although this does not appear to have had any noticeable effects on energy balance or body composition. It is also likely that the high plasma corticosterone concentrations in sham-operated lean mice during the glucose challenge led to higher levels of plasma glucose than would be observed in non-stressed lean mice.

In conclusion, it still appears likely that the composition of the diet consumed by obese mice plays an important role in determining the outcome of adrenalectomy. This effect of diet composition is not simply due to differences in the proportion of fat in the diet; instead, more subtle differences in the amount and type of other dietary components must be important.

Chapter 6. General summary and conclusions.

These studies have described two treatments which normalized efficiency of energy retention of obese mice - namely, long term cold acclimation, and adrenalectomy of mice fed stock diet. The effects of these two treatments on body composition were quite different, however. Cold-acclimated obese mice had even less skeletal muscle than obese mice at normal room temperature, but a similar proportion of body fat. Adrenalectomy on the other hand, almost normalized skeletal muscle weights of obese mice, and lowered their proportion of body fat. These effects of adrenalectomy were not simply due to the absence of the adrenal gland however, since feeding semipurified diets to obese mice blocked almost all of the effects of adrenalectomy on energy balance and body composition. Further, these effects of cold-acclimation and adrenalectomy on body composition were probably both mediated in large part by the actions of corticosterone and insulin.

Both cold acclimation and adrenalectomy of stock-fed obese mice resulted in the normalization of efficiency of energy retention. However, these two treatments had opposite effects on plasma corticosterone concentrations - cold-acclimation resulted in a 50-150% increase in plasma corticosterone, while adrenalectomy reduced corticosterone concentrations to almost 0. The effects of these two treatments on insulin sensitivity also differed. Although cold-acclimation lowered plasma insulin concentrations somewhat in obese mice, they probably remained resistant to insulin due to the continued presence of hyperinsulinemia (relative to lean mice)

BAT activity, bypassing the mechanisms involving corticosterone and insulin, thus lowering the efficiency of energy retention.

The effects of cold-acclimation and adrenalectomy on skeletal muscle of obese mice were probably both mediated in large part by corticosterone and insulin. In cold-acclimated obese mice, muscle growth was reduced even more than that of control obese. This is consistent with the elevated plasma corticosterone concentration and reduced insulin sensitivity present in these mice. Adrenalectomy of obese mice fed stock diet almost normalized muscle weights, consistent with their reduced plasma corticosterone concentrations and increased insulin sensitivity. As with the results for energy balance, the results for muscle weights of adrenalectomized obese mice fed semipurified diets demonstrate that the absence of adrenal secretions alone was not sufficient to improve muscle gain. The return of insulin sensitivity was blocked in these mice - this was probably responsible, at least in large part, for the failure of adrenalectomy to improve muscle gains in obese mice fed semipurified diets.

These studies focused primarily on the effects of cold-acclimation and adrenalectomy on obese mice with very little mention of their lean counterparts. The observation that these treatments had less effect on energy balance and body composition of lean mice is almost as interesting as the more dramatic effects of these treatments on obese mice. It is clear that lean mice have a more tightly controlled system for maintaining their normal energy balance and body composition. Plasma corticosterone concentrations were altered at the most by a factor of 2 by either treatment, and

and the high plasma corticosterone concentrations. Adrenalectomized obese mice fed stock diet were probably more sensitive to insulin, due to the almost normalization of plasma insulin concentration and the very low corticosterone concentrations. The finding of Ohshima et al. (56) that skeletal muscle of adrenalectomized obese mice had normal insulin sensitivity supports this suggestion. Insulin is required for normal thermogenic function of BAT (63). In addition, small amounts of glucocorticoids are needed for normal BAT function, but chronic administration of corticosterone to intact mice (resulting in higher than normal plasma corticosterone concentrations) reduces the capacity of BAT for heat production (13,25). Thus, the effects of adrenalectomy on energy balance of stock-fed obese mice could have been due directly to the reduced concentrations of corticosterone, resulting in increased BAT activity and reduced efficiency of energy retention, as well as to the improvement in insulin sensitivity, which could stimulate BAT thermogenesis. The importance of the restoration of insulin sensitivity can be seen from the results in obese mice fed semipurified diets. According to the glucose challenge data, insulin sensitivity was not restored to adrenalectomized obese mice fed the semipurified diets - likewise, energy balance was not normalized in these mice.

The effects of cold-acclimation on energy balance of obese mice were probably not mediated by corticosterone and insulin, since the changes in the concentration of corticosterone and insulin sensitivity were opposite to what one would predict based on the energy balance data. Cold-acclimation could have directly stimulated

insulin varied by even less. Energy gain, muscle gains and the proportion of body fat in these mice were maintained very close to that of controls after cold-acclimation or adrenalectomy, probably at least partly as a result of this maintenance of hormonal balance. Thus, the lean mouse can maintain its normal energy balance and body composition in the face of wide fluctuations in environmental and physiologic conditions.

In contrast to the lean mouse, the obese mouse demonstrated the capacity for large changes in efficiency of energy retention, and muscle gain. Throughout these marked changes in energy balance and muscle gain, however, the obese mouse did defend its obese body composition to some degree, as demonstrated by the maintenance of an elevated energy density in all of the experiments reported here. Interactions between corticosterone and insulin appear to be important in mediating these changes in energy balance and body composition in obese mice.

References

1. Agius, L., B.J. Rolls, E.A. Rowe, and D.H. Williamson. Increased lipogenesis in brown adipose tissue of lactating rats fed a cafeteria diet. The possible involvement of insulin in brown adipose tissue hypertrophy. FEBS Letters 113:45-48, 1981.

2. Bas, S., E. Imesch, D. Ricpuier, F. Assimacopoulos-Jeannet, J. Seydoux, and J.-P. Giacobino. Fatty acid utilization and purine nucleotide binding in brown adipose tissue of genetically obese (ob/ob) mice. Life Sci. 12:2123-2130, 1983.

3. Bergen, W.G., M.L. Kaplan, R.A. Merkel, and G.A. Leveille. Growth of adipose and lean tissue mass in hindlimbs of genetically obese mice during preobese and obese phases of development. Am.J.Clin.Nutr. 28:157-161, 1975.

4. Bieri, J.G., G.S. Stoewsand, and G.M. Briggs. Report of the American Institute of Nutrition ad hoc committee on standards for nutritional studies. J.Nutr. 107:1340-1348, 1977.

5. Bray, G.A., and D.A. York. Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. Physiol. Rev. 59:719-809, 1974.

6. Bray, G.A., D.A. York, and R.S. Swerdloff. Genetic obesity in rats. I. The effects of food restriction on body composition and hypothalamic function. Metabolism 22:435-442, 1973.

7. Bruce, B.K., B.M. King, G.R. Phelps, and M.C. Veitia. Effects of adrenalectomy and corticosterone administration on hypothalamic obesity in rats. Am.J.Physiol. 243:E152-E157, 1982.

8. Chlouverakis, C. Induction of obesity in obese-hyperglycemic mice on normal food intake. Experientia 26:1262-1263, 1970.

9. Cleary, M.P., J.R. Vasselli, and M.R.C. Greenwood. Development of obesity in Zucker obese (fa/fa) rat in absence of hyperphagia. Am.J.Physiol. 238:E284-E292, 1980.

10. Davidson, J.M., L.E. Jones, and S. Levine. Feedback regulation of adrenocorticotrophin secretion in "basal" and "stress" conditions: acute and chronic effects of intrahypothalamic corticoid implantation. Endocrinology 82:655-663, 1968.

11. Davis, J.R., A.R. Tagliaferro, J.S. Roberts, and J.O. Hill. Effects of early cold adaptation on food efficiency and dietary induced thermogenesis in the adult rat. Physiol. Behav. 29:135-140, 1982.

12. Davis, T.R.A., and J. Mayer. Imperfect homeothermia in the hereditary obese-hyperglycemic syndrome of mice. Am.J.Physiol. 177:222-226, 1954.

13. Deavers, D.R., and X.J. Musacchia. The function of glucocorticoids in thermogenesis. Fed. Proc. 38:2177-2181, 1979.

14. Debons, A.F., E. Siclari, K.C. Das, and B. Fuhr. Gold thiogluose-induced hypothalamic damage, hyperphagia, and obesity: Dependence on the adrenal gland. Endocrinology 110:2024-2029, 1982.

15. Debons, A.F., K.C. Das, B. Fuhr, and E. Siclari. Anorexia after adrenalectomy in gold thioglucose-treated obese mice. Endocrinology 112:1847-1851, 1983.

16. Desautels, M., F. Zaror-Behrens, and J. Himms-Hagen. Increased purine nucleotide binding, altered polypeptide composition and

thermogenesis in brown adipose tissue mitochondria of cold-acclimated rats. Can.J.Biochem. 56:378-383, 1978.

17. Dubuc, P.U. Effects of limited food intake on the obese-hyperglycemic syndrome. Am.J.Physiol. 230:1474-1479, 1976.

18. Dubuc, P.U. Non-essential role of dietary factors in the development of diabetes in ob/ob mice. J.Nutr. 111:1742-1748, 1981.

19. Dubuc, P.U., P.W. Mobly, and R.J. Mahler. Elevated glucocorticoids in obese-hyperglycemic mice. Horm. Metab.Res. 7:102-104, 1975.

20. Fanelli, M.T., and M.L. Kaplan. Effects of high-fat and high-carbohydrate diets on the body composition and oxygen consumption of ob/ob mice. J.Nutr. 108:1491-1500, 1978.

21. Feldman, D. Evidence that brown adipose tissue is a glucocorticoid target organ. Endocrinology 102:2091-2097, 1978.

22. Fellenz, M., J. Triandafillou, C. Gwilliam, and J. Himms-Hagen. Growth of interscapular brown adipose tissue in cold-acclimated hypophysectomised rats maintained on thyroxine and corticosterone. Can.J. Biochem. 60:838-842, 1982.

23. Findlay, J.A., K.A. Rookledge, A. Beloff-Chain, and J.D. Lever. A combined biochemical and histological study on the islets of Langerhans in the genetically obese hyperglycaemic mouse and in the lean mouse, including observations on the effects of streptozotocin treatment. J. Endocrinol. 56:571-583, 1973.

24. Foster, D.O., and M.L. Frydman. Nonshivering thermogenesis in the rat. II.Measurements of blood flow with micrespheres point to

brown adipose tissue as the dominant site of the calorigenesis induced by noradrenaline. Can.J.Physiol.Pharmacol. 56:110-122, 1978.

25. Galpin, K.S., R.G. Henderson, W.P.T. James, and P. Trayhurn. GDP binding to brown adipose tissue mitochondria of mice treated chronically with corticosterone. Biochem.J. 214:265-268, 1983.

26. Garthwaite, T.L., D.R. Martinson, L.F. Tseng, T.C. Hagen, and L.A. Menahan. A longitudinal hormonal profile of the genetically obese mouse. Endocrinology 197:671-676, 1980.

27. Genuth, S.M. Hyperinsulinism in mice with genetically determined obesity. Endocrinology 84:386-391, 1969.

28. Goldberg, A.L., S.B. Martel, and M.J. Kushmerick. In vitro preparations of the diaphragm and other skeletal muscles. Methods Enzymol. #9:82-94, 1975.

29. Grundleger, J.L., V.Y. Godbole, and S.W. Thenen. Age-dependent development of insulin resistance of soleus muscle in genetically obese (ob/ob) mice. Am.J. Physiol. 239:E363-E371, 1980.

30. Halberg, F., P.G. Albrecht, and J.J. Bittner. Corticosterone rhythm of mouse adrenal in relation to serum corticosterone and sampling. Am.J.Physiol. 197:1083-1085,1959.

31. Herberg, L., and H.K. Kley. Adrenal function and the effect of a high-fat diet on C57BL/6J and C57BL/6J-ob/ob mice. Horm.Metab.Res. 7:410-415, 1975.

32. Heroux, O.,G.E. Johnson, and K.V. Flattery. Seasonal changes in catecholamine content and composition in interscapular brown fat in rats fed a commercial chow or a semi-purified diet. Can.J.Physiol.Pharmacol. 50:30-36, 1972.

33. Hogan, S., and J. Himms-Hagen. Abnormal brown adipose tissue in obese (ob/ob) mice: response to acclimation to cold. Am.J.Physiol. 239:E301-E309, 1980.

34. Holt, S., and D.A. York. The effect of adrenalectomy on GEP binding to brown-adipose-tissue mitochondria of obese rats. Biochem.J. 208:819-822, 1982.

35. Hollifield, G., and W. Parson. Body composition of mice with gold thioglucose and hereditary obesity after weight reduction. Metabolism 7L179-183, 1958.

36. Hooper, A.C.B. Muscles and bones of large and small mice conpared at equal body weights. J.Anat. 127:117-123, 1978.

37. Ip, C., H.M. Tepperman, P. Holohan, and J. Tepperman. Insulin binding and insulin response of adipocytes from rats adapted to fat feeding. J.Lipid Res. 17:588-599, 1976.

38. Kahn, C.R., I.D. Goldfine, D.M. Neville, Jr., and P. deMeyts. Alterations in insulin binding induced by changes in vivo in the levels of glucocorticoids and growth hormone. Endocrinology 103: 1054-1066, 1978.

39. Kaplan, M.L., and G.A. Leveille. Core temperature, 02 consumption and early detection of ob/ob genotype in mice. Am.J.Physiol. 227:912-915, 1974.

40. King, B.M., A.R. Banta, G.N. Tharel, B.K. Bruce, and L.A. Frohman. Hypothalamic hyperinsulinemia and obesity: role of adrenal glucocortocoids. Am.J.Physiol.245:E194-E199, 1983.

41. Knehans, A.W., and D.R. Romsos. Reduced norepinephrine turnover in brown adipose tissue of ob/ob mice. Am.J.Physiol. 239:E301-E309, 1980.

42. Larson, B.A., Y.N. Sinha, and W.P. Vanderlaan. Serum growth hormone and prolactin during and after the development of the obese-hyperglycemic syndrome in mice. Endocrinology 98:139-145, 1976.

43. Lavau, M., S.K. Fried, C. Susini, and P. Freychet. Mechanism of insulin resistance in adipocytes of rats fed a high-fat diet. J.Lipid Res. 20:8-16, 1979.

44. Lin, P.Y., D.R. Romsos, and G.A. Leveille. Food intake, body weight gain and body composition of the young obese (ob/ob) mouse. J.Nutr. 107:1715-1723, 1977.

45. Lin, P.Y., D.R. Romsos, J.G. VanderTuig, and G.A. Leveille. Maintenance energy requirements, energy retention and heat production of young obese (ob/ob) and lean mice fed a high-fat or high-carbohydrate diet. J.Nutr. 109:1143-1153, 1979.

46. Lin, M.H., J.G. VanderTuig, D.R. Romsos, T. Akera, and G.A. Leveille. Heat production and Na+,K+-ATPase enzyme units in lean and obese (ob/ob) mice. Am.J.Physiol. 238:E193-E199, 1980.

47. Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall. Protein measurement with the Folin phenol reagent. J.Biol.Cham. 193:265-275, 1951.

48. Marchand-Brustel, Y., B. Jeanrenaud, and P. Freychet. Insulin binding and effects in isolated soleus muscle of lean and obese mice. Am.J.Physiol. 234:E348-E358, 1978.

49. Marchington, D., N.J. Rothwell, M.J. Stock, and D.A. York. Energy balance, diet-induced thermogenesis and brown adipose tissue in lean and obese (fa/fa) Zucker rats after adrenalectomy. J.Nutr. 113:1395-1402, 1983.

50. Mayer, J., and R.J. Barnett. Sensitivity to cold in the hereditary obese-hyperglycemic syndrome of mice. Yale J.Biol.Med. 26:38-45, 1953.

51. Mondon, C.E., and D.R. Drury. Glucose tolerance in mildly and moderately diabetic rats exposed to cold. Proc.Soc.Exp.Biol.Med. 113:805-809, 1963.

52. Mook, D.G., J.C. Fisher, and J.C.Durr. Some endocrine influences on hypothalamic hyperphagia. Horm.Behav. 6:65-79, 1975.

53. Munro, H.N., and A. Fleck. Analysis of tissues and body fluids for nitrogenous constituents, in, Munro, H.N. (ed.): Mammalian Protein Metabolism, vol 3. Academic Press, New York, 1969.

54. Naeser, P. Function of the adrenal cortex in obese-hyperglycemic mice (gene symbol ob). Diabetologia 10:449-453, 1974.

55. Odedra, B.R., S.S. Dalal, and D.J. Millward. Muscle protein synthesis in the streptozotocin- diabetic rat. A possible role for corticosterone in the insensitivity to insulin infusion in vivo. Biochem.J. 202:363-368, 1982.

56. Ohshima, K., N.S. Shargill, T.M. Chan, and G.A. Bray. Adrenalectomy reverses insulin resistance in muscle from obese (ob/ob) mice. Am.J.Physiol. 246:E193-E197, 1984.

57. Olefsky, J.M., J. Johnson, F. Liu, P. Jen, and G.M. Reaven. The effects of acute and chronic dexamethasone administration on insulin binding to isolated rat hepatocytes and adipocytes. Metabolism 24:517-527, 1975.

58. Poe, R.H., and T.R.A. Davis. Cold exposure and acclimation in alloxan-diabetic rats. Am.J.Physiol. 202:1045-1048, 1962.

59. Powley, T.L., and S.A. Morton. Hypophysectomy and regulation of

body weight in the genetically obese Zucker rat. Am.J.Physiol. 230:982-987, 1976.

60. Rannels, S.R., and L.S. Jefferson. Effects of glucocorticoids on muscle protein turnover in perfused rat hemicorpus. Am.J.Physiol. 238:E564-E572, 1980.

61. Rannels, S.R., D.E. Rannels, A.E. Pegg, and L.S. Jefferson. Glucocorticoid effects on peptide-chain initiation in skeletal muscle and heart. Am.J. Physiol. 235:E134-E139, 1978.

62. Rothwell, N.J., and M.J. Stock. Similarities between cold- and diet- induced thermogenesis in the rat. Can.J.Physiol.Pharmacol. 58L842-848m 1980.

63. Rothwell, N.J., and M.J. Stock. A role for insulin in the diet-induced thermogenesis of cafeteria fed rats. Metabolism 30:673-678, 1981.

64. Saito, M., and G.A. Bray. Diurnal rhythm for cortocosterone in obese (ob/ob), diabetes (db/db) and gold thioglucose-induced obesity in mice. Endocrinology 113:2181-2185, 1983.

65. Saito, M., and G.A. Bray. Adrenalectomy and food restriction in the genetically obese (ob/ob) mouse. Am.J.Physiol. 246:R20-R25, 1984.

66. Santidrian, S., P. Marchon, X.H. Zhao, H.N. Munro, and V.R. Young. Effect of corticosterone on rate of myofibrillar protein breakdown in adult male rats. Growth 45:342-350, 1981.

67. Santidrian, S., M. Moreyra, H.N. Munro, and V.R. Young. Effect of corticosterone and its route of administration on muscle protein breakdown, measured in vivo by urinary excretion of N-methylhistidine in rats: Response to different levels of

dietary protein and energy. Metabolism 30:798-804, 1981.

68. Sclafani, A., P.F. Aravich, and M. Landman. Vagotomy blocks hypothalamic hyperphagia in rats on a chow diet and sucrose solution, but not on a palatable mixed diet. J.Comp.Physiol.Psych. 95:720-734, 1981.

69. Shapira, J.F., I. Kircher, and R.J. Martin. Indices of skeletal muscle growth in lean and obese Zucker rats. J.Nutr. 110:1313-1318, 1980.

70. Seydoux, J., A. Chinet, G. Schneider-Picard, S. Bas, E. Imesch, F. Assimacopoulos-Jeannet, J.D. Giacobino, and L. Girardier. Brown adipose tissue metabolism in streptozotocin-diabetic rats. Endocrinology 113:604-610, 1983.

71. Seydoux, J., E.R. Trimble, F. Bouillaud, F. Assimacopoulos-Jeannet, S. Bas, D. Ricquier, J.P. Giacobino, and L. Girardier. Modulation of B-oxidation and proton conductance pathway of brown adipose tissue in hypo- and hyperinsulinemic states. FEBS Letters 166:141-145, 1984.

72. Solomon, J., G. Bradwin, M.A. Cocchia, D. Coffey, T. Condon, W. Garrity, and W. Grieco. Effects of adrenalectomy on body weight and hyperglycemia in five months old ob/ob mice. Horm.Metab.Res. 9:152-156, 1977.

73. Solomon, J., and J. Mayer. The effect of adrenalectomy on the development of the obese-hyperglycemic syndrome in ob/ob mice. Endocrinology 93:510-513, 1973.

74. Steel, R.G.D., and J.H. Torrie. Principles and Procedures of Statistics. McGraw-Hill Book Co., New York, New York. 1960.

75. Susini, C., and M. Lavau. In-vitro and in-vivo responsiveness of

muscle and adipose tissue to insulin in rats rendered obese by a high-fat diet. Diabetes 27:114-120, 1978.

76. Swerdloff, R.S., R.A. Batt, and G.A. Bray. Reproductive hormonal function in the genetically obese (ob/ob) mouse. Endocrinology 98:1359-1364, 1976.

77. Tepperman, J. Hormonal regulation of calcium homeostasis, in, Metabolic and Endocrine Physiology, Year Book Medical Publishers, Inc., Chicago, 1980.

78. Thurlby, P.L., and P. Trayhurn. Regional blood flow in genetically obese (ob/ob) mice. The importance of brown adipose tissue to the reduced energy expenditure on non-shivering thermogenesis. Pflugers Arch. 385:193-201, 1980.

79. Thurlby, P.L., and P. Trayhurn. The role of thermoregulatory thermogenesis in the development of obesity in genetically obese (ob/ob) mice pair-fed with lean siblings. Brit.J.Nutr. 42:377-385, 1979.

80. Trayhurn, P., and W.P.T. James. Thermoregulation and non-shivering thermogenesis in the genetically obese (ob/ob) mouse. Pflugers Arch. 373:189-193, 1978.

81. Trayhurn, P., Jones, P.M., M.M. McGuckin, and A.E. Goodbody. Effects of overfeeding on energy balance and brown fat thermogenesis in obese (ob/ob) mice. Nature 295:323-325, 1982.

82. Trayhurn, P., P.L. Thurlby, and W.P.T. James. A defective response to cold in the obese (ob/ob) mouse and the obese Zucker (fa/fa) rat. Proc.Nutr.Soc. 35:133A, 1976.

83. Trostler, N., D.R. Romsos, W.G. Bergen, and G.A. Leveille. Skeletal muscle accretion and turnover in lean and obese (ob/ob)

mice. Metabolism 28:928-933, 1979.

84. VanderTuig, J.G., K. Ohshima, T. Yoshida, D.R. Romsos, and G.A. Bray. Adrenalectomy increases norepinephrine turnover in brown adipose tissue of obese (ob/ob) mice. Life Sci. 34:1423-1432, 1984.

85. VanderTuig, J.G., D.R. Romsos, and G.A. Leveille. Maintenance energy requirements and energy retention of young obese (ob/ob) and lean mice housed at 33 and fed a high-carbohydrate or a high-fat diet. J.Nutr. 110:35-41, 1980.

86. Welton, R.F., R.J. Martin, and B.R. Baumgardt. Effects of feeding and exercise reginens on adipose tissue glycerokinase activity and body composition of lean and obese mice. J.Nutr. 103:1212-1219, 1973.

87. Wilson, H., J.J. Borris, and R.C. Bohn. Steroids in the blood and urine of female mice bearing an ACTH-producing pituitary tumor. Endocrinology 62:135-149, 1958.

88. Wittmers, L.E. Jr., and E.W. Haller. Effect of adrenalectomy on the metabolism of glucose in obese (C57BL/6J-ob/ob) mice. Metabolism 32:1093-1100, 1983.

89. Woodward, C.J.H., P. Trayhurn, and W.P.T. James. Cost of maintenance and growth in genetically obese (ob/ob) mice. Proc.Nutr.Soc. 36:115A, 1977.

90. York, D.A. and G.A. Bray. Dependence of hypothalamic obesity on insulin, the pituitary and the adrenal gland. Endocrinology 90:885-894, 1972.

91. Zaragoza-Hermans, N., and J.-P. Felber. Studies on the metabolic effects induced in the rat by a high-fat diet. I.Carbohydrate

metabolism in vivo. Horm.Metab.Res. 2:323-329, 1970.