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Ye-Min Nei

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DIETARY GLUCOSE INCREASES PLASMA INSULIN AND DECREASES BROWN ADIPOSE TISSUE THERMOGENIC ACTIVITY IN ADRENALECTOMIZED OB/OB MICE

By

Ye-Min Nei

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ABSTRACT

DIETARY GLUCOSE INCREASES PLASMA INSULIN AND DECREASES BROWN ADIPOSE TISSUE THERMOGENIC ACTIVITY IN ADRENALECTOMIZED OB/OB MICE

By

Ye-Min Nei

Adrenalectomy reduces plasma insulin and increases brown adipose tissue metabolism in ob/ob mice fed high starch diets, but has minimal influence on these parameters in ob/ob mice fed high glucose diets. The purpose of my study was to determine if consumption of a high glucose diet would increase plasma insulin concentrations and decrease brown adipose tissue metabolism in adrenalectomized ob/ob mice that had previously been fed a high starch diet. Adrenalectomized ob/ob mice consumed more energy and gained more weight without an increase in oxygen consumption when switched from a high starch diet to a high glucose diet. Within 2 days after the switch to the high glucose diet, plasma insulin concentrations increased by 70 %. Brown adipose tissue metabolism, as assessed by GDP binding to brown adipose tissue mitochondria, was decreased 4 days after the diet switch. Plasma insulin and brown adipose tissue metabolism were unaffected in adrenalectomized lean mice switched from the high starch to the high glucose diet. The high glucose diet-induced increase in plasma insulin concentrations in adrenalectomized ob/ob mice may contribute to the observed depression in brown adipose tissue metabolism.

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I. LITERATURE REVIEW

A. INTRODUCTION

Genetic factors are one of the primary factors cause of obesity. Since it is very difficult to study the metabolic causes of obesity in man, many studies use genetically obese animal models. The symptoms manifested by ob/ob mice include hyperphagia, hyperinsulinemia, hyperglycemia, high circulating levels of corticosterone and impaired thermoregulatory thermogenesis (13,34,64). Lowered oxygen consumption and hyperinsulinemia are observed in ob/ob mice as early as 5 days (8) and 6 days (21) after birth. Hyperglycemia and hyperphagia are observed during the third or fourth week of life (13,55,111).

Adrenalectomy reduces food intake by 35-60% and body weight gain by 50-90% in several obese animal models (57,84). Adrenalectomy also reduces the high efficiency of energy retention in these animals. This reduction is not due exclusively to reduced energy intake. The reduction in efficiency of energy retention is more likely caused by reduced intake combined with increased energy expenditure per Kcal consumed, possibly resulting from increased activity of brown adipose tissue. Brown adipose tissue (BAT) has one main function, heat production. It is important in the

regulation of body temperature and energy balance, particularly in small mammals. Brown adipose tissue possesses mechanisms for regulating energy expenditure in nonshivering thermogenesis (NST) or diet-induced thermogenesis (DIT). Ob/ob mice show impaired cold tolerance, reduced DIT during overfeeding, and a depressed thermogenic response to noradrenaline, which is due to decreased oxygen consumption of BAT. The primary mechanism responsible for thermogenesis in brown fat appears to be the uncoupling of the mitochondrial proton conductance pathway from ATP synthesis (64). The activity of this pathway, assessed from the binding of purine nucleotides to isolated BAT mitochondria, is markedly depressed in both ob/ob and diabetic db/db mice.

Effects of adrenalectomy on energy balance in ob/ob mice are diet dependent (91). Adrenalectomized ob/ob mice fed high starch diets reduce their high efficiency of energy retention to values similar to those observed in lean mice; whereas, adrenalectomized ob/ob mice fed a high glucose diet continue to retain a high proportion of dietary energy and develop obesity. The only difference between the high starch and high glucose diets is the source of carbohydrate. It would appear that dietary glucose somehow lowers the metabolic rate of adrenalectomized ob/ob mice , but direct measurements of metabolic rate have not been reported.

B. Energy Balance in Genetically Obese Animals

Obesity results from a positive imbalance between energy intake and energy expenditure. Control of food intake is one of the most complex biological problems yet to be understood. Intake or expenditure can be manipulated to get balance, but they are not totally independent of each other. Energy balance can be achieved in normal weight subjects or in overweight subjects provided energy output equals input level. In humans, food intake and energy expenditure are not finely balanced within a day, but good balance is normally achieved over weeks or months. Smaller animals regulate better on a day to day basis than humans. Time to attain balance might be associated with the relationship of energy expenditure to body weight. Smaller animals have relatively larger surface areas per unit body weight than larger animals, so smaller animals have higher metabolic rates per gram body weight. Rats die within one week without food whereas humans can survive for several months without food.

Energy input, energy expenditure and energy stores are closely related to each other. It is unclear precisely how this system is regulated even in normal weight animals or subjects. I review the components of the system below.

1. Regulation of Food Intake

Precise measures of food intake in humans are very difficult to obtain except subjects are housed in metabolic wards. Food recall or diet records provide general estimates of energy intake, but lack the sensitivity to detect small treatment differences. Some subjects may for various reasons underestimate and overestimate intake. In controlled conditions, it is questionable if the data represent free-living intakes. Food intake is much easier to measure in animals than in humans. We can get clues from animals studies which can apply in human studies.

It has generally been assumed that food intake is a more important controlling factor than energy expenditure in energy balance. Defective appetite control (i.e, hyperphagia) is often assumed to be the primary cause of obesity in humans and experimental animals (54,55,62,70,89).

Many investigators have studied the adjustment of food intake in response to variations in the caloric density of food (4,30,62,69,70). Mature rats can compensate for diluted diet within a range from 2.95 kcal/ml to 5.05 kcal/ml (69). Monkeys fed diluted diets ranging from 0.5 to 1.35 kcal/ml maintain a constant caloric intake by adjusting oral intake in response to changes in caloric density (30). In a human study (142),

normal weight subjects were given a 200 ml preload containing aspartame or glucose (50 gm) with equal sweetness. Then, each subject was given excess to food after one hour. A comparison was made between equi-sweet preloads of aspartame, glucose and water control. The glucose group exhibited less hunger and desire to eat than aspartame and water control groups during the test meal. Total energy intakes, preload plus meal, were the same for all three (aspartame, glucose, and water) groups. These data show that a very good compensation exists to control food intake within specific range of caloric density in normal animals and subjects. However, the ability to compensate is affected by diet composition, variety, palatability and motivation.

Offering rats multichoice cafeteria diets ,which are an assortment of energy rich foods processed for human consumption, produces hyperphagia and obesity compared to feeding a standard stock diet (62,70,73,89). Cafeteria diets selected by rats are normally high in fat content with the same amount of carbohydrate as the control diet (70). It has been suggested that a variety of flavored food items is a contributing factor to cause dietary obesity in animals. However, Naim (62) indicated that variety in diet flavor did not induce hyperphagia in rats fed a nutritionally controlled purified diet. Both high fat and high sucrose diets con-

taining a variety of flavors in a cafeteria diet, and a high fat diet without added flavors resulted in higher energy intake. Thus, the effect of a variety of food flavors on hyperphagia in rats may have a minor effect in purified diets compared to the effect of the fat in diets (62). Consequently, energy density may play an important role in the hyperphagia and obesity induced by high-fat diets. This also appears to be true in human studies (56,58).

Young female subjects fed high energy and low energy lunch by changing the carbohydrate and fat ratio, did not adjust total energy intake. Males adjusted mean daily energy intake by 11% when given the high-calorie lunch to compensate for over intake (58). Lissner also found that female subjects consumed a 15.4% surfeit on the high-fat diet, resulting in significant increase in body weight (56). The situation is more complex because different type of fat fed causes different responses, at least in rats. Normally, rats consume more solid fat (shortening, lard or tallow) than liquid fat (corn oil). If corn oil is emulsified then consumption increases (71).

Many manipulations used in the research of food intake cause changes in eating pattern including rate of food intake, meal size and meal frequency (15,73). Popplewell reported that reduction in the permitted rate of food intake contributed to a clear reduction of meal size and an increase in

meal frequency in rats (15). Rogers and Blundell have very detailed investigations of eating patterns in rats. Offering a cafeteria diet to rats causes an immediate increase in meal size and meal frequency. Palatability has a major influence on meal size, while variety affects both meal size and meal frequency (73). They also found that the food preferred during the second meal was different from that consumed in the first meal; mixed meals were consumed more than single item meals. Overriding satiety signals happened as body weight increased with a resultant decrease in meal frequency, but meal size remaining high. Obesity has an influence on feeding primarily through a decrease in meal frequency with elevating meal size, which are true for most models of obesity. These data from animal research in eating patterns are good references to apply for human studies.

Many studies have been done which show that variation in food intake is the major cause of changes in body fat or weight. Obese animals eat more than nonobese animals (54). Also, adult obese mice and moderately diabetic rats self-selected a higher proportion of energy from fat and a lower proportion from protein and carbohydrate than did lean (4,59,75). Obese (ob/ob) mice fed a high-fat diet were 41% more efficient than ob/ob mice fed a high-carbohydrate diet, and 38 to 71% more efficient than lean mice fed high-carbohydrate or high-fat diets respectively

(55). After 4 weeks of age, ob/ob mice consume more food and gain more weight than lean mice (54). All these observations suggest that hyperphagia plays an important role in the later development of obesity in ob/ob animals.

2. Energy Expenditure

Studies (2,19,76,83,100,105) on energy expenditure in obese animals have focused on obese (ob/ob) mice, obese-diabetic (db/db) mice, and obese (fa/fa) rats. These obese animals exhibit high retention of dietary energy. Obese (ob/ob) mice contain more body fat than lean littermates as early as 7 days after birth (8). Milk intake has been measured from 7 to 21 days of age in ob/ob and lean pups, and ob/ob pups did not consume more milk than lean pups (54). Similar findings have been reported in obese (fa/fa) rat pups (10). It is clear that the early appearance of high energy content in these ob/ob mice and fa/fa rats occurs before hyperphagia is evident and therefore must result from low energy expenditure.

Oxygen consumption is lower in preobese ob/ob pups than in lean littermates; low oxygen consumption could be detected as early as 5 days of age in ob/ob mice (8). Because ob/ob pups contained 38% more fat than lean pups at 1 week of age, the reduced oxygen consumption may be

due to the less lean body mass. Ob/ob and lean pups suckled dams fed a high-carbohydrate diet or a high-fat diet for 2 weeks. The body weight and body fat of obese pups suckling dams fed the high-carbohydrate diet were the same as the lean pups suckling dams fed the high-fat diet. But, obese pups still consumed less oxygen than did the lean pups. Thus, the low energy expenditure in these ob/ob pups can not be explained by differences in body composition (55). The fact that obesity develops in ob/ob mice even when energy intake is limited to that of ad lib fed lean siblings also demonstrates that energy expenditure is low in ob/ob mice (54,98).

Low energy expenditure in young obese (ob/ob) mice could result from a reduced maintenance energy requirement or from an improved ability to retain energy consumed above maintenance. The maintenance energy requirement is 40% less in 3- to 6-week old (ob/ob) mice than lean mice housed at 25 to 30 ^{o}C and fed either a high-fat or a high-carbohydrate diet (55). The utilization of energy above maintenance in ob/ob mice fed the high-fat or the high- carbohydrate diet was 71% and 38% more efficient than that in lean mice, respectively (55). Thus, the 40% lower maintenance energy requirement of these obese mice is a major contribution to the high efficiency of energy retention. In genetically ob/ob mouse,

a decreased oxygen consumption and body temperature, and an increased body fat concentration appear within the first three weeks of age. This indicates that the initial development of obesity in the ob/ob mutant is entirely due to the high efficiency of energy retention caused by a low energy expenditure.

C. Thermogenesis and Obesity

Genetically obese animals with low energy expenditure have defects in either cold-induced thermogenesis or diet-induced thermogenesis. I will discuss these two components of energy expenditure and compare the differences between genetically obese and lean rodents.

1. Cold-Induced Thermogenesis:

Since small animals have a large surface area/volume ratio, they have greater metabolic rates than large animals, particularly when small animals are housed below their lower critical temperature. Cold-acclimated rats remain lean even thought they exhibit extreme hyperphagia. They use the excess food energy for cold-induced thermogenesis to maintain body temperature (80). One of the earliest abnormalities observed in ob/ob mice is the failure to maintain body temperature when exposed to temperatures below the thermoneutral zone (32–33 ^{o}C). Trayhurn et al (103) showed

that 12-d-old ob/ob mice exposed to an environmental temperature of 15–20 ^{o}C had a marked fall in body temperature within 15 minutes. These researchers used this finding to identify animals bearing the ob/ob genotype before they could be distinguished visually. Db/db mice and fa/fa rats exposed to cold show the same impaired metabolic response to cold exposure as ob/ob mice (43,100). These observations indicate that the high energy efficiency in these mice and rats may be explained in part by a reduced energy expenditure for thermoregulatory thermogenesis (42,58,59). Some studies have examined (44,98,101) efficiency of energy retention in obese (ob/ob) mice at warm environmental temperature (33–34 ^{o}C). When the environmental temperature is decreased the metabolic rate of the ob/ob mice increases less than in lean mice (101). Maintenance energy requirement of obese mice housed at 25 to 30 ^{o}C were the same as those of obese mice housed at 33 ^{o}C (108). Similar findings were observed in db/db mice (100,105). The capacity for non-shivering thermogenesis was reduced by 50% in the ob/ob mice compared to lean mice at 31 ^{o}C (101). Taken together, a defect in thermoregulatory thermogenesis is a major factor resulting in high efficiency of dietary energy retention in young obese mice housed at normal room temperature (20–28 ^{o}C). However, ob/ob mice housed at 33 ^{o}C still become obese even if pair fed (74,98). Similar findings have been observed in db/db

mice (105). Factors other than defective thermoregulatory thermogenesis must also be involved.

2. Diet Induced Thermogenesis

Energy expenditure is associated with the level of food intake. Energy expenditure increases as energy intake increases. Two components of diet-induced thermogenesis are obligatory and adaptive thermogenesis. The definition of obligatory thermogenesis, which has also been termed heat increment or SDA, is the energy costs associated with digestion, absorption, and the immediate metabolism of the ingested nutrients. Adaptive thermogenesis is defined as the increase in energy expenditure associated with a meal that is over and above the obligatory component. It is technically difficult to evaluate the relative contributions of obligatory and adaptive thermogenesis to diet-induced thermogenesis. Most researchers focus on total diet-induced thermogenesis without attempting to separate the two components.

Rothwell and Stock fed a cafeteria diet for 3 weeks to induce overeating in rats (81). Cafeteria-fed rats consumed 80% more energy than did stock-fed control rats, but less than 10% of this excess intake was stored as body energy (81). The association of lowered energy efficiency with

increased diet-induced thermogenesis in the overfed animals was confirmed by 20-30% higher rates of resting oxygen consumption than in stock-fed rats (81). Cafeteria diet induction of diet-induced thermogenesis could occur within 2.5 days (77). The increase in diet- induced thermogenesis is associated with an elevation of BAT wet weight, DNA, total protein and 3 times higher GDP binding in isolated BAT mitochondria of cafeteria-fed rats (36).

Rats fed a low-protein diet ate considerably more than their controls fed an adequate protein diet. Nevertheless, rats fed a low-protein diet retained much less energy (92,107) indicating that protein content of the diet influences diet-induced thermogenesis. Leblanc and Brondel (53) suggest that palatability of the meal is another factor causing diet- induced thermogenesis which may be via sensory stimulation to activate the sympathetic nervous system. Eight female subjects were fed either a highly palatable meal (HPM) or a nonpalatable meal (NPM) which was presented as desiccated biscuits by mixing all the ingredients of the highly palatable meal. Resting metabolic rate (RMR) during 90 min after ingestion of HPM was 50% higher than that of NPM control group.

For the 5 week post-weanling period, obese mice converted 3 to 4 times more dietary energy to body energy than did lean mice, whereas

obese mice consumed only 20 to 40% more energy (54). Trayhurn also studied the effects of cafeteria diets compared with stock diets in 4 week old lean and obese (ob/ob) mice (104). After 3 weeks, energy intake of lean mice fed a cafeteria diet was 69% more than that of lean animals fed the stock diet. Although energy intake increased, the cafeteria-fed lean mice only showed a 19% increase in energy gain and no change in body weight gain. Energy intake of the cafeteria-fed obese (ob/ob) mice was 49% higher than in those of stock-fed obese (ob/ob) mice. In contrast to lean mice, the cafeteria-fed obese (ob/ob) mice gained 88% more energy than stock-fed obese (ob/ob) mice. Energy efficiency of the cafeteria-fed obese (ob/ob) mice was much higher than the stock-fed groups. They also found that cafeteria-fed lean mice, but not ob/ob mice, showed an increase of metabolic rate in BAT. After this study was published Triandafillou and Himms-Hagen (106) examined fa/fa rats to find out whether a cafeteria diet would activate BAT thermogenesis in these obese animals. Either a stock diet or a cafeteria diet was given to young lean and fa/fa rats housed at 28 ^{o}C for 2 to 3 weeks. GDP-binding in BAT mitochondria was lower in fa/fa rats than in lean rats fed a stock diet, and cafeteria-fed fa/fa rats failed to activate BAT as it did in lean rats. These data suggest that DIT is reduced in obese compared with the lean rats, as previously observed in obese mice. Diet induced thermogenesis in brown adipose in response to

overeating leads to an attenuation of obesity in normal rodents, whereas defective diet induced thermogenesis in BAT is one of the reasons for high metabolic efficiency in the genetically obese rodents.

3. Brown Adipose Tissue

Brown adipose tissue is located in small deposits throughout the body, including the interscapular, subscapular and axillary regions, at the nape of the neck, along the length of the great vessels in the thorax and abdomen and between the ribs. Brown adipocytes have several small lipid droplets and are packed with large mitochondria. In contrast white adipocytes have a single large lipid droplet and relatively few small mitochondria. Non-shivering thermogenesis in brown adipose tissue is mediated by the sympathetic nervous system. Brown adipose tissue has an abundant sympathetic innervation secreting norepinephrine by the nerve ending to regulate BAT metabolic activity. As already indicated brown adipose may be important in regulation of energy balance although it represents only 1-3% of body weight in rats (24,33).

3.1. Mechanism of thermogenesis in brown adipose tissue

Brown adipose tissue mitochondria have a unique loosely coupled respiration (32,34,37). The principal mechanism of heat production for

non-shivering thermogenesis in brown adipose tissue is uncoupling of oxidative phosphorylation; respiration proceeds without ATP synthesis with resultant heat production. The mechanism of uncoupling involves a proton conductance pathway which makes the inner mitochondria membrane more permeable to protons (32,34,37).

The thermogenic function of brown adipose tissue mitochondria is related to a specific protein (32,000 D polypeptide which is named differently as thermogenin, uncoupling protein, nucleotide binding protein, and GDP-binding protein). Thermogenin is located in the outer surface of the inner mitochondrial membrane. The main sequence of non-shivering thermogenesis in BAT involves a stimulus (i.e. cold temperature or cafeteria diet) which activates the sympathetic nervous system to release norepinephrine from sympathetic nerves ending. Thus, norepinephrine binds to β-adrenergic receptors on BAT cells and activates adenyl cyclase with a resultant increase in cAMP. The resulting increase in cAMP causes protein kinase activation of a hormone-sensitive lipase and accelerates lipolysis which serves as the intracellular signal to switch on the proton-conductance pathway, and as the fuel for oxidation in mitochondrial (32,34,37). The precise mechanisms for control of the stimulation and inhibition of non- shivering thermogenesis are still unknown.

3.2. Assessment of thermogenesis in brown adipose tissue

The only technique to measure the quantitative contribution of BAT to whole body energy expenditure is assessment of BAT blood flow with radioactively labelled microspheres and tissue oxygen consumption (24). Foster and Frydman found that the 25-fold increase of blood flow to BAT that occurs in response to maximum norepinephrine-induced stimulation of metabolic rate in cold acclimated rats accounts for 60% of the overall increase in metabolic rate (24).

Measurement of blood flow to BAT is a very difficult technique and not realistic to do in large numbers of animals. Investigators have thus used other methods to assess BAT function. Most studies have generally used some of the following four basic measurements: tissue weight, protein content, cytochrome oxidase activity and mitochondrial GDP binding as an index of thermogenic state of BAT. The wet weight of BAT is only a rough index of the triacylglyerol content and generally this parallels the amount of lipid stored in white adipose tissue. Tissue weight is a useless measure of metabolic capacity. Measurement of total protein, DNA, or a mitochondrial marker enzyme, such as cytochrome oxidase, provides a better index of the thermogenic capacity, but not of the activity, of the tissue. Binding of GDP to brown adipose tissue mitochondria is the most

widely used index of the activity of the proton conductance pathway. GDP binding is expressed per unit of mitochondrial protein. Chronic cold exposure leads to an increase in GDP binding and uncoupling protein concentration (1,102). When the tissue is acutely stimulated by activation of its sympathetic nerves, GDP binding is rapidly increased (94,102). This increase in binding is associated with ultrastructural changes of isolated mitochondria without an increase in uncoupling protein concentration. This acute increase in GDP binding indicates an "unmasking" of existing nucleotide binding sites. It is important that more direct experimental approaches be used to understand the molecular basis for this opponent unmasking and alteration in thermogenic activity of the tissue.

The recent development of an immunoassay for direct quantitative measurement of UCP (uncoupling protein) provides the only specific means of identifying BAT. Measurement of the amount of the UCP is a good index of the potential thermogenic capacity of BAT, but not of its actual thermogenic activity. Another useful index of the thermogenic activity of BAT is the measurement of NE turnover which provides information about the activity of the sympathetic nervous system, the key regulator of the BAT thermogenesis.

3.3 Brown adipose tissue thermogenesis in genetically obese animals

Himms-Hagen and Desautels provided evidenced for a defect in mitochondria of BAT in ob/ob mice that may explain why these mice exhibit a diminished thermogenic response to acute, severe, cold exposure (35). GDP binding to isolated BAT mitochondria of ob/ob mice was lower than in lean mice. There is considerable evidence now that the impaired thermogenesis in ob/ob mice is due at least in part to a defect in this pathway in the mitochondria of BAT. Acute cold-exposure in mice causes a 45% increase in GDP binding (35). A similar alteration is found in rats (94,95). GDP binding of BAT mitochondria in obese (ob/ob) mice was 50% lower than in mitochondria of lean mice at 28 ^{o}C. Additionally, an acute exposure at 4 ^{o}C for 3 h increased GDP binding in BAT mitochondria from lean mice, but failed to increase binding in obese mice (35).

GDP binding to BAT mitochondria was 60% lower at 14 days of ob/ob than lean mice (28). The lower GDP binding also is found in diabetic-obese (27) mice. Defective control of sympathetic nervous system in BAT is likely a major factor in the low thermogenic activity of BAT. Sympathetic nervous system activity, indicated by norepinephrine turnover, is 40% lower in BAT of preobese (2 wk) (49) and 60% lower in adult (8

wk) obese mice (49,50) than in same age of lean littermates housed at 25 ^{o}C and fed a stock diet. Reduction of nonshivering thermogenesis shown by low rates of norepinephrine turnover and low GDP binding appears before the development of obesity indicating that it is not a secondary phenomenon.

Trayhurn (97) compared blood flow in BAT of obese (ob/ob) and lean mice. Lean mice showed about a 40-fold increase in blood flow to BAT in response to norepinephrine injection, whereas the increase in blood flow to BAT in obese mice was only half that of lean mice (97). Because ob/ob mice increase their metabolic rate and blood flow less after norepinephrine injection than do lean mice, it has been speculated that an alteration in BAT metabolism contributes to the increased efficiency of energy retention in ob/ob mice.

D. Relationship between Insulin, Thermogenesis, Central Nervous System, and Obesity

Hyperinsulinemia in ob/ob mice is observed at a very early age (21). Insulin plays an important role in control of food intake and BAT thermogenesis. Thus, many experiments have focused on the role of insulin in development of obesity. First, I will mention insulin secretion. Then, the

relationship between insulin, thermogenesis, and food intake are discussed.

1. Regulation of Plasma Insulin Concentration

1.1. Glucose stimulation of insulin secretion

Many factors modulate insulin secretion. However, glucose is the primary stimulus for insulin secretion. The relationship between plasma insulin and plasma glucose is sigmoidal. Glucose-induced insulin release has been studied in vivo within glucose ranges from 50 mg/dl to 300 mg/dl. Plasma glucose below a threshold about 50 mg/dl does not cause insulin secretion, and at a level of 300 mg/dl a maximum insulin secretion is reached in humans (46). However, the exact mechanism whereby glucose stimulates insulin secretion is unclear. Lavine found that ob/ob mouse islets were more hypersensitive and hyperresponsive to glucose than were lean controls (5,52). Similar alternations have been found in humans (46).

Hyperinsulinemia in ob/ob mice is partially due to this hypersecretion in response to glucose after about 30 days of age, but not at the earlier time point. Hyperinsulinemia occurred as early as 6 days (21) and hyperglycemia is not evident until the mice are about 30 days of age (111). Since hyperinsulinemia precedes the onset of hyperglycemia in ob/ob and db/db mice (16,21,22,111), glucose is not the only cause of insulin

hypersecretion.

1.2. Nervous system stimulation of insulin secretion

Neural transmitters are released from nerve terminals of the autonomic nervous system, which includes sympathetic and parasympathetic nerve, near β cells of pancreatic islets (61). Sympathetic fibers innervating the pancreas release norepinephrine, and the adrenal medulla releases epinephrine into circulation to provide additional control of insulin secretion. Norepinephrine and epinephrine can react with either α (inhibitory) or β (stimulatory) receptors, but their overall effect is to play an inhibitory role in insulin secretion in rats or mice (61). However, the mechanism of norepinephrine inhibition of insulin secretion is still not clear.

Acetylcholine released from parasympathetic vagus nerve terminals is responsible for cephalic phase insulin secretion which can be blocked by vagotomy (93). Parasympathetic nervous system response to a meal may stimulate insulin secretion over 26% (6). The mechanism of acetylcholine potentiation of meal-induced insulin secretion is still not completely understood.

2. Insulin and Thermogenesis

The complex and numerous effects and interactions of insulin on nutrient metabolism have important implications in energy balance regulation. Recently it was suggested that insulin was an important mediator of carbohydrate-induced thermogenesis and acts as a central satiety signal in the regulation of body weight (51,79,82). However, insulin can have different effects depending on central or peripheral action (14,82).

Effects of insulin added in vitro with labeled glucose C^{14}– to slices of rat interscapular brown adipose tissue were examined. Insulin dramatically increased glucose uptake up to 60-fold and increased oxygen consumption (90). Cafeteria-fed rats require insulin to produce diet-induced thermogenesis and to response to noradrenaline. Twelve hours after injection of PZI insulin (8 U/rat) into insulin deficient diabetic rats resting metabolic rate and noradrenaline response values increase to equal those of non-diabetic cafeteria rats (79). Rothwell also found that diabetic rats fail to maintain body temperature when exposed to 5 ^{o}C. Giving insulin to diabetic rats causes a recovery of cold-induced thermogenesis (79). These data indicate an insulin requirement for diet-induced thermogenesis and cold-induced thermogenesis. Subjects continuously infused with insulin and glucose causing hyperinsulinemia with normoglycemia had an

increased metabolic rate of 7.6%. After propranolol administration, energy expenditure decreased by 4% indicating that one mechanism whereby insulin induced thermogenesis was through β adrenergic receptors. Cold-adapted rats exhibit hypermetabolism with lower insulin levels compared with control rats housed at 20 ^{o}C (18). This suggests a negative correlation between plasma insulin concentrations and thermogenesis. Cunningham found that cold-adapted rodents are highly sensitive to insulin with considerably improved glucose tolerance (18). The exact role plasma insulin plays in control of BAT thermogenesis remains unclear.

3. Hyperinsulinemia in Ob/Ob Mice

Hyperinsulinemia appears in ob/ob mice as early as 6 days of age (21). The early hyperinsulinemia has been implicated as a primary cause of excess adiposity in ob/ob mice. Insulin promotes adipocyte hypertrophy and proliferation, increases activity of lipogenic enzymes, and decreases lipolysis. Hyperinsulinemia in ob/ob mice is due to either an increased secretion or a decreased clearance. Karakash (45) used streptozotocin to destroy pancreatic islets and decrease insulin secretion. In these animals with lower plasma insulin concentrations, hepatic insulin clearance increased. These data indicate that the decreased insulin clearance observed in ob/ob mice is likely a secondary consequence of

hyperinsulinemia. Thus, insulin secretion likely plays a major role of hyperinsulinemia in ob/ob mice. Tassava (96) also found that pancreatic islets from ob/ob mice were hyperresponsive to acetylcholine compared to islets from lean mice. Obese mice develop severe insulin resistance by 6 weeks of age (3). This may contribute to their impaired thermogenesis since increases in insulin sensitivity are seen in cafeteria-fed rats (79) and rats fed a low protein diet (78) compared with stock-fed controls.

4. Central Nervous System and Insulin

Although insulin has some direct actions on metabolism of BAT, its effects on thermogenesis are probably mediated centrally. Insulin is present in most areas of the brain but concentrations are variable. Insulin receptors are present in the hypothalamus and insulin may exert its effects at periventricular sites (17). Continuous infusion of insulin into the CSF (cerebrospinal fluid) of rats decreased food intake and reduced body weight (14). CSF insulin might be a satiety signal for body weight regulation. An obvious site of action for insulin is within the VMH (ventromedial hypothalamus). Electrical stimulation of the VMH resulted in a decrease in food intake. Whereas lesions VMH caused hyperphagia and obesity in rat. Some workers (68) found that electrical stimulation of VMH showed an increased thermogenesis in BAT. These data suggest that

the VMH exerts an inhibitory effect on energy intake and a stimulatory effect on thermogenesis and energy output. As a result, insulin can increase sympathetically-mediated thermogenesis probably via its central actions. Obese ob/ob animals may have a defect in the brain in response to the hyperinsulinemia resulting in low thermogenesis.

E. Relationship between Adrenalectomy, Glucocorticoid, and Diet Compositions

Cushing's syndrome is the clinical manifestation of the metabolic effects of hypercortisolism. Truncal obesity is commonly seen in Cushing's syndrome patients. Also, many obese people have signs and symptoms of Cushing's syndrome (88). Genetically obese (ob/ob) mice have increased plasma corticosterone concentrations at 3 weeks of age which is before they are visually obese. Thus, glucocorticoid must also be considered as a contributing factor.

1. Adrenalectomy

Adrenalectomy completely or partially normalizes most abnormalities of the ob/ob mice and fa/fa rats. These include hyperphagia, body weight gain and obesity, hyperinsulinemia, insulin resistance, and reduced brown adipose tissue mitochondrial GDP binding (40,41,57,66,84). Thus

hyperadrenocorticism of the ob/ob mice has been assumed to associated with the development of obesity.

2. Glucocorticoids

Many studies have shown that excess glucocorticoids cause increases in total body fat in adults (38) and in obese experimental animals (12,25). Chronic administration of glucocorticoid to adrenalectomized ob/ob mice and fa/fa rats reverses the effects of adrenalectomy. (25,99). There is evidence that obese animals are very sensitive to glucocorticoid administration. Freedman studied fa/fa rats adrenalectomized at 4 weeks of age and given daily injections of different concentrations of glucocorticoid for 30 days. As glucocorticoid doses increased, food intake and plasma insulin markedly increased in obese fa/fa rats without altering these measures in the lean rats (25). Adrenalectomized fa/fa rats responded to glucocorticoid at low doses (25). Thus, they suggested that genetically obese animals may be hypersensitive to glucocorticoid. Hypersensitive to glucocorticoids was also demonstrated by Tokuyama et al after 2 weeks implantation of corticosterone-containing pellets at 8.5 weeks of adrenalectomized ob/ob mouse (99). At low physiological levels of serum corticosterone (10 $\mu g/dl$), ob/ob mice in contrast to lean mice obviously increased body weight gain, food intake, serum insulin, and decreased BAT mitochondrial

GDP binding (99). Increase of food intake in lean mice only occurred at very high levels of corticosterone (30 $\mu g/dl$). All these results indicates that the ob/ob mouse is hypersensitive and hyperresponsive to physiological levels of corticosterone resulting in hyperphagia, hyperinsulinemia, and increased weight gain. The mechanisms whereby glucocorticoids cause obesity are not clear. One hypothesis is that glucocorticoids act through the central nervous system to restore hyperphagia (20).

3. Diet Compositions and Energy Balance

The effects of adrenalectomy in normalizing energy balance are diet dependent in ob/ob mice. In long term studies (3 weeks), adrenalectomy prevents the abnormalities in ob/ob mice fed a high-carbohydrate stock diet, whereas adrenalectomized ob/ob mice still exhibited the obese syndrome when they were fed a high-fat diet or a high glucose diet (29,47, 48,91,109). I only focus on a comparison of high-starch diet and high-glucose diet effects in adrenalectomized ob/ob mice in this thesis.

A major difference between a high-carbohydrate stock diet and a high-glucose diet is the source of carbohydrate. Several studies found that adrenalectomized ob/ob mice reduced their high efficiency of energy retention by decreased energy intake and increased energy expenditure through

a increase of thermogenic activity in BAT to levels comparable to lean mice when they were fed a high-starch diet. There is only a minimal effects on energy efficiency or thermogenic activity in BAT when adrenalectomized ob/ob mice fed a high-glucose. Plasma insulin concentrations are similar in both lean and adrenalectomized ob/ob mice fed a high-starch diet; however there is a less pronounced effect in adrenalectomized ob/ob mice fed a high-glucose diet (48,109). Warwick also showed that there is no additional effect due to dietary fiber when adrenalectomized ob/ob mice were fed the starch plus wheat bran diet (109). These results indicated that dietary glucose somehow lowers the metabolic rate of adrenalectomized ob/ob mice.

4. Time Sequences of Response to Diet

No studies have examined how quickly dietary glucose causes these parameters to change in adrenalectomized ob/ob mice. However, some clues can be obtained from other studies to presume that dietary effects maybe happen within a few days. When young obese rats were adrenalectomized GDP binding of BAT mitochondria was normalized within 7 days after adrenalectomy. A initial increase of GDP binding in obese rats appeared rapidly (within 24 hr after adrenalectomy), followed by a slower rate of increase during the next 6 days (39). Acute cold ($4\ ^{o}C$) exposure

of 1 to 3 hr of young rats caused a rapid change in GDP binding (67,102,106). Similar findings were showed in lean mice (35). When young fa/fa and lean rats were injected with noradrenaline, a rapid increase of GDP binding occurred within 30 min (67). These studies suggest that thermogenic actively of BAT can change within hours after a treatment is started.

Glucocorticoid treatment of adrenalectomized fa/fa rats for 24 h increases plasma insulin concentrations about 5 times compared to lean controls (23). An increase of plasma insulin and a decrease of GDP binding has also been observed within 15 h after glucocorticoid injection in both adrenalectomized ob/ob and lean mice (31). Adrenalectomized ob/ob mice are, however, more sensitive and responsive to the rapid action of corticosterone than adrenalectomized lean mice in terms of insulin secretion and BAT thermogenesis depression. From the above data it appears that adrenalectomy, cold temperature, and hormones (noradrenaline and glucocorticoid) can change GDP binding to BAT mitochondria and plasma insulin concentrations within one day.

F. Objectives and Hypothesis

My objectives were 1) to determine if replacement of dietary starch with glucose affects oxygen consumption, brown adipose tissue metabolism and plasma insulin concentrations in adrenalectomized ob/ob mice and 2) to describe the time sequence of changes in oxygen consumption, brown adipose tissue metabolism and plasma insulin concentrations in adrenalectomized ob/ob mice.

I hypothesized that thermogenic activity of brown adipose tissue (BAT) decreases and plasma insulin increases when adrenalectomized ob/ob mice are switched from a high-starch diet to a high-glucose diet.

II. MATERIALS AND METHODS

A. Animals and Diets:

Male obese (ob/ob) and lean (ob/+ or +/+) littermates were obtained from our breeding colony of C57Bl/6J-ob/+ mice. They were weaned at 21 days of age and housed in solid-bottom plastic cages with wood shavings as bedding in a room maintained at 23–25 ^{o}C with lights on from 07:00 to 19:00 h daily. Mice were offered a stock diet (Wayne Lab- Blox, Continental Grain, Chicago, IL) and water ad libitum. At 26 days of age, ob/ob and lean pairs were separated from their littermates and housed individually. At 28 days of age mice were bilaterally adrenalectomized or sham-operated through dorsal incisions while under ether anesthesia. Each adrenal gland was gently lifted and a curved scissors was used to remove each gland along with a small amount of adipose tissue. Incisions were closed with stainless steel wound clips. The total surgical procedure was completed within 6 min. Sham-operated mice underwent the same procedure except the adrenal glands were left intact. Physiological saline (0.9% NaCl) was given to adrenalectomized mice after surgery. All mice had semipurified diet and water/saline ad libitum after surgery.

Two semipurified diets were fed. The high-glucose diet contained in g per 100 g: 65 glucose, 20 casein, 0.3 methionine, 5.0 corn oil, 3.5 mineral mixture (7), 1.0 vitamin mixture (7), 0.2 choline chloride, and 5.0 cellulose. The high-starch (corn starch) diet was formulated on an equal energy basis by replacing 65 g of glucose with 59.2 g starch. These diets contained 66, 22, and 12% of metabolizable energy as carbohydrate, protein, and fat, respectively.

B. Experimental Design:

Experiment 1: Two sets of mice were used. Each set contained four groups, sham and adrenalectomized ob/ob, and sham and adrenalectomized lean mice. One set of mice served as controls and was fed the high-starch diet throughout the 16 day experiment. Another set of mice was fed the starch diet for 12 days, and then the high-glucose diet for the last 4 days of the 16 day experiment. Food intake, body weight and oxygen consumption were measured on day 11, 12, 13, 14 and 16. Oxygen consumption was measured at 0830-1100 h daily. Mice were decapitated at 11:00 h on day 16 to obtain trunk blood in heparinized beakers for plasma corticosterone, insulin and glucose assays.

Experiment 2: Only adrenalectomized mice were used. Adrenalectomized ob/ob and lean mice were either fed the high-starch diet throughout the day feeding trial, or fed the high-starch diet for 12 days and then switched to the high-glucose diet for 2 or 4 days. Mice were killed at 11:00 h on day 14 or 16 by decapitation to determine BAT thermogenic activity by measuring GDP binding to isolated BAT mitochondria. Blood was also collected in heparinized beakers for plasma corticosterone, insulin and glucose assays.

C. Experimental Assays:

Oxygen consumption: Mice were placed on a wire-mesh floor in the glass bottle that contained soda lime to remove expired carbon dioxide. The bottle was closed and immersed in a water bath maintained at $25 \pm 1\ ^{o}C$. After a 5 min adaptation period, 6 estimates of oxygen consumption were recorded within the next 5 to 10 min (111). Data were calculated as ml oxygen consumed/hr/g body weight at STP (standard temperature and pressure).

Hormone and glucose assays: Plasma for the corticosterone assay was diluted 1:9 with borate buffer/BSA (bovine serum albumin), then incubated at $60\ ^{o}C$ for 30 min to denature corticosterone-binding proteins.

Corticosterone was extracted with ethanol. After evaporating 50 μl of the ethanol extract in a vacuum oven, plasma corticosterone concentrations were determined by radioimmunoassay (Endocrine Sciences, Tarzana, CA) with modification. Only those adrenalectomized mice with plasma corticosterone concentrations less than 1 $\mu g/dl$ were included. The lowest detectable plasma corticosterone concentration in this assay was 0.15 $\mu g/dl$ plasma. Adrenalectomized ob/ob and lean mice fed the high-starch diet and then switched to the high-glucose diet had plasma corticosterone values of 0.74 ± 0.04 and $0.53 \pm 0.08 \mu g/dl$ plasma, respectively. Plasma corticosterone concentrations averaged 16.5 ± 4.1 and 2.7 ± 0.6 ug/dl plasma in sham- operated ob/ob and lean mice when diets were switched from the high-starch diet to the high-glucose diet, respectively. Adrenalectomized ob/ob and lean mice fed the high-starch diet had 0.59 ± 0.04 and $0.51 \pm 0.06 \mu g$ corticosterone/dl plasma, respectively. Sham-operated ob/ob and lean mice fed the high-starch diet throughout the 16 day trial had plasma corticosterone concentrations of 16.8 ± 2.7 and 4.6 ± 0.7 ug/dl plasma, respectively.

Plasma glucose concentrations were determined by the glucose oxidase-peroxidase method (Boerhinger-Mannheim, Indianapolis, IN). Plasma insulin concentrations were measured by radioimmunoassay with

rat insulin as the standard and antiporcine insulin serum (Nova Research Laboratories, Bagsvaerd, Denmark) with modifications to accommodate reduced sample volumes.

GDP binding to BAT mitochondria: Mice were killed and interscapular and subscapular BAT depots were rapidly removed, combined, weighed, and cut in pieces, then homogenized with 5% wt/vol in ice-cold buffer containing 250 mM sucrose and 5 mM K-TES (potassium-N-tris-methyl-2-aminoethane sulfonic acid) pH 7.2. Mitochondria were isolated in the sucrose buffer by the procedure of Cannon et al (63). H^3-GDP binding to BAT mitochondria was determined by the method of Nicholls (9) with modifications. Binding of H^3–GDP to BAT mitochondria was determined by incubation for 10 min at 25 ^oC in a media containing 100 mM sucrose, 20 mM K-TES, 1 mM EDTA, 2 μM rotenone, 100 μM potassium atractyloside, 2.2 x 10^9 dpm/ml H^3–GDP (New England Nuclear, 8.2 Ci/mmole), 10 μm unlabeled GDP, and 5.5 x 10^8 dpm/ml C^{14}–sucrose (New England Nuclear, 498.7 mCi/mmole). At the end of the incubation mitochondria were separated by centrifugation, and the mitochondria pellet was dissolved in Beckman tissue solubilizer-450 and counted in a liquid scintillation counter. C^{14}–sucrose was included as a marker of trapped media in the final mitochondria pellet. Specific binding

of H^3–GDP was calculated by subtraction of H^3–GDP trapped and non-specific H^3–GDP binding which was obtained from binding of H^3–GDP in the presence of 200 μM unlabeled GDP. Protein content of mitochondrial preparations and BAT homogenates (after extraction of lipids with acetone-petroleum ether) were measured by a modified Lowry method (11,13).

D. Statistical Analysis:

Two-way factorial analysis of variance was used to analyze data. The Bonferroni two-tailed t-test was employed in selected groups to detect significant difference between treatment and control groups. Student t-test was applied to examine diet effects in adrenalectomized ob/ob mice (26). Data were presented as means ± SE, and all significant effects were at $P < 0.05$.

III. RESULTS

Food intake, body weight gain and oxygen consumption of mice fed the high-starch diet during the first 12 days after surgery are shown in Fig 1. Sham-operated ob/ob mice consumed more energy and gained more body weight than lean mice (Fig. 1). Adrenalectomy reduced energy intake 26% and body weight gain 37% in ob/ob mice without affecting lean mice (Fig. 1). Oxygen consumption, expressed as ml/hr/gm body weight, was lower in sham-operated ob/ob mice than in lean mice, and adrenalectomy increased oxygen consumption 20% in ob/ob mice without affecting lean mice (Fig. 1). Sham-operated and ADX ob/ob mice consumed similar amount of oxygen when values were expressed per mouse.

Sham-operated ob/ob mice had higher food intake and body weight gain and lower oxygen consumption than lean mice independent of diet (Fig. 2). Food intake and oxygen consumption increased in sham-operated lean mice after their diet was switched from a high-starch diet to a high-glucose diet for 4 days.

Adrenalectomized ob/ob mice, but not adrenalectomized lean mice, consumed more energy and gained more weight when switched from the

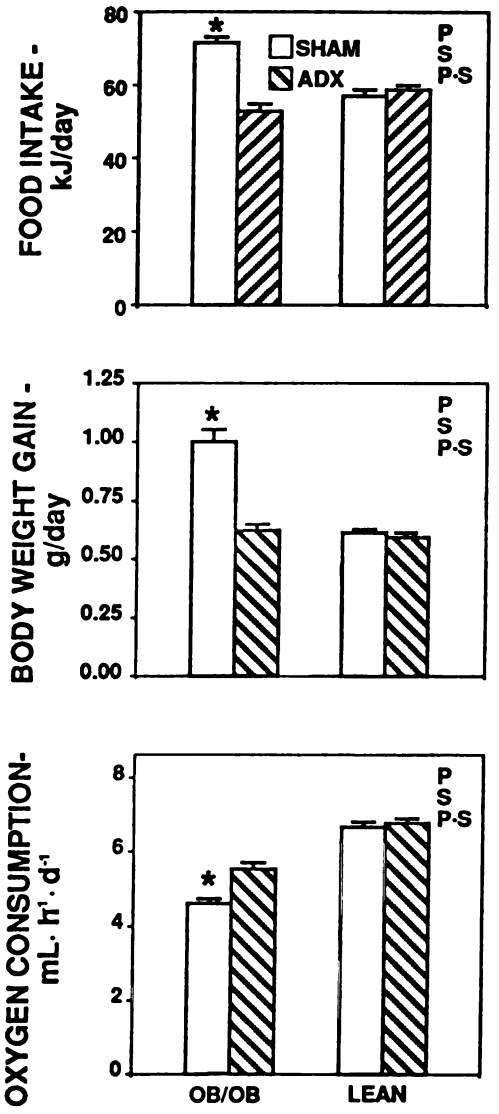

Fig. 1. Food intake, body weight gain, and oxygen consumption of sham-operated (SHAM) and adrenalectomized (ADX) ob/ob and lean mice fed a high starch diet for 12 days. Body weights at 12 days after surgery averaged 22.85 ± 0.54, 19.97 ± 0.38, 19.01 ± 0.22, and 18.36 ± 0.34 in sham-ob/ob, ADX-ob/ob, sham-lean, and ADX-lean mice, respectively. Oxygen consumption was measured between 08:30 and 11:00 on day 12. Each bar represents means ± SE for 20-34 mice. Asterisks indicate significant differences ($P<0.05$) between sham-operated ob/ob mice and adrenalectomized ob/ob mice. P indicates significant effect ($P<0.05$) of phenotype; S indicates significant effect ($P<0.05$) of surgery and P · S indicates significant ($P<0.05$) phenotype surgery interaction.

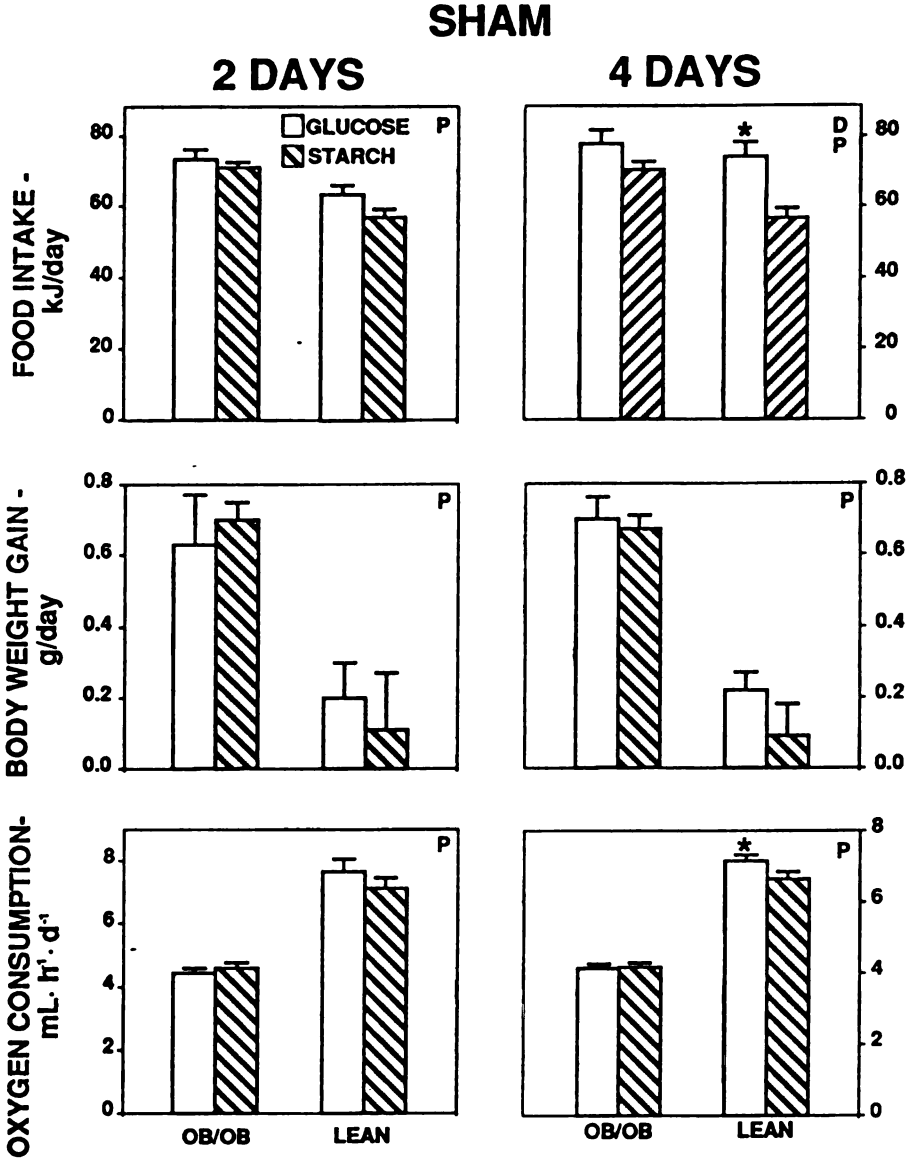

Fig. 2. Food intake, body weight gain, and oxygen consumption of sham-operated (SHAM) mice switched from a high-starch diet to a high-glucose diet for 2 or 4 days. Left panels: sham-operated (SHAM) ob/ob and lean mice were fed the high starch diet for 14 days, or were fed the high starch diet for 12 days and then switched to the high glucose diet for 2 days. Right panels: SHAM mice were fed the high-starch diet throughout the 16 day feeding trial, or switched to the high-glucose diet for the last 4 days of the 16 day experiment. Bars for food intake and oxygen consumption represent the means (11-12 mice per treatment group) ± SE on day 14 (left panels) and 16 (right panels). Body weight gains were calculated as the average of difference between body weight on day 14 and day 12 (left panels) or day 16 and day 12 (9-12 mice per group; right panels). Asterisks indicate a significant effect ($P<0.05$) of diet within phenotype. D indicates significant effect ($P<0.05$) of diet and P indicates significant phenotype effect.

high- starch diet to the high-glucose diet for 4 days (Fig. 3). Adrenalectomized ob/ob mice fed glucose did not increase their oxygen consumption even though energy intake was elevated (Fig. 3).

Diet did not influence plasma glucose or insulin concentrations in sham-operated mice (Fig. 4). As expected, plasma insulin concentrations were much higher in ob/ob mice than in lean mice.

Plasma glucose concentrations in adrenalectomized mice were not influenced by diet (Fig. 5). Plasma insulin concentrations, however, were approximately 2 times higher in adrenalectomized ob/ob mice, but not in adrenalectomized lean mice, switched to a high-glucose diet for 2 or 4 days than in the respective adrenalectomized control mice fed a high-starch diet (Fig. 5). Adrenalectomy lowered the high plasma insulin concentrations in ob/ob mice (Fig.4 & Fig.5) as has been observed before (48,91,109).

Adrenalectomized ob/ob mice had a higher protein content of BAT than that of adrenalectomized lean mice (Fig. 6). BAT protein content was unaffected by diet. GDP binding to isolated BAT mitochondria, which is an indicator of thermogenic activity of BAT, was lowered 26% in adrenalectomized ob/ob mice, but not in adrenalectomized lean mice,

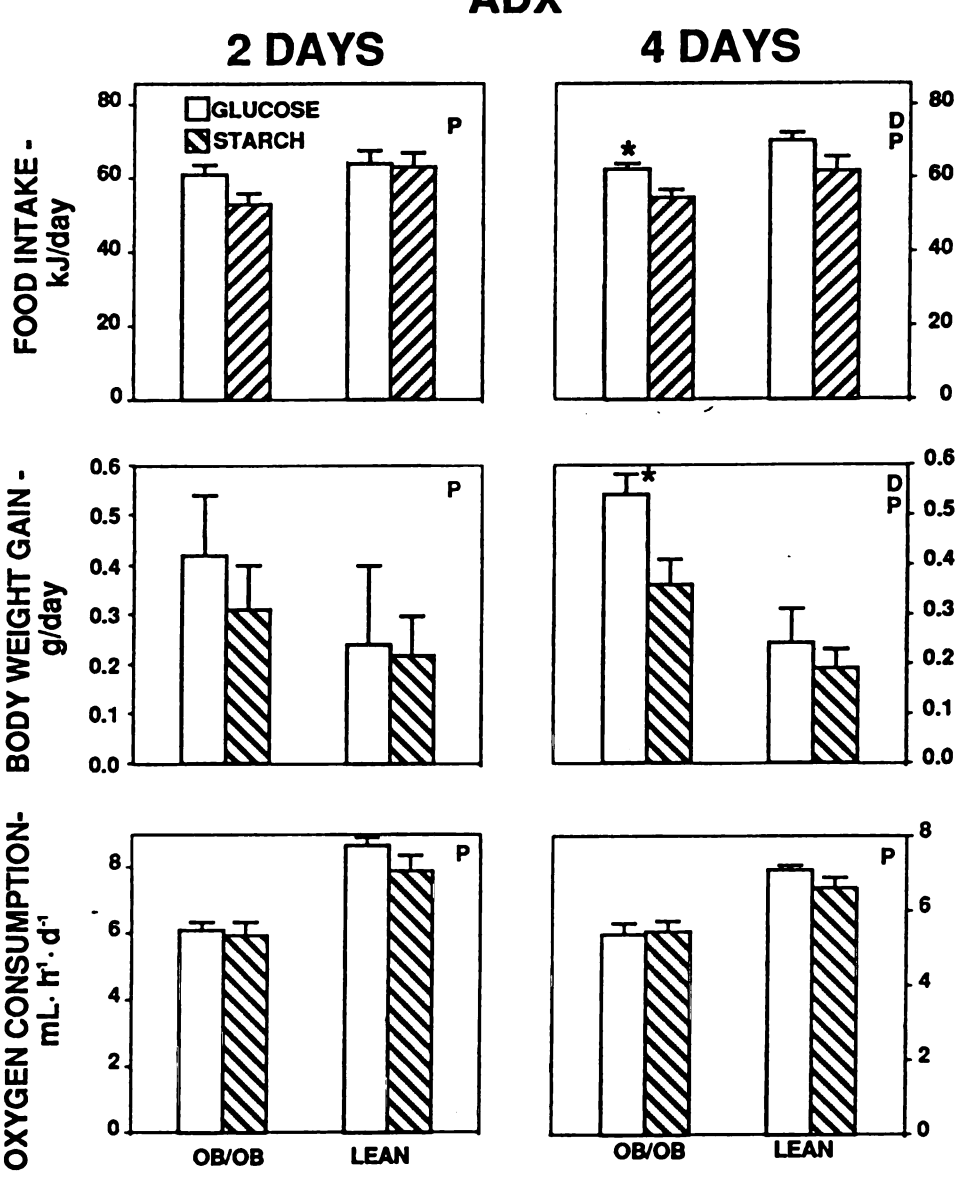

Fig. 3. Food intake, body weight gain, and oxygen consumption of adrenalectomized mice switched from a high-starch diet to a high-glucose diet for 2 or 4 days. Left panels: adrenalectomized (ADX) ob/ob and lean mice were fed the high-starch diet for 14 days, or were fed the high starch diet for 12 days and then switched to the high glucose-diet for 2 days. Right panels: ADX mice were fed the high-starch diet throughout the 16 day feeding trial, or switched to the high-glucose diet for the last 4 days of the 16 day experiment. Bars for food intake and oxygen consumption represent the means (8-12 mice per treatment group) ± SE on day 14 (left panels) and 16 (right panels). Body weight gains were calculated as the average of difference between body weight on day 14 and day 12 or day 16 and day 12 (9-21 mice per group). Asterisk indicates a significant effect ($P<0.05$) of diet within phenotype treatment. D indicates significant effect of diet and P ($P<0.05$) indicates significant effect of phenotype.

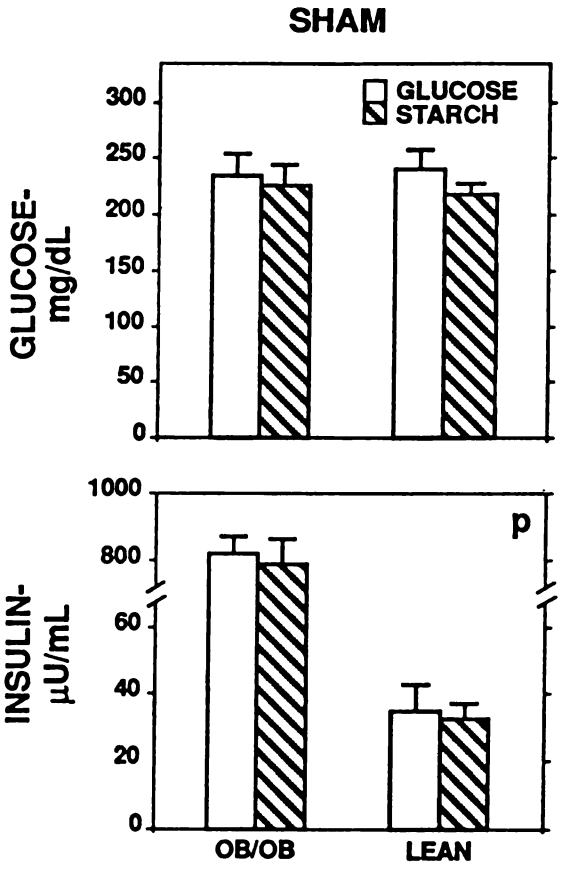

Fig. 4. Plasma glucose and insulin concentrations of sham-operated (SHAM) ob/ob and lean mice fed the high-starch diet for 16 days, or fed the high- starch diet for 12 days and then switched to the high-glucose diet for 4 additional days. Each bar represents means ± SE for 5-10 SHAM ob/ob and lean mice. P indicates significant effect ($P<0.05$) of phenotype.

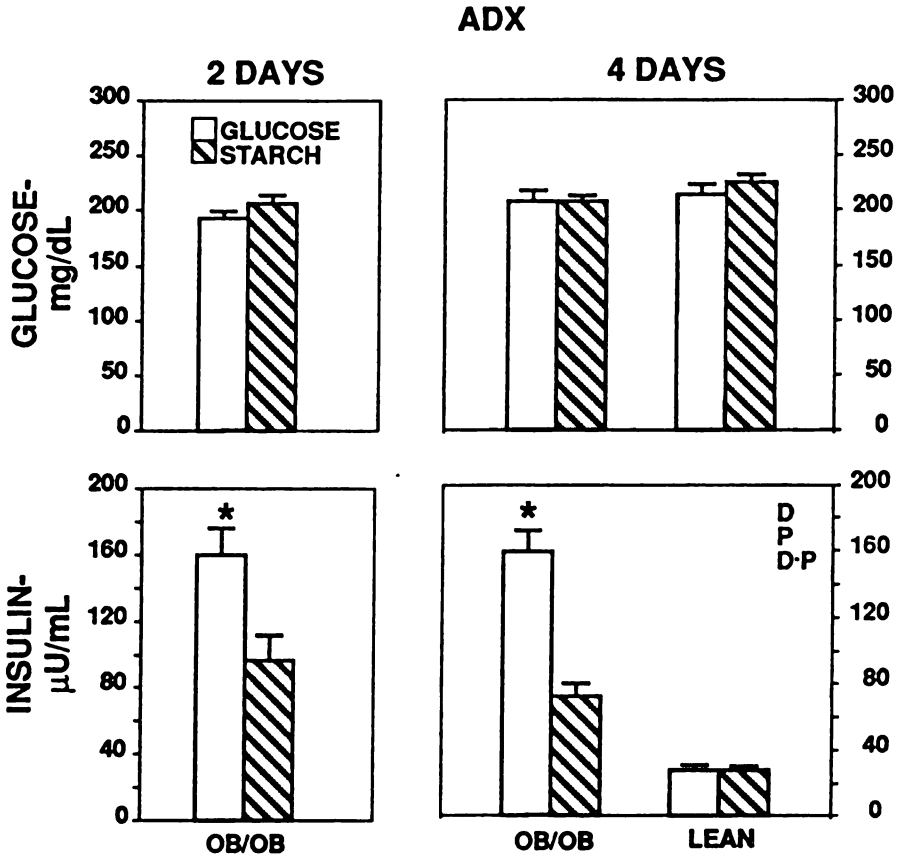

Fig. 5. Plasma glucose and insulin concentrations of adrenalectomized mice switched from a high-starch diet to a high-glucose diet for 2 or 4 days. Left panels: adrenalectomized (ADX) ob/ob mice were fed the high-starch diet for 14 days, or were fed the high-starch diet for 12 days and then switched to the high-glucose diet for 2 additional days. Right panels: ADX mice were fed the high-starch diet for 16 days, or were fed the high-starch diet for 12 days and then switched to the high-glucose diet for 4 additional days. Each bar represents means ± SE for 20-26 ADX ob/ob and lean mice. Asterisk indicates a significant effect ($P<0.05$) of diet within phenotype treatment. D, significant effect ($P<0.05$) of diet; P, significant effect ($P<0.05$) of phenotype; D · P, significant ($P<0.05$) diet phenotype interaction.

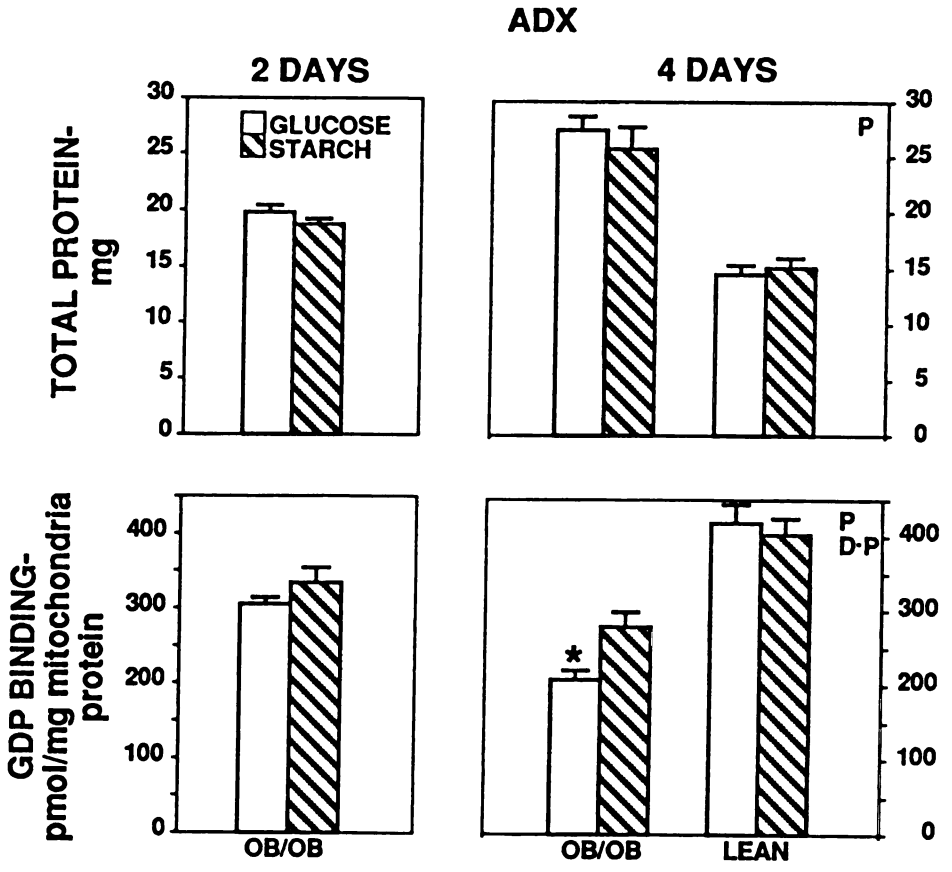

Fig. 6. Total protein and GDP binding to isolated brown adipose tissue (BAT) of adrenalectomized ob/ob and lean mice fed a high starch diet for 14 (left panels) to 16 (right panels) days, or fed the high starch diet for 12 days and then switched to a high glucose diet for 2 (left panels) or 4 (right panels) additional days. BAT represents combined interscapular and subscapular BAT depots. Each bar represents means ± SE for 10-16 mice. Asterisk indicates a significant effect ($P<0.05$) of diet within phenotype. P indicates significant effect ($P<0.05$) of phenotype and D · P indicates significant ($P<0.05$) diet phenotype interaction.

switched from the high-starch diet to the high-glucose diet for four days; no changes in GDP binding were noted within 2 days after switching from the high-starch to the high-glucose diet (Fig. 6). Adrenalectomized lean mice had higher GDP binding to BAT mitochondria than adrenalectomized ob/ob mice (Fig. 6).

IV. DISCUSSION

A. Thesis Discussion

Adrenalectomy reduced food intake, body weight gain and plasma insulin concentration in ob/ob mice fed a high-starch diet, as others have reported (48,91,109). Energy expenditure, as measure by oxygen consumption and expressed as ml/hr/gm body weight, was higher in adrenalectomized ob/ob mice than in sham ob/ob mice even though adrenalectomized ob/ob mice consumed less food. Thus, the decrease in efficiency of dietary energy retention observed in adrenalectomized ob/ob mice is caused by both decreased energy intake and increased energy expenditure per unit body weight.

Effects of adrenalectomy are diet dependent (48,91,109). Chronic consumption of a high-glucose diet can partially block the effects of adrenalectomy in ob/ob mice. I investigated the mechanism of this response by determining if dietary glucose could reverse effects of adrenalectomy in ob/ob mice fed a high starch diet. Consumption of a high glucose diet for only 4 days caused an increase in food intake and body weight gain without changing oxygen consumption in adrenalectomized ob/ob mice. Plasma insulin also increased and GDP binding to

isolated BAT mitochondria decreased in these mice within 4 days after switching to the high-glucose diet. Thus, dietary glucose can within several days reverse effects of adrenalectomy in ob/ob mice fed a high-starch diet. These responses are genotype dependent as the switch from dietary starch to glucose, did not change these parameters in adrenalectomized lean mice.

The mechanisms responsible for dietary glucose-induced increases in plasma insulin and decreases in GDP binding to BAT mitochondria in adrenalectomized ob/ob mice are not entirely clear. Plasma insulin was elevated at the earliest time point examined (2 days after the diet switch) without significant influences of the diet switch on food intake or plasma glucose concentrations. The possibility that diet-induced differences in glucose concentration within the gastrointestinal tract alter gastrointestinal hormones release and/or neural signals that control insulin secretion needs to be explored.

The BAT response to the diet switch in adrenalectomized ob/ob mice took longer (4 days) than did the insulin response (2 days). This raises the possibility that the decrease of thermogenic activity in BAT may be caused by the earlier increase in plasma insulin. It has been demonstrated the injection of insulin into the carotid artery decreases the sympathetic

efferent firing rate to BAT (87). Moreover, several reports have shown that BAT thermogenesis is depressed in insulin-deficient diabetic rats (79) and in insulin-resistant obese animals (82). Thus, insulin resistance in obese animals plays an important role in thermogenesis of BAT. At 4 weeks of age ob/ob mice, before insulin resistant development, have normal responses of thermogenic activity in BAT on acute exposure to cold. Whereas, the response to cold is greatly blunted when insulin resistance has developed at 5-week-old of ob/ob mice (60). Several studies (85,86) found that insulin may also act directly on the hypothalamus to regulate sympathetic activity. Sakaguchi (85,86,87) has demonstrated that injection of insulin into the ventromedial hypothalamus or paraventricular nucleus decreases sympathetic firing rate to BAT. Destruction of neurons in the VMH abolished these effects caused by insulin injection. From the above data I suggest that insulin may be one modulator for the hypothalamic control of sympathetic nervous system to BAT in ob/ob mice. Further research is needed to explore these areas.

My hypothesis is that the glucose component of the diet may act through gastrointestinal receptors or hormones to cause alteration of autonomic neurotransmitter to the pancreatic β-cell in adrenalectomized ob/ob mice showing a two-time increase of plasma insulin. Then, insulin acts

through the hypothalamic control of sympathetic nervous action to BAT. This effect is mediated in part by the VMH to decrease the firing rate of sympathetic nerves to BAT resulting in a decrease in GDP binding and an increase in food intake and body weight gain.

B. Future Research

To further understand my data and to keep searching for the mechanisms whereby diet composition and adrenal secretions contribute to obesity, I propose the following studies.

1. Norepinephrine turnover

Norepinephrine turnover, which is an indicator of sympathetic nervous system activity, should be measured in adrenalectomized ob/ob mice fed a high-starch diet and then switched to a high-glucose diet for 2 to 4 days. According to my results which showed a lower thermogenic activity in brown adipose tissue of adrenalectomized ob/ob mice fed a high-glucose diet for 4 days, I predict that consumption of the glucose diet decreases stimulation of the sympathetic nervous system to BAT. Thus, measurement of norepinephrine turnover in BAT can give further information in this area.

2. Peripheral insulin injection

I speculate that the glucose diet-induced lowering of BAT metabolism is caused by the earlier elevation in plasma insulin concentration. To test the hypothesis that hyperinsulinemia is the key regulator causing low thermogenic activity in BAT, I would inject long-acting insulin into adrenalectomized ob/ob mice fed the starch diet. I would measure GDP binding to BAT mitochondria after 2 to 4 days. Insulin might act directly on BAT or indirectly via the central nervous system. Therefore, I would determine if peripheral insulin injection decreased the firing rate of sympathetic nervous system to BAT in adrenalectomized ob/ob mice. If this occurred I would further suspect that insulin may act at central nervous system which then modulates sympathetic nervous system to BAT. To explore this hypothesis, I would inject insulin into the hypothalamus and measure the firing rate of sympathetic nerves in BAT. If I found that hypothalamic injection of insulin reduces the efferent rate of sympathetic activity to BAT in adrenalectomized ob/ob mice but not in lean mice, this would suggest that insulin may be one modulator for hypothalamus to control sympathetic nerves efferent firing rate to BAT. A dose response curve for insulin could also be measured to provide data on sensitivity of the hypothalamus to insulin.

3. Vagotomy or cholinergic blocker

Consumption of a high-glucose diet caused an increase in plasma insulin concentration in adrenalectomized ob/ob but not in lean mice. The mechanisms responsible are still not clear. There are at least four possibilities : 1) nervous system regulates insulin secretion; 2) glucose directly acts on pancreas β cells; 3) glucose stimulates gut hormones secretion which acts on pancreas to modulate insulin secretion; or 4) glucose metabolites stimulate insulin secretion. I think that nervous system regulation of insulin secretion is the most likely pathway according to recent findings (65,85,86,87). To address the possibility that dietary glucose affects plasma insulin in adrenalectomized ob/ob mice via altered neural regulation of insulin secretion I would use vagotomy or cholinergic antagonist (atropine). Adrenalectomized ob/ob mice and lean mice either fed a high-starch diet or fed a high-starch diet and then switched to a high-glucose diet would be vagotomized or injected with atropine. A disadvantage of vagotomy would be that the surgery would decrease of food intake and may interfere with the results. I would therefore use atropine as the first approach.

4. Body composition

Energy density of gain (an indicator of relative proportions of fat and lean tissue gain) and efficiency of energy retention can give us a better idea of how quickly the dietary glucose can reverse adrenalectomy effect in energy efficiency within a short time. Generally speaking, it is difficult to detect differences in these parameters within a short time. However, my results showed that adrenalectomized ob/ob mice gained more body weight when switched from the high-starch diet to the high-glucose diet. It would be interesting to determine if detectable change in body composition would be evident within 4 days after the diet switch.

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