Brown adipose tissue of hypothalamic knife-cut rats :: effects of high-carbohydrate and high-fat diets.

Joan M. Hamilton
University of Massachusetts Amherst

Follow this and additional works at: https://scholarworks.umass.edu/theses
BROWN ADIPOSE TISSUE
OF HYPOTHALAMIC KNIFE-CUT RATS:
EFFECTS OF HIGH-CARBOHYDRATE
AND HIGH-FAT DIETS

A Thesis Presented
By
JOAN MARTHA HAMILTON

Submitted to the Graduate School of the
University of Massachusetts in partial fulfillment
of the requirements for the degree of
MASTER OF SCIENCE
September 1984
Psychology
BROWN ADIPOSE TISSUE
OF HYPOTHALAMIC KNIFE-CUT RATS:
EFFECTS OF HIGH-CARBOHYDRATE
AND HIGH-FAT DIETS

A Thesis Presented
By
JOAN MARTHA HAMILTON

Approved as to style and content by:

George N. Wade, Chairperson of Committee

John W. Donahoe, Member

Richard M. Gold, Member

Seymour M. Berger, Department Head
Department of Psychology
ACKNOWLEDGEMENT

There are many people without whose contributions this thesis would not have been possible.

I am indebted to George Wade, my advisor and thesis committee chairman, for unrestrained financial support and for his policy of noninterference which encouraged independence and provided ample opportunity for benefitting from my mistakes.

I am grateful to Dick Gold for serving on my committee and for the considerable time and effort he devoted to me and my work.

I am thankful to John Donahoe for serving on my committee. His contributions were both intended and unintended. His advice was always meaningful and willingly given, and his presence as a scientist and scholar was inspirational.

The technical assistance of Jay Alexander and his helpers, most notably Lisa Grochmal and Steve Grochmal, and of Lynn Bengston is gratefully acknowledged. In addition, I am thankful to Jay for his friendship which helped me to maintain the proper perspective on life in graduate school.

Extremely helpful discussion and advice was eagerly offered by Sue Anne Assimon, Tim Bartness, Hank Heller,
John McElroy, Anita Sylvan, and Sandra Woods. I am very grateful to all of them.

For his indefatiguable support, I thank my husband, Peter.
LIST OF FIGURES

1. Experiment 1 body weight ........................................... 26
2. Experiment 1 body weight change, food intake, and feed efficiency ........................................ 29
3. Experiment 1 cumulative body weight change, food intake, and feed efficiency ........................................ 31
4. Experiment 1 resting oxygen consumption ........................................ 34
5. Experiment 1 white fat pad weight ........................................ 36
6. Experiment 1 brown fat pad weight ........................................ 39
7. Experiment 1 brown fat protein and DNA ........................................ 41
8. Experiment 2 body weight during scheduled pair feeding ........................................ 58
9. Experiment 2 body weight change, food intake, and feed efficiency during scheduled pair feeding ........................................ 60
10. Experiment 2 cumulative body weight change, food intake, and feed efficiency during scheduled pair feeding ........................................ 63
11. Experiment 2 body weight during ad libitum feeding ........................................ 66
12. Experiment 2 body weight change, food intake, and feed efficiency during ad libitum feeding ........................................ 68
13. Experiment 2 cumulative body weight change, food intake, and feed efficiency during ad libitum feeding ........................................ 71
14. Experiment 2 thermic effect of feeding ........................................ 74
15. Experiment 2 white fat pad weight ........................................ 77
16. Experiment 2 brown fat pad weight ........................................ 80
17. Experiment 2 brown fat protein and DNA ........................................ 83
18. Experiment 2 brown fat cytochrome c oxidase activity ........................................ 86
# TABLE OF CONTENTS

## ACKNOWLEDGEMENT

... iii

## LIST OF FIGURES

... v

## Chapter

### I. INTRODUCTION

1

- Mechanism of Heat Production in Brown Adipose Tissue: 4
- Thermogenesis and Obesity: 6
- Specific Aims: 15

### II. GENERAL METHODS

16

- Subjects: 16
- Surgery: 16
- Diets: 17
- Resting Oxygen Consumption: 17
- Thermic Effect of Feeding: 18
- Biochemical Assays: 19
- Histology: 20
- Statistical Analysis: 20

### III. EXPERIMENT 1

22

- Procedure: 24
- Results: 25
  - Histology: 25
  - Body weight and food intake: 25
  - Oxygen consumption: 33
  - White adipose tissue: 33
  - Brown adipose tissue: 38
- Discussion: 44

### IV. EXPERIMENT 2

51

- Procedure: 53
- Results: 57
  - Histology: 57
  - Body weight and food intake: scheduled pair feeding: 57
  - Body weight and food intake: ad libitum feeding: 65
CHAPTER I

INTRODUCTION

The first documented evidence of the absence of weight gain following increased food intake appeared in 1902 (67). The phenomenon has since been re-examined and clinically established (64, 91). The ensuing search for a mechanism revealed that the discrepancy between calories ingested and weight gained could not be fully accounted for by increased activity, malabsorption, or altered body composition, and thus led to the suggestion that overeating must produce excess heat (65). The effector of this heat production was not readily apparent, but was ultimately revealed by the search for the mechanism of heat production which occurs in response to chronic cold exposure. Several lines of evidence in this field culminated in the proposal that the adaptive heat production known as nonshivering thermogenesis seen in cold-acclimated animals was a result of brown adipose tissue activity.

The early link of nonshivering thermogenesis to brown adipose tissue activity was provided by the discovery that the gradual shift from shivering to nonshivering thermogenesis which accompanies acclimation to cold was
associated with hypertrophy and hyperplasia of brown adipose tissue (13,70,95). Evidence directly implicating brown adipose tissue activity in nonshivering thermogenesis came first from work with hibernating mammals, animals found to have a relatively large amount of brown adipose tissue (94). Positive identification of brown adipose tissue as the primary site for nonshivering thermogenesis in the cold-acclimated rat followed several years later when Foster and Frydman (21,22) demonstrated a greatly enhanced blood flow to the interscapular brown fat pad in response to cold acclimation and infused noradrenaline.

The simultaneous discovery of the role of catecholamines in the development and regulation of nonshivering thermogenesis contributed to the establishment of brown adipose tissue as an effector organ of nonshivering thermogenesis. A greatly increased calorigenic response to injected catecholamines was found to coincide with the changes in brown adipose tissue morphology which follow cold acclimation (14,44,45,56). Surgical removal of interscapular brown adipose tissue reduces the calorigenic response to norepinephrine (38,57). Taken together, these findings pointed to the importance of sympathetic-mediated brown adipose tissue activity in cold-induced nonshivering thermogenesis.
The relevance of these findings in cold-induced nonshivering thermogenesis to the search for an effector of the heat production following overeating lies in the demonstration that changes in diet can influence sympathetic activity. Specifically, fasting suppresses activity of the sympathetic nervous system in rats (113), while overfeeding stimulates it (112). Since thermogenic activity of brown adipose tissue has been shown to be influenced by the sympathetic nervous system in cold-exposed animals, then diet-induced changes in the sympathetic nervous system might also effect changes in the thermogenic activity of brown adipose tissue. More recent reports have confirmed that overfeeding (82,83,114) and fasting (114) affect the sympathetic activity of brown adipose tissue. An increase in brown adipose tissue thermogenesis with overfeeding could explain the disproportionate relationship between weight gain and caloric intake.

Rothwell and Stock (78) subsequently demonstrated that diet-induced thermogenesis was indeed a quantitatively important component of energy expenditure. Rats overeating on a cafeteria diet gained less weight than expected; that is, the cafeteria-fed rats had a lower efficiency of weight gain (weight gained per calorie ingested) than controls did
on the stock diet. This feed inefficiency resulted from increased energy expenditure as indicated by increased oxygen consumption. Furthermore, both an enhanced thermogenic response to catecholamines and changes in brown adipose tissue metabolism and growth characterized the adaptation to chronic overfeeding. The similarities between diet-induced and nonshivering thermogenesis in the calorigenic response to catecholamines and in changes of brown adipose tissue firmly established the significance of brown adipose tissue in diet-induced thermogenesis.

**Mechanism of Heat Production in Brown Adipose Tissue**

The most generally accepted mechanism of heat production in brown fat is that of a proton leakage or conductance pathway as proposed by Nicholls (68). Mitochondrial respiratory activity normally pumps protons out of the mitochondrion, generating a proton gradient across the inner mitochondrial membrane that can be used to provide energy for the formation of adenosine 5'-triphosphate (ATP) from adenosine 5'-diphosphate (ADP). Thus respiration is linked to ATP formation. In the heat-producing brown fat mitochondria, however, there is an uncoupling of ATP production from respiration which results in the liberation of energy as heat. Uncoupling results
from a dissipation of the proton gradient which appears to be due to the presence of a 32,000-Dalton molecular weight polypeptide in the inner mitochondrial membrane. This protein effectively creates a hole or pathway through which protons enter the mitochondrion. Activity of the proton conductance pathway can be modulated by the binding of purine nucleotides, such as guanosine 5'-diphosphate (GDP). Presence of functional proton conductance pathways can therefore be assessed by measuring the mitochondrial capacity for GDP binding. GDP binding is positively correlated with proton conductance and, thus, with brown adipose tissue thermogenesis. The proton conductance pathway appears to be stimulated ultimately by the norepinephrine-induced activation of adenylate cyclase. Adenylate cyclase on the inner surface of the plasma membrane causes an increase in adenosine 3',5'-phosphate (cyclic AMP) which in turn stimulates triglyceride lipolysis, resulting in the provision of fatty acids for mitochondrial oxidation and in some way increasing proton conductance.

Direct control of brown adipose tissue thermogenesis is mediated by the sympathetic nervous system. This is evidenced by the well-documented sympathetic innervation of interscapular brown adipose tissue (see 87 for a review)
and the demonstration that changes in brown adipose tissue norepinephrine turnover parallel adaptive changes in heat production in rats (114). In addition, denervation of interscapular brown adipose tissue reduces the level of norepinephrine in the tissue and decreases the thermogenic response to cold (20).

**Thermogenesis and Obesity**

The relationship between caloric intake and energy expenditure has important implications for the regulation of body weight. Adjustments in diet-induced thermogenesis may contribute to maintenance of a constant body weight. Conversely, increased metabolic efficiency (less energy expended per calorie ingested) might predispose an individual to obesity.

The possibility that changes in brown adipose tissue activity may be important to obesity was first demonstrated in genetically obese (ob/ob) mice (see 101 for review). These animals display both an impaired development of nonshivering thermogenesis with cold exposure (102) as well as an obesity syndrome characterized by hyperphagia (60). However, development of the obesity occurs in the absence of hyperphagia (60). The combination of impaired cold-
induced nonshivering thermogenesis and obesity without hyperphagia suggests impaired diet-induced thermogenesis. This was confirmed by pair feeding and measuring metabolic rate at a variety of environmental temperatures (99,102).

Ob/ob mice have decreased thermogenesis in brown adipose tissue. Brown fat mitochondrial binding of purine nucleotides is depressed and is not increased with acute cold exposure, as it is in lean animals (3,39). Interestingly, however, ob/ob mice are able to improve thermogenic capacity with cold acclimation through almost normal brown adipose tissue growth, mitochondrial proliferation, and alteration in mitochondrial properties (42). Brown adipose tissue of ob/ob mice also responds abnormally to injected catecholamines (2,4,86) and has reduced norepinephrine turnover (52), indicating decreased sympathetic activity. The importance of brown fat to the reduced thermogenesis of ob/ob mice is further illustrated by reduced regional blood flow and in vivo brown adipose tissue oxygen consumption which have revealed that 93% of the observed differences in nonshivering thermogenesis between lean and obese mice are attributable to brown adipose tissue (100). In addition, cafeteria-fed ob/ob mice have a reduced capacity for diet-induced thermogenesis which parallels reduced adaptive changes in brown adipose
tissue (103). Thus, the contribution of reduced brown adipose tissue thermogenesis to the abnormally low energy expenditure observed in ob/ob mice is confirmed.

Impaired brown adipose tissue thermogenesis may be the primary factor in the development of this genetic obesity (101). Excessive energy accumulation in ob/ob mice begins to appear at about 12 days of age, yet food intake is comparable to that of lean mice for the first 4 weeks of life (98). A concurrence of decreased brown fat mitochondrial GDP binding and excess fat deposition at 10 to 14 days of age has been observed (32), and indicates a decreased expenditure of energy in young normophagic ob/ob mice.

Genetically obese Zucker (fa/fa) rats present a similar model of obesity which appears to be partially attributable to defective brown adipose tissue thermogenesis. As with ob/ob mice, obese Zucker rats have reduced cold tolerance (104) and fail to show the normal thermogenic response to food (76,77,79,80). Unlike ob/ob mice, however, fa/fa rats do not reliably show an impaired thermogenic response to injected catecholamines (63,75,76,111). Nevertheless, sympathetic nervous system-mediated brown adipose tissue thermogenesis does appear to be abnormal (76). It has been suggested that while the
peripheral thermogenic mechanism of Zucker fa/fa rats can function normally in response to catecholamines, the centrally-mediated sympathetic response to food is defective (75).

Excessive adiposity develops in suckling fa/fa rats during the first week of life, before the onset of hyperphagia (8,25). Although an abnormal increase in the activity of white adipose tissue lipoprotein lipase (an enzyme responsible for fat uptake) accompanies the onset of obesity (8), decreased thermogenesis at this developmental stage may also contribute to the excess energy accumulation (26). As with ob/ob mice, early, pre-hyperphagia occurrence of suppressed thermogenesis in fa/fa rats suggests a primary role for decreased energy expenditure in the development of the obesity.

More recent work with dietary obesity attests to the influence of diet-induced thermogenesis on body weight. Rats overeat and gain weight when offered a stock diet supplemented with a sweet sucrose solution (47). However, the weight gained falls short of that predicted by the ingested calories (34). Furthermore, the brown fat of these sucrose-fed rats exhibits an enhanced lipid synthesis which resembles that seen in cold-exposed rats (33,34). Although this is not direct evidence for the involvement of
brown fat in the increased energy expenditure in sucrose-fed rats, brown adipose tissue thermogenesis is strongly suggested by the similar metabolic response of this tissue to cold exposure and sucrose overfeeding (34). Sucrose feeding in rats also increases interscapular brown adipose tissue norepinephrine turnover (114). In contrast, sucrose feeding in golden hamsters produces no changes in caloric intake, body weight, carcass composition, or resting oxygen consumption (107). However, feeding golden hamsters a high-fat diet does lead to significant body weight gain and increased carcass fat content, which occur in the absence of increased caloric intake (107). These obese, normophagic high-fat-fed hamsters have significantly reduced energy expenditure, as measured by resting oxygen consumption (107). In light of these findings, then, it is clear that changes in energy expenditure resulting from changes in diet can significantly contribute to the regulation (or lack thereof) of body weight.

Obesity resulting from damage to the hypothalamus is of special relevance to the role of energy expenditure in obesity. The independence of food intake and obesity in ventromedial hypothalamic (VMH)-lesioned rats has long been recognized. In 1964 Han and Young (36) first demonstrated a failure to prevent weight gain in adult VMH-lesioned
rats by pair-tube-feeding. Although weanling VMH-lesioned rats are not hyperphagic and do not gain excess weight (6,7,35,49), they do have an increased body fat content (5). VMH lesions in adult rats occasionally do not cause overeating, but do cause significantly greater accumulations of body fat (73). These differences between carcass energy gain and energy intake suggest increased metabolic efficiency.

Lesions of the VMH which produce hyperphagia and obesity have been shown to result in reduced activity of the sympathetic nervous system. Rats with VMH lesions have smaller submaxillary salivary glands and reduced serum glucagon levels, both indicative of reduced sympathetic nervous system activity (46). Pair-feeding to maintain food intake at levels comparable to intact controls did not correct the glucagon levels or salivary gland weights (46). Work with unilateral lesions of the VMH and selective sympathectomies provided more direct evidence for a link between the VMH and peripheral actions of the sympathetic nervous system. Rats with unilateral VMH lesions exhibited a reduced lipolytic response in the contralateral retroperitoneal fat pad to an acute fast (10). This reduction was comparable to that which follows unilateral denervation of the retroperitoneal fat pads (10). Thus,
lesions of the VMH mimicked the effect of abdominal sympathectomy on lipid mobilization.

These findings combined with recent evidence for VMH-mediated sympathetic control of brown adipose tissue (71,90,105, also unpublished results cited in 101) suggest a role for impaired brown fat thermogenesis in hypothalamic obesity. Changes in brown adipose tissue of rats with VMH lesions have recently been confirmed. Within 3 days after VMH lesions in adult female rats the metabolic reactivities of interscapular brown adipose tissue to nerve stimulation and norepinephrine administration are depressed (88). Four to five weeks after lesions, the brown fat of lesioned rats has decreased DNA concentration and content, indicating tissue involution. More recent work comparing VMH-lesioned, cafeteria-fed, and stock diet-fed female rats revealed a defective diet-induced brown adipose tissue response in lesioned rats (74). Both cafeteria-fed and VMH-lesioned rats increased their interscapular brown adipose tissue mass as compared to control rats. In cafeteria-fed rats the increased mass represented growth of active tissue, while in VMH-lesioned rats lipid filling accounted for most of the increase. Consequently, whereas cafeteria-fed rats maintained basal and noradrenaline-stimulated specific heat production rates of the tissue
comparable to controls and thereby increased total heat production of the interscapular pad, VMH-lesioned rats had decreased specific heat production and total heat production which was equivalent to that of their respective control group. The failure of hyperphagic lesioned rats to exhibit an increased thermogenic capacity comparable to that of hyperphagic cafeteria-fed rats strongly suggests a defective diet-induced adaptation in hypothalamic obesity in rats.

Hogan, et al. (41) have also examined the brown adipose tissue of VMH-lesioned rats. In contrast to Seydoux, et al. (88), they found normal content of DNA, protein and cytochrome oxidase (a mitochondrial respiratory enzyme), but an enhanced lipid content, three weeks after lesioning. Thermogenic activity of the tissue as measured by GDP binding was normal in lesioned male rats, but was low in lesioned female rats. Exposure to cold increased GDP binding, indicating normal thermogenic responsiveness in both males and females. Rohner-Jeanrenaud, et al. (74) have more recently replicated this finding in 3-week cold-acclimated VMH-lesioned female rats with noradrenaline-stimulated heat production of interscapular brown fat being comparable to that of controls. Hogan, et al. (41) point out that since the lesioned animals are hyperphagic, one
would expect to see changes in brown adipose tissue characteristic of rats overeating a palatable diet, such as tissue growth (78) and increased GDP binding by brown fat mitochondria (40). The failure of the lesioned animals to exhibit these changes in brown adipose tissue suggests that impaired diet-induced thermogenesis in VMH-lesioned rats contributes to the obesity seen in these animals.

Parasagittal hypothalamic knife cuts alongside the paraventricular nucleus (PVN) produce hyperphagia and obesity which is similar to that produced by VMH electrolytic lesions (29), but produce less neural damage, less disruption in nonfeeding behaviors, and fewer endocrine and metabolic disorders than do VMH electrolytic lesions (see 85 for a review, 11). Investigation of brown adipose tissue of PVN knife-cut rats therefore has the potential for revealing more information about the precise location of hypothalamic control over brown fat and its possible contribution to hypothalamic obesity.

After initiation of this project Coscina, et al. (16) reported the brown adipose tissue responses of hypothalamic knife-cut obese rats to acute cold exposure. As with the VMH-lesioned rats discussed above (see ref. 41), knife-cut obese rats had normal brown fat at room temperature and showed normal responses to acute cold
exposure, as measured by protein and DNA content, cytochrome oxidase activity, and mitochondrial GDP binding. Coscina, et al. (16) suggest that the normal activity of the tissue at room temperature in hyperphagic knife-cut rats may indicate an impaired ability to respond to overeating, as has been suggested to occur in VMH lesion obesity (41).

**Specific Aims**

This study was conducted in order to assess the effects of hypothalamic knife cuts on brown adipose tissue and energy expenditure. The rats were offered diets of varying macronutrient composition in order to investigate whether adaptive thermogenic responses to increases in dietary carbohydrate or fat are impaired by knife cuts.
CHAPTER II
GENERAL METHODS

Subjects

Female CD Sprague-Dawley rats weighing 200-250 g were purchased from Charles River Breeding Labs (Wilmington, MA) and housed individually in wire hanging cages with unrestricted access to food pellets (Purina Rodent Chow 5001) and tap water unless stated otherwise. Room lights were on 12 hours per day, lights on as indicated for each experiment. Room temperature was maintained at 21-23°C.

Surgery

Experimental rats received bilateral parasagittal knife cuts alongside the paraventricular nucleus according to the method of Gold, et al. (29,30). Briefly, the rat was mounted in the stereotaxic apparatus with the incisor bar 3.0 mm below the interaural line. A guide cannula containing the caudally oriented retracted wire knife was lowered 7.0 mm from the dura at 8.4 mm anterior to ear bar zero and 0.9 mm lateral to the midline. The wire was then extended 3.0 mm and the knife lowered until it reached the base of the skull (as indicated by a caudal deflection of
the guide cannula), where it was gently bounced 2 to 3 times to insure a complete cut at this point. The knife was then raised 3.0 mm from the base of the skull and finally lowered to the original starting position, after which the wire was retracted into its guide cannula. Sham surgery consisted of lowering the guide cannula 7.0 mm below the dura, with no extension of the cutting wire. Surgery was performed under chloropent anesthesia (3.0 ml/kg).

Diets

Three different diets were used: Purina Rodent Chow 5001 pellets (23.4% protein, 49% carbohydrate, 4.5% fat; 3.3 kcal/g), Purina Rodent Chow 5001 pellets supplemented with a 32% (w/v) sucrose solution (32% sucrose = 1.28 kcal/g), and Purina Mouse Chow 5018 pellets (17.5% protein, 53.5% carbohydrate, 11% fat; 3.8 kcal/g). The three diets are referred to as Chow, Sucrose, and Fat, respectively.

Resting Oxygen Consumption

Resting oxygen consumption was measured at room temperature in an open-loop system using an Applied Electrochemistry S3-A oxygen analyzer. Room air was drawn
through a cylindrical acrylic chamber in which the animal was held and then passed through soda lime and CaSO₄ (Drierite) to remove CO₂ and H₂O before entering the oxygen analyzer. The air flowed at a constant rate of 750 ml/min. In order to avoid potential confounding by the thermic effect of feeding (see Experiment 2), food was removed from the home cage 1 hour prior to testing. Measurements were taken on awake, immobile animals. A mean of 5 separate readings at least 30 seconds apart was used for the computation of resting oxygen consumption, expressed as ml oxygen consumed/min/kg·75 (51). Five to six 30- to 60-minute adaptation sessions in the chamber preceded data collection.

**Thermic Effect of Feeding**

The thermic effect of feeding was assessed by measuring oxygen consumption for 2 hours following ingestion of a meal. The equipment used was essentially as described above for the measurement of resting oxygen consumption except that the data were collected by a computer. Samplings were taken every minute, and the final analysis was based on the average amount of oxygen consumed per minute over the 2-hour postprandial period.
**Biochemical Assays**

Interscapular brown adipose tissue (IBAT) was rapidly dissected from the body after decapitation and kept on ice while cleaned of adhering white fat and muscle, and then weighed. For the determination of protein and DNA content, the tissue was homogenized in 0.25 M sucrose containing 1 mM EDTA, pH 7.4 and lipid was extracted from the homogenate using ethanol. The washed pellet was then dissolved in 0.3 M KOH. Aliquots of this were taken for protein determination according to the method of Lowry, *et al.* (61). Six percent perchloric acid was added to the remaining homogenate in 0.3 M KOH, and aliquots were taken for the determination of DNA content according to the method of Burton (12).

IBAT mitochondria were partially purified using a procedure described by Slinde, *et al.* (93). IBAT was homogenized at 50 mg tissue/ml in a medium containing 0.25 M sucrose, 0.2 mM EDTA, and 1.0 mM HEPES, pH 7.2. The homogenate was centrifuged 10 min at 6,000 rpm in an SS-34 rotor of a Sorvall RC2-B refrigerated centrifuge, and the supernatent stored at -70°C for later assay. Activity of the mitochondrial enzyme cytochrome c oxidase was measured using a modification (P. Mason, personal communication) of the spectrophotometric method of Wharton and Tzagoloff
(110). Briefly, partially purified mitochondria were resuspended in a 75 mM NaPi buffer, pH 7.0, containing 0.5% w/v Tween-80 (Sigma). Oxidized cytochrome c was reduced by the addition of dithionite. Mitochondria were then added to the reduced cytochrome c and change in absorbancy at 550 nm over a 2-minute period was monitored. Activity of the enzyme was expressed relative to whole homogenate protein content.

**Histology**

Frozen coronal sections were stained with cresyl violet in order to determine knife cut position. Animals whose cuts did not meet the criteria established by Gold, et al. (29) were not used in the analyses.

**Statistical Analysis**

Two- and three-way analyses of variance (ANOVAs) were used to test for significant overall main and interaction effects. Post hoc pairwise comparisons were made using Duncan's Multiple Range Test (50) for independent groups and Bonferroni t tests (66) for matched groups. The alpha-level was .05 for each collection of multiple comparisons. Using Duncan's procedure, an overall alpha-level of .05
translates to a mean protection level of 0.77, which is the minimum probability of finding no erroneous significant pairwise differences among six means (the maximum number of means compared in this study) (50).
CHAPTER III
EXPERIMENT 1

The possible contribution of decreased energy expenditure to the obesity seen following knife-cut hypothalamic damage in adult female rats was investigated. Females were used rather than males because the relatively slower growth rate and greater body weight changes of females allows for more readily detectable knife-cut-induced weight gain (27). The rats received parasagittal knife cuts or sham surgery and then were allowed ad libitum access to one of three different diets as described above.

Several reasons exist for the use of these different diets in answering the question of whether changes in brown adipose tissue activity and energy expenditure are factors in the PVN obesity syndrome. It has already been suggested that hypothalamic obese rats may have impaired diet induced thermogenesis (16,41,74). Deficits in diet-induced thermogenesis are more likely to be detected when overeating a sucrose-supplemented diet since sucrose feeding in normal rats enhances energy expenditure and produces changes in brown adipose tissue metabolism (33,34,114). The effects of dietary fat on energy expenditure and brown fat are unclear. A high fat test
meal has been shown to produce a significant increase in postprandial resting oxygen consumption of rats maintained on a lower fat stock diet (80). This finding is in agreement with the observation that fat stimulates sympathetic nervous system activity in rats (55). However, a decreased in vitro brown adipose tissue respiration rate following a high fat test meal has been observed in rats which had intermittent access to the high-fat diet prior to testing (23). It has also been reported that varying the percentage of fat in the diet does not affect interscapular brown fat pad weight in rats (97). In golden hamsters, dietary fat has a clear suppressive effect on actual energy expenditure, but the thermogenic capacity of the fat-fed hamster is simultaneously enhanced (107). These findings, together with the observation that PVN knife-cut rats may resist hyperphagia and obesity on a standard chow diet, but will readily overeat and gain weight when offered a more palatable higher fat diet (28), suggest a possible role for dietary fat-induced changes in brown adipose tissue in PVN knife-cut obesity.

Energy expenditure was assessed by measuring resting oxygen consumption. Measurement of interscapular brown adipose tissue protein and DNA provided an index of tissue growth. Retroperitoneal white adipose tissue (RWAT) weight
served as an indicator of relative adiposity.

**Procedure**

Fifty rats were assigned to six treatment groups in a 2 X 3 factorial design so that the mean body weights of each group were matched and variability within each group was equalized. Animals received either bilateral knife cuts or sham surgery, followed by *ad libitum* access to one of the three experimental diets. Cumulative food intake to the nearest 0.1 g (solid food spillage accounted for) and body weight to the nearest 1.0 g was measured every 3 days up to the time of measuring resting oxygen consumption. Room lights were on from 7 am to 7 pm.

Resting oxygen consumption was measured 22 to 24 days after surgery. Testing was conducted during the light portion of the light cycle between 10 am and 2 pm when activity levels were at their lowest.

After testing, animals had *ad libitum* access to their experimental diets until they were decapitated 33 to 39 days after surgery. The brain was rapidly removed and placed into 10% buffered formalin for later histological verification of knife-cut placement. Interscapular brown adipose tissue (IBAT) was removed, weighed and prepared for determination of protein and DNA content. Finally, the
left RWAT was removed and weighed.

Results

Histology

Histological examination of coronal sections resulted in the elimination of 7 of the 24 knife-cut rats from the analyses. Through the course of the experiment one control rat escaped and its data were also eliminated. The number of animals remaining in each group was: Chow-Sham, n=7; Sucrose-Sham, n=8; Fat-Sham, n=8; Chow-Cut, n=4; Sucrose-Cut, n=7; Fat-Cut, n=6.

Body weight and food intake

Body weights of the six treatment groups for the duration of the experiment are shown in Fig. 1. Four days after surgery, the Sucrose-Cut and Fat-Cut groups had significantly greater mean body weights than the three sham groups. The Chow-Cut group was not significantly different from any other group. By day 7, the Fat-Cut group was significantly heavier than any other group. When resting oxygen consumption was measured at day 22-24, the body weight difference between the Fat-Cut and Sucrose-Cut groups was no longer significant, but both were still greater than the sham groups. The mean body weight of the
Figure 1. Mean ± S.E.M. body weights of adult female rats in Experiment 1 which received bilateral parasagittal hypothalamic knife cuts or sham surgery and were allowed ad libitum access to a stock chow diet, a higher carbohydrate sucrose-supplemented chow diet, or a higher fat diet. Rats were killed 33 to 39 days after surgery. Size of each treatment group is indicated in parentheses following symbol identification.
Chow-Cut group was intermediate to the sham and other cut groups. At the time of decapitation (33-39 days after surgery), the Fat-Cut and Sucrose-Cut groups were still heavier than any of the sham groups, and were also both significantly heavier than the Chow-Cut group. The Chow-Cut group was significantly heavier than the Chow-Sham at sacrifice.

Three-day total food intakes are plotted in Fig. 2 along with corresponding changes in body weight. The ratio of these values is expressed in the bottom panel as feed efficiency. Food intakes were greatest in the Fat-Cut and Sucrose-Cut groups throughout the 19-day period. On day 19 the Fat-Cut and Sucrose-Cut groups had significantly greater intakes than the Chow-Cut and Sucrose-Sham groups. Also, the Sucrose-Sham group exhibited significantly greater caloric intakes than both of the other two sham groups. An initial significant difference between the cut and sham groups in feed efficiency was no longer present 19 days after surgery.

Cumulative measures of changes in body weight, food intake, and feed efficiency are presented in Fig. 3. Although the energy intakes of the Fat-Cut and Sucrose-Cut groups were equivalent, the cumulative weight gain of the Fat-Cut group was significantly greater beginning on day 7
Figure 2. Mean body weight change, food intake, and feed efficiency expressed as g change in body weight per kcal eaten of rats described in Figure 1. Each point represents 3-day totals for the day indicated and the preceding two days.
Figure 3. Mean cumulative body weight change, food intake, and feed efficiency of rats described in Figure 1.
and continuing through day 19. This difference is reflected by the significantly greater cumulative feed efficiency of the Fat-Cut group on days 7-19. Cumulative feed efficiencies of the Chow-Cut and Sucrose-Cut groups did not differ and were consistently significantly greater than the three sham groups from day 7. Despite a significantly greater overall (i.e., day 19) energy intake in the Sucrose-Sham group relative to the other two sham groups, the cumulative body weight gain of the three groups did not differ. This difference, however, is not reflected by a lower cumulative feed efficiency, possibly because of the greater but nonsignificant overall weight gain by the sucrose-fed group.

**Oxygen consumption**

Figure 4 depicts the results of the resting oxygen consumption measurements. There were no significant overall effects of surgical or dietary treatment, and no significant differences between any two treatment groups.

**White adipose tissue**

Retroperitoneal white fat pad wet weights and pad weight as percent body weight are presented in Fig. 5. Comparison of pad weights across groups corresponds well with body weight at sacrifice (Fig. 1, days 33-39). Pad
Figure 4. Mean + S.E.M. resting oxygen consumption of rats described in Figure 1. Oxygen consumption was measured 22 to 24 days after surgery in 2-hr fasted rats during the light portion of the light:dark cycle. Each rat's oxygen consumption represents the average of 5 separate readings at least 30 seconds apart taken when awake and immobile. Group sizes are indicated at the base of each bar.
Figure 5. Mean ± S.E.M. left RWAT pad wet weight and pad weight as percent body weight of rats described in Figure 1.
Brown adipose tissue

IBAT wet weight and IBAT weight as percent body weight are shown in Fig. 6. Differences in IBAT pad weights were similar to those found in the white fat pad (see Fig. 5). Overall, pad weights of the cut groups were heavier than the sham groups, but within each diet group this difference was significant in the sucrose and fat conditions only. The effect of diet on pad weight was not the same for the two surgery conditions. Sucrose- and fat-fed keloid cut animals had significantly greater pad weight than the Chow-Cut animals, whereas only sucrose-fed sham animals had significantly increased pad weights. When expressed as percent body weight, a significant main effect of surgery can be attributed to a difference between the two fat-fed groups. Again, the diet effect was different in the two surgery conditions. In the cut groups, both sucrose and fat feeding resulted in a greater percentage of IBAT than chow feeding; but in the sham groups only the sucrose diet had this effect.

IBAT protein and DNA contents are given in Fig. 7. Total protein content was significantly affected by diet, but there was no overall effect of surgery. The only noteworthy difference in pairwise comparisons was a significantly greater protein content in the Sucrose-Sham
Figure 6. Mean ± S.E.M. IBAT pad wet weight and pad weight as percent body weight of rats described in Figure 1.
Figure 7. Mean ± S.E.M. IBAT protein and DNA content of rats described in Figure 1. Total protein and DNA contents of the pads are shown in the upper panels. Lower panels show concentration of protein and DNA per mg tissue.
group as compared to the Chow-Sham group. Significant main effects of both surgery and diet were present when protein content was expressed per mg tissue. Within each of the three diet conditions, the sham animals had significantly greater protein concentrations than the cut animals. Protein concentration was greater in the Chow-Cut group than in the other two cut groups, but this difference was not significant. Among the sham groups, the only significant difference was a greater IBAT protein concentration in the chow vs. sucrose condition.

Total DNA content was significantly affected by diet, but not by surgery. However, the only significant differences between diet treatments within each surgery condition was a greater DNA content in the Sucrose-Sham group compared to either the Chow-Sham or Fat-Sham group. Relative DNA content, on the other hand, was significantly affected by both the diet and surgery treatments. A significant effect of the dietary treatments, however, was only present within the cut groups with the Chow-Cut group displaying a higher DNA concentration than both the Sucrose- and Fat-Cut groups. IBAT DNA concentration was significantly reduced by knife-cut surgery in both the sucrose and fat diet conditions.
Discussion

The degree of obesity induced by parasagittal knife cuts alongside the paraventricular nucleus was clearly influenced by the diet offered, as has been previously reported (28, 53, 92). Both the higher fat Mouse Chow diet and the higher carbohydrate sucrose-supplemented Rat Chow diet produced weight gains which were significantly greater than those achieved by knife-cut rats fed Rat Chow only. However, the relationship between energy intake and body weight gain across the different diets was dissimilar in the two surgery conditions. Although the intakes of the sucrose- and fat-fed knife-cut groups did not differ, the fat-fed group gained more weight per kcal ingested (see Figs. 2 and 3). This relationship was quite different in the sham condition where intake of the sucrose group was greater than that of the fat group, but body weight changes were comparable. Thus in both knife-cut and sham animals there appeared to be a thermogenic response to overconsumption of the sucrose-supplemented diet. However, hyperphagic fat-fed knife-cut rats failed to exhibit such a thermogenic response. Cumulative feed efficiency of the Fat-Cut group was greater than both its chow-fed knife-cut and fat-fed sham control groups. The increased feed
efficiency of the fat-fed knife-cut group was not a direct effect of the diet since consumption of the fat diet by the shams resulted in no change in feed efficiency when compared with the chow-fed sham control group. However, it is possible that overconsumption of the fat diet is necessary for expression of a lowered feed efficiency. Unfortunately, the appropriate control group to test this suggestion (i.e., a sham hyperphagic fat-fed group) was not included in this experiment. A defect in the thermogenic response to overfeeding in the fat-fed knife-cut groups is supported, however, by the observation that dietary fat stimulates the sympathetic nervous system in the rat (55).

Another possible explanation for the lack of a thermogenic response to overconsumption of the fat diet relies on the role of dietary protein in diet-induced thermogenesis. Although results have varied as a function of the age of the animals, whether the protein component of the diet is near or below the requirement, and on the other components of the diet, there are reported observations in mature animals which consistently suggest that a relatively low-protein diet may lead to nonconservative utilization of the other energy-yielding components of the diet (17, 62, 81, 96). Throughout the 19 days of continuous data collection the fat-fed cut group consistently consumed more
of its total calories as protein than did the sucrose-fed cut group (on average, 123% greater protein calorie intake; data not shown). The greater protein content of the fat diet compared to that of the sucrose-supplemented diet may have inhibited diet-induced thermogenesis in the hyperphagic fat-fed knife-cut animals. However, without an hyperphagic fat-fed sham control group acceptance of this explanation is precluded and the question of whether knife-cut animals lack an adaptive thermogenic response to overconsumption of a high fat diet remains open for discussion.

Vilberg and Keesey (106) have very recently shown that metabolic deficits which have been reported in food-restricted and/or pair-weighted VMH-lesioned rats can be attributed to a decline in the more metabolically active protein component of the carcass. Furthermore, since ad libitum-fed obese VMH rats do not exhibit carcass protein losses, the obesity may not be due to a reduced resting metabolic rate (106). Their observation may help to explain the resting oxygen consumption results obtained in this experiment. The obese knife-cut rats were feeding ad libitum and presumably incurred no losses of carcass protein since they reportedly increase their linear growth (29). The increases in body weight observed in the present
study were predominantly a result of increased carcass fat (see Fig. 5). Given Vilberg and Keesey's (106) results, resting oxygen consumption would therefore not be expected to be lowered by PVN knife-cut-induced obesity in freely feeding rats. This expectation is further supported by the observation that at least within the interscapular brown fat pad, total protein and DNA were unaffected by knife-cut surgery (see Fig. 7). The observed concentrations of brown adipose tissue protein and DNA demonstrate that the increases in pad weight in the knife-cut groups are attributable to increases in lipid content (Figs. 6 and 7). The failure to find a significant knife-cut-induced change in resting oxygen consumption in this experiment is therefore consistent with Vilberg and Keesey's (106) reported results in VMH-lesioned rats and with expectations based on brown adipose tissue protein and DNA content in these animals.

However, since sucrose feeding has been reported to enhance energy expenditure and brown adipose tissue metabolism (33,34,114), an increase in resting oxygen consumption in the sucrose-fed groups would be expected. Resting oxygen consumption was slightly, but not significantly, elevated in the sucrose-fed animals. To the extent that interscapular brown fat protein and DNA content
indicate brown adipose tissue activity, the significant increases in both these indices with overconsumption of sucrose in the sham animals conflict with the failure to find significant increases in resting oxygen consumption. On the other hand, interscapular brown adipose tissue protein and DNA were not significantly increased by the sucrose diet in the hyperphagic knife-cut rats, and this was paralleled by the lack of change in resting oxygen consumption. The use of unequal size groups and the high degree of variability may have rendered the resting oxygen consumption measure insensitive to changes in brown fat thermogenesis in the final analysis. This possibility is suggested both by the failure of the resting oxygen consumption results to support the previously reported effects of dietary sucrose on energy expenditure and brown adipose tissue (33,34,114), and by the lack of agreement between resting oxygen consumption and measures of interscapular brown adipose tissue protein and DNA.

Knife-cut surgery induced hyperphagia in both sucrose- and fat-fed animals, but there was no concomitant increase in interscapular brown adipose tissue protein or DNA as was observed in the hyperphagic Sucrose-Sham group. This suggests a defective brown adipose tissue response to hyperphagia which may in turn indicate impaired diet-
induced thermogenesis in freely feeding obese PVN knife-cut rats. Recent work with VMH-lesioned rats (41,74) and PVN knife-cut rats (16) (as previously discussed in the Introduction) has also led other investigators to suggest that impairment of adaptive diet-induced thermogenesis contributes to the obesity seen in these hypothalamic-damaged rats. Although the results of this experiment neither directly nor compellingly indicate defective brown adipose tissue thermogenesis as a significant contributor to the obesity seen in PVN knife-cut rats, the possibility should not be ruled out.

Unfortunately, more direct statements about changes in brown adipose tissue thermogenesis in these obese rats are not possible for a number of reasons. The resting oxygen consumption results contained a great deal of variability that may very well have masked the statistical significance of treatment effects. In addition, it is possible that adaptive changes in diet-induced thermogenesis may not be reflected in daytime resting oxygen consumption. The measure may be further confounded by knife-cut-induced disruption of meal patterns and circadian rhythms of feeding (31) which carry with them rhythms in energy expenditure and utilization (58). Although increases in interscapular brown adipose tissue DNA and protein are
indicative of tissue growth, a positive correlation with thermogenic activity should not be assumed. In conclusion, however, it is reasonable to propose that the results of this experiment indicate a defective brown adipose tissue response to hyperphagia in rats made obese by parasagittal hypothalamic knife-cuts alongside the paraventricular nucleus.
CHAPTER IV

EXPERIMENT 2

The increase in metabolic rate produced by the consumption of food in man was first discovered in the eighteenth century by Lavoisier (51). Decreases in this response to feeding have been postulated as possible contributors to the increased metabolic efficiency seen in obese humans and animals. As early as 1924 a reduced increment in the postprandial metabolic rate was demonstrated in obese human subjects (108). This finding has been more recently replicated by several investigators (48, 72, 84, 87). A role for this component of energy expenditure in the regulation of energy balance is supported by work with rats. As with obese humans, genetically obese Zucker rats exhibit a decrement in the metabolic response to a meal (76, 77, 80). Conservation of energy in fully fasted rats is partially achieved by a diminished thermic response to refeeding (77). Hyperphagic cafeteria-fed rats dissipate some of the extra ingested energy through an enhanced thermic response to feeding (77). Thus, adjustments in the metabolic response to feeding appear to contribute to the control of energy balance. Normal, lean animals partially correct for errors...
in energy intake by appropriately adjusting the thermic response to a meal. Obese animals fail to make these corrective adjustments, and favor energy storage over expenditure.

These differences in acute metabolic responses to feeding in normal and obese humans and animals parallel the general diet-induced thermogenesis patterns as described previously, and thus lead to the suggestion that deficits in the obese animal’s metabolic response to feeding are a result of decreased brown adipose tissue activity. Indeed, work in this area has shown that although the acute metabolic response to feeding is in part due to the energy costs of nutrient digestion and assimilation, brown adipose tissue thermogenesis accounts for a significant portion of it (24). Furthermore, decreased brown adipose tissue activity as measured by interscapular brown adipose tissue temperature has been shown to be associated with a decreased metabolic response to feeding in obese Zucker rats (80). The thermic effect of a meal thus appears to represent a significant component of diet-induced thermogenesis.

Given the potential importance of the role of the thermic effect of feeding in the regulation of energy balance, it is reasonable to investigate whether obesity
resulting from hypothalamic damage may be partially attributable to a suppression of this response. Therefore, oxygen consumption following the ingestion of a meal was measured. Since it is possible that the obese state itself may alter this response, the measurement of the thermic response was made on food-restricted knife-cut animals as well as on ad libitum feeding obese knife-cut animals.

In addition to measuring the protein and DNA content of interscapular brown adipose tissue, estimation of brown adipose tissue mitochondrial activity was obtained by assaying the tissue for the activity of the respiratory enzyme, cytochrome c oxidase. The combined weight of the right retroperitoneal and parametrial white fat pads was used as a measure of relative adiposity.

Procedure

To prevent hyperphagia and consequent obesity, knife-cut animals were pair-fed with controls. It was anticipated that pair-fed knife-cut rats would eat their daily ration in a much shorter time period than the controls. Since differences in meal patterning can lead to differences in fuel metabolism (43) and metabolic efficiency (15,18), access to food in all groups was limited to 6 hours per
day. Adaptation to the scheduled feeding was continued until intakes and body weights were stabilized.

Fifty animals were adapted to a 6-hour daily feeding schedule. An unrestricted supply of chow (Purina Rodent Chow 5001) pellets was available between 12 noon and 6 pm; water was freely available throughout the entire day. Room lights were on from 12 midnight to 12 noon. Food intake and body weight were measured every third day. After intakes on the scheduled feeding were stabilized, the rats were assigned to six experimental groups as described for Experiment 1. Rats scheduled for knife cuts were paired with sham controls in the corresponding diet group, matched for body weight and food intake. The sham member of each pair received surgery one day before the cut member so that the sham's previous day's ad libitum intake during the 6-hour feeding period could be subsequently allotted to the knife-cut animal. Food was withheld overnight after surgery, and schedule feeding of chow was resumed the day after surgery. The sucrose and fat diets were introduced to the appropriate groups the following day.

After all sham groups had achieved a constant daily food intake (20-24 days after surgery), the thermic effect of a meal was assessed. Since a complete overnight fast has been shown to abolish the thermic response to a meal
presumably because fasted animals store incoming energy - 1/4 of the daily meal was provided at the usual meal time (i.e., 12 noon) and 1/4 given again 4 hours before testing and removed 2 hours before testing. At this time a 3 g chow (Purina Rodent Chow 5001) pellet was offered in the home cage, 30 minutes was allowed for ingestion, and then oxygen consumption measured for the following 2 hours. Testing was conducted during the light portion of the light:dark cycle between 1 am and 10 am.

A 2-hour test period required that group size be limited to 4 rats in this phase of the experiment. In order to increase the likelihood of including successful knife-cut animals in this phase of the experiment, the 4 rats within each cut group which had the greatest ad libitum food intake on the first post operative day and their sham pairmates were chosen.

Following testing of the thermic response to food, all animals were allowed unrestricted access to their respective diets and the light:dark cycle was shifted so that lights were on from 8 am to 8 pm. Cumulative food intakes and body weights were measured twice weekly. After 9 weeks of unrestricted feeding, the thermic effect of a meal was again measured in the same animals tested previously. Although food was partially restricted before
testing (1/4 of the previous 3 or 4 days' average daily intake was provided at 8 am on the day prior to testing, and another 1/4 was available between 4 and 2 hours before testing), most animals did not consume their 3 g chow pellet within 30 minutes of presentation. Therefore, a 1:1 wet mash (1 part water to 1 part powdered chow) adulterated with 0.1% saccharin was offered and found to be readily consumed. All animals to be tested were allowed 24-hour access to the saccharin-flavored wet mash 1 week prior to testing in order to avoid any neophobia-induced inhibition of ingestion of the test meal. At the time of testing 6.16 g of the test meal (containing 3 g powdered chow) was given in the home cage and oxygen consumption was measured for 2 hours beginning 30 minutes after introduction of the test meal. Testing was conducted during the light portion of the light:dark cycle, between 10 am and 7 pm.

Animals were decapitated within 2 weeks after testing and the brain, right retroperitoneal and parametrial white fat, and interscapular brown adipose tissue (IBAT) removed. White fat pads were weighed and discarded, and IBAT was weighed and prepared for the measurement of protein and DNA content. IBAT mitochondrial activity was also measured using the assay for cytochrome c oxidase.
Results

Histology

Several animals' data were eliminated from the final analyses as a result of misplaced knife-cuts. Group sizes were as follows: Chow-Sham, n=8; Sucrose-Sham, n=8; Fat-Sham, n=8; Chow-Cut, n=5; Sucrose-Cut, n=9; Fat-Cut, n=8.

Body weight and food intake: scheduled pair feeding

Body weight and food intake data for the scheduled pair feeding phase of Experiment 2 are presented in Figs. 8-10. After day 1, the cut groups all weighed more than the sham groups, largely owing to the one day of ad libitum feeding allowed during the first day after surgery. Across the 25 days after surgery there were no significant effects of diet on body weight within each of the two surgery conditions, although the tendency for the sucrose-fed group to be heaviest was present in both surgery conditions.

Body weight gain (Fig. 9) was initially greater for the cut groups as compared to each of their sham controls, but this gap diminished with time so that by day 13 there were no significant differences among any of the groups. Food intake (Fig. 9) was greatest in the sucrose-fed groups throughout the period of scheduled feeding. Pair feeding
Figure 8. Mean ± S.E.M. body weights of adult female rats in Experiment 2 which received bilateral parasagittal hypothalamic knife cuts or sham surgery. Access to food was restricted to 6 hrs per 24 hrs, beginning at lights off. Knife-cut rats were pair-fed to sham controls within each diet condition. The diets are the same as those described in Figure 1 for Experiment 1. Group sizes are indicated in parentheses after symbol identification.
Figure 9. Mean body weight change, food intake, and feed efficiency of rats described in Figure 8. Each point represents 3-day totals for the day indicated and the 2 preceding days.
was generally successful; that is, intakes were not significantly different between cut and sham groups within each diet condition except on day 4 when the intake of the Sucrose-Cut group exceeded that of the sham sucrose-fed group. The cumulative effect of this initial overfeeding, however, was not significant (see Fig. 10). Spillages were difficult to anticipate and, consequently, differences in food intakes of paired animals did occur. Feed efficiencies were higher in the knife-cut groups 4 days after surgery, but this was significant in the sucrose condition only. Throughout the remaining period of scheduled feeding there were no notable significant differences in feed efficiency.

Cumulative measures depicted in Fig. 10 reveal an overall greater weight gain in the cut groups which was significant in the sucrose-fed group only. Cumulative food intakes were greatest in the sucrose-fed animals, as was the case with 3-day intakes (plotted in Fig. 9). Sucrose- and chow-fed knife-cut animals displayed a greater cumulative feed efficiency than their respective controls over most of the 25 days after surgery. This significant difference appears to have been partially sustained by a greatly enhanced efficiency present at day 4, since feed efficiencies calculated for days 22-25 only (Fig. 9) were
Figure 10. Mean cumulative body weight change, food intake, and feed efficiency of rats described in Figure 8.
not significantly different between the surgical treatments within each diet condition. Fat-fed knife-cut animals had significantly greater cumulative feed efficiencies than their sham controls on days 13-22.

Body weight and food intake: ad libitum feeding

Body weight and food intake measures for the period of ad libitum feeding which followed the scheduled pair feeding are presented in Figs. 11-13. The significant difference in body weight between cut and sham animals which was present after 25 days of scheduled pair-feeding (Day 0, Fig. 11) was enhanced throughout the period of ad libitum feeding (Fig. 11). At the time of sacrifice (days 76-79), the sucrose- and fat-fed groups within each surgery condition were heavier than the chow-fed group, but this difference was significant between the sucrose- and chow-fed sham groups only.

Three- or four-day total body weight gain and food intake through day 63 of ad libitum feeding are presented in Fig. 12 along with corresponding feed efficiency. Body weight gain was greatest at the onset of ad libitum feeding, and decreased dramatically by day 8. Over the entire course of this phase of the experiment the decrease in body weight gain was greater in cut animals so that by
Figure 11. Mean ± S.E.M. body weight of rats described in Figure 8. *Ad libitum* feeding followed 25 to 28 days of scheduled pair feeding. Rats were killed after 76 to 79 days of *ad libitum* feeding.
Figure 12. Mean body weight change, food intake, and feed efficiency of ad libitum feeding rats described in Figure 11. Each point represents 3- or 4-day totals for the day indicated and the preceding 2 or 3 days.
69 DAYS OF AD LIBITUM FEEDING

ΔBW (g)

FOOD INTAKE (Kcal)

FEED EFFICIENCY (ΔBW/Kcal)

DAYS OF AD LIBITUM FEEDING

○ Chow-Sham (8)
▲ Sucrose-Sham (8)
□ Fat-Sham (8)
● Chow-Cut (5)
△ Sucrose-Cut (9)
■ Fat-Cut (8)
day 33 only the sucrose-fed group was gaining weight at a faster rate than its sham control group. On day 63 no differences in body weight gain were present. Knife-cut groups maintained greater food intakes than each of their respective sham groups throughout the recorded 63 days of ad libitum feeding. Feed efficiencies were initially higher in all of the knife-cut groups (day 5); but this general distinction did not persist as feed efficiencies in the knife-cut groups decreased more over time than did those of the sham groups. However, within each diet condition, knife-cut animals more often than not had higher feed efficiencies than their sham counterparts through day 33.

Figure 13 contains cumulative measures of body weight gain, food intake, and feed efficiency. Cumulative weight gains were greatest in the sucrose- and fat-fed cut groups and this difference increased over days. The Chow-Cut group paralleled the other two cut groups in weight gain through day 12, after which its rate of gain decreased so that it fell intermediate to the other two cut groups and the three sham groups in cumulative weight gain. The decrease in rate of weight gain after day 22 in the cut groups is nicely illustrated by the cumulative measure of change in body weight. The initial greater food intake in
Figure 13. Mean cumulative body weight change, food intake, and feed efficiency of rats described in Figure 11.
the knife-cut groups as compared to the shams was enhanced throughout the 36 days of data collection, thus reflecting the consistently greater intake by the cut groups (as seen in Fig. 12). Cumulative food intake was not differentially affected by diet in the two surgery conditions. Chow- and fat-fed groups' intakes within each surgery condition did not differ and were both significantly less than that of the sucrose-fed groups' intakes. Cumulative feed efficiencies of the cut groups were generally higher than those of the sham groups, but this difference did not achieve significance until day 22. However, the fat-fed cut group maintained a higher cumulative feed efficiency than its sham control group throughout the 36 days of continuous data collection.

**Thermic effect of feeding**

The thermogenic responses to a meal during both scheduled pair feeding and *ad libitum* feeding are shown in Fig. 14. Owing to the high degree of variability in this measure and the very small sample size, apparent differences were largely statistically nonsignificant. A repeated measures (restricted vs. *ad libitum* feeding) ANOVA revealed a significant overall effect of diet and a significant feeding (restricted vs. *ad libitum*) by surgery
Figure 14. Mean ± S.E.M. oxygen consumption following ingestion of a meal in a subgroup of rats described in Figures 8 and 11. Oxygen consumption was measured during the light portion of the light:dark cycle. Each mean represents the average amount of oxygen consumed per min over a 2-hr postprandial period. Group sizes are indicated at the base of each bar. The same rats were tested first under conditions of scheduled pair feeding (20-24 days after surgery), and then again after 9 wks of ad libitum feeding.
interaction. During restricted feeding the sham group within each diet condition consumed more oxygen than the cut group, but the Duncan Multiple Range Test revealed no significant pairwise comparisons within each diet condition. However, it is noteworthy that between diet conditions, both the sham and cut sucrose-fed groups had significantly greater thermic responses to food than their respective chow-fed control groups. During the period of ad libitum feeding there were no significant differences between any two groups. Comparing each group across restricted and ad libitum feeding revealed a significant increase in the thermic response to a meal in the ad libitum feeding Chow-Cut group only. After 9 weeks of ad libitum feeding the general trend observed during restricted feeding for sham groups to consume more oxygen following a meal than cut groups had disappeared.

**White adipose tissue**

Figure 15 contains white fat pad weights and the relative contribution of the measured fat pad weights to total body weight. Pad weights were significantly increased by knife-cut surgery, but there was no main effect of diet. However, within the sham groups pairwise comparisons revealed that fat pads of the chow-fed group
Figure 15. Mean ± S.E.M. wet weight of the combined right parametrial and retroperitoneal white fat pads and the combined weight as percent body weight of rats described in Figure 11.
weighed significantly less than those of the sucrose-fed group. When expressed relative to body weight, there was a significant main effect of surgery and a significant surgery by diet interaction. Across all diet conditions the knife-cut animals had greater relative adiposity, but this was not significant in the sucrose condition. Within the sham treatment groups, both the sucrose and fat diets produced significantly greater relative adiposity. This distinction was not present within the cut groups where there were no differences between groups.

Brown adipose tissue

Absolute and relative IBAT pad weights are presented in Fig. 16. Knife-cut animals had significantly heavier fat pads in all three diet conditions. IBAT pads of sucrose- and fat-fed groups in both surgery conditions were heavier than those in chow-fed groups, with sucrose-fed groups having the heaviest pads overall. However, the greater pad weight of the sucrose-fed sham group as compared to that of the fat-fed sham group was not significant. Dietary and surgical treatments both resulted in significant main effects on IBAT mass as percent body weight. IBAT pads of knife-cut groups represented a greater percentage of body weight than those of sham
Figure 16. Mean ± S.E.M. IBAT pad wet weight and pad weight as percent body weight of rats described in Figure 11.
groups, but this was not significant within the chow diet condition. Sucrose- and fat-fed groups in both diet conditions had greater relative IBAT mass than chow-fed groups, but the difference between the Fat-Sham and Chow-Sham groups was not significant. Sucrose feeding resulted in greater relative IBAT mass than fat feeding regardless of surgical treatment.

IBAT protein and DNA contents are shown in Fig. 17. Total protein was significantly affected by dietary, but not by surgical, treatment. Sucrose feeding resulted in significantly greater IBAT protein when compared with chow-fed groups in both surgery conditions; but when compared with fat-fed groups, only the sucrose-fed cut groups had significantly greater total protein content. IBAT protein content per mg tissue was significantly decreased by knife-cut surgery in all diet conditions. Although neither diet nor surgery by diet effects were significant, there was significantly less protein per mg tissue in the Sucrose-Sham group as compared to the other two sham groups.

Analysis of IBAT DNA content revealed a significant main effect of surgery only. However, the only significant difference contributing to this main effect was the greater IBAT DNA content in the Fat-Cut group as compared to the Fat-Sham group. Although the sucrose- and fat-fed knife-
Figure 17. Mean ± S.E.M. protein and DNA content of IBAT of rats described in Figure 11. Total protein and DNA contents of the pad are shown in the upper panels. Lower panels show concentration of protein and DNA per mg tissue.
cut groups had greater DNA content than their chow-fed control group (a trend not seen in the sham groups), these differences were not significant. IBAT DNA concentration was significantly affected by both surgical and dietary treatments. In addition, there was a significant interaction of the two treatments on DNA concentrations. DNA concentration was decreased by knife-cut surgery in all diet conditions, but this was not significant in the sucrose-fed groups. In sham animals, both the sucrose and fat diets produced significantly lower IBAT DNA concentrations than the chow diet. Furthermore, relative DNA content in IBAT of the Sucrose-Sham group was significantly less than that of the Fat-Sham group. Although this general trend for the chow-fed group to have greater DNA concentration than the fat-fed group, which was greater than that of the sucrose-fed group, was present in the knife-cut animals, the differences were very small and nonsignificant.

Total cytochrome c oxidase activity per IBAT pad (Fig. 18) was significantly affected by surgical treatment, but not by diet nor by a significant interaction of the two treatments. Although the knife-cut animals in the chow and fat diet conditions had less enzyme activity than their sham controls, post hoc comparisons were not significant.
Figure 18. Mean ± S.E.M. IBAT cytochrome c oxidase activity of rats described in Figure 11. Total enzyme activity of the IBAT pad is shown in the upper panel. The lower panel shows enzyme activity per mg tissue.
However, significantly less enzyme activity was found in the fat-fed cut group when compared with the sucrose-fed cut group, which was not the case for the corresponding sham groups. There were significant main effects of both surgery and diet (P = .052) on specific activity of the enzyme (i.e., activity per mg IBAT protein). Specific activity was depressed in knife-cut animals; however, this effect was not significant when pairs were compared within each diet condition. Sucrose feeding reduced specific enzyme activity compared to chow feeding in the sham animals, but not in the cut animals. The lowered specific activity in the fat-fed vs. chow-fed animals was not significant in either surgery condition.

Discussion

Pair feeding for 25 days after surgery revealed no consistent effects of surgery or diet on efficiency of weight gain (Figs. 9 and 10). Knife-cut surgery resulted in an elevated cumulative feed efficiency, but this was largely due to greater intakes on the day following surgery when ad libitum feeding was allowed. The knife-cut-induced increase in feed efficiency did not persist beyond four days after surgery (Fig. 9). Feed efficiency was not
altered by the greater caloric intake of the sucrose-fed groups as weight gains were proportional to those exhibited by the other diet groups. The caloric intakes of the sucrose groups in this experiment were lower than those of the sham sucrose-fed group in Experiment 1 (compare Figs. 2 and 9) and may have been insufficient to stimulate thermogenesis. In contrast to the findings of Experiment 1, fat-fed knife-cut rats did not exhibit an enhanced cumulative feed efficiency (compare Figs. 3 and 10). Thus, although knife-cut rats have normal feed efficiency when hyperphagia on a higher fat diet is prevented, feed efficiency is increased when they are hyperphagic.

Regardless of surgical treatment, both the sucrose and fat diets increased postprandial oxygen consumption during restricted feeding. However, the thermic effect of a meal was significantly lower in knife-cut rats (Fig. 14). Thus, pair-fed knife-cut rats resemble food-restricted animals in their more conservative utilization of ingested energy (1, 9, 19, 54, 59, 69, 109). Whether this might be a result of food restriction-induced carcass protein losses as was found in pair-weighted VMH-lesioned rats (106) is unanswerable. Unfortunately, separate groups of animals were not tested in restricted and ad libitum feeding situations. Consequently, terminal measures of white and
brown fat were not obtained in pair-fed animals. Based on the results obtained, however, it is possible to conclude that although the adaptive thermogenic response to dietary sucrose and fat was retained by food-restricted knife-cut rats, the thermic response to food is diminished when compared with ad libitum feeding sham controls.

Allowing unrestricted access to food after the period of scheduled pair feeding resulted in differences in food intake and body weight gain between the treatment groups (Figs. 12 and 13) which differed slightly from those seen in Experiment 1 (Figs. 2 and 3). Unlike in Experiment 1, knife-cut fat-fed animals consistently ate fewer calories than the sucrose-fed knife-cut group. However, body weight gain was similar in the two groups. Overall, this again results in a greater efficiency of weight gain in the fat-fed group. This increased feed efficiency was intermittent, however, and the cumulative measure was not always significantly elevated. Another way of viewing this difference between the sucrose- and fat-fed cut groups is to focus on the relatively decreased efficiency of weight gain in the sucrose-fed group. Thus it appears as though the Sucrose-Cut group successfully expended calories ingested in excess of those taken in by the Fat-Cut group. These dissimilarities in the relationship of food intake to
body weight are not always supported by similar differences in the feed efficiency ratio and may be symptomatic of an inadequacy of the popularly used measure. Hill, et al. (37) have recently failed to show a positive correlation between feed efficiency and other measures of energy expenditure such as resting metabolic rate, postprandial metabolic rate, and in vitro brown adipose tissue oxygen consumption. Furthermore, they reported a great deal of individual variability in the capacity for adaptive thermogenesis even among animals of the same strain, age, and sex.

Oxygen consumption after ingestion of a meal during ad libitum feeding was unaffected by diet or surgery treatments (Fig. 14). It appears that the knife-cut rats were able to recover from the food restriction-induced suppression of postprandial thermogenesis. By the time testing was performed, food intakes of the knife-cut groups were still significantly greater than their sham control groups' intakes (see Fig. 12). Since the animals were overeating, increases in the thermic response to a meal would have been expected. Thus, as in Experiment 1, defective adaptive diet-induced thermogenesis is indicated in hyperphagic knife-cut rats.

Interscapular brown fat pad weight was significantly
increased in the knife-cut animals (Fig. 16). In addition, total DNA content was elevated by knife-cut surgery in both the sucrose- and fat-fed groups (Fig. 17). This is in contrast to the findings of Experiment 1 where increased brown fat pad wet weight could be attributed to lipid filling (Figs. 6 and 7). The increases in DNA observed in Experiment 2 may indicate growth of active tissue. However, the increase in tissue DNA was not accompanied by a parallel increase in diet-induced thermogenesis as measured by postprandial oxygen consumption. Also, total cytochrome c oxidase activity, a mitochondrial marker and index of brown adipose tissue thermogenic activity, indicates that thermogenesis was suppressed by knife-cut surgery in the chow and fat diet conditions (Fig. 18).

Disagreement among the various endpoints casts doubt upon the validity of the measures used and makes it difficult to draw meaningful conclusions. However, it is worthwhile to summarize the general trends observed. Energy expenditure was decreased in pair-fed knife-cut animals. Although feed efficiencies were not consistently decreased, the thermic response to feeding was significantly reduced by knife-cut surgery. Energy expenditure and efficiency of energy utilization were not affected by the diet offered in pair-fed and control rats.
Hyperphagic knife-cut rats appear to lack the diet-induced thermogenic response to overeating which intact hyperphagic rats exhibit (74,78). Postprandial oxygen consumption did not increase as would be expected in the hyperphagic knife-cut rats. In addition, activity of the brown adipose tissue respiratory enzyme cytochrome c oxidase was suppressed or unchanged by knife-cut surgery. An increase in activity of this enzyme would be expected in brown fat of hyperphagic rats as it is an index of thermogenic activity of the tissue. Taken together, these results are suggestive of a lowered level of energy expenditure in knife-cut rats which is apparent during both restricted and ad libitum feeding.
CHAPTER V

SUMMARY

Several investigators have suggested that defective diet-induced brown adipose tissue thermogenesis may contribute to hypothalamic obesity (16, 41, 74, 88). This study was conducted to test further this hypothesis in rats made obese by parasagittal knife-cuts alongside the paraventricular nucleus. Since diet can influence energy intake, utilization, and expenditure, rats in this study were fed diets containing differing amounts of protein, carbohydrate, and fat in order to address more completely the question of whether deficits in diet-induced thermogenesis may play a role in the obesity induced by PVN knife-cuts. In addition, hyperphagia in some knife-cut rats was prevented by restricting their intake to that of sham controls. Energy balance was estimated by recording of body weight and food intake and the computation of feed efficiency. Resting and postprandial oxygen consumption were measured, and brown fat assayed for indices of tissue growth and thermogenic activity. Although the results were not clearcut and in some cases were conflicting, they did offer support for the suggestion that hypothalamic knife-cut rats may have diminished diet-induced thermogenesis.
Several problems with the study should be noted. Treatment effects were often obscured by variability. The current knowledge about how variations in dietary protein can cause changes in energy utilization suggests the need for control of the protein component in the diets used in this study. Since carcass protein content has been shown to correlate with resting metabolic rate, carcass analysis would have allowed for more confident interpretations of the results. It is important to realize that although the feed efficiency ratio provides an index of efficiency of weight gain, it does not indicate how ingested calories are stored (e.g., in fat or lean body mass) or by what route(s) they are expended (e.g., activity, basal metabolism, cold- or diet-induced nonshivering thermogenesis, or fecal and urinary losses). As mentioned previously, terminal measures in the food-restricted knife-cut rats would have been helpful. Finally, more direct assessment of the effects of PVN knife-cuts on energy balance in ad libitum feeding rats would have been possible with the use of hyperphagic sham control rats.
REFERENCES


12. Burton, K. A study of conditions and mechanisms of the diphenylamine reaction for the colorimetric


19. Forsum, E., P.E. Hillman and M.G. Nesheim. Effect of energy restriction on total heat production, basal


24. Glick, Z., R.J. Teague and G.A. Bray. Brown adipose tissue; increased by a single low protein, high


64. Miller, D.S. and P. Mumford. Gluttony. 1. An


77. Rothwell, N.J., M.E. Saville and M.J. Stock. Factors influencing the acute effect of food on oxygen con-


89. Shetty, P.S., R.T. Jung, W.P.T. James, M.A.


103. Trayhurn, P., P.M. Jones, M.M. McGuckin and A.E.


