MECHANISMS FOR COMPENSATORY GROWTH OF ADIPOSE TISSUE IN LIPECTOMIZED RATS

by

JIE LU

(Under the Direction of Dorothy Hausman)

ABSTRACT

Studies were designed to examine the regulation of body weight/fat by lowering body weight/fat through surgical removal of bilateral epididymal fat pads of adult male Wistar rats.

Food intake during the first 4 weeks and energy expenditure on day 7-10 and day 28-31 post surgery were not different between lipectomized and sham operated rats. The carcass composition of lipectomized and sham operated rats was not significantly different 16 weeks post surgery, indicating a compensatory adipose tissue growth in lipectomized rats.

The compensatory growth of adipose tissue was fat pad specific: both mesenteric and retroperitoneal fat pads, but not inguinal or perirenal fat pads were significantly heavier in lipectomized rats than in sham operated rats. Lipectomized rats had more total, small and large adipocytes at 4 weeks, and more total and large adipocytes at 16 weeks, than sham operated rats in the retroperitoneal fat depot, suggesting that both hyperplasia and hypertrophy contributed to the compensatory growth. Leptin and insulin did not appear to facilitate signaling of the loss of fat content, as serum concentrations of these putative adiposity signals were not different between lipectomized and sham operated rats 2 or 4 weeks after lipectomy.

In vitro testing indicated that serum from lipectomized rats stimulated the proliferation of preadipocytes more than that from sham operated rats. In contrast, media conditioned by exposure to fat pads from lipectomized rats did not increase preadipocyte proliferation. This suggests that the blood borne factors stimulating proliferation of adipocytes are from tissues other than fat tissue. On the other hand, serum from lipectomized rats did not induce differentiation of preadipocytes more than that from sham operated rats, indicating that mechanism(s) other than blood borne factors may induce hypertrophy in compensating adipose depots.

The possible involvement of the sympathetic nervous system in compensatory growth was tested by measuring norepinephrine concentrations in retroperitoneal fat pads, and no significant difference was observed between lipectomized and sham operated rats.

Rats restore body fat level reduced by lipectomy through compensatory adipose tissue growth indicating that body weight/fat is regulated. Compensatory growth of adipose tissue is mediated, in part, by blood borne factors.

INDEX WORDS: Obesity, Lipectomy, Weight loss, Energy expenditure, Food intake, Adipocyte, Adiposity signals, Proliferation, Differentiation, Primary cell culture, Serum, Paracrine factors, Norepinephrine

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DEDICATION

I dedicate this dissertation to my parents, Yuheng Lu and Renhui Wang, and my lovely wife Haojie Li. Thank you for the encouragement to explore every possibility. You instilled in me the power of love and importance of education.

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TABLE OF CONTENTS

Page

CHAPTER I

INTRODUCTION

Obesity is a disease characterized by increased adipose tissue mass. Obesity is a major health problem in both developed countries and developing countries. It is estimated that nearly half a billion of the world's population are overweight or obese (24). In the United States of America, there is an obesity epidemic. More than half of all U.S. adults were considered overweight or obese in 2001 (21). China witnessed a tripling of the incidence of being overweight among men in the past 8 years, and a doubling among women (27). Obesity has been shown to be a risk factor for many diseases (23), and the economic cost of obesity is enormous (1).

Obesity does not develop overnight. Instead body weight is regulated physiologically (10,15,20). The development of obesity is caused by an imbalance between energy intake and energy expenditure. The excess in energy between intake and expenditure is stored as lipid in adipose tissue. Adiposity factor(s), which reflect the fat content (energy balance condition), informs the brain of the status of body weight/fat (3,12,26). The central nervous system responds to these putative adiposity signals by changing food intake and energy expenditure. Adipose tissue enlargement is caused by adipocyte hyperplasia (increase of cell number) and/or hypertrophy (increase of cell size) (4,13). The development of adipocytes is affected by many variables such as blood borne factors which originate from both fat tissue and non fat tissue, and paracrine factors that

are secreted by adipose tissue and regulate the proliferation and/or differentiation of preadipocytes (11). The sympathetic nervous system may also play a role in the regulation of adipose tissue (6,14,18).

The mechanism by which body weight is regulated is an important question in the fight against obesity. Lipectomy (liposuction), the surgical removal of adipose tissue, is sometimes utilized to reduce the fat content of obese patients or for the purpose of plastic surgery (8). Lipectomy is also a good means to investigate mechanisms of body weight regulation (20). In many species such as Golden-mantled ground squirrels, Siberian and Syrian hamsters (18,19), rats (2,7,9,16,22,25), mice (5) and pigs (17), the partial removal of body adipose tissue induces the growth of non-excised fat pads to the degree that the total fat mass is not different between the lipectomized animals and the sham controls. This suggests a compensatory growth of adipose tissue after lipectomy. The mechanisms of compensatory growth of adipose tissue after lipectomy are not well understand, however. Food intake and energy expenditure after lipectomy has been investigated in many studies, and the results are controversial. There are many other aspects regarding compensatory growth that need to be investigated, such as the time pattern of cellularity change in compensatory grown fat depots, potential signals conveying the information of body weight/fat reduction of lipectomized animals, and the roles of serum borne factors and/or adipose tissue originated paracrine factors, and the sympathetic nervous system in compensatory growth.

The hypothesis tested in the current study is that compensatory adipose tissue growth after lipectomy is due to hyperplasia and/or hypertrophy, and is mediated by blood borne factors and/or the sympathetic nervous system. Studies with lipectomized

rats were designed to answer the following questions. First, what characterizes the positive energy balance after lipectomy? Second, do insulin and leptin facilitate the compensatory growth after lipectomy? Third, what is the pattern of cellularity changes in fat pads during compensatory growth? Fourth, are blood borne factors involved in the compensatory growth? Fifth, do paracrine factors secreted from adipose tissue stimulate the compensatory growth? Sixth, is the sympathetic nervous system involved in the compensatory growth after lipectomy? The findings from these studies will bridge the gap in understanding the mechanisms of compensatory growth after lipectomy, and of the regulation of body weight/fat.

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CHAPTER II

REVIEW OF THE LITERATURE

BRIEF INTRODUCTION OF OBESITY

The definition of obesity is having a very high amount of body fat in relation to lean body mass, or a Body Mass Index (BMI) of 30 or higher for adult. BMI is a measure of an adult's weight in relation to his or her height, that is the adult's weight in kilograms divided by the square of his or her height in meters.

Obesity is a major health problem in developed countries. From 1985 to 2001, there has been a dramatic increase in obesity in the United States. In 1985, no one state had an obesity rate greater than 14%. However, by year 2001, 20 states had rates of 15–19%; and 29 states had rates of 20-24 %, and one state had an obesity rate higher than 25%. Along with the spread of the obesity epidemic, the prevalence of overweight in U.S. adults increased by 61% from 1991 to 2000. More than half of all U.S. adults were considered overweight (BMI 25 to 29.9) or obese in 2001 (136).

Obesity is also a problem in children. Data from the 1999-2000 National Health and Nutrition Examination Survey (NHANES) (144) showed that the prevalence of overweight children was 15.5% among 12- through 19-year-olds, 15.3% among 6- through 11-year-olds, and 10.4% among 2- through 5-year-olds, compared with 10.5%,

11.3%, and 7.2%, respectively, in 1988-1994 (NHANES III). This is about a 40% increase in the incidence from 1988-94 to 1999-2000.

Obesity is no longer considered a by-product of modern life in developed countries, instead it is spreading into developing countries as well (134). Take China as an example. China is experiencing increasing social and economic development and urbanization, with accompanying improvements in nutrition and longevity. Consequently, there has been a tripling of the incidence of overweight men in the past 8 years, and a doubling of overweight women (213). In Shanghai, China, about 29.5% of 2776 randomly selected adults (20-94 years of age, both men and women) were overweight, and 4.3% were obese (95). It is estimated that nearly half a billion of the world's population are considered to be overweight or obese (160).

The health risk of obesity lies in its relation with many diseases (142). Obesity is more than a cosmetic problem; instead, it is a health hazard. Obesity is a risk factor for diseases such as hypertension, high blood cholesterol, dyslipidemia, Type 2 (non-insulin dependent) diabetes, insulin resistance, hyperinsulinemia, coronary heart disease, angina pectoris, congestive heart failure, stroke, gallstones, cholescystitis and cholelithiasis, gout osteoarthritis, obstructive sleep apnea and respiratory problems, some types of cancer (such as endometrial, breast, prostate, and colon), complications of pregnancy, poor female reproductive health (such as menstrual irregularities, infertility, irregular ovulation), bladder control problems (such as stress incontinence), uric acid nephrolithiasis and psychological disorders (such as emotional suffering, depression, eating disorders, distorted body image, and low self esteem) (142,198). It is estimated that the number of annual deaths attributed to obesity among US adults is approximately

280,000 (5). In 1995, the estimated healthcare costs of obesity were $99.2 billion, which represented about 6% of the national health expenditure in the United States (205). In 2000, the economic cost of obesity in the United States was about $117 billion (1).

Obesity does not develop overnight, instead it is a chronic condition. Overall there are an array of factors that are involved in obesity development. In short, behavioral, environmental, and genetic factors may contribute to obesity. Obesity results from an energy imbalance, which means energy (caloric) intake is too high and/or energy expenditure is too low. However, the etiology of obesity is not so simple. Body weight is influenced by genes, metabolism, behavior, environment, culture, and socioeconomic status.

As the first law of thermodynamics indicates, weight gain is a result of more calorie consumption than the amount of energy used. In the United States, many foods are available which tend to be high in calories, and portion size has increased. All those changes contribute to increased energy consumption (22,158). At the same time, energy expenditure is not increased accordingly. On average, most Americans are sedentary. This imbalance between energy intake and expenditure will inevitably cause the accumulation of energy, in the form of increased fat mass. Genetics also plays a role in obesity. In animals, specific gene mutations cause obese animals, such as *ob/ob* mouse (220), *db/db* mouse (39,43,45,93) and Zucker fatty rat (43,150). In humans, genes can directly cause obesity in disorders such as Bardet-Biedl syndrome (140) and Prader-Willi syndrome (115). However, genes do not always predict future health. Genes and behavior may interact to affect the development of obesity. In some cases multiple genes may increase one's susceptibility for obesity and require outside factors, such as an

abundant food supply or little physical activity (2). Some illnesses such as Cushing's disease, and polycystic ovary syndrome (30,122) may lead to obesity, though they are rare.

BODY WEIGHT/FAT SIGNALS FOR REGULATION

The study of the etiology of obesity is the first step towards the prevention and treatment of obesity. There are many methods to study obesity in the laboratory. Studies about energy balance regulation, adiposity signals, weight reduction and regain and fat cell proliferation and differentiation all contribute to the understanding of body weight regulation. Following is a review of (1) how the body weight status is detected, (2) how animals adjust food intake and energy expenditure to maintain body weight, and (3) what lipectomy studies tell us about body weight regulation.

Body weight, like body core temperature and body fluid osmolarity, is regulated physiologically. Overweight, obesity or underweight occurs if the regulation fails. The mechanism by which the body regulates its weight is a challenging question to scientists. How does the body get information about its body weight/body fat level? This is one of the questions that need to be answered before we can determine a practicable means to prevent or treat obesity. The following is a review on how the body "senses" its body weight/body fat condition.

Several theories (see Stubbs' review (189)) about energy balance regulation have been postulated, such as the glucostatic hypothesis (133,189,197), the thermostatic hypothesis (89), the aminostatic hypothesis (135), and the lipostatic hypothesis (104).

Each hypothesis has some supporting evidence. However, here we will review the lipostatic hypothesis only.

Originally, the idea of body weight regulation was proposed by Kennedy (104). He postulated the lipostatic hypothesis, which says that the young rat adjusts its food intake so precisely to its energy needs that its fat stores remain almost constant. He further suggested that the obesity produced by medial hypothalamic lesions resulted from an impairment in the lipostatic mechanism that normally operates to prevent excess fat mass deposition. Hervey (86) further proposed a mechanism for lipostatic regulation. By measuring a fat-soluble hormone as an indicator of fat mass, he postulated that such a hormone would act as a physiological signal that monitors adipose tissue mass by the dilution principle. Keesey et al. (103) extended this idea and proposed the body weight set point theory, which suggests that the medial and lateral hypothalamus jointly determine a body weight set point which could be adjusted by external factors such as diet palatability. Under conditions of energy balance, the daily resting energy expenditure of animals, ranging in size from small birds to rodents to large mammals, was a fixed function of their "metabolic mass"-- body weight raised to the 0.75 power. All animals expend energy at comparable rates when expenditure is expressed to their metabolic mass (kleiber's equation) only when at their physiological regulated body weight or set point. Body weight set point is that particular weight at which the energy expenditure of the animal is consistent with the interspecies Kleiber value (102,106). There is only one set point at a time, but it can be changed over the individual's life span or altered by dietary or surgical intervention. Thus, animals adjust their food intake and resting energy expenditure following weight change so as to maintain their normal body

weight. Overfeeding or caloric restriction regimes, which increase or decrease body weight of animals, demonstrate this body weight regulation theory. Following release from these regimes animals change their food intake and resting energy expenditure so as to maintain the pre-intervention "regulated" body weight (102,132,204).

No matter how the body regulates its total weight or total fat, the first information needed before the regulation occurs is the status of its weight/fat condition. There must be some signal(s) to inform the brain of body weight/fat change. An adiposity signal is a signal that informs the brain of the energy balance and homeostasis. The characteristic of the input signals has not been well defined. Based on the literature reviewed below, it is clear that both circulating factors and neural factors may act as adiposity signals.

1. Humoral factors

Parabiosis experiments indicate that blood borne factors play an important role in the control of total body fat. In the parabiosis model two rats or mice are joined surgically so that they share a common blood supply, but not a common nervous system (44,46,80). When the circulatory systems of *ob/ob* and wild-type mice were joined, the weight of *ob/ob* decreased and that of wild-type mice was not changed. The joined circulation of *db/db* and wild-type mice did not change the body weight of either of the *db/db* or the wild-type. When the circulatory systems of *db/db* and *ob/ob* mice were joined, however the weight of *ob/ob* decreased, while the *db/db* mice remained obese. This study suggested that the *ob/ob* mice lack a circulating anorexic factor whereas the *db/db* mice have an impaired response to this factor.

For a blood borne factor to act as an adiposity signal, it needs to satisfy the following criteria. First, the circulating concentration of that factor must be relatively constant and proportional to adipose tissue mass. Second, the factor must be able to pass the blood brain barrier. Third, the brain must express specific receptor(s) for that factor and respond correspondingly (mostly, by adjusting food intake and energy balance) to maintain body weight at the regulated level. Insulin and leptin are two circulating factors that meet all the criteria for an adiposity signal as reviewed below.

(1). Insulin as an adiposity signal:

A large body of literature supports the hypothesis that circulating insulin enters the brain to produce anorexic responses, including reducing food intake. Much of the extensive literature can be found in review articles (16,83,171,210,211) and is summarized here.

Insulin is secreted by the islets of Langerhans in the pancreas. Insulin has a well documented role of regulating nutrient substrate metabolism by insulin-sensitive tissues such as skeletal muscle and fat. Specifically insulin promotes anabolic metabolism in peripheral tissues. When more energy is taken in than is expended, the excess calories are stored in adipose tissue in the form of lipid. After receiving the information from adiposity signals, including insulin, of the changed energy homeostasis, the brain adjusts food intake and energy utilization, trying to restore adipose tissue mass to a normal regulated level (170).

Even though insulin is not secreted by adipocytes, it still conveys the status of energy homeostasis and adiposity to the central nervous system (CNS). Circulating insulin concentration is proportional to adiposity, and obesity is associated with elevated

levels of insulin, both basal and in response to a glucose challenge (9,23,71,99,152). Bagdade et al. (9) reported that the fasting serum insulin level in obese subjects was significantly higher than that in normal weight controls, and was positively correlated with body weight for both groups (r: 0.72). Bernstein et al. (23) observed that the serum basal insulin level was positively correlated with body weight for rats with a wide range of body weights induced by over-feeding or under-feeding. Kahn et al. (99) also observed a positive correlation between circulating insulin and adiposity. Goodpaster et al. (71) reported that obese men and women had higher insulin levels compared with lean controls. Polonsky et al. (152) investigated the pattern of insulin secretion of normal and obese human subjects over a 24-hour period. They observed that basal insulin secretory rates were consistently elevated in the obese, and that the secretory rates in the 4 hours after breakfast, lunch and dinner were also increased in obese subjects compared with normal body weight subjects. Although the amplitude of secretory pulses were greater in obese subjects compared with that of normal weight subjects, the number and timing of the pulses were similar in both groups. Interestingly, a positive correlation between cerebrospinal fluid (CSF) insulin levels and adiposity was also observed. Owen et al. (147) reported that obese humans have elevated CSF immunoreactive insulin (IRI) concentrations as compared with normal weight controls.

Insulin rapidly crosses the blood brain barrier (BBB) (21,94). Using a compartmental model with three components (plasma-->intermediate compartment--> CSF) during rapid changes of plasma and CSF insulin levels, Baura et al. (21) found that the uptake of insulin from plasma through the intermediate compartment into CSF decreased progressively with increasing plasma levels. In contrast, the rate constants for

insulin removal from the intermediate compartment and from CSF did not vary with plasma insulin. This suggested that insulin rapidly crosses the BBB via a receptor-mediated, saturable transport process across brain capillary endothelial cells. Obesity reduces the transport of serum insulin into the CSF. In high fat diet induced obese dogs, weight gain is associated with and may be causally related to the reduced transport of insulin from the blood into the CSF (100). The uptake of insulin from plasma into CSF appears to be reduced in Zucker fatty rats compared with lean controls, though insulin infusion induced elevated CSF IRI in both groups (185). Thus, a lowered uptake of insulin from blood into CSF and a decreased IRI in brain was observed in Zucker rats and obese dogs. In contrast, a study with dietary induced obese female Osborne Mendel rats that received 24-h insulin infusions, and with hyperinsulinemic Wistar rats, found that the transport of insulin into the brain is not damaged by moderate diet-induced obesity or by hyperinsulinemia per se (94).

What is the relationship between peripheral and CSF insulin levels? Most studies found a positive correlation between peripheral insulin levels and CSF insulin concentrations in normal weight men (199) and rats (184,185,188). Wallum et al. (199) reported that CSF insulin concentrations increased during peripheral infusions of insulin in normal weight men. CSF immunoreactive insulin significantly increased about 3 fold while plasma IRI increased 20 fold during a 4.5 hour infusion of insulin at approximately postprandial insulin levels. Under fasting and refeeding, basal CSF-immunoreactive insulin levels were positively correlated with plasma IRI levels in rats (188). In addition, chronic insulin infusion induced elevated CSF IRI in both Zucker fatty rats and lean rats (185).

Immunoreactive insulin concentrations in both plasma and CSF in obese Zucker rats are higher than those in lean Zucker and age-matched normal weight Wistar rats, and there is a positive correlation between CSF insulin and plasma insulin levels in these animals (184). Interestingly, the concentration of immunoreactive insulin in most brain sites including the hypothalamus, olfactory bulb and cerebral cortex is significantly lower in obese and heterozygous Zucker rats than in lean controls (18). This discrepancy of insulin levels between the CSF and brain may suggest a decreased binding of insulin in Zucker rats. The high affinity insulin binding is decreased in the olfactory bulb and liver of obese and heterozygous Zucker rats compared with the lean controls, though total binding is not different in the hypothalamus, which suggests a possible gene-related change in insulin binding in Zucker rats (58). The inconsistencies of CSF and brain insulin is unlikely to be the result of impaired transport of CSF insulin to the brain, because it has been shown that CSF IRI diffuses from CSF into the hypothalamus (20). It is possible that brain insulin content and CSF insulin level may reflect different aspects of brain insulin physiology. In contrast to most studies cited above, Ono et al. found that the CSF insulin level did not increase in response to the rising plasma insulin level which increased following infusion of glucose over 4.5 hours in rats (146). In summary, most studies suggest that the insulin concentration in the CSF is positively related to its concentration in plasma, which in turn is proportional to adiposity. Therefore, CSF insulin level is very likely to be proportional to adiposity.

Insulin receptors are expressed in the hypothalamus, particularly in the arcuate nucleus (ARC) and dorsomedial nucleus (DMN), which play important roles in the regulation of food intake and energy homeostasis (19). Membrane binding studies

indicate that insulin receptors exist at various concentrations in the brain, with the highest concentration in the hypothalamus (84,85,87). Using autoradiographic techniques, Corp et al. (50) showed that the arcuate nucleus and dorsomedial nucleus had the highest concentration of insulin receptors within the hypothalamus of rats.

What is the change of insulin level after fat removal? There are no reported studies designed to test whether CSF insulin level is decreased after fat removal. Most human studies suggest a decrease of fasting circulating insulin level by weight reduction. For example, weight loss by caloric restriction significantly decreased serum insulin levels and increased insulin sensitivity for both obese men and premenopausal women. Insulin sensitivity was correlated with adiposity before and after weight loss (71). Purnell et al. reported similar results in older obese men (153). Weight loss by dexfenfluramine caused a loss in visceral fat, and decreased fasting insulin level in moderately overweight men with type 2 diabetes (127). In moderately obese patients, a decrease in abdominal fat by a two month intensive exercise intervention was associated with a decrease in insulin level and an increase in insulin sensitivity, though body weight was not decreased (138). York et al. (215) reported a significant reduction in insulin level in rats that lost weight, which was basically a reduction of body fat. In contrast, one study published in 1976 indicated that the plasma insulin level was not changed after the removal of a substantial amount of fat (110). In that experiment, 24% of the total body fat of non-obese adult Sprague-Dawley rats was surgically removed. During the 12-week follow-up, the plasma insulin level was not significantly different between lipectomized rats and sham rats. One thing to keep in mind is that compensatory hypertrophy or hyperplasia of

adipose tissue in the lipectomized rats compared with sham-operated controls was not observed in that study.

In summary, blood insulin levels are proportional to adiposity, and insulin is transported into the brain, which has specific receptors for it. Intracerebroventricular insulin administration reduces food intake and body weight in a dose-dependent manner. Insulin could be regarded as an adiposity signal involved in the regulation of body weight/fat.

(2). Leptin as an adiposity signal:

A major step towards verifying the hypothesis that circulating endocrine factors act in the brain to maintain body weight by adjusting food intake and energy expenditure was the discovery of leptin (ob protein) in 1994 (220). These investigators reported the cloning and sequencing of the mouse ob gene and its human homologue. The Ob gene encodes a 4.5 kb adipose tissue mRNA with a highly conserved 167-amino-acid frame. Initial studies on leptin were focused on its functions as a central satiety agent. Leptin reflects, and informs the brain of, adiposity, and affects food intake and energy expenditure. It has been discovered recently that leptin has functions unrelated to satiety. Leptin also plays a role in the reproductive system, immune functions, insulin sensitivity and stress responses. The wealth of research on leptin has been extensively reviewed (10,14,16,33,55,64,78) and is summarized here.

The primary source of circulating leptin is adipose tissue (220) though it is also expressed in the stomach (8), placenta (118), and muscle (200). Leptin circulates in the blood, probably bound to a family of binding proteins, and is transported through the blood brain barrier and acts on central neural networks that regulate energy balance (33).

Leptin is believed to provide a communication link from fat tissue to the brain (33). Leptin decreases food intake and increases energy expenditure. Many studies indicate that leptin's anorexic effects on energy balance are mediated by the CNS (4,169,175,186,193).

Leptin concentration is proportional to body fat content. Leptin mRNA expression and protein secretion correlate with percentage of body fat in mice (63,125), rats (63,145) and humans (49,69,125,173,219). For example, Considine et al. (49) observed that the ob mRNA content of adipocytes was about twice as high in obese male subjects as in normal-weight subjects. Geldszus et al. (69) reported that serum leptin levels were positively correlated with BMI for both obese and lean female subjects. Furthermore, leptin concentrations in CSF are strongly positively correlated to the plasma level (173).

Evidence has been provided for a specific transport system for leptin (ob protein) to cross the BBB and enter the brain of mice, rats and humans (76). The rate of transport can be decreased by high plasma concentrations of leptin. It has been showed that there is a central binding site for labeled leptin in the choroid plexus in mice (191) and rats (76,177). The leptin receptor (OB-R) is expressed in the choroid plexus, the hypothalamus as well as in all peripheral tissues. OB-R exists in multiple forms; OB-Ra, OB-Rb, OB-Rc, OB-Rd, OB-Re, OB-Rf . OB-Rb is the long form with the complete intracellular domain, and all other forms are short forms with a truncated intracellular domain (39,76,116,191). The long form is thought to be the form that signals and mediates the biological effects of leptin. Initial in situ hybridization studies have demonstrated that the mRNA for the long form OB-R receptor is localized in the

hypothalamus as well as in peripheral sites (27,76,191). Furthermore, mutation of OB-Rb receptor causes obesity in rats (203) and mice (39,116). Recently, it was suggested that leptin transport across the blood-brain barrier of the Koletsky rat is not mediated via the known leptin receptor, as this animal does not express any known functional leptin receptor (12). These investigators observed that the rate of transport of radio-labeled leptin through the BBB after leptin perfusion via a carotid artery cannula was not different between Kolestsky rats and wild type controls. In this study, leptin was transported completely across the BBB, the transport was saturable, and leptin had the same distribution among brain regions as previously found in normal weight mice (highest transport into the hippocampus and hypothalamus, lowest in the frontal cortex). This study indicates that another leptin transporter may exist.

What is the function of leptin, as an adiposity signal, in energy balance regulation? Administration of leptin to *ob/ob* mice results in marked decreases in food intake and body weight, normalized body temperature and neuroendocrine status (including hypogonadism, hypercorticosteronemia, and low levels of thyroid hormone), and increased energy expenditure (34,77,148,181,218). Leptin is thought to signal the brain about the overall nutritional state of the individual, rather than to serve as a short-term inhibitor of feeding (62). A comprehensive review has been written on the other physiological functions of leptin, such as its involvement in reproductive function, immune function and stress response (78).

How does leptin change after body weight reduction? When obese subjects lose weight, both serum leptin concentration and ob mRNA content of adipocytes decline. Chen et al. (40) found that the serum leptin concentration was significantly reduced, and

the correlation between leptin and BMI at baseline disassociated on the second day after suction lipectomy. The reduction lasted for at least 14 days after lipectomy. Talisman et al. (190) found similar results. They observed that leptin levels at 1 week postoperatively were significantly lower than the preoperative levels for patients from whom more than 2700 ml fat had been aspirated. The reduced leptin levels were correlated with a decrease in appetite. At 6 weeks post surgery, leptin returned to preoperative levels while there was still a loss of an average of 7% body weight. Considine et al. (49) found that both serum leptin concentration and ob mRNA content of adipocytes markedly declined after 10% weight reduction over 8~12 weeks. Serum leptin increased slightly during the 4 weeks maintenance of the lower weight, in which the body weight did not change. The ob mRNA at the low weight period is not significantly different from the value before weight loss. Geldszus et al. (69) found that weight reduction of obese female subjects by dieting induced a rapid decrease of serum leptin levels starting from 3 weeks after dieting compared with that at baseline. Serum leptin levels were significantly lower in the obese weight reduced subjects than in the non-dieting BMI-matched healthy control subjects at 3, 6 and 12 weeks. Serum leptin levels increased in the weight reduced group after the end of the diet regime (12 weeks), but remained significantly lower than the BMI-matched controls, even after 7 weeks' normal energy diet. Other studies also reported a decrease of leptin level after weight loss for both humans (71) and mice (125).

Although leptin is thought to be an adiposity signal informing the brain of the body fat content, leptin does not seem to play an important role in the compensatory adipose tissue growth after lipectomy. Leptin deficient *ob/ob* mice exhibit compensatory adipose tissue growth after lipectomy (42). This was recently confirmed by Harris et al.

(79) who found similar body mass and total carcass lipid in sham and lipectomized *ob/ob* mice, wild type mice or BL/6J *db/db* mice (which have short-form and circulating leptin receptors, but not long form leptin receptor OB-Rb) 16 weeks after surgical removal of the epididymal fat.

What makes the body weight regulation mechanism more complicated is the interaction between leptin, sensory nerves and sympathetic efferent nerves. Injection of leptin into either side of the epididymal fat pad activates sensory afferent nerves, and the increased sensory afferent nerves induce an increase of sympathetic efferent nerve drive in the fat pad (143). The involvement of sensory nerves in adiposity signaling is reviewed below.

There may be other adiposity signals in addition to insulin and leptin. Leptin alone does not explain all of the findings from parabiosis experiments (65). Another (or more than one) as yet unidentified factor(s) may be involved in energy balance regulation (see review by Fruhbeck et al. (65)).

2. Afferent sensory nerves

The CNS could be informed of total body fat not only by humoral factors, but through the neural system as well, or by combined neural/humoral signals. Adipose tissue is innervated by sensory nerves, as studies(13,61,70) with anterograde tract tracers and immunocytochemistry detected the presence of substance P in white adipose tissue.

One piece of evidence supporting sensory innervation of white adipose tissue as an adiposity signal is the results from unilateral lipectomy. Mauer et al. (130) investigated compensatory fat mass increases after side-specific body fat removal,

unilaterally or bilaterally. This study suggests that the regulation center can detect not only the total fat deficit, but also the side of the deficit. Since the blood borne factors are evenly distributed, it seems unlikely that the detection of the laterality could be achieved by blood borne signals.

Studies of damaged sensory nerves in adipose tissue also indicate the involvement of sensory nerves in fat tissue development. Capsaicin, which is a sensory nerve toxin, decreases body fat if applied globally (52). The decrease may not be attributed to the dysfunction of the sensory nerves in adipose tissue, however. Global application of capsaicin destroys sensory nerves in many sites which have a substantial influence on fat mass, such as those influencing food intake (157) and thermogenesis by brown adipose tissue (90). Interestingly, sensory denervation increases sympathetic drive (29,154), and sympathetic nervous system may affect the development of adipose tissue. It has been shown that norepinephrine (NE) inhibits the proliferation of preadipocytes (97) and local sympathetic denervation of white adipose tissue induces preadipocyte proliferation and increases fat mass and adipose number (51,217).

In conclusion, body weight/body fat is regulated by a sophisticated and coordinated system, the input signals of which include at least circulating humoral factors like insulin and leptin and the sensory neurons. There are still many questions to be answered.

MAINTENANCE OF ENERGY BALANCE: ADJUSTMENT IN FOOD INTAKE AND ENERGY EXPENDITURE AFTER BODY WEIGHT/FAT LOSS

According to the first law of thermodynamics, energy intake will equal energy expenditure plus energy storage, in order to maintain energy balance. When energy intake is more than energy expenditure, extra energy storage, in the form of fat tissue, will occur. When adequate food is available in the environment, most adult individuals maintain relatively stable amounts of stored fat (adiposity) over long intervals (174,209). In terms of energy balance, stable adiposity means that energy intake and expenditure are matched to one another despite a considerable variety of eating patterns (54). When energy intake is less than energy expenditure, fat tissue will be mobilized to provide energy for the body. The regulation of energy balance involves a complex and coordinated system. If body weight is reduced by any means such as surgical liposuction or a low energy diet, the body has certain mechanism(s) (see review II: body weight/fat signals for regulation) to detect the body weight/fat weight change, and both sides of the energy balance equation (energy intake and energy expenditure) will be adjusted. The following is a review of energy balance changes after body weight/fat weight change.

1. Regulation of meal size and number

During evolution, the body evolved both short-term and long-term regulation mechanisms to keep energy in balance over a long period of time. Short-term regulation is through a change in meal size and meal number.

The average number of meals per day varies widely among and within animal species. The mean number is affected by many factors such as food availability, light cycle, stress, predators or social competitors and social situations. However, animals readily adjust their eating behavior while maintaining long-term energy homeostasis. Either the size or the time of meals is adjustable, and animals can adjust either or both to

accommodate changes in energy balance (212). To date, many peptide hormones such as cholecystokinin (CCK), bombesin, gastrin-releasing peptide, neuromedin B and glucagon (66,68,111,139) secreted by the gut have been shown to decrease meal size. Those satiety factors accumulate during eating and eventually terminate the meal.

The gut-brain signaling peptide CCK is the most studied satiety factor. CCK originates from the gut, and the administration of exogenous CCK reduces meal size in rats (6,35) and in humans (67). Satiety peptides not only alter meal size themselves, but also can combine with other signals to influence meal size. Low-dose CCK-8 (a CCK analogue) and mild gastric distension reduce meal size synergistically (167) and gastric load reduces the threshold dose of the CCK analogue required to inhibit food intake (168). Satiety factors do not produce nausea in animals (92) and humans (67,139). Instead, the satiety peptides signal the brain through peripheral nerves (such as vagal afferent fibers) (167) and receptors in the brain (59). In chronic decerebrated rats, in which all connections between the lower brainstem and the forebrain are damaged, CCK decreases sucrose intake in the rats, indicating that the neuronal circuit is in the lower brainstem (73). Though satiety peptides are potent in decreasing meal size, they do not cause body weight reduction, because the animals compensate for the decreased meal size with an increase in the number of meals (202). West et al. reported that 6 days of CCK-8 infusion in the rat induced a reduction by at least 44% in average meal size, while daily meal number increased by 162% for all 6 days. In other words, satiety factors affect food intake over a short period of time, that is individual meals, which coupled with the change of meal number, has less potent effects on energy balance over a long period of time. The long-term signals for food intake are not satiety signals but adiposity

signals which are proportional to adipose mass, and inform the brain of the energy balance status (see review II "Body Weight/Fat Signals for Regulation"). The food intake regulation lies on the integrated interaction between satiety factors and adiposity signals.

2. The central mechanism of long-term food intake control

As reviewed in " Body Weight/Fat Signals for Regulation ", the adiposity signals insulin and leptin inform the brain of the body energy status. The brain adjusts food intake and energy expenditure according to these input signals to maintain long-term balance between energy intake and expenditure. The mechanism on how leptin and insulin affect food intake and energy expenditure is currently the subject of intense study.

Insulin reduces food intake and body weight in a dose-dependent manner when administered directly into the CNS (207,208). Chronic intracerebroventricular insulin administration causes a dose-dependent reduction in food intake and body weight in baboons (208). Furthermore, insulin antibody injected in the VMH induces a transient hyperphagia (187). The reduction in food intake by insulin is not due to malaise. Intraventricular (IVT) insulin treatment causes a significant reduction in body weight but has no effect on indices of malaise such as taste aversion, oxytocin secretion, or sodium appetite in response to furosemide treatment in the rats (38). A large body of evidence indicates that the central anorexic effects of insulin are mediated by the arcuate nucleus (ARC). Insulin, like leptin (reviewed below), inhibits ARC Neuropeptide Y (NPY) expression, NPY protein level and food intake (37,172,176,182) but its effects on other hypothalamic signaling systems are not as well described. Another mechanism of

insulin's effect on food intake may be through enhancement of the effectiveness of satiety factors that regulate meal size such as CCK. CCK-8 (a CCK analogue) infusion via the lateral ventricles given prior to a 30-min meal decreased meal size to a significantly greater degree when insulin was chronically infused than when cerebrospinal fluid (control) was infused in baboons (60). A similar result was observed in the rat as well (156). These results support the hypothesis that insulin can interact with other meal-regulatory peptides to regulate food intake.

Recombinant leptin reverses the hyperphagia and obese phenotype of the *ob/ob* mouse and also reduces food intake and body mass in normal lean rodents (34,77,148). The decreased food intake after infusion of leptin is not induced by conditioned taste aversion (192). Instead, current evidence suggests that the hypothalamus is the primary target for leptin action in energy homeostasis. Receptors for leptin are found throughout the body, as well as in many areas of the brain. However, the long-form of the leptin receptor (OB-Rb) is expressed in particularly high levels in several cell groups of the medial hypothalamus, including the arcuate, ventromedial, and dorsomedial nuclei (17,27,76). Leptin activation of Ob-Rb initiates intracellular signal transduction via JAK-STAT pathway in the hypothalamus. Furthermore, activation of the Ob-Rb receptor by leptin generates expression of an intracellular signal transduction protein, SOCS-3, that is dramatically increased in the ARC following leptin treatment (26).

Neuropeptide Y (NPY) is expressed ubiquitously in many areas of the brain. One group of NPY neurons in ARC expresses leptin receptors, and is therefore under the influence of leptin. These neurons project to the paraventricular nuclei (PVN), where NPY is secreted. Decreased leptin levels activate these ARC neurons and stimulate

release of NPY into the PVN (169,186). NPY increases food intake, body weight gain (101,183), and reduces energy expenditure (25).

The melanocortins are a family of peptides including adrenocorticotropic hormone (ACTH) and melanocyte–stimulating hormone (MSH). Proopiomelanocortin (POMC), the precursor of MSH, is expressed in ARC neurons and is adjacent to neurons synthesizing NPY (15,105). Both NPY- and POMC-positive neurons contain the leptin receptor (Ob-Rb) (15,41). Leptin increases hypothalamic POMC expression in the ARC (175,193). Alpha-MSH stimulates melanocortin (MC) receptors, MC3 and MC4 (166). Agouti-related protein (AgRP), which is expressed in ARC, is an antagonist of MC3/MC4 receptors. The administration of AgRP into the brain stimulates food intake and weight gain (159). Leptin also prevents metabolic/gastrointestinal responses to caloric restriction by activating hypothalamic CRH- and POMC-containing pathways, and these peripheral responses to CNS leptin administration contribute to leptin's anorexigenic action (196).

In brief, when energy stores are lowered, such as in food deprivation or restriction, decreased adiposity signals such as insulin and leptin inform the brain of the energy deficiency status. NPY (which increases food intake and reduces energy expenditure) and AgRP (which antagonizes -MSH) in the ARC are increased on one hand, and alpha- MSH (which reduces food intake and increases energy expenditure) is inhibited on the other hand. As a result, the reduced adiposity signal increases food intake and decreases energy expenditure, thus maintaining the energy homeostasis in the long term. Conversely, in imposed positive energy balance, the opposite responses

would be expected to decrease food intake and increase energy expenditure. Body weight will be altered when this regulation does not function well.

3. The effect of lipectomy on food intake

What is the food intake response to lipectomy? It is logical to expect an increase of food intake after lipectomy because of the observation of compensatory adipose tissue growth. An increase in food intake after lipectomy was observed in a few studies. For example, Liebelt et al. (120) found that after removing the gonadal fat tissue, the inguinal fat depot had a higher lipid content while the total body lipid was similar to that of the sham mice, which suggested compensatory adipose tissue growth. This was accompanied by an increased food intake in the lipectomized as compared to the sham-operated mice. However, most studies suggest that food intake after lipectomy is not increased during the period that the compensatory adipose tissue growth occurs (53,165). For example, Dark et al. (53) showed that food intake of lipectomized animals did not increase while the body mass was completely restored. Four months after surgical removal of a substantial portion of adipose tissue of golden-mantled ground squirrels, food intake of the lipectomized squirrels was not significantly increased compared to that of the sham operated squirrels. Over this period the total body mass did not differ between lipectomized and sham operated squirrels.

Bartness et al. (206) found that Siberian hamsters increased food hoarding after lipectomy. More interestingly, the hamsters decreased food hoarding to pre-lipectomy levels once their untouched fat pads had increased to compensate for the body fat removed. However, animals do not necessarily eat all the food they hoard as suggested

by Cabanac et al. (32), who found that 2-hour food intake was constant and did not vary as a function of body weight loss. At the same time, the amount of food hoarded was a linear increasing function of body weight loss below the hypothetical "set point" for body weight. This study suggests that the main response to starvation is food hoarding rather than increased food intake. A study with golden hamster by Phillips et al. (149) showed similar results.

4. Energy expenditure

Energy expenditure by animals consists of three parts: basic resting energy expenditure (basal metabolism), specific dynamic action of food (thermogenesis due to food) and the calories needed for growth, repair and physical activities (31). Energy expenditure and the influence of leptin on energy expenditure are briefly reviewed here.

Thermogenesis is comprised of obligatory and regulated thermogenesis. Obligatory thermogenesis corresponds to resting energy expenditure and minimal biological functions essential for life in the absence of food, in resting conditions, and at thermoneutrality. Age, sex, thyroid activity, environmental temperature, sleep, diet and menstrual cycle all modify the basal metabolic rate (BMR) (31). With exposure to cold and food intake, regulated thermogenesis (adaptive thermogenesis) occurs. Brown adipose tissue, in addition to liver and muscle, is the primary thermogenic tissue and plays a pivotal role in regulating energy expenditure in rodents. Ablation of brown adipose tissue induced mild obesity in male transgenic mice (123). The thermogenic function of brown adipose tissue in responding to cold temperature and excess food intake is under the control of catecholamines acting through adrenoceptors

(88,89,141,161,161,162,162). Activation of the ß$_3$ - adrenergic receptor activates uncoupling protein (UCP) in mitochondria of brown adipose tissue (107) and induces synthesis of new UCP (36,151,216). UCP uncouples respiration and oxidative phosphorylation in mitochondria, allowing high rates of substrate oxidation and heat production instead of generating ATP. Overexpression of UCP prevents genetic obesity (108). Decreased thermogenic response to noradrenaline was found in constitutionally obese humans (98) and genetically obese mice (194).

Thyroid hormone plays a major role in modulating resting energy expenditure, while the sympathetic nervous system and noradrenaline are the major regulators of regulated thermogenesis (88). Leptin has also been found to exert an influence on energy expenditure.

Leptin infusion into *ob/ob* mice decreases body weight and fat tissue weight. However, the decrease of body weight can not be attributed to decreased food intake alone, as PBS treated *ob/ob* mice pair-fed to leptin-treated *ob/ob* mice had a smaller decrease in body weight (77,119). Thus, like food intake, energy expenditure is also under the influence of leptin. It was reported that leptin administration increases oxygen consumption in *ob/ob* mice (148) and normal weight F-344 X BN rats (163). Leptin increases oxygen consumption and uncoupling protein expression in brown adipose tissue. UCP mRNA levels were significantly increased after leptin infusion in both ad libitum-fed and pair fed normal weight rats compared to their controls (163). In addition, leptin increases sympathetic drive to brown adipose tissue in *ob/ob* mice (47). The increase in energy expenditure by leptin administration extends into recovery after withdrawal of leptin as reported by Gullicksen et al. (74). They observed a sustained

increase of heat production through the 21 days of recovery after ICV injection of leptin for 4 days in normal weight Sprague-Dawley rats compared with control rats.

5. Energy expenditure after body weight reduction

Food intake is one arm of the energy balance equation, and the other arm is energy expenditure. If food intake is not increased after lipectomy while there is compensatory adipose tissue growth and thus an accumulation of extra energy, the other possible mechanism by which this occurs is through a decrease in energy expenditure.

Resting metabolic rate (RMR) is the component of energy expenditure that contributes the largest proportion of total daily energy expenditure, however, the relationship between RMR and body weight is controversial. Ravussin et al. (155) observed that 24-hour energy expenditure (adjusted for body composition, age, and sex) in American Indians was correlated with the rate of change in body weight over a two-year follow-up period. For persons with a low adjusted 24-hour energy expenditure, the estimated risk of gaining more than 7.5 kg in body weight was fourfold higher than that for persons with a high 24- hour energy expenditure. However, another prospective study in whites found that RMR was not positively related to weight change (179).

What is the change of energy expenditure after weight reduction? Some studies suggested a decrease in energy expenditure after weight loss. Van Dale et al. (195) found that resting metabolic rate decreased after 14 weeks of exercise combined with dietary energy restriction. In that study, 20 women with an average initial body mass index of 33.5 underwent a 14 week exercise training and dietary regime. Frequent dieters (yo-yo) and women without a dietary history (non-yo-yo) were matched into the following

groups: diet- exercise yo-yo (DE-Y), diet-exercise non-yo-yo (DE-NY), and diet-non- yo-yo group (D-NY). After 14 weeks, significant differences in weight loss and fat loss were revealed between D and DE groups but not between yo-yo and non-yo-yo dieters. RMR decreased in all groups but there was a significantly smaller decline after 14 wk for the DE groups. Leibel et al. (117) reported that maintenance of a reduced body weight is associated with compensatory changes in energy expenditure. After a 10% reduction in body weight for pre-obese and normal weight persons, 24 hour total energy expenditure, resting energy expenditure and nonresting energy expenditure decreased significantly. Bessard et al. (24) found that 24 hour and basal energy expenditure dropped significantly after weight reduction in obese women, even though they were still higher in previous obese people than in controls.

In contrast, several studies (113,201,214) suggested no change in RMR of previously obese people after weight reduction. After adjusting RMR for lean mass, fat mass, age and sex, Wyatt et al. (214) found no significant difference in RMR between weight-reduced obese subjects and weight-matched controls. Weinsier et al. (201) reported that for 24 overweight women undergoing dieting to lose weight, the body composition-adjusted RMR and T(3):rT(3) decreased during the energy restriction phase, but returned to baseline in the normal-weight, energy-balanced state. RMR among weight-loss women was not significantly lower than that of controls, and a lower RMR did not predict greater 4-year weight regain.

The discrepancy of the RMR change after weight reduction of previously obese people may be due to a lack of statistical power to detect small differences in RMR from studies with small sample size. A meta- analysis (7) of resting metabolic rate in formerly

obese subjects from 15 published studies with a total of 152 obese subjects and 237 control subjects found that the RMR of formerly obese subjects was 3-5% lower than that from control subjects. The lower RMR may be a genetic or acquired characteristic of these previously obese subjects, and may actually induce the obesity. Another possibility is that the lower RMR may be a metabolic change resulting from the weight reduction. No matter what is the case, the lower RMR may contribute to the high rate of the weight regain in those now normal weight but formerly obese subjects.

Although meta-analysis is a powerful tool to detect small differences, caution needs to be exercised when interpreting results from it. The meta-analysis included studies that are different in many aspects: subject age, gender, original body weight, the amount of body weight reduction, and the period of study. Study on the RMR change pattern around the weight change including before, during and after weight reduction of obese subjects will be more helpful for us to better understand the mechanism of high rate of weight regain after weight loss by obese persons. Bessard et al. (24) found that before weight reduction, total 24 hour and basal energy expenditures were significantly greater in obese subjects compared with their age-matched controls. After weight loss, both energy expenditure parameters were significantly decreased, though they were still greater in the obese group than in the controls. Therefore the thermogenic response to weight reduction favors the regain of weight. The major shortcoming of this study is that it had only 6 obese subjects.

One study found an increase in stimulated thermogenesis after weight reduction. Blaak et al. (28) reported that the nonselective beta-agonist isoprenaline-induced whole body thermogenesis tended to increase as a result of weight loss by a 5 week very low

calorie diet intervention. However, the respiratory exchange ratio during isoprenaline infusion was not altered before and after weight reduction, indicating the source of fuel used for energy expenditure was not changed by weight reduction.

The contradictory results of these studies reflect the complexity of energy regulation after body weight reduction. Study design may also have a big influence on the results. The compensatory adipose tissue growth after lipectomy or body weight regain after weight reduction occurs during a long period of time. However, in most studies, the measurement of the food intake and energy expenditure did not cover the whole span of time, which may explain the negative results. Furthermore, since the digestion efficiency and absorption efficiency may differ with the same amount of food consumed, the total energy intake may be different even when measured food intake is the same.

LIPECTOMY: A MEANS TO STUDY BODY WEIGHT/FAT REGULATION

Lipectomy, which is partial removal of white adipose tissue by surgery or aspiration, decreases the percentage of body fat. It is also a common medical procedure (suction lipectomy, liposuction). It is commonly practiced to reduce fat content of obese patients or for the purpose of plastic surgery. Liposuction induces positive life style outcomes (72). Among 332 patients who had liposuction, a larger portion were more confident, had an increase in self-esteem and were more comfortable in clothes. However, one question needs to be answered. That is, what is the body's response to localized removal of fat and the long-term efficacy of this procedure? The answer to this question seems controversial. Lambert et al. (112) found that resting energy expenditure,

response to glucose feeding, site specific fat cell size, and sum of four skinfold thicknesses were not significantly different at one to two month post-operation, compared with that prior to surgery. This study had few subjects (seven non-obese women) and the follow up time was short (one to two months after liposuction). In a review of 631 consecutive liposuction cases over 12 years by the same senior surgeon between 1986 and 1998, Commons et al. (48) found a 2- to 6-inch drop in skin-fold thickness compared with that at preoperation and that 80 percent of patients maintained stable postoperative weights one year after liposuction. In contrast to the studies reported for humans, studies with the many species of animal showed compensatory adipose tissue growth or re-growth after lipectomy.

In Golden-mantled ground squirrels, Siberian and Syrian hamsters (128,131), rats (11,57,114,120,137,165), mice (42) and pigs (124), the partial removal of body fat induces the growth of non-excised fat pads to the degree that the total fat mass is not different between the lipectomized animals and the sham controls. In 1965, Liebelt *et al*. (120) found that after removing the gonadal fat organ, the inguinal fat depot had a higher lipid content while the total body lipid was similar to that of the sham mice, which suggested compensatory adipose tissue growth. Schemmel at al. (165) reported in 1971 that 32 weeks after surgical removal of one or both inguinal and epididymal fat pads from weaning Osborne Mendel male obese rats, the percentage of body fat was similar for lipectomized rats and sham operated rats. It is further noted that the fat content of body organs (liver, heart, muscle, skin) other than adipose tissue were not significantly different between lipectomized and sham operated rats (114), which indicates that the compensatory growth was confined to adipose tissue in fat pads specifically.

In contrast, some studies with rats (56,110) did not show compensatory adipose tissue growth after lipectomy. Kral et al. (110) surgically removed 24% of the total body fat of non-obese adult Sprague-Dawley rats, and found the reduction persisted for at least 12 weeks. They did not find altered food intake, weight gain, or compensatory hypertrophy or hyperplasia of adipose tissue compared with sham-operated controls. In a study with obese humans, Kral et al. (109) did not find evidence of regeneration of adipose tissue in situ after removal of 12-18% of the total number of fat cells.

Why did these studies on changes of body fat mass after lipectomy have contradictory results? Several factors may affect the response. The animal age at surgery and thus adipose tissue developmental stage is different in these studies. The study duration is also inconsistent. For studies with non-compensatory adipose tissue growth, the animals may not have been allowed enough time for the compensatory adipose tissue growth to occur. The amount of fat removed varied greatly in different studies, which may have a big influence on the outcome. The fat pads removed were also different. Another difference between these studies may be the degree of damage to testes and subsequent ability to secrete testosterone. It has been reported that there is an inverse relationship between obesity and blood level of free testosterone in man (3,75,178,180). Furthermore, administration of testosterone to obese men (126) or hypogonadal rats (91) reduces visceral fat mass.

An increase in fat mass can be achieved by an increase of either fat cells size (hypertrophy) or fat cell number (hyperplasia) or both. Fat cell number increases in the compensating fat pad of ground squirrels (53), Siberian hamsters (129,130) and Sprague-Dawley rats (114). For example, Mauer et al. (129) found that lipectomized hamsters

showed compensatory mass increases in retroperitoneal adipose tissue, and that the increase was due to hyperplasia. The expansion of adipose tissue is the result of fat cell hypertrophy followed by almost unlimited hyperplasia for almost all animal obese models and obese humans. Adipocyte enlargement proceeds early and then plateaus, then most of fat expansion is caused by adipocyte replication (96,164). Adipose tissue of humans, rodents, and pigs contains adipocyte precursors which may proliferate and be recruited to become new adipocytes (81,121). Hausman et al. (82) has recently reviewed the proliferation of white adipocytes. Many peptides (such as insulin-like growth factor-1, angiogension II, tumor necrosis factor α, macrophage colony-stimulating factor, transforming growth factors, insulin-like growth factor binding proteins) and lipid signals (prostaglandins, arachadonic acid, etc) may play a promotion or inhibition role in the proliferation of preadipocytes. Since preadipocytes express the receptors for insulin-like growth factor -1, tumor necrosis factor α and angiogension II, and respond to them at a physiological level, these factors may play an important role in the proliferation of preadipocytes.

Although it is commonly observed that compensatory adipose tissue growth occurs after body weight reduction including by lipectomy, the mechanism underlying the growth is not clear. What is the energy balance after lipectomy? Is the growth caused by hyperplasia or hypertrophy? Do blood borne factors contribute to the compensatory adipose tissue growth? Does the sympathetic nervous system play a role? The answer to all these questions will help in the understanding of the regulation of body weight/fat, and thus benefit the study of obesity.

In summary, most studies confirm that there is compensatory adipose tissue growth after lipectomy. However, the mechanism is barely studied. Research on the mechanism underlying compensatory adipose tissue growth will contribute to the understanding of regulation of body weight/fat, and help the battle against obesity development.

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CHAPTER III

BLOOD BORNE FACTORS UNDERLYING THE COMPENSATORY ADIPOSE TISSUE GROWTH IN LIPECTOMIZED RATS[1]

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ABSTRACT

The regulation of body weight/fat was studied by investigating mechanisms for compensatory adipose tissue growth after removal of bilateral epididymal fat pads from male adult Wistar rats. Food intake during the first 4 weeks and energy expenditure on day 7-10 and day 28-31 post surgery were not different between lipectomized and sham operated rats. The carcass composition of lipectomized and sham operated rats was not significantly different 16 weeks after surgery. The compensatory growth was fat pad specific: both mesenteric and retroperitoneal fat pads, but not inguinal and perirenal fat pads, were heavier in lipectomized rats than in sham operated rats. Compensatory growth of retroperitoneal fat pads is contributed by hyperplasia and hypertrophy initially and by hypertrophy predominately in later stages. Serum levels of leptin and insulin were not different between lipectomized and sham operated rats. Serum from lipectomized rats, but not media conditioned by exposure to retroperitoneal fat pads from lipectomized rats, stimulated proliferation of preadipocytes *in vitro* more than that from sham operated rats, while serum from lipectomized rats did not induce differentiation of preadipocytes *in vitro* more than that from sham operated rats. Norepinephrine concentrations, an indicator of sympathetic nervous system drive, were not different in retroperitoneal fat pads of lipectomized rats as compared with sham rats at 2 and 4 weeks after surgery. Thus, compensatory adipose tissue growth after lipectomy occurs by both hyperplasia and hypertrophy, and may be mediated, in part, by blood borne factors that are derived from tissues other than adipose tissue.

Key words: obesity, adipocyte proliferation, norepinephrine, paracrine factors, adiposity signals

INTRODUCTION

Body weight, like body core temperature and body fluid osmolarity, is regulated physiologically. The idea of body weight regulation was proposed originally through the lipostatic hypothesis of Kennedy (35), which says that the young rat adjusts its food intake so precisely to its energy needs that its fat stores remain almost constant. He further suggested that the obesity produced by medial hypothalamic lesions resulted from impairment in the lipostatic mechanism that normally operates to prevent excess fat mass deposition. Keesey et al. (34) extended this idea and proposed the body weight set point theory, which suggests that the medial and lateral hypothalamus jointly determine a body weight set point which could be adjusted by external factors such as diet palatability. Overweight, obesity or underweight may occur if the regulation mechanism fails.

The mechanism by which the body regulates its weight is unknown and needs to be addressed before we can determine a practical means to prevent or treat obesity. One means to study this mechanism is to investigate the body responsiveness after total body fat content is altered experimentally. Whenever there is a forced deviation from the "regulated" body weight/adiposity, a series of responses will occur, trying to restore the regulated set level against the deviation force. Lipectomy, which is partial removal of white adipose tissue by surgery or aspiration, decreases the percentage of body fat and fat cell number. Studies with many species have shown that there is compensatory growth of the non-excised fat tissues or re-growth of the excised fat tissues after lipectomy. In

hamsters (46,50), rats (4,18,40,42,52,58), mice (12) and pigs (43), the partial removal of adipose tissue induces the growth of non-excised fat pads to the degree that the total fat mass is not different between the lipectomized animals and the sham controls. The mechanism(s) by which the total body fat level recovers after lipectomy are not well understood, however. Food intake and energy expenditure has been investigated in many studies, but with conflicting results. In addition, potential signals conveying the information of body weight/fat reduction of lipectomized animals and the pattern of cellularity change in compensatory grown fat depots have not been defined.

Adipose tissue expansion is caused by adipocyte hyperplasia and/or hypertrophy and is affected by many variables including blood borne factors which originate from both fat and non-fat tissues and paracrine factors that are secreted by adipose tissue and regulate the proliferation and/or differentiation of preadipocytes (26). In addition to blood borne factors or adipose tissue derived paracrine factors, the sympathetic nervous system also influences the development of adipose tissue, as norepinephrine has been shown to inhibit proliferation of preadipocytes (33), and local denervation of the sympathetic nervous system induces an increase of fat mass (13). The potential involvement of humoral and neural factors in the compensatory adipose tissue growth after lipectomy has not been explored.

The hypothesis of the current study is that compensatory adipose tissue growth after lipectomy is due to hyperplasia and/or hypertrophy, and is mediated by blood borne factors and/or the sympathetic nervous system. We aimed to test, first, if food intake and energy expenditure respond to lipectomy; second, if insulin and leptin facilitate the compensatory growth after lipectomy; third if hyperplasia and hypertrophy contribute to

compensatory growth; fourth, if blood borne factors are involved in the compensatory growth; fifth, if factors secreted from adipose tissue act as paracrine factors to stimulate the compensatory growth; and sixth, if the sympathetic nervous system is involved in the compensatory growth.

MATERIALS AND METHODS

Adult male Wistar rats were obtained from Harlan Sprague Dawley (Indianapolis, Indiana). The rats were single housed in plastic cages with Tek-fresh bedding from Harlan, and had free access to tap water and chow diet (LabDiet® 5012 from PMI® Nutrition International, LLC) throughout the experiment. The room temperature was kept between 74 to 75 ^{0}F. The light/dark period was 12/12 hours. There was one week for the rats to adjust to the new environment before surgery. Experimental protocols were approved by the University of Georgia Animal Care and Use Committee and conducted according to National Institutes of Health and US Department of Agriculture guidelines.

Surgical procedures

Experiment 1 (Pilot study). Male Wistar rats were distributed into two groups matched for body weight (means ± SE: 292 ± 4 grams), 8 rats in the lipectomy group, and 4 rats in the sham group. Isoflurane (IsoFlo®, Abbott Laboratories, North Chicago, IL) was used as anesthesia for all surgeries. Rats were positioned on their back for surgery. For rats in the lipectomy group, a single small longitudinal incision of about 1.5 cm in length was made in the skin of the abdominal area, and a second incision was made

in the peritoneal wall. The epididymal fat pads (with the testes) were pull out of the cavity, and bilateral epididymal fat pads were separated from the testes and removed carefully. Caution was exercised to minimize the possible damage to the testes and the spermatic artery to prevent ischemic changes in the testes. The intact testes were returned to the cavity. Both incisions were sutured with silk sutures at the end of surgery. The sham operation was similar to that for the lipectomized rats, however the fat pads of rats in the sham surgery group were not excised. At the end of 14 weeks after surgery, the rats were killed by decapitation, samples from retroperitoneal fat pads were taken for cellularity measurement, and body composition was analyzed.

Experiment 2. Male Wistar rats were distributed into two groups (lipectomy or sham) matched for body weight (320-330 grams). Surgery procedures were performed as described above. Following surgery, food intake and body weight were measured daily at the same time each day for up to 4 weeks. Energy expenditure was measured on two sets with 12 rats per set (6 lipectomy and 6 sham) at one week after surgery and four weeks after surgery. At 2, 4 or 16 weeks after surgery, lipectomized rats and their sham controls were killed by decapitation (10-19 rats in each group at each time point). Trunk blood was collected, and serum separated by centrifugation at 2500 RPM for 20 minutes in a refrigerated centrifuge, and stored at -80 ^{o}C. Serum insulin and leptin levels were measured by radioimmunoassay (Linco Research, Inc. St Charles, Missouri). Inguinal, retroperitoneal, mesenteric, perirenal, epididymal fat pads and testes were dissected and weighed. Fat tissue samples from inguinal and retroperitoneal fat pads were taken for cellularity measurements and conditioned media were made from inguinal and retroperitoneal fat pads (see below). The gastrointestinal tract was cleaned and returned

to the carcass and carcasses from rats killed at 16 weeks after surgery were analyzed for body composition (see below).

Energy expenditure

Heat production was determined by continuous indirect calorimetry. A computer-controlled open circuit calorimetry system for rats (Oxymax®, Columbus Instrument Co., Columbus, OH) was used. The energy expenditure of 12 rats was measured at a time. Oxygen consumption, carbon dioxide production, RQ, and heat production were measured for each rat at 16.5-min intervals, 24 h/day except during a short period (approximately 45 min) of daily weighing of rats, food, water, and fecal mass. Measurements were taken for day 8-10 and day 28-31 after surgery. Airflow was controlled and measured using a mass flowmeter for each chamber. Gas composition of incoming outdoor air and exhaust gas were measured using an infrared gas analyzer for carbon dioxide, and an electrochemical oxygen sensor battery system, based on limited diffusion metal air battery for oxygen. The gas analyzers were calibrated daily using cylinders of primary gas standard mixtures with known concentrations of CO_2, O_2, and N_2. The percentages heat production (HP) resulting from fat and carbohydrates oxidized were based on the RQ (7). Heat production calculations were based on the Brouwer equation (8), HP = 3.820 O_2 consumption (liters) + 1.150 CO_2 production (liters). The heat generated is expressed based on the metabolic body size, which is body weight in kilograms raised to the 0.75 power ($kg^{0.75}$). The data for RQ and HP were averaged over 24 h.

Carcass composition

Carcass composition was analyzed by the method of Hartsook (25) as modified by Harris (24). In brief, the frozen carcass (including gastrointestinal tract, but not inguinal and retroperitoneal fat pads--which had been taken to make conditioned media) was autoclaved in an individually sealed jar at 140 ^{0}C for 40 minutes. After cooling, the carcass was chopped and homogenized with an equal weight of water in a blender with a homogenizer. Triplicate 6-8 gram aliquots of homogenate were transferred to crucibles and dried at 70 ^{0}C for 7 days to determine water content. The crucibles were then held at 650 ^{0}C overnight to determine ash. Triplicate 6-8 gram aliquots of homogenate were analyzed for fat content by chloroform: methanol extraction. Carcass protein was calculated as carcass weight minus water, fat and ash content.

Adipose tissue cellularity

Fat cell size and number were determined through electronic quantification using the method of Hirsch and Gallian (28) as modified by Cartwright (11). Duplicate adipose tissue samples were fixed in a solution containing 0.12 M osmium tetroxide in 50 mM collidine (2,4,6-trimethylpyridine) buffer. Samples were fixed for at least 1 week at room temperature, rinsed with 0.9% NaCl, and placed in 8 M urea for several days to facilitate separation of cells from the tissue. Fixed adipocytes were rinsed with 0.9 % NaCl through a 240 µm nylon screen and then collected on a 20 µm nylon screen. Samples of cells were analyzed on a Coulter electronic particle counter (multisizer II; Coulter Electronics Limited, Beds England) to determine cell number and size distribution.

Number of adipocytes per pad was calculated by multiplying the mean cells per milligram of sample by the total mass of the corresponding depot.

Conditioned media preparation

Retroperitoneal and inguinal fat pads were quickly removed, further dissected to remove visible blood vessels, finely minced, rinsed three times in fresh 37°C Hanks' balanced salt solution (HBSS), blotted on P8 filter paper, and weighed. Ten ml DMEM/F12 Ham's medium containing 72 mM gentamicin sulfate, 120 mM cefazolin, and 27 mM amphotericin B was added per one gram tissue, and samples were incubated for 4 h at 37°C in a humidified 5% CO_2 atmosphere after which the adipose tissue conditioned media were filtered from the minced adipose tissue through P8 filter paper (porosity: coarse) and stored frozen at -80°C.

Primary cell culture

Inguinal fat pads were excised aseptically from pentobarbital-anesthetized, male young Sprague-Dawley rats (80-100 g body weight). Adipose tissues from two rats were pooled, and stromal-vascular cells and preadipocytes were isolated as described by Ramsay et al. (57). Briefly, tissues were minced and incubated with 5 ml/g tissue of digestion buffer (0.1 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid [HEPES] with 1000units/ml collagenase) for 2 hours in a 37°C shaking water bath (110 rpm). Undigested tissue was removed by filtering through 240 and 20µm nylon mesh. Filtered cells were resuspended in Dulbecco's modified Eagle's medium (DMEM)/F-12 Ham's medium (containing 72 mM gentamicin sulfate, 120 mM cefazolin, and 27 mM

amphotericin B) and centrifuged at 600g for 10 min to separate the fat cells from the pelleted stromal-vascular cells. Aliquots of S-V cells were stained with Rappaport's stain and counted on a hemocytometer. For proliferation assays, cells were seeded on 12.5 cm^2 tissue culture flasks (with canted neck and 0.2 um vented seal cap) with 2 ml of plating medium (DMEM/F12 Ham's medium, 10% fetal bovine serum, antibiotics--72 mM gentamicin sulfate, 120 mM cefazolin, and 27 mM amphotericin B) at a density of 4.8 X 10^3 cell/cm^2. For the differentiation assay, cells were seeded on 12 well plates with 2 ml of plating medium at a density of 1.7 X 10^4 cell/cm^2. Cells were cultured at 37 °C in a humidified 5% CO^2 atmosphere.

Proliferation assay

On day 1 after seeding, the plating medium was removed, and cultures were rinsed and replaced with DMEM/F-12 Ham's with antibiotics until treatment media were applied on day 2. Proliferation of preadipocytes and S-V cells in response to test media was determined through the use of [^{3}H] labeled thymidine incorporation during the exponential growth phase (11). Cultures were treated with basal control medium (DMEM/F-12 Ham's with antibiotics, 0.5% porcine serum) or test media (for proliferation assay testing conditioned media: 25% adipose tissue conditioned media, 75% DMEM/F12, 0.5% porcine serum; for proliferation assay testing rat serum: 0.5% or 2.0% rat serum, 0.5% porcine serum, remainder DMEM/F12 Ham's) containing 0.50 µCi/flask [^{3}H] thymidine for days 2-5 of culture. On day 5, the flasks were rinsed and refed with lipid filling medium (10% porcine serum, 1.0 nM porcine insulin, and 10 U/ml heparin in DMEM/F12 Ham's with antibiotics) to promote lipid accretion in the

preadipocytes. Lipid filling medium was changed every other day through day 13. On day 15, the cells were enzymatically harvested using Hanks' balanced salt solution containing 0.5 % BSA, 0.5 mg/ml trypsin, and 125 units/ml collagenase. The lipid-filled mature adipocytes (preadipocytes now fully differentiated) and the non-lipid-filled S-V cells were separated by density gradient centrifugation through Percoll as described by Novakofski et al. (54). The incorporation of [^{3}H] thymidine in both cell fractions was determined by scintillation counting.

Differentiation assay

SV cells were plated in 12 well plates at a density of 1.7 X 10^4 cell/cm^2 as described above (DMEM/F12 Ham's with antibiotics plus 10% FBS as plating medium). On day 1, plating medium was removed and the dishes were rinsed with DMEM/F12 Ham's with antibiotics prior to adding DMEM/F12 Ham's with 0.5% pig serum. On day 4, dishes were rinsed with DMEM/F12 Ham's with antibiotics before adding test media. Treatment media were DMEM/F12 Ham's with antibiotics containing 0.5% serum from lipectomized or sham operated rats and either 2 nM triiodothyronine (T_3 : Sigma Chemical Co., St. Louis, MO), 1 nM insulin, 0.6 ng/ml transferin and 0.6 pg/ml sodium selenite (1 nM ITTS. ITS, Sigma) or 2 nM T_3, 10 nM insulin, 6.0 ng/ml transferin and 6.0 pg/ml sodium selenite (10 nM ITTS). Treatment media were changed every 2 days. Cultures were harvested on day 10 into sucrose buffer (0.25 M, pH 7.4). The harvested cells were homogenized by sonic dismemberment, centrifuged and supernant assayed for glycerol-3-phosphate dehydrogenase activity (GPDH; EC 1.1.1.8) using the method of Wise et al. (64) as modified by Ramsay et al. (57), except for the following changes: 35

µl sample supernant and 175 µl GPDH assay solution were added into 96 well plates, and subsequent reaction monitored using a Labsystems Multiskan MCC/340 (Fisher Scientific, Pennsylvania). Soluble protein concentrations of the homogenates were also determined (Bio-Rad, Melville, NY) and data are expressed as unit of enzyme activity/mg protein, with one unit of enzymatic activity corresponding to the oxidation of 1 nmol NADH/min.

Norepinephrine content

Norepinephrine content of the retroperitoneal fat pads was measured using reverse-phase HPLC with electrochemical detection according to the methods of Meffors (51) as modified by Youngstrom et al. (69). Briefly, tissue was weighed and minced. 100 mg samples were added to 790 µl of PCA/AA buffer (0.2 M perchloric acid with 1 ng/ml ascorbic acid) in microcentrifuge tubes and 10 µl of 50 ng/ml dihydroxybenzoacetic acid (DHBA) was added to each sample and served as an internal standard. Tissue was further minced and then sonicated for 3 min on ice (3 times, 30 seconds on and 30 seconds off). Norepinephrine was extracted from the remaining infranatant using alumina (approximately 150 mg per sample). The extracted samples were assayed using an ESA (Chelmsford, MA) HPLC system with electrochemical detection (guard cell, +35 mV; cell 1, +10 mV; cell 2, -30 mV). The mobile phase was Cat-A-Phase II (ESA, Inc). Standard solutions were prepared at concentrations of 3.125, 6.25, 12.5 and 25 ng/ml from commercially supplied standard kits (ESA, Inc) and were run at the beginning, in the middle and at the end of each set of unknowns. NE content in the samples was expressed as ng/g tissue.

Statistical analysis

For all statistical analysis, differences were accepted as significant at the $p<0.05$ level. Daily body weight and food intake measures were modeled separately with repeated-measures Analysis of Variance (ANOVA). Post hoc comparisons of treatment means at different time points were affected by t-test. Differences in single time point measures of energy expenditure, and relative weight of fat pad and testes were determined by one-way ANOVA, using Statistica software (Statistica, StatSoft, Tulsa, OK). Differences in serum leptin and insulin levels, proliferation activity and GPDH activity were determined by MANOVA, using Statistica software.

RESULTS

The pilot studies showed that the surgical removal of epididymal fat pads of the Wistar rats was successful. All animals recovered from the surgery quickly and remained healthy throughout 14 weeks of follow-up. At the end of 14 weeks after surgery, lipectomized rats had heavier mesenteric and perirenal fat pads than sham operated rats, and the amount of lipid, protein, ash and water in the carcass was not significantly different between lipectomized and sham operated rats (data not shown). Following are results from the second experiment.

The weight of epididymal fat pads lipectomized was 2.96 ± 0.32 (means $\pm$ SE) grams, which accounted for 0.876 ± 0.093 (means $\pm$ SE) percent of the total body weight. Surgery caused a transient weight loss in both lipectomized and sham operated rats.

However, body weight was not significantly different between the two groups at any time throughout the experiment (Fig. 1).

Lipectomized rats ate less compared with the sham rats during the first 2 days after surgery, but food intakes were not different from day 3 through the remainder of the period measured (Fig. 2). The amount of heat produced adjusted by the body size ($Kcal/Kg^{0.75}$/24 Hours) was not significantly different between the lipectomized and sham rats on days 8-10 and days 28-31 post surgery (Fig. 3). RQ was not significantly different between the lipectomized and sham rats on days 8-10 and days 28-31 post surgery (data not shown).

The carcass components were not significantly different between lipectomized and sham operated rats at the end of 16 weeks post-surgery (Fig. 4), which suggested that the lipectomized rats had compensatory growth of adipose tissue during the 16 weeks. Retroperitoneal and inguinal fat pads were not included with the digested carcass, as samples from these two fat depots were used to prepare conditioned media. Had these two fat pads been included, no significant difference between lipectomized and sham operated rats would have been expected.

At the end of 2 weeks after surgery, there was no significant difference in the relative weight of the inguinal, retroperitoneal, mesenteric and perirenal fat pads between lipectomized and sham operated rats (Fig. 5A), though there was a tendency ($P = 0.07$) that lipectomized rats had heavier mesenteric fat pads than sham rats. At the end of 4 weeks after surgery, lipectomized rats had significantly heavier mesenteric fat pads than the sham rats ($P = 0.02$), however the relative weights of inguinal, retroperitoneal, and perirenal fat pads were similar between lipectomized and sham rats (Fig. 5B). At the end

of 16 weeks after surgery, among the five fat pads dissected, lipectomized rats had significantly higher relative retroperitoneal and mesenteric fat pad weights than the sham rats, while the relative weights of inguinal and perirenal fat pads were similar between the two groups (Fig. 5C). As expected, the weights of the epididymal fat pads were markedly less in lipectomized rats than in sham rats at the end of 2, 4 and 16 weeks post surgery (Fig. 5). A small amount of epididymal fat pad tissue was detected in the lipectomized rats. This may have regenerated or been left from the surgery, though the latter is less likely. Testes weights were also significantly less in lipectomized rats than in sham rats at the end of 2, 4 and 16 weeks post surgery (Fig. 5).

As insulin and leptin are generally regarded to be adiposity signals to inform the brain of energy balance status, their concentrations at 2 or 4 weeks after surgery were measured. There was no significant difference in serum leptin concentration (Fig. 6B) at 2 or 4 weeks after surgery between lipectomized and sham rats. Likewise, circulating leptin concentrations were not different for lipectomized rats at 2 weeks as compared with 4 weeks post-surgery, or between the sham operated rats at 2 weeks vs. 4 weeks. At the end of 2 weeks after surgery, serum insulin levels from lipectomized rats were significantly higher than those from sham rats (Fig. 6A), and the difference disappeared at the end of 4 weeks. At both 2 and 4 weeks, leptin concentrations were positively associated with the weight of all five dissected fat pads (R^2: 0.44, $P< 0.001$). The correlation between insulin and the weight of all five dissected fat pads was not significant (R^2: 0.007, $P=0.596$).

To determine what accounted for compensatory growth in adipose tissue mass, cell size and number determination was made for one of the compensating fat pads, the

retroperitoneal. Lipectomized rats had more adipocytes in retroperitoneal fat pads 4 and 16 weeks after surgery compared with sham rats, while the numbers were not different 2 weeks after surgery (Table 1). The distribution pattern of adipocytes in retroperitoneal fat pads (Fig. 7A) was similar between lipectomized rats and sham rats at the end of 2 weeks after surgery. Four weeks after surgery, lipectomized rats had significantly more adipocytes in the size range between 30 - 52 uM and 94 - 240 uM than did sham rats (Fig. 7B). This suggests that both hypertrophy and hyperplasia occurred. At the end of 16 weeks, there were more large adipocytes (size range between 116 - 240 uM) in retroperitoneal fat pads from the lipectomized rats than from the sham rats (Fig. 7C). This indicates that hypertrophy was the major change associated with the increased size of the retroperitoneal fat pads of the lipectomized rats at 16 weeks post surgery.

To test what may be responsible for changes in adipose tissue cellularity after lipectomy, media were conditioned by exposure for 4 hours to fat tissue from lipectomized or sham operated rats, thus factors secreted from adipose tissue were collected in the media. Conditioned media were prepared from retroperitoneal fat pads of the rats killed at the end of 2 or 4 weeks after surgery, as these rats were expected to have an active response to the lipectomy, and the time course of the response may be observed by comparison between 2 and 4 weeks. The ability of conditioned media to stimulate the proliferation of preadipocytes in an in vitro primary cell culture system was examined. Conditioned media collected from retroperitoneal fat at the end of 4 weeks after surgery increased the incorporation of thymidine into preadipocytes (Fig. 8A) and stromal-vascular cells (Fig. 8B) at a higher degree than that collected at the end of 2 weeks after surgery. However, there was no significant difference in the proliferation activity of

conditioned media collected from lipectomized rats as compared with that from sham rats at either 2 or 4 weeks (Fig. 8).

Since factors secreted by adipose tissue did not stimulate the proliferation of preadipocytes. We asked the question whether blood borne factors contribute to the hyperplasia observed in retroperitoneal fat pads of lipectomized rats. Serum from lipectomized rats stimulated the proliferation of preadipocytes to a higher degree than serum from sham rats at both 2 and 4 weeks after surgery (Fig. 9A). Serum from lipectomized rats 2 weeks post surgery also increased the incorporation of thymidine into stromal-vascular cells as compared with serum from sham rats (Fig. 9B).

The influence of serum on preadipocyte differentiation in rat preadipocyte primary culture was tested by measuring GPDH activity, a late marker of adipocyte differentiation. Primary cell cultures were treated with 0.5% rat serum and either 1 nM (Fig. 10A) or 10 nM (Fig. 10B) ITTS. GPDH specific activity of the culture was not different when serum was collected from lipectomized as compared with sham operated rats, at either 2 or 4 weeks post surgery (Fig. 10). Serum collected from lipectomized or sham operated rats at 2 or 4 weeks combined with 1 or 10 nM ITTS stimulated differentiation of preadipocytes in this *in vitro* system more than did 1 or 10 nM ITTS alone and a significant increase in GPDH activity was observed with 10 nM ITTS compared with 1 nM ITTS treatment in the absence of serum (data not shown).

To test whether the sympathetic nervous system plays a role in adipose tissue compensatory growth after lipectomy, norepinephrine levels in retroperitoneal fat pads, as an indicator of the sympathetic nerve drive, were measured. The norepinephrine

concentration in retroperitoneal fat pads at the end of 2 or 4 weeks post surgery was not significantly different between lipectomized and sham operated rats (Fig. 11).

DISCUSSION

The current study confirmed that male Wistar rats had a compensatory growth of adipose tissue at the end of 16 weeks after lipectomy to the degree that the body compositions of lipectomized and sham rats were not significantly different. Compared with sham operated rats, the lipectomized rats grew larger retroperitoneal and mesenteric fat pads to compensate for the removal of epididymal fat pads. Both hypertrophy and hyperplasia contributed to the compensatory growth of adipose tissue. To our knowledge, this is the first study to show that serum may contribute to the compensatory growth by stimulating the proliferation of preadipocytes. Serum collected from lipectomized rats at 2 or 4 weeks after lipectomy stimulated thymidine incorporation into newly proliferated preadipocytes in an *in vitro* primary cell culture system more than that collected from sham rats. On the other hand, serum from lipectomized rats did not trigger the differentiation of preadipocytes to a higher degree than that from sham rats. This indicates that blood borne factors may not contribute to the hypertrophy of adipose tissue compensatory growth. The role the sympathetic nervous system plays in the compensatory growth was investigated primarily by measuring the NE level, which was not changed by lipectomy. However, this does not rule out that other sympathetic nervous system components may be involved.

Compensatory growth after lipectomy is the growth of non-excised fat pads to compensate for the deficit of fat removed by lipectomy to the degree that the total fat

mass is not different between the lipectomized animals and the sham controls. Lipectomized rats showed a tendency to have heavier mesenteric fat pads two weeks after surgery than sham operated rats. After four weeks, lipectomized rats had significantly heavier mesenteric fat pads and a tendency for heavier retroperitoneal fat pads than did the sham controls. This suggests that compensatory growth was in progress at that time. Carcass components were not significantly different between lipectomized and sham rats at the end of sixteen weeks post-surgery, which suggests that the lipectomized rats had fully compensated for the excised fat during the sixteen weeks. Most lipectomy studies report compensatory growth of adipose tissue. In 1965, Liebelt *et al*. (42) found that 38 days after lipectomy the inguinal fat depot had a higher lipid content after removing the gonadal fat, while the total body lipid was similar to that of the sham mice, which suggested compensatory growth. Schemmel at al. (58) reported that 32 weeks after surgical removal of one or both inguinal and epididymal fat pads from weaning Osborne Mendel male obese rats, the percentage of body fat was similar for lipectomized rats and sham operated rats. Compensatory growth after lipectomy was also found in Golden-mantled ground squirrels, Siberian and Syrian hamsters (46,50), rats (18,42,52,58), mice (12) and pigs (43). In contrast, several studies with rats (17,36) did not show compensatory growth after lipectomy. For example, Kral et al. (36) surgically removed 24% of the total body fat of nonobese adult Sprague-Dawley rats, and found that the reduction persisted for at least 12 weeks. They did not find altered food intake, weight gain, or compensatory hypertrophy or hyperplasia of adipose tissue compared with sham-operated controls.

Why did these studies on changes of body fat mass after lipectomy have contradictory results? Several factors may affect the response. The animal age at surgery and thus adipose tissue developmental stage is different in these studies. The study duration is also inconsistent. For studies with non-compensatory growth, the animals may not have been allowed enough time for the compensatory growth to occur. The amount of fat removed varied greatly in different studies, which may have an influence on the outcome. The fat pads removed were also different. Another difference between these studies may be the degree of damages to testes and subsequent ability to secrete testosterone. It has been reported that there is a reverse relationship between obesity and blood level of free testosterone in man (1,21,60,61). Furthermore, administration of testosterone to obese man (44) or hypogonadal rats (29) reduces visceral fat mass. In the current study, the weight of testes in the lipectomized rats was less than that in the sham controls, which indicates a possible lower concentration of testosterone. The lower testosterone concentration may in turn contribute to the growth of adipose tissue. However, compensatory growth of adipose tissue after lipectomy can not be explained solely by the possible decreased testosterone level, because removal of fat pads other than epididymal fat pad (i.e., inguinal fat pad) also triggers compensatory adipose tissue growth (12,18,46), while there is no change in testosterone level. The compensatory growth of lipectomized castrated animals also suggests that testosterone is not necessary for adipose tissue compensation after epididymal lipectomy (4,47).

In our study, we found compensatory growth at the end of 16 weeks after lipectomy, therefore it is logical to expect a positive energy balance as the compensatory growth develops. We measured both food intake and energy expenditure. The amount of

food consumed by the lipectomized rats was not different compared with that of sham rats during the first four weeks after surgery. This is in agreement with the majority of studies which suggest that food intake after lipectomy is not increased during the period when the compensatory growth occurs (14,58). In contrast, Liebelt *et al*. (42) found an increased food intake in lipectomized mice as compared to sham-operated mice. As food intake is generally not different between lipectomized and sham rats, and there is an excess of calories accumulated in the form of adipose tissue, therefore in theory, energy expenditure must be decreased to have positive energy balance for the compensatory growth. We did not observe a reduction in body weight adjusted heat production during days 8-10 and days 28-31, however. There are several possible reasons why we failed to observe increased food intake or/and decreased energy expenditure in our lipectomized rats. First, we only determined food intake for the first four weeks post surgery and energy expenditure at two discrete time points for 7 days total. Approximately 3 grams of epididymal fat had been removed. At the end of 4 weeks post surgery, only the percentage of mesenteric fat pad was higher in lipectomized rats than in controls, and the relative weights of inguinal, retroperitoneal and perirenal fat pads were not different between treatments. This suggests that compensatory growth of adipose tissue had not occurred by the time when food intake was measured. Secondly, the changes in food intake and energy expenditure may have be too small to be detected with about 10 rats in each group and measurements taken only during a small portion of the 16 weeks during which the compensatory growth occurred. It is likely that a small positive energy balance each day will accumulate to a large amount of excess energy over 16 weeks. The energy cost of depositing 1 gram of fat in rats is approximately 53 kJ metabolizable energy (55),

and the metabolizable energy of the chow diet used in the experiment is 13.06 kJ/gm. In theory, a growth of 3 grams of fat tissue would equal to 159 kJ, which is equivalent to 12.17 gram chow diet. This means an additional 0.11 gram of diet consumed by the lipectomized rats per day will eventually lead them to gain an extra 3 grams fat tissue at the end of 16 weeks. When the daily food intake is about 25-30 grams, a difference as small as 0.11 gram in daily food intake is hard to be detected in 4 weeks with 10 rats in each group. If we had measured the food intake and energy expenditure through the entire 16 weeks, with more rats in each treatment, it is more likely that the food intake difference between lipectomized and sham rats might have been observed.

How were the rats informed of body fat content? Are there specific factors that signal body weight or fat content? A body of studies suggest that leptin (3,5,6,9,16,19,22) and insulin (6,27,59,65,66) may function as adiposity signals. Many studies report a decreased leptin level after weight loss. Thus in the present study, lower levels of serum leptin were predicted for the lipectomized rats as compared with the sham operated rats at 2 and/or 4 weeks post-surgery as compensation for lipectomy induced fat deficit was not complete at this time (as suggested by the relative weights of the dissected fat pads). The comparison of leptin levels in lipectomized rats was with those of the corresponding sham controls, rather than with basal pre-surgery values, since leptin concentration is correlated with BMI and body weight and body weight of the rats steadily increased over time. Based on this comparison, no differences were observed between lipectomized and sham rats at either 2 or 4 weeks post surgery which indicates that the deficit in fat weight was not reflected by a change in leptin level. The finding of compensatory growth without a detectable difference in leptin concentration in our study

suggests that leptin does not facilitate the compensatory growth after lipectomy. This is also confirmed by studies with *ob/ob* mice (12,23) which do not produce leptin. Harris et al. (23) found similar body mass and total carcass lipid in sham and lipectomized *ob/ob* mice, wild type mice or BL/6J *db/db* mice (without long form leptin receptor) by 16 weeks after surgical removal of the epididymal fat. As in the current study, this indicates that leptin is unlikely to be the signal communicating the loss of body weight.

Another potential adiposity signal is insulin. Many human studies suggest a decrease of fasting insulin level by weight reduction (20,45,53,56), although there are also reports that plasma insulin level is not changed after lipectomy (36). Based on the majority of evidence, a decrease of serum insulin level had been expected in the present study at 2 or 4 weeks post surgery, as the compensatory growth was not completed at that time (as suggested by the relative weights of dissected fat pads). However, we found that the insulin levels at the end of 2 weeks post surgery were increased, rather than decreased, in lipectomized rats as compared with the sham rats, and not different between lipectomized and sham rats at 4 weeks after surgery. Interestingly, insulin concentrations were not correlated with the total weight of all five fat pads dissected from the lipectomized or sham operated rats. One thing to keep in mind is that the rats in the current study were not fasting when the serum was collected. However, all rats were killed in the morning, and the lipectomized and sham rats were killed randomly. Therefore, the influence of eating on the difference of levels of insulin and leptin between lipectomized and sham operated rats would be small. Like leptin, insulin does not appear to be signaling the loss of body fat in this study.

Are there any other factors that inform the reduction of body weight? There may be signals for negative energy balance. Adiponectin (adipocyte complement related protein 30: ACRP 30; AdipoQ), an adipocytes derived protein, may convey the loss of fat content. Adiponectin is secreted exclusively by mature adipocytes in mice and rats (32). Adiponectin levels has been shown to be negatively correlated with BMI in Japanese men and women (2) and Caucasian and Pima Indian populations (63). The expression of adiponectin decreases in obese *ob*/*ob* mice (32), *db*/*db* mice (67), obese monkeys (31) and obese humans (2,30,32,68). Furthermore, weight reduction of obese patients by gastric partition surgery increases plasma levels of adiponectin (68). Adiponectin may be an adiposity signals conveying the fat deficit by lipectomy. Measuring serum adiponectin levels in lipectomized and sham operated rats may help to detect if adiponectin facilitates the compensatory adipose tissue growth after lipectomy.

The cell size distribution of adipocytes of our lipectomized rats suggests that both hypertrophy and hyperplasia contributed to their compensatory growth. This is consistent with previous studies by others. An increased fat cell number has been reported in the compensating fat pad of ground squirrels (14), Siberian hamsters (48,49) and Sprague-Dawley rats (40). To investigate the mechanism by which this occurs, we tested the activity of serum and conditioned media from lipectomized or sham operated rats on stimulating proliferation and differentiation of preadipocytes in primary culture, and found that serum from the lipectomized rats stimulated preadipocyte proliferation. This suggests that a factor(s) circulating in the blood may trigger the compensatory adipose tissue growth. However, we have not yet identified the exact factor(s). Since insulin has mitogenic effects, it is possible that the elevated insulin level accounted for the increased

proliferation activity of serum. This seems unlikely, however, as serum from lipectomized rats at both 2 and 4 weeks post surgery had a greater capacity to stimulate proliferation of preadipocytes, while insulin level from lipectomized rats increased only at the end of 2 weeks compared with that from sham operated rats. There are many blood borne factors that can stimulate the proliferation of preadipocytes such as insulin-like growth factor-1, angiotension II, tumor necrosis factor α, macrophage colony-stimulating factor and transforming growth factors (26). Further study is warranted on the specific factor(s) in serum that stimulates the proliferation of preadipocytes and thus may be involved in compensatory growth of adipose tissue after lipectomy.

Do the blood borne proliferation factors originate from adipose tissue? In other words, do adipose tissue derived paracrine factors play an important role in the compensatory growth after lipectomy? If the blood borne proliferation factors originate from adipose tissue, such factors should have been collected in the conditioned media prepared by 4 hours of exposure to fat from the retroperitoneal pads of the lipectomized rats. Conditioned media prepared from lipectomized rats at the end of 2 and 4 weeks post surgery should have stimulated the proliferation of preadipocytes more than that from sham operated rats. We found that conditioned media from lipectomized rats did not stimulate proliferation of preadipocytes *in vitro* more than that from sham operated rats while serum collected from lipectomized rats at the same time points did stimulate proliferation. This suggests that the proliferation factors present in serum may not be adipose tissue derived or at least are not from the retroperitoneal fat depot. Caution needs to be exercised when applying the results from *in vitro* study to what occurs *in vivo* physiologically, however. Preadipocytes from different fat depots differ in their inherent

capacity to proliferate (26). In the current study, the conditioned media was prepared from retroperitoneal fat depots of adult Wistar rats, and the primary cell culture used in the proliferation assay was from inguinal fat pads of young male SD rats. It would be closer to physiological condition if the conditioned media from retroperitoneal fat depots were applied to primary cell culture from the same origin.

Protein-adjusted-GPDH activity was used as a marker of late adipocyte differentiation. GPDH is an important enzyme in triglyceride synthesis, and is generally proportional to lipid filling. Serum from lipectomized rats did not show an increased activity to stimulate differentiation of preadipocytes more than serum from sham at 2 or 4 weeks post surgery. The cellularity data suggested that hypertrophy of adipocytes occurred in lipectomized rats 4 weeks post lipectomy, yet this is not reflected in ability of serum to stimulate differentiation of preadipocytes, therefore mechanism(s) other than serum factors may mediate the lipid filling of adipocytes.

Another potential system for regulating adipose tissue growth is the sympathetic nervous system. Norepinephrine has been showed to inhibit the proliferation of preadipocytes (33), and local surgical denervation of the retroperitoneal fat pad in rats induces preadipocyte proliferation (13). Mauer et al. (49) suggested that animals respond differently to the same amount of fat deficit induced on different sides of the body. This response is unlikely to be achieved by evenly distributed blood borne factors, but may be mediated by the sympathetic nervous system. Thus, we measured the norepinephrine level of the retroperitoneal fat pads, as a gross indicator of sympathetic drive. We did not find differences in the NE concentration in retroperitoneal fat pads of the lipectomized rats compared with those of sham rats at 2 or 4 weeks post surgery, while retroperitoneal

fat depot showed a trend to compensate at the end of 4 weeks and had significantly compensated at 16 weeks post surgery. However, similar NE concentrations in lipectomized and sham rats do not exclude a possible role for the sympathetic nervous system in the compensatory growth. Changes in the sympathetic system other than NE, such as altered expression of adrenoceptors may contribute to the compensatory growth. The effect of norepinephrine on adipocyte development depends on which adrenoceptors it activates (stimulating when binding to $\beta_{1,2,3}$; inhibits when binding to α_2) (37,37), therefore, the ratio of the α_2 to $\beta_{1,2,3}$ receptors dictates whether lipolysis (more $\beta_{1,2,3}$ than α_2) or anti-lipolysis (more α_2 than $\beta_{1,2,3}$) rules. Differential receptor ratios have been documented across white adipose tissue within many species such as rats (10), humans (38,41), dogs (62), rabbits (39) and hamsters (15). The different ratio of $\beta_{1,2,3}$ to α_2 may play a role in the compensatory growth of lipectomy even when norepinephrine levels are unchanged.

We confirmed a compensatory growth of adipose tissue after surgical removal of bilateral epididymal fat pads. Both hyperplasia and hypertrophy contribute to this compensatory growth. It seems neither leptin nor insulin act as adiposity signals in this case to induce changes in food intake or energy expenditure. In fact, we did not observe a measurable increase in food intake in the first 4 weeks after surgery or a decrease in energy expenditure. We found that a blood borne factor(s) may underlie the compensatory adipose tissue growth after lipectomy. Serum from lipectomized rats has an increased activity to stimulate proliferation of preadipocytes, which would contribute to the hyperplastic growth of adipocytes. At the same time, serum from lipectomized rats did not induce differentiation of preadipocytes more than that from sham rats, which

suggests other mechanism for the hypertrophic growth of adipocytes. We did not detect an involvement of paracrine factor(s) in the compensatory growth, as conditioned media from lipectomized rats did not stimulate the proliferation of preadipocytes more than that from sham rats. It is possible that sympathetic nervous system plays a role in compensatory adipose tissue growth, though we did not detect a difference in norepinephrine concentration.

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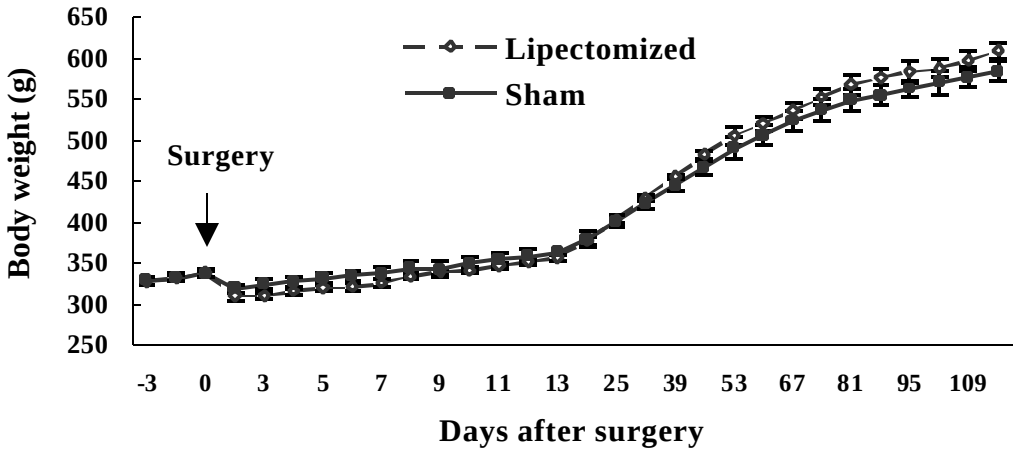

Figure 1. Body weights of lipectomized and sham operated rats. Data are means ± SE for groups with 16 rats per treatment.

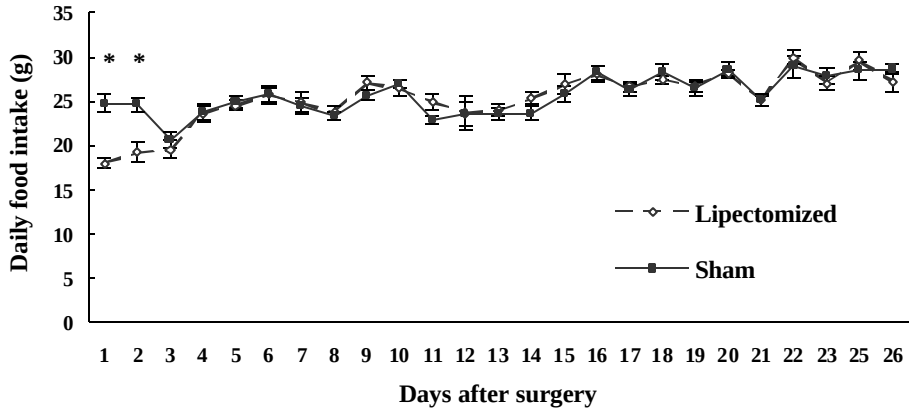

Figure 2. Food intakes for the first 25 days post surgery. Data are means ± SE for groups with 12 rats per treatment. There was no significant difference between lipectomized and sham operated rats, except a lower food intake in the first 2 days for lipectomized rats.

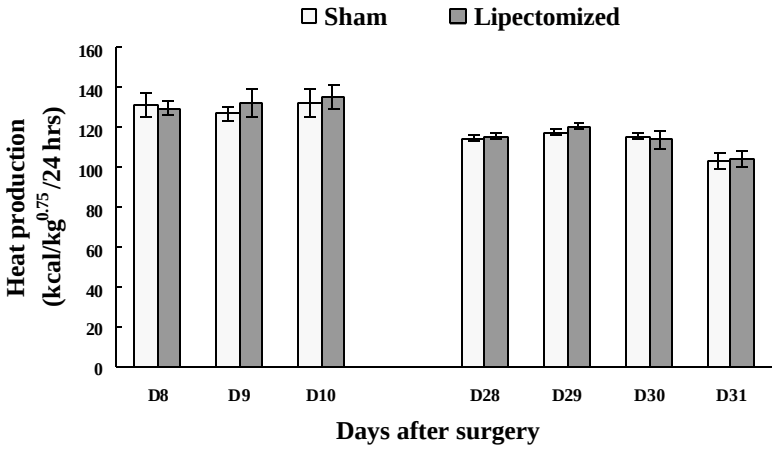

Figure 3. Adjusted heat production (Kcal/$Kg^{0.75}$/24 hour) during day 8-10 and 28-31 after surgery. Data are means ± SE for groups of 12 rats in each treatment. There was no significant difference between lipectomized and sham operated rats.

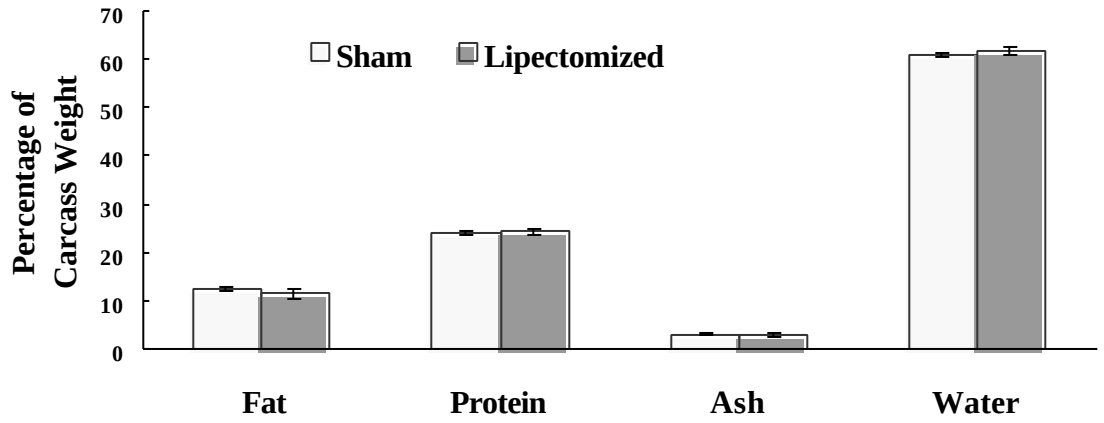

Figure 4. Body composition at the end of 16 weeks after surgery. Data are means + SE for groups with 16 rats in each treatment. There was no significant difference in percentage of fat, protein, ash and water in the animal carcass between lipectomized and sham operated rats.

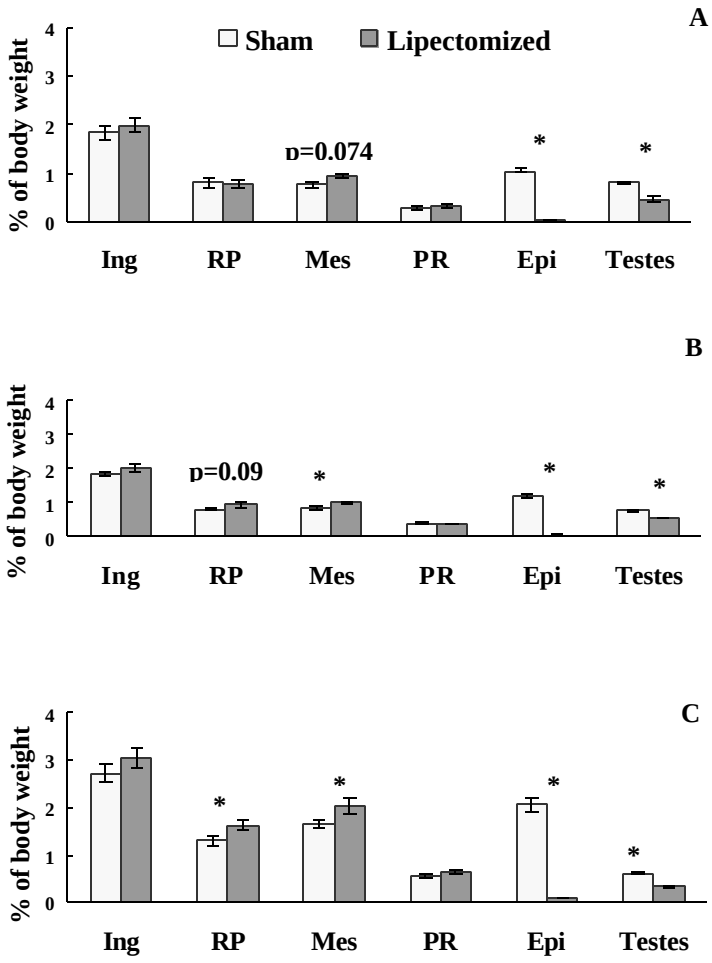

Figure 5. Relative weights of different fat depots and the testes in rats at the end of 2 (A), 4 (B) and 16 (C) weeks after surgery. Data are means ± SE for groups of 16 rats in each treatment. An asterisk (*) indicates a significant difference ($P < 0.05$) between sham-operated and lipectomized rats. Abbreviations as follows: Ing, inguinal; RP, retroperitoneal; Mes, mesenteric; PR: perirenal; Epi, epididymal.

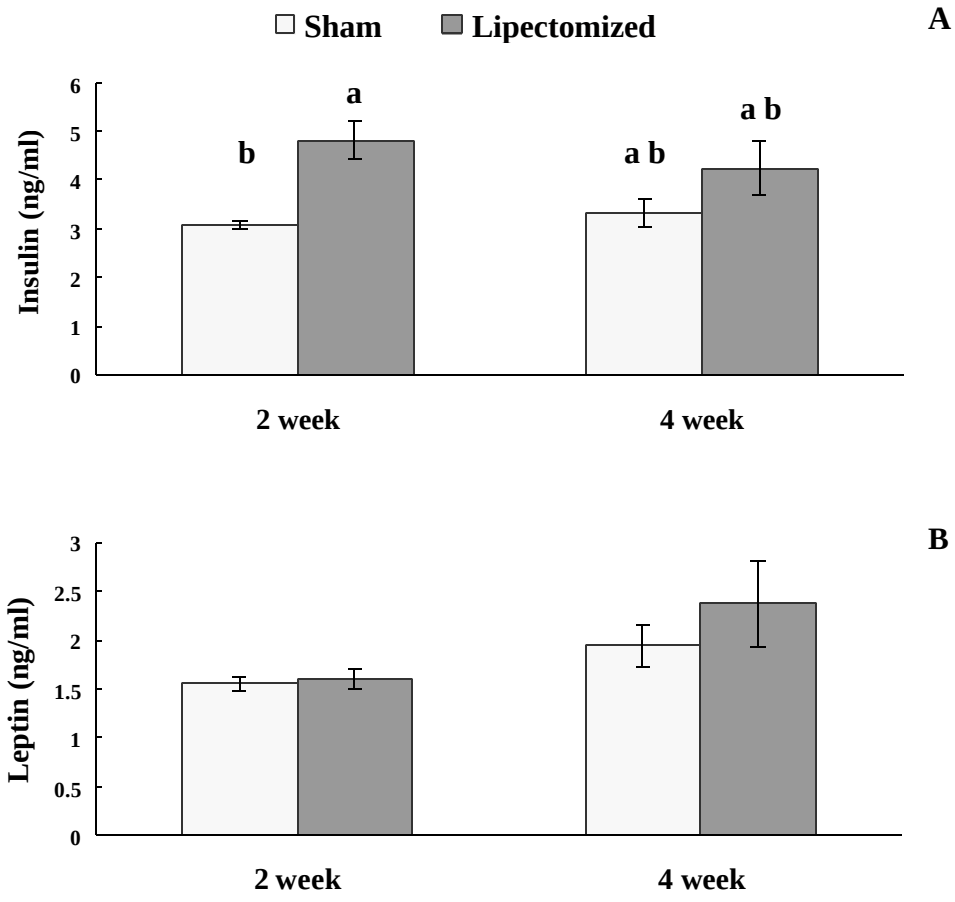

Figure 6. Insulin (A) and leptin (B) concentration in serum of lipectomized and sham operated rats at 2 or 4 weeks after surgery. Data are means ± SE for 9-11 rats in each group. Values not sharing a common superscript are significantly different ($P<0.05$).

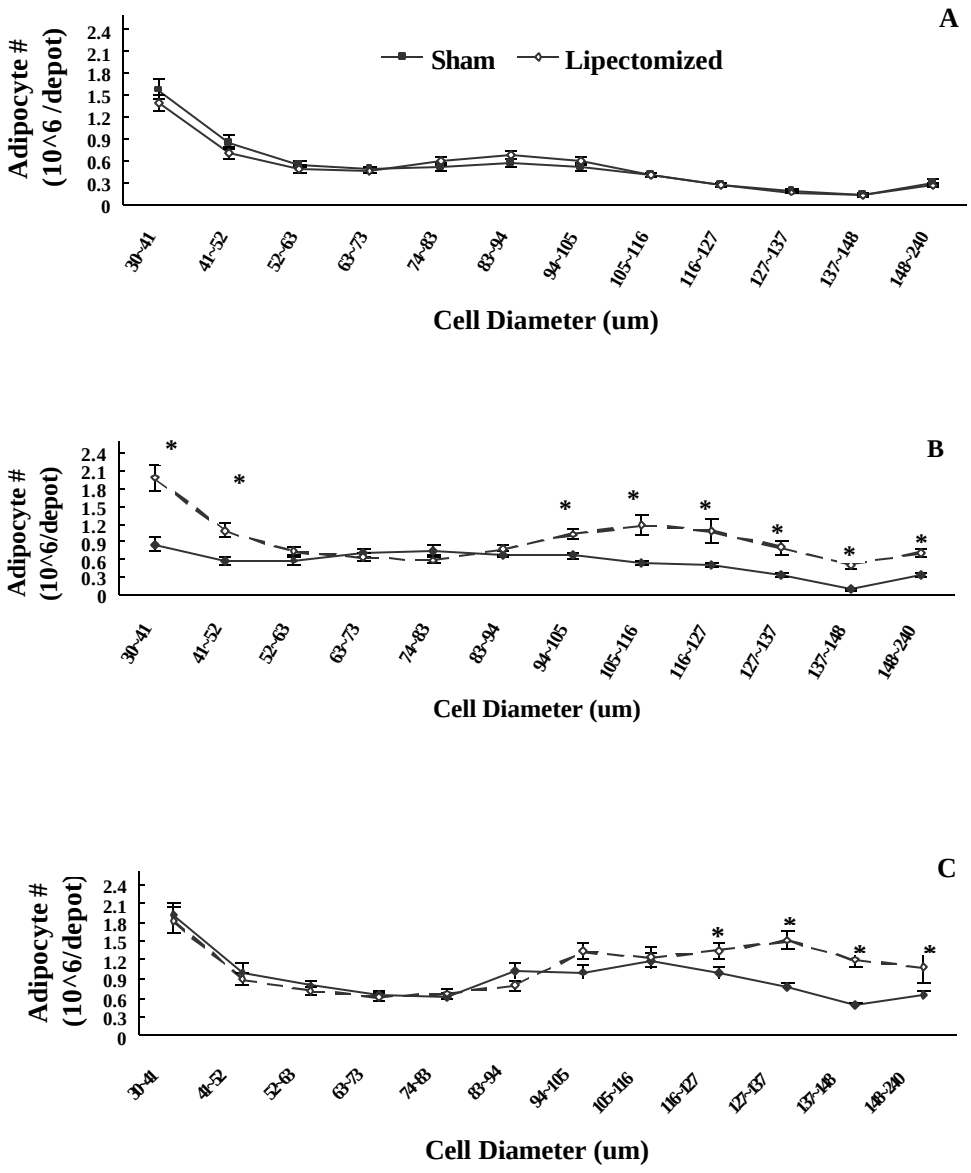

Figure 7. Size distribution of adipocytes in retroperitoneal fat pads at the end of 2 (A), 4(B) and 16(C) weeks after lipectomy surgery. Data are means ± SE of 8~10 rats in each group. An asterisk (*) indicates a significant difference ($P < 0.05$) between sham-operated and lipectomized rats.

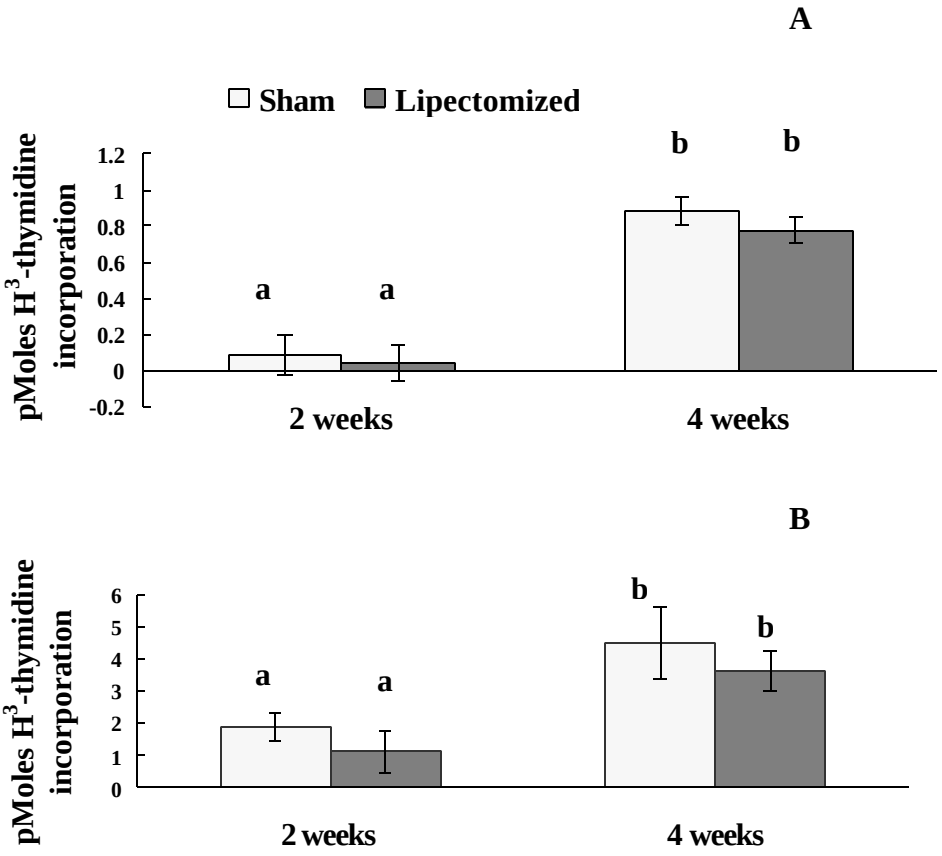

Figure 8. [H^3] Thymidine incorporation into the preadipocyte (A) and stromal-vascular (B) fractions of rat primary cell culture treated with 25% adipose-tissue conditioned media collected from the retroperitoneal depot of lipectomized and sham operated rats 2 or 4 weeks post surgery. Data were corrected to adipocyte number representing activity per 1,000,000 adipocytes present in the fat depot used for preparation of the conditioned media. Data are means ± SE for 10~12 rats each group. Values not sharing common superscripts are significantly different ($P<0.05$).

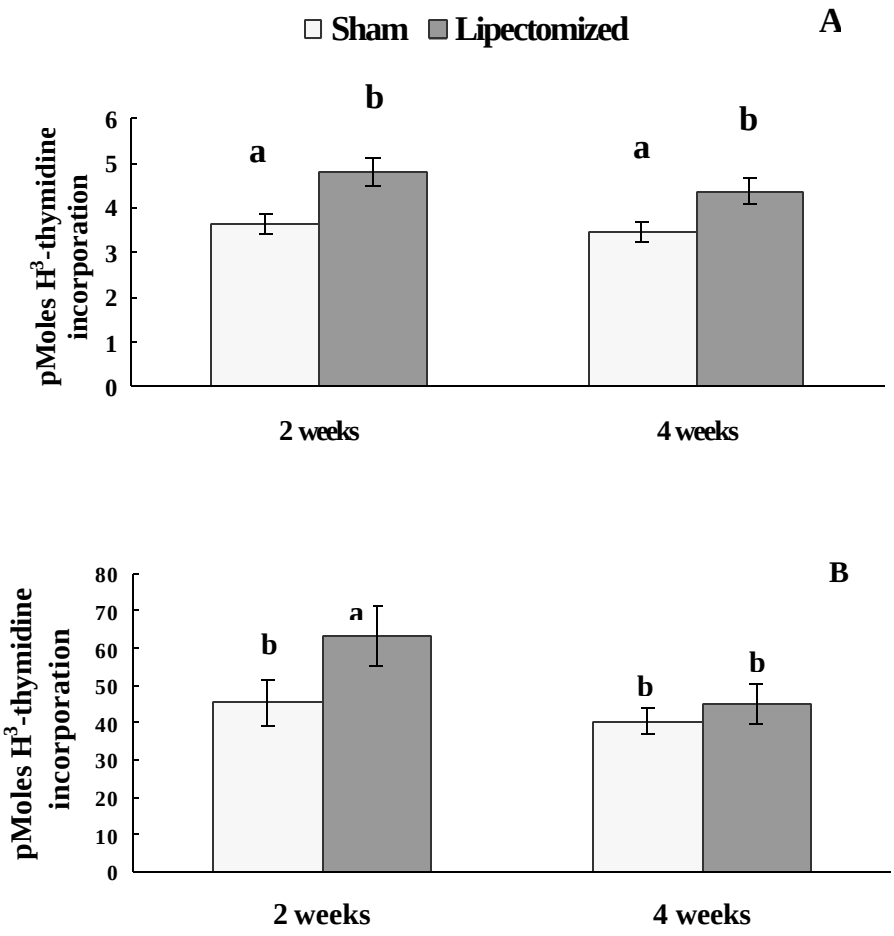

Figure 9. [H^3] Thymidine incorporation into the preadipocyte (A) and stromal-vascular (B) fraction of rat primary cell culture treated with 0.5% serum from lipectomized and sham operated rats 2 or 4 weeks after surgery. Data are means ± SE for 9~12 rats each group. Values not sharing a common superscript are significantly different ($P<0.05$).

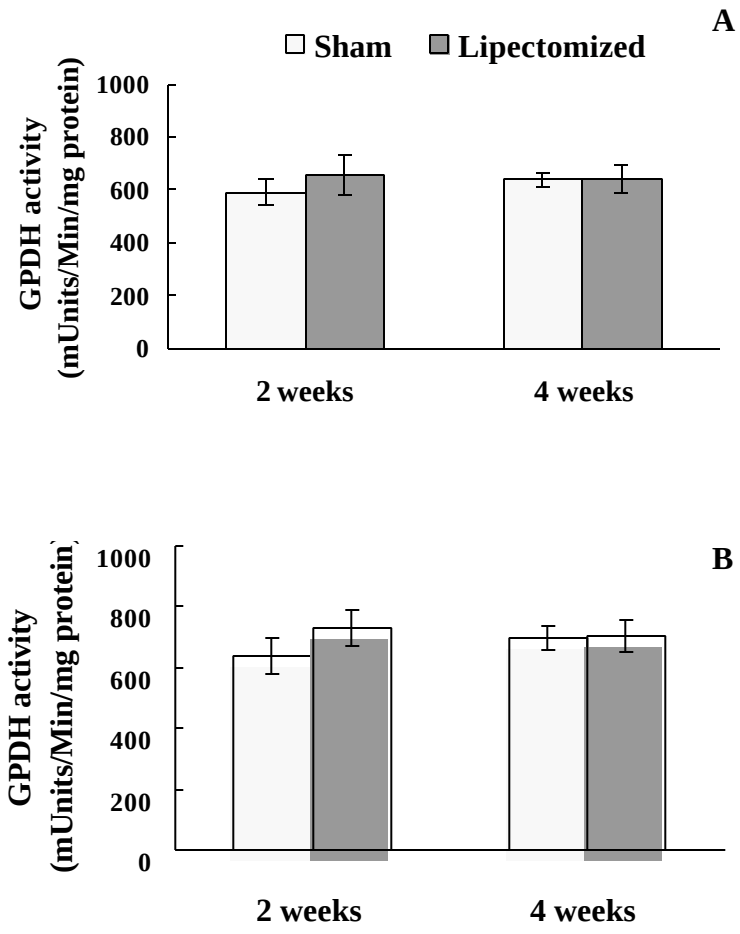

Figure 10. Primary cell cultures were treated with 0.5% rat sera, which were collected either 2 or 4 weeks post surgery, and with either 1 nM (Fig. 10A) or 10 nM (Fig. 10B) ITTS. Protein-adjusted-GPDH activities (mUnits/min/mg protein) were not different in cultures treated with sera from lipectomized as compared with sham rats. Data are means ± SE for 8-11 replicate treatments with serum pooled from 4 rats in each group.

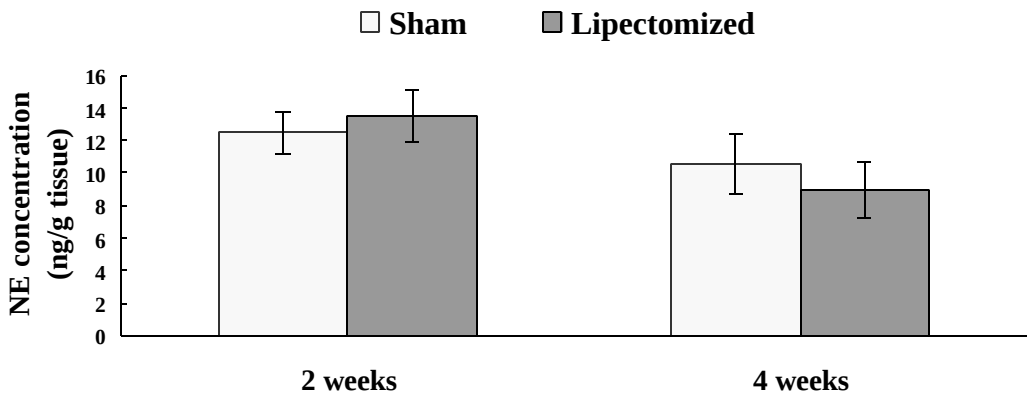

Figure 11. Norepinephrine concentration in retroperitoneal fat pads of lipectomized and sham operated rats at the end of 2 or 4 weeks after surgery. Data are means ± SE for 9-12 rats each group. No significant differences between lipectomized and sham rats were observed.

Table 1. The total number of adipocytes in retroperitoneal fat pads at the end of 2, 4 and 16 weeks after lipectomy surgery.

Weeks after surgery	Treatment	
	Lipectomized	Sham operated
2	6.107 +/- 0.583	6.476 +/- 0.599
4	11.184 +/- 1.138 *	6.714 +/- 0.647
16	13.267 +/- 1.059 *	11.113 +/- 1.015

Data are means ± SE for groups of 8~10 rats in each treatment. Data are in the unit of 10^6. An asterisk (*) indicates significant difference ($P < 0.05$) between lipectomized and sham-operated rats.

CHAPTER IV

SUMMARY AND CONCLUSION

To study the regulation of body weight/fat and identify mechanisms of compensatory adipose tissue growth after lipectomy, bilateral epididymal fat pads were surgically removed or sham operated from adult male Wistar rats. Energy balance, putative adiposity signals, the pattern of change over time in adipocyte size and number in a compensating fat pad and the involvement of blood borne factors, paracrine factors and/or the sympathetic nervous system were investigated to identify potential mechanisms regulating the compensatory growth.

Energy balance was examined by measuring food intake and energy expenditure. Food intake during the first 4 weeks and metabolic-body-weight-adjusted heat production on day 7-10 and day 28-31 post surgery were not different between lipectomized and sham operated rats. A positive energy balance would be more likely to be detected, had energy balance been measured for the whole sixteen weeks with bigger sample size in each treatment. Although a positive energy balance was not detected during the above time points, the lipectomized rats showed a compensatory growth of adipose tissue. Mesenteric fat pads were significantly heavier and retroperitoneal fat pads had a tendency to be heavier in lipectomized rats than that in sham operated rats four weeks after surgery. At the end of 16 weeks after surgery mesenteric and retroperitoneal fat pads of lipectomized rats were significantly heavier than those in sham operated rats, and carcass

composition of lipectomy rats was similar to that of sham operated rats, which confirmed a full compensatory growth of adipose tissue.

Serum insulin and leptin levels were measured as signals conveying the reduction of fat content. Leptin levels were not significantly different between lipectomized and sham operated rats at either 2 or 4 weeks post surgery, while insulin levels was higher in lipectomized rats at 2 weeks post surgery than that in sham operated rats. Thus, the putative adiposity signals insulin and leptin did not appear to facilitate the compensatory adipose tissue growth.

The enlargement of compensating fat pads may be due to hyperplasia and/or hypertrophy of adipocytes. Two weeks after surgery, the total number and cell size distribution of adipocytes in retroperitoneal fat pads were similar between lipectomized and sham operated rats. Four weeks post surgery, lipectomized rats had more total, small and large adipocytes than sham operated rats. At the end of 16 weeks, more total and large adipocytes were measured in lipectomized rats than in sham operated rats. This was in agreement with the changes in relative weight of retroperitoneal fat pad. Changes in adipocyte number and size distribution indicated that compensatory growth occurs by both hyperplasia and hypertrophy initially, and hypertrophy predominately at the late stage.

What are the mechanisms that induce hyperplasia and hypertrophy? Serum collected from lipectomized rats at two or four weeks after surgery stimulated proliferation of preadipocytes in an *in vitro* primary cell culture system more than that from sham operated rats, suggesting blood borne factor(s) may underlie the hyperplasia observed in compensating fat pads. Did the proliferative factors originate from fat pads? Media conditioned by exposure to retroperitoneal fat tissue did not show an elevated activity to stimulate proliferation, indicating that the proliferation-factors in the blood

were not from adipose tissue. On the other hand, serum from lipectomized rats did not stimulate differentiation of preadipocytes more than that from sham operated rats, indicating mechanism(s) other than blood borne factors may be responsible for the fat cell hypertrophy. Norepinephrine concentration in retroperitoneal fat pads was measured as an indicator of the activity of the sympathetic nervous system. No difference in norepinephrine concentration was observed between lipectomized and sham operated rats at 2 or 4 weeks post surgery. It is unclear if other components of the sympathetic nervous system play a role in the compensatory adipose tissue growth after lipectomy.

In summary, rats respond to body weight/fat loss induced by lipectomy by restoring total body fat level through compensatory enlargement of non-excised fat pads. Compensatory adipose tissue growth is due to both hyperplasia and hypertrophy. Blood borne factors that increase cell proliferation may underlie the enlargement of compensating fat pads. Further studies are warranted to identify other adiposity signals signaling the deficit of fat content and the specific blood borne factors mediating compensatory adipose tissue growth.