AN INVESTIGATION OF DIET-INDUCED THERMOGENESIS DURING OVERFEEDING, THERMOGENIC RESPONSIVENESS TO EPHEDRINE ADMINISTRATION, AND DIET-INDUCED THERMOGENESIS FOLLOWING PROPRANOLOL ADMINISTRATION

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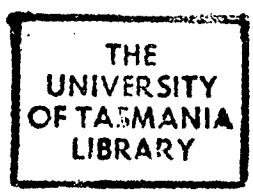

ABSTRACT

The current obesity literature places strong emphasis upon the influence that Diet-Induced Thermogenesis (DIT) has on weight regulation and the possible mediating role of Brown Adipose Tissue (BAT) in DIT. This focus arises from animal research which establishes the significance of DIT and BAT in the energy balance of rodents.

Recent studies indicate differential postprandial DIT between lean and obese individuals. The contribution of resting state DIT to energy balance is equivocal, a situation in part due to disagreement regarding the extent of excess energy needed to trigger the response, and individual differences in responding. At present, only catecholamine stimulation studies suggest the active presence of BAT in adults.

This thesis addresses four research questions arising from the obesity literature: (i) Does the DIT response to overfeeding in either postprandial or resting state conditions show any change over five days overfeeding? (ii) Is a subject's responsiveness to a standard test of thermogenic capacity (i.e. stimulation by the sympatheticomimetic drug ephedrine) predictive of their DIT response to overfeeding? (iii) Is BAT involved in mediating the DIT response during the postprandial period? and (iv) Can the DIT response to overfeeding be blocked by the beta-adrenergic antagonist propranolol? These questions were investigated in two studies. Experiment 1, testing seven subjects, examined questions (i) to (iii), Experiment 2 examined question (iv) with the three strongest responders from the first experiment. DIT and response to sympathetic stimulation were measured through metabolic rate

(O_2 consumption and HR) increase. BAT activity was assessed via skin temperature change at sites of probable BAT deposits.

The results suggest that in certain individuals resting state DIT can be achieved following a single day's overfeeding. The data also suggest that dietary history may underpin DIT response ability. As only a positive trend was found between MR stimulated by ephedrine and overfeeding respectively, the relationship between these responses remains equivocal. The BAT hypothesis was challenged by a failure to observe skin temperature increase following both ephedrine administration and overfeeding, and the failure of propanolol to inhibit DIT. The results are interpreted as clarifying the occurrence of resting state DIT in respect to short term overfeeding, and calling into question speculation regarding BAT's role in thermogenesis.

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"Every case of obesity is the end product of some form of energy imbalance. For the individual, too much food has been put in - or too little energy expenditure has been incurred"

(Wallace, 1980).

CHAPTER 1 INTRODUCTION

Obesity is not a unitary disorder, as the term may be taken to imply, rather, it arises from idiopathic combinations of metabolic, endocrine, psychological and genetic factors (James, 1976).

This thesis investigates human metabolic responses to overeating, the sympatheticomimetic drug ephedrine, and overeating during beta-adrenergic blockage with propranolol. The research is addressed within the context of examining metabolic correlates to obesity.

1.1 THERMOGENESIS

Thermogenesis can be defined as an increase in resting metabolic rate (RMR) due to physical stimuli (e.g. food, cold or drug infusion) or psychological states (e.g. fear, following Garrow, 1981). Two thermic responses characterize homeotherms: diet-induced thermogenesis (DIT), the thermic effect of food consumption, and thermoregulatory or cold-induced thermogenesis.

DIT can be separated into two conceptual components: specific dynamic action (SDA), the absorbative phase increase in metabolic rate (MR), and luxusconsumption, a postulated additional thermic response occurring during the resting state.

Thermoregulatory thermogenesis is made up of two independent components: shivering, a muscular reaction usually accompanying exposure to sudden cold, and non-shivering thermogenesis (NST), a more subtle capacity

for heat production which is routinely assessed by measuring the rise in RMR following norephinephrine infusion at thermoneutrality (Jansky, 1973).

The research reported herein examines DIT responding. As will be discussed in the following section, varying conceptualizations of SDA and luxusconsumption exist in the literature. Because of this situation an often employed convention will be adopted to describe the two DIT components.

It should be appreciated that metabolic efficiency in catabolizing ingested nutrient is reflected by the extent of energy liberated as heat (DIT) and the extent directed to bodily functions or storage. The animal literature has established that thermogenesis plays a significant role in the energy balance and hence body weight of certain rodents, and that brown adipose tissue (BAT) underpins capacity for thermogenic responding (Foster & Frydman, 1978a, 1978b; Himms-Hagen & Desautels, 1978; Himms-Hagen, Trianadillou & Gwillian, 1981). This research forms the empirical basis for two distinct lines of speculation regarding human functioning, viz: (i) that thermogenesis is a significant factor in human energy balance, and (ii) that BAT may be the major metabolic operative of thermogenesis in humans.

1.2.1 DIET-INDUCED THERMOGENESIS: SPECIFIC DYNAMIC ACTION

Although the biochemical/physiological mechanisms responsible for the effect are unclear, most authorities state that SDA represents wasted free energy which accompanies metabolism of nutrient (Mitchell, 1964: Jensen, 1976). For example, the hexokinase reaction which allows glucose to be metabolised is thermodynamically inefficient and involves

the liberation of approximately 5,500 calories per mole in the conversion of ATP and ADP (Hoar, 1975). High protein foods result in the largest increment in heat production (up to 30% of caloric value ingested), followed by fats (approximately 8%), and carbohydrates (approximately 5%, Jensen, 1976). However, in a mixed nutrient meal each foodstuff exerts a depressant effect upon the SDA of the others. A general rule-of-thumb is to expect approximately a 10% total increase in RMR over the postprandial period following a mixed nutrient meal supplying 1000Kcal (Garrow, 1978).

Recently a new conceptualization of SDA has emerged. Glick, Teague and Bray (1981) observed an increased respiratory rate in the BAT of rats following consumption of a single meal, thus leading them to suggest that BAT activity mediates SDA. These researchers further speculate that luxusconsumption is simply the summation of heightened individual SDA's during prolonged overeating. It should be appreciated, however, that the animals in Glick et al's (1981) study had been overfed on a palatable cafeteria diet for two weeks prior to the test meal, which itself was a cafeteria variety. As will be discussed in Chapter 2, it is not surprising that these rats demonstrated a considerable BAT mediated thermic response to a large meal (i.e. Chapter 2, Section 2.1). To provide convincing evidence of BAT's role in SDA, thermogenesis mediated by the organ must be shown to follow a normal sized meal.

1.2.2 DIET-INDUCED THERMOGENESIS: LUXUSCONSUMPTION

It appears that the term was first proposed by Newman in 1902 to describe a sustained increase in heat production, following the absorbative phase, to maintain an energy balance threatened by

excess food consumption (Sims, 1976). Opinions now differ as to how the response should be defined and if it in fact exists. Two areas of disputation will be examined, (i) the time period elapsing between overfeeding and the onset of the response, and (ii) the metabolic operative responsible for the response. Each area will be considered in turn.

Following a review of fifteen 'key studies' Garrow (1978) posited that the commonality factor shared between research which evidenced luxusconsumption is a 23Mcal or more energy overload. He therefore concluded that a threshold of overfeeding in that order must be exceeded before luxusconsumption occurs, and considers that several days of excess intake are required to bring in the response. This proposal has recently been challenged by Dauncey (1980) who observed a mean increase in RMR of 12%, 14 hours after a single days overfeeding regimen which increased subjects normal daily energy intake by approximately 60%. One clear finding in the literature is wide individual differences in resting state responding (e.g. Apfelbaum, Bostsarron & Lacatis, 1971; Norgan & Durin, 1980; Dauncey, 1980, see Chapter 3, Section 3.2.2) and it is reasonable to suggest that this situation has in part fuelled disagreement regarding the period-to-onset or time course question.

In respect to the metabolic operative underpinning human thermogenesis, speculation centering on BAT has largely superseded earlier theroies concerning trans-cellular ionic pumping (Sims, 1976; Reference Note 1) or futile cycling in muscle tissue (James & Trayhurn, 1976; Newsholme, 1980). While histological surveys have established that BAT is present in adult humans (Heaton, 1972),

evidence regarding its influence on energy balance is tenuous, resting singly upon catecholamine stimulation studies (i.e. Rothwell & Stock, 1979; James & Trayhurn, 1981a).

The Cambridge group of researches (i.e. James, Dauncey, Jung, Shetty & Trayhurn, 1979) suggest that luxusconsumption is a variable component in the SDA response which can be maintained during the resting state (see Figure 1). Consistant with an earlier proposal by Miller (1975), the response is seen to depend upon the food composition of a meal - specifically, the fat content. Most importantly, this adaptive component of DIT is not posited to be simply indexed to fat content, rather, it is suggested to depend on BAT responsiveness to fat influx (James & Trayhurn, 1981a). Thus, a link is proposed between dietary fat intake, genetically predetermined BAT responsiveness, and propensity to obesity. Two lines of evidence have been presented to support this position, however, both pertain solely to a luxusconsumption response during the absorbative phase (i.e., James & Trayhurn, 1981b; Zed & James, 1982). A recent second proposal concerning the role of BAT in DIT is presented by Cawthorne (1982) following examination of animal data provided by Rothwell & Stock (1981). In pointing out a stratification of oxygen utilization attributable to BAT during norephinephrine infusion and in RMR conditions, he suggests that while consumption of a large meal provides the stimulus for high level BAT thermogenesis, futile cycling in skeletal muscle may mediate most DIT in the resting state.

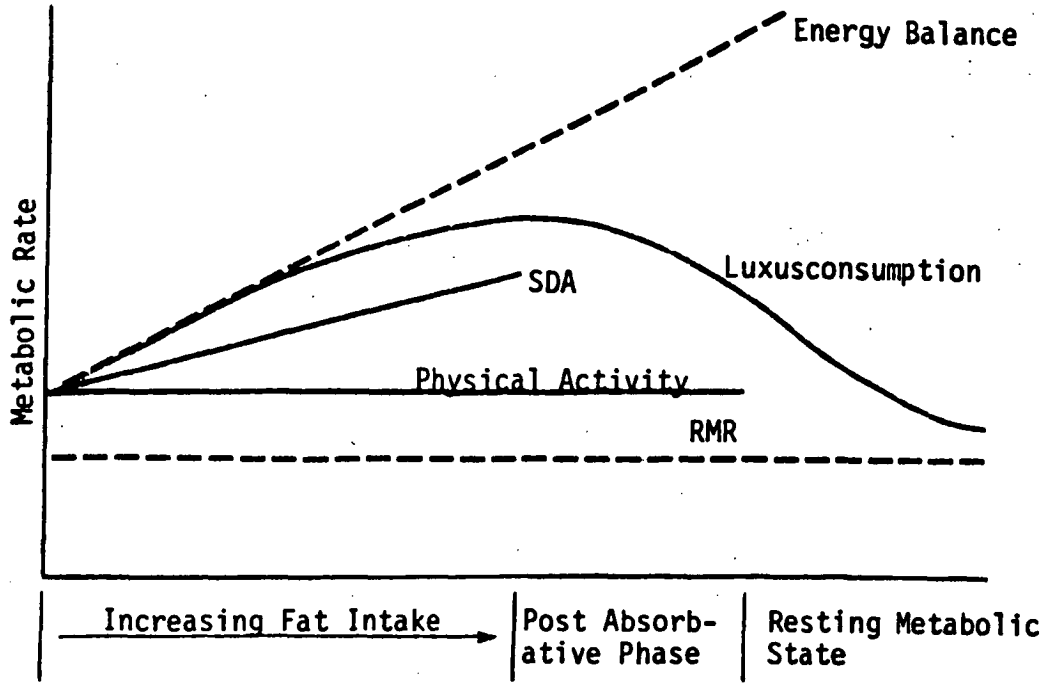

Figure 1. The Cambridge model of diet-induced thermogenesis; an SDA response limited to the absorbative phase and a luxusconsumption response which can be maintained in the resting state.

1.2.3 DIET-INDUCED THERMOGENESIS: DEFINITIONAL CONVENTION

As discussed above, the literature is inconclusive regarding the period-to-onset of a luxusconsumption response. Further, in respect to models which posit an immediate luxusconsumption response during the absorbative phase (i.e. Miller, 1975; James et al, 1979), it is not possible using current empirical techniques appropriate for use with humans to distinguish the relative increase in MR attributable to either SDA or luxusconsumption. In view of this situation, this thesis will follow the convention adopted by many researchers (e.g. Shetty, Jung & James, 1979; James & Trayhurn, 1981a; Morgan, York, Wasilewska & Portman, 1982) and simply label thermogenesis occuring either following a meal or during RMR conditions as postprandial or resting state DIT respectively.

1.3 BROWN ADIPOSE TISSUE AND WEIGHT REGULATION

As indicated, although theories of BAT's actual role in DIT differ, models of its influence on energy balance are all somewhat similar with possibly the most developed model being that proposed by the Cambridge group. Because of its development, this model will be briefly summarized to indicate how metabolic processes are seen to effect weight regulation.

The central tenent of the Cambridge model is that obesity-prone individuals have a genetically predisposed subnormal thermogenic response to food and other stimuli (e.g. caffeine), though a normal RMR when corrected for body composition (James et al, 1979). This subnormal response ability arises from defective BAT functioning, the obesity-prone individual thereby being restricted in the wastage of excess energy as heat. Due to the failure of this homeostatic mechanism, body weight increases (both white fat and lean body mass) with a concomitant rise in MR (Tzankoff & Norris, 1977) until energy balance vis a vis dietary intake is reached (James, Davies, Bailes & Dauncey, 1978). The model has been consistently presented in an evolutionary context through speculations that an individual with a diminished thermogenic capacity (i.e. an efficient storer of energy) will be better equipped for survival in conditions of uncertain food supply but rendered metabolically susceptible to obesity when exposed to a palatable western diet (James & Trayhurn, 1976; 1981b).

1.4 THE AIM OF THE THESIS

With respect to the current data base and orientation of the obesity literature this thesis reports two experiments investigating the following issues:

Experiment 1

1. Recent data presented by Dauncey (1980) has challenged Garrow's (1978) conclusion regarding the time course of DIT with caloric overload. This issue will be addressed via the daily compilation of metabolic rate responding, during both postprandial and resting state conditions, to a five day overfeeding regimen.

2. Possibly the most consistent result in the literature is wide individual variability to caloric overload. It is apparent that this factor may be a significant hindrance to agreement on the time course of resting state DIT. Recent research by Morgan et al (1982) indicates some similarity between mean DIT responding and thermic response to a sympathetic comimetic drug (as assessed by changes in MR). This suggested concomitance between DIT and drug stimulation will be investigated with the intention of piloting a ready technique for determining individual thermogenic responsiveness.

3. As indicated, although considerable speculation is evident regarding BAT activity in humans, to date demonstrations of what is suggested to be BAT mediated thermogenesis has relied upon drug stimulation. In an attempt to further corroborate the role of BAT in DIT, skin temperature changes at probable BAT sites will be assessed during overfeeding and sympathetico-mimetic stimulation.

Experiment 2

1. It is established that BAT is thermogenically activated by the sympathetic nervous system (Schinazu & Tukahashi, 1980;

Perkins, Rothwell, Stock & Stone, 1981). As such, the relative thermogenic contribution of BAT can be strongly suggested through any decrease in DIT caused by sympathetic antagonist ingestion. An assessment of postprandial DIT during beta-adrenergic blockage will therefore be undertaken with strong thermogenic responders identified in the first experiment.

1.5 AN OVERVIEW OF THE INVESTIGATIVE STRATEGY OF THE THESIS

As the animal literature has been highly influential in generating speculation concerning both the effect of thermogenesis on human energy balance and BAT's role in the response, Chapter 2 will provide a review of the animal research and also brief discussion on the control and operation of BAT. Three lines of investigation pursued with humans are then examined in Chapter 3: (i) heritability studies, (ii) DIT research, and (iii) NST research. The evidence regarding the occurrence and activity of BAT in humans is reviewed in Chapter 4. The research questions to be investigated by this thesis in two experiments are stated at the conclusion of Chapters 3 and 4 respectively.

CHAPTER 2.

ANIMAL RESEARCH ON THERMOGENESIS AND BROWN ADIPOSE TISSUE

In terms of energy balance, obesity may be described as a state where energy intake has exceeded expenditure over a sustained period resulting in substantial excess energy stores, mainly in white fat. As such, the condition may arise from either an abnormally high energy intake, an unusually low energy expenditure, or some combination of both factors. Research has established that the latter scenario causes obesity in most genetically obese rodents.

2.1 DIET-INDUCED THERMOGENESIS AND ENERGY BALANCE IN NORMAL AND GENETICALLY OBESE RODENTS.

Genetically normal rats usually limit their intake of a standard laboratory diet to maintain a lean body state. However, as demonstrated by Rothwell and Stock (1979), when offered palatable food items in addition to a stock diet (i.e. cafeteria feeding) they will consume up to 80% more energy. Despite hyperphagia, weight gain in the Rothwell and Stock (1979) study was only in the order of 30% over a prolonged period when compared to normally fed litter mates. This ability to buffer excess energy intake is related to increases in DIT, with cafeteria fed animals evidencing a 20% to 30% rise in RMR. Rothwell and Stock (1979) observed that an increased RMR is sustained for approximately 3 days following withdrawal of the cafeteria diet even though the animals were hypophagic and remained so until a lean body state was again achieved.

Employment of a radio-actively labelled microsphere technique to measure regional blood flow has established that development of BAT deposits is responsible for heightened DIT in cafeteria fed animals

(Rothwell & Stock, 1981a; Brooks, Rothwell & Stock, 1982). Indeed, hyperphagia has been induced without weight gain due to increases in both the mass and cellular activity of the organ (Brooks, Rothwell, Stock, Goodbody & Trayhurn, 1980). It also appears that some rodent strains have a diminished capacity for DIT apparently due to thermogenically incompetent BAT (Rolls, Rowe & Turner, 1980; Brockway & Lobley, 1981). Further, it should be appreciated that small differences in genetic and/or environmental background within the same strain can profoundly influence DIT capacity (Rothwell & Stock, 1979).

Obese mutants appear unable to dissipate excess energy accrued through overeating via increased DIT. Trayhurn, Jones, McGuckin and Goodbody (1982) found that while cafeteria feeding will result in substantial extra overeating in already hyperphagic genetically obese ob/ob mice, no functional change in BAT is stimulated.

2.2 NON-SHIVERING THERMOGENESIS AND ENERGY BALANCE IN NORMAL AND GENETICALLY OBESE RODENTS

The ob/ob and db/db (diabetic) mouse will perish of hypothermia when exposed to a temperature of $4^{o}C$ whereas lean mice can survive indefinitely in this environment. The hypothermia of both mutants is due to defective NST. In normal temperature zones (20^{o} - $25^{o}C$) mutants display a metabolic rate approximately 20% lower than normal (Trayhurn & James, 1978). Furthermore, the distinct diurnal rhythm in body temperature shown by lean mice, though similar in both phase and amplitude, is lower at every point throughout the 24 hour cycle by approximately $2^{o}C$ in ob/ob and db/db mice (Trayhurn & James, 1980).

By reducing MR, and hence core temperature, obese mutants reduce demand for NST thereby diverting energy to be stored as fat (see Thurlby & Trayhurn, 1979, for caloric savings in obese mice across the normal temperature zone).

Utilizing the microsphere technique, Foster & Frydman (1978a & b) established that 60-65% of norephinephrine induced NST in lean rats, and a similar percentage for both cold-acclimatized and cold-exposed, warm-acclimatized animals, was attributable to BAT. Subnormal NST in ob/ob mutants is totally accounted for by defective BAT thermogenesis (Thurlby & Trayhurn, 1980). Nevertheless, as pointed out by Cawthorne (1982), the absolute capacity of the obese for NST is in excess of that required to maintain normal body temperatures in an environmental temperature zone between 20°C and 30°C. Cawthorne (1982) speculates that the inability to regulate body temperature may be due to either of two types of metabolic defect. Firstly, a failure of the thermogenesis initiation system to produce norephinephrine, or secondly, if norephinephrine is present, resistance to sub-maximal concentrations of the transmitter in the thermogenic system itself or with respect to vasodilatation in the microvascular bed of the BAT.

2.3 THE CONTROL AND OPERATION OF BROWN ADIPOSE TISSUE

The physiology of BAT is that of a biological furnace; numerous lipid globules containing high-energy fats surrounded by a multitude of mitochondria for the oxidative burning of fatty acids - the predominant substrate produced during the lipolysis of triglycerides (Nicholls, 1979). BAT typically has abundant beta-adrenergic innervation, with fibers forming a cocoon like network around each cell. It appears that this innervation originates mainly

from ventromedial hypothalmic nuclei, an area considered to be part of the sympathetic neural output from the hypothalamus (Shimazu & Takahashi, 1980). Electrical stimulation of the ventromedial hypothalamus produces a thermic response of similar magnitude and duration to that seen following norephinephrine infusion (Perkins, Rothwell, Stock & Stone, 1981). Further, the effect of electrical stimulation can be all but neutralized through administration of the beta-adrenergic antagonist propranolol. Sympathetic innervation of the organ also mediates dilation of BAT viscera to promote O_2/CO_2 exchange during thermogenesis (Nicholls, 1979). Although the organ may make up only 1% of body weight, in some animals it is capable of receiving up to one third of cardiac output while maintaining a high oxygen extraction rate (Himms-Hagen & Desautels, 1978).

As indicated above, it appears that the thermogenic capacity of BAT depends upon its preceding activity history. In normal animals, both cold-acclimatization and overfeeding enhances the response ability of BAT to sympahtetic stimulation. Rothwell and Stock (1979) observed that cafeteria fed rats were more sensitive to norephinephrine infusion over a dosage range of 5-40 mg per 100 g body weight. Further, at the lowest dosage level only the cafeteria animals exhibited a thermogenic response. These data suggest that BAT will provide its greatest contribution to homeostasis following a priming period.

Although a detailed description of BAT respirative functioning is not warranted in this thesis it should be appreciated that BAT metabolism is considered unqiue in its treatment of ATP, the basic energy source for all metabolic processes. As proposed by Nicholls

(1979), it appears that the inner mitchondrial membrane of BAT possesses a specialized proton conductance pathway which dissipates the proton gradient generated by respiration, thereby allowing prodigious respiration and hence heat production (for a detailed treatment of this process see Nicholls, 1979; or Cawthorne, 1982).

2.4 IMPLICATIONS FOR PRESENT RESEARCH

Animal investigations, conducted principally with rodents, strongly indicate that thermogenesis is a major factor in the energy balance of homeotherms. In mutant rodents predisposed to obesity, it is established that subnormal NST and DIT in large part contribute to a positive energy balance state. Conversely, genetically normal rodents are to an extent protected from obesity when hyperphagic by increased DIT. In both mutant and normal animals, BAT has been shown as the major metabolic operative of thermogenesis.

This evidence has prompted speculation regarding the influence of thermogenesis on human energy balance and the contribution of BAT to human thermogenesis. In order to highlight parallels between animal data and human responding, and so warrant further research in this area, it is necessary to consider four lines of human research. Firstly, because the animal literature has made extensive use of genetically obese mutants as a basis for speculation regarding a human predisposition to obesity, it is necessary to review the literature concerning constitutional metabolic differences between the lean and obese. A requirement closely allied to this point is the need to specifically discuss studies examining differential DIT ability between lean and obese individuals since models

which posit a metabolic predisposition to obesity at present place central emphasis upon subnormal DIT in the obese. As would be expected, literature investigating differential NST ability must also be reviewed. It should be apparent that, to be of clinical importance, differential thermogenic responding between the lean and obese must be of sufficient magnitude as to have a significant effect on weight regulation. A fourth area of literature requiring review are studies dealing with the occurrence of BAT in humans and its contribution to thermogenesis. Each of these research areas will be in turn discussed to build up a composite scheme of support for the research issues investigated by this thesis.

CHAPTER 3

HUMAN RESEARCH ON THERMOGENESIS

As stated at the conclusion of Chapter 2, four lines of research will be considered, these are: (i) heritability studies, (ii) DIT research, (iii) NST research, and (iv) literature pertaining to the occurrence and activity of BAT in man. As the first three areas of research centre upon thermogenesis vis a vis human energy balance they will each be considered in turn in this chapter. Chapter 4 will specifically examine the occurrence of BAT in man and its role in human thermogenesis.

3.1 HERITABILITY SUTDIES

To date, only one heritability study has been published which specifically examines genetic factors in relation to energy balance, i.e. Griffiths and Payne, 1976. Research investigating the heritability of obesity *per se* is available. However, these data do not bear upon thermogenic considerations and will not be reviewed.

The Griffiths and Payne (1976) study compared energy intake and expenditure in groups of equivalent body size 4 - 5 year old children distinguished according to their parents being either normal or overweight. Results indicate that children from obese parents expended 24% less kilocalories per kilogram body weight daily than their peers and had a 16% lower RMR per kilogram body weight. Of major interest is the finding that these standard weight for height children were maintaining energy balance by limiting themselves to 76% of the daily kilocalorie per kilogram intake of their peers. It is reasonable to conclude that familial pressure to consume a normal diet would render such individuals overweight.

Additional support for the contention that metabolic efficiency is genetically determined comes from DIT research by Shetty, Jung and James (1979) and a norephinephrine infusion study conducted by Jung, Shetty, James, Barrand and Callingham (1979). In both experiments obese subjects were selected with regard to a family history of obesity, the Jung et al (1979) study also including lean, post-obese individuals selected with respect to this criterion. The studies reveal a diminished thermic response ability in obese, and more importantly, lean post-obese subjects when compared to lean counterparts, thereby suggesting that this impairment may be constitutive.

3.2 DIET-INDUCED THERMOGENESIS RESEARCH

Early research on energy balance indicated that man possessed the ability to dissipate excess nutrient as heat (i.e. Neumann, 1902 - cited in Sims, 1976: Gulick, 1922). Subsequent studies, however, were unable to substantiate this conclusion (e.g. Wiley & Newburgh, 1931; Passmore & Durin, 1955) and it appears that the concept was in disrepute until the 1960's (following Sims, 1976). Two overfeeding studies and a thermogenesis study were instrumental in reopening the debate. Ashworth, Creedy, Hunt, Mahon and Newland (1962) intragastrically supplemented subjects normal diet by 1000 or 2000 Kcal/day for periods up to 36 days with no suppression of voluntary food intake and a much less than predicted gain in weight. Ten years later, in what is considered the classic overfeeding study, Sims, Danforth, Horton, Bray, Glennon and Salans (1973) enticed inmates of Vermont Prison to consume intakes of approximately

7,000 - 10,000 Kcal/day for around 8 months. The results of this experiment clearly established a wide across subject variability in ability to gain weight through excessive overeating. In between these two studies, research by Miller, Mumford and Stock (1967) provided one explanation for the observed failure to gain weight by showing that both the energy cost of exercise and postprandial DIT are increased with excess nutrition.

Because Miller et al (1967) observed large increases in postprandial DIT with overfeeding, in some cases up to 900% total period, they discounted the influence on homeostasis of 7-8% rises in RMR. Recent research suggests however, that variability in RMR, as well as MR during the postprandial period, significantly effects an individual's susceptibility to obesity. In examining recent DIT studies this thesis will adopt the format of dividing data presentation into either postprandial or resting state research. It should be appreciated that postprandial research has mainly focused upon thermogenic responsiveness following normal dietary intake, whereas resting state studies have examined thermogenesis in relation to overfeeding.

3.2.1 Diet-Induced Thermogenesis during the Postprandial Period

Research indicates that obese individuals show a diminished thermic response during the postprandial period following a normal meal.

In a study comparing the DIT produced by carbohydrate ingestion (50 g

oral glucose load), Pittet, Chappuis, Acheson, De Techtermann and Jequier (1976) observed MR increases of approximately 13.0% and 5.2% in control and obese groups respectively. This differential post-prandial response pattern was later confirmed following a mixed nutrient liquid meal, with both an obese and post-obese group evidencing a reduced MR (Shetty, Jung & James, 1979). As subjects in the obese group were selected on the basis of a family history of obesity the suggestion of genetic predisposition to diminished DIT is raised by this research. No indication is given regarding selection criteria in the post-obese group. Of particular interest to this thesis is Shetty et al's (1979) finding that plasma concentrations of norephinephrine was largest in obese and post-obese subjects, thereby suggesting that subnormal DIT does not result from inadequate sympathetic stimulation.

The MR results obtained by Shetty et al (1979) are substantiated by York, Morgan and Taylor (1980) in a study assessing DIT in males of habitually high or low energy intake. Mean body fat percentage of the low intake group (22.5%) indicates this group to be obese with respect to the threshold limit of 22.0% body fat proposed by Lesser, Deutsch and Markobsky (1971). York et al (1980) observed that ingestion of a liquid meal providing either 1,000 Kcal or 500 Kcal produced an increase in MR of 28.6% and 21.6% respectively in the high intake group and 20.0% and 8.2 % respectively in the low intake group. This diminished thermic ability, most apparent following a small meal,

strongly suggests the importance of metabolic efficiency as a factor influencing body weight.

3.2.2 Diet-Induced Thermogenesis during Resting State Conditions

Only three studies directly compare RMR responding between the lean and obese. Because of considerable individual differences in both subject populations no conclusions can be drawn from these data regarding differential response patterns in the respective subject groups.

In a nine day overfeeding study with two obese women, Passmore, Strong, Swindells and el Din (1963) observed a mean 2.6% increase in resting oxygen consumption in one subject, the other evidencing no change. This research was subsequently extended by Strong, Shirling and Passmore (1967) with the presentation of four days overfeeding data on sixteen subjects which included recalculation of Passmore et al's (1963) results. Representation of Passmore et al's (1963) data for the first four days of overfeeding indicates no difference for either subject between control and experimental conditions. Resting measurements on additional obese subjects in the Strong et al (1967) study were unfortunately not presented. However, data provided on five subjects selected due to leanness evidenced a mean change in resting metabolism ranging from -2.3% to +5.9%. The trend suggested by this research, for lean subjects to demonstrate small resting thermic responses which are not shared by the obese, is contradicted by Glick, Schwartz, Magazanik and Modan (1977) in a five day overfeeding study involving four lean and four obese subjects. While the four lean subjects evidenced a mean change in resting metabolism of approximately

-3.5%, 0%, +8.0% and +9.5% respectively, their obese counterparts demonstrated mean changes of -11.0%, -3.0%, +11.0% and +14.5% respectively.

In a recent direct calorimetry study Zed and James (1982) overfed lean and obese subjects for six days with a fat supplement of approximately 1,000 Kcal/day. Two 24 hour measurement periods indicate a greater thermic response in the lean (mean 129.6 Kcal) than the obese (mean 50.9 Kcal). Although it is possible that differential responding is a consequence of postprandial DIT no clarification is made by the authors as to whether RMR in subject groups was also altered.

While it remains unclear if overfed obese individuals are disposed to show only slight increases in RMR, it is reasonable to conclude that wide individual differences exist in this parameter with respect to lean subjects. For example, supplementation of eight subjects' normal diet by 1,500 Kcal/day for fifteen days produced an increase in RMR ranging from 12 to 29% (Apfelbaum, Bostzarron & Lacatis, 1971), while similar supplementation for forty days with six subjects resulted in RMR changes ranging from -1 to 17% (Norgan & Durin, 1980). As indicated, a common paradoxical occurrence in the literature is the observation of diminished or static RMR with overfeeding. This finding apparently defies explanation in terms of inadequate adaption and sampling time with indirect calorimetry techniques (i.e. see Garrow, 1978; Dauncey & Ingram, 1979) since it also manifests in direct calorimetry research. In the study conducted by Dauncey (1980 , which employed direct calorimetry techniques) changes in RMR with overfeed-

ing ranged from 0 to 25%. It should be appreciated that Dauncey's 1980 data conflicts with the conclusion drawn by Garrow (1978) concerning the period elapsing between overfeeding and the onset of resting state DIT (as discussed in Chapter 1, Section 1.3.2). The occurrence of individual differences has received scant attention in the literature. With respect to such findings, it is reasonable to suggest that widely varying manifestations of resting state DIT are a potential stumbling block for concensus regarding the time course of the response.

3.3 NON-SHIVERING THERMOGENESIS RESEARCH

Although it has been known for some time that man is capable of NST, the response has only recently been recognized as a factor differentially influencing energy balance across individuals. A further recent appreciation has been the potential effect on energy balance of the replacement capacity of DIT for NST.

Research by Hey (1975) clearly demonstrates that newborn infants readily evidence NST to mild cooling of the environment, a response employed to overcome the sudden environmental temperature drop occuring at birth. Although adults usually adopt means other than NST to maintain body temperature, experimentation indicates that the capacity is potentially available. Davis (1961) has demonstrated NST in repeatedly cold exposed volunteers and follow-up research shows that such individuals evidence an increase in RMR of approximately 20% to norephinephrine infusion (Joy, 1963).

Data presented by Blaza and Garrow (1980) indicates that the obese do not show NST to temperature changes which are encounted in every-

day life. This is in contrast to lean subjects who increase heat loss by an average of 7% (Blaza & Garrow, 1980; Dauncey, 1981). Dauncey (1981) argues that such energy expenditure is a significant consideration in energy balance, calculating (if all other factors are equal) that this degree of NST for 10% of a year for 10 years would produce an 8 kg loss in body weight. In a similar analysis Cawthorne (1982) suggests that one factor leading to obesity in urban society acculturated Australian aborigines, with exposure to a constant food supply, is their reduced need to expend energy on thermoregularoty thermogenesis.

3.3.1 Speculation Regarding the Effect on Energy Balance of the Replacement Capacity of Diet-Induced Thermogenesis for Non-Shivering Thermogenesis

Commonality between NST and DIT in animals (as indicated in Chapter 2) has led to speculation of a similar situation in man. Of central interest is the influence that an interaction between NST and DIT can have on energy balance. Research with pigs and rats indicates that DIT can replace NST (Close & Mount, 1978; Rothwell & Stock, 1980). Dauncey (1981) has confirmed this effect with humans by showing that MR during 3 hours following a meal was not significantly different at 22^{o}C from that at 28^{o}C. As shown in preceding sections of this chapter, obese individuals evidence depressed NST and DIT. The implication drawn by Rothwell and Stock (1980) and Dauncey (1981) is that the replacement capacity of DIT for NST may be as significant a consideration in energy balance as either response considered singularly.

3.4 CLINICAL SIGNIFICANCE OF DIET-INDUCED AND NON-SHIVERING THERMOGENESIS RESEARCH

As noted in Chapter 2, Section 2.4, to be of clinical significance impaired thermogenesis must be of sufficient magnitude as to be appreciable in terms of weight regulation. In respect to NST, Dauncey (1981) indicates that ineffective responding may produce a 'credit' of approximately 0.8 kg per year. By itself, this degree of inclination toward a positive energy balance may not prove difficult to arrest. However, when considered in respect to concomitant dimished DIT, the propensity can be seen as a significant weight regulation liability. As noted, the obese evidence the greatest impairment to thermic responding following a small meal. In the York et al (1980) study, obese subjects expended only 2.58 Kcal on DIT following a 500 Kcal meal as compared to 31.48 Kcal for their lean counterparts. On the basis of this difference Morgan et al (1982) calculate that the response difference between populations per 24 hours, given that postprandial DIT lasts for 16 hours in any 24 hour feeding cycle, amounts to a short fall of approximately 21% in heightened MR for the obese. In terms of energy balance, these subjects were expending approximately 203 Kcal/day less in DIT. Although no precise composite picture of the total energy balance laibility wrought by diminished thermogenesis can be offered, it is apparent that the net effect can be substantial in some cases over a prolonged period.

3.5 RESEARCH QUESTIONS

A number of disputation points exist in the human thermogenesis literature. As noted in Chapter 1, Section 1.3.2, one basic controversy centres upon the time course of DIT with overfeeding.

The conclusion reached by Garrow (1978) that resting state DIT occurs only after several days nutrient overload has been placed in doubt by data presented by Dauncey (1980). Aside from possible methodological problems, a probable factor contributing to this situation is wide individual differences in diet-induced thermic responding (as discussed in Section 3.2.2). As such, two concerns in the literature requiring resolution are, (a) the time course of DIT following nutrient overload, and (b) a discrimination technique for identifying the thermogenic responsiveness of individuals.

In respect to these issues, an experiment was conducted (Experiment 1) which addressed the following research questions :

Research Question Number 1

Does the DIT response to overfeeding in either postprandial or resting state conditions show any change over five days overfeeding?

Research Question Number 2

Is a subject's responsiveness to a standard test of NST capacity (i.e. stimulation by a sympatheticomimetic drug) predictive of their DIT response to overfeeding?

In respect to sympatheticomimetic responding, it should be appreciated that, while an increase in MR due to stimulation of the sympathetic nervous system is a standard index of NST, a number of researchers have employed sympathetic stimulation to demonstrate human DIT potential (i.e. Rothwell & Stock, 1979; Morgan et al, 1982).

CHAPTER 4.

BROWN ADIPOSE TISSUE, ITS PRESENCE AND ACTIVITY IN HUMANS

In Chapter 2, thermogenesis, principally mediated by BAT, was shown as a significant factor in the energy balance of rodents. The preceding chapter established that thermogenesis is also a significant factor in human energy balance. Discussion will now turn to an examination of evidence supporting speculation that BAT is a major metabolic operative of thermogenesis in humans.

4.1 THE PRESENCE OF BROWN APIPOSE TISSUE IN HUMANS

Full-term newborn infants have significant deposits of BAT (15-40 g) and it is assumed that this organ is responsible for their NST ability at birth (Davis, 1980). Because the influence of BAT on adult energy balance was discounted prior to the recent upsurgence of animal data, no research is presently available which specifically tests BAT's thermogenic capacity in mature individuals. However, histological data do provide support for speculation concerning BAT's active presence in man.

On the basis of light microscopic examination, Heaton (1972) concluded that the wide distribution of active BAT present during the first decade of life diminishes in later years, particularly in peripheral areas, to 'thermogenic jacket' concentrations found mainly around the deeper organs of the body. Of particular interest to this study is her observation of a high BAT incidence in the neck of all age groups sampled. Although the study did not evaluate thermogenic potential it should be appreciated that only a small quantity of active BAT (approximately 30 g) is required to produce the NST response observed in cold exposed subjects (as described in Chapter 3,

Section 3.3, following Cawthorne, 1982).

4.2 THE ACTIVITY OF BROWN ADIPOSE TISSUE IN HUMANS

To date, only drug administration research has been employed to investigate BAT thermogenesis. These studies can be separated into two distinct groups. One line of research centres upon the determination of localized thermic activity at probable BAT sites. These data will be reviewed first. The second line of research to be examined concerns differential responding between lean and obese individuals.

Rothwell and Stock (1979) thermogramically observed localized skin temperature increases around the scapula and in the neck of two subjects following ingestion of ephedrine at a dosage level of 1 mg/kg body weight. No indication of temperature increase magnitude was given in this report or in a later more detailed account of the experiment (see Rothwell & Stock, 1981b). The study has recently been critisized by Hervey and Tobin (1981) who argue that observed temperature increase may be simply the result of vasodilation, and not BAT activity. In reply to this challenge James and Trayhurn (1981a) report a temperature increase of approximately $0.6^{o}C$ in back tissue (previously identified as thermically active) following infusion of norephinephrine at 0.1 mg/kg body weight/minute. As this temperature increase was determined in respect to referent electrodes, placed in other back regions, which evidenced either no temperature change or a drop in temperature, the authors contend that the effect does not reflect vasodilatation associated with gener-

alized heat production.

Additional support for speculation that BAT mediates human thermogenesis can be construed from research conducted by Jung, Shetty and James (1979) examining the effect of beta-adrenergic blockage on the RMR of obese individuals receiving either a high or low energy diet. A significant fall in the RMR of overfed subjects orally administered propranolol to a maximum dosage of 80 mg/6 hours was observed. This result can be interpreted as indicating the cessation of BAT activity via antagonist intervention since propranolol has been shown to almost totally inhibit ephedrine induced respiration in brown adipocytes (Bukowiecki, Sahjah & Follea, 1982).

Two studies have investigated thermogenic stimulation capacity in the lean and obese. In the first, Jung, Shetty, James, Barrand and Callingham (1979) intravenously infused norephinephrine (0.1 ug/kg ideal body weight/minute, for 45 minutes) into resting lean, obese and post-obese subjects, the latter two groups being selected with regard to familial history of obesity. Although similar plasma norephinephrine levels were achieved in all three subject groups, lean subjects evidenced a mean rise in RMR of 21.2% while obese and post-obese subjects both recorded a mean rise of only 9.6%. Equivalent free fatty acid concentrations across the groups indicate that availability of substrate energy sources was not a factor influencing thermogenesis. This result, considered in conjunction with norephinephrine equivalence, suggests that a sympathetically activated thermogenic system may be defective in the obese. Further, because sub-normal responding was also observed in lean post-obese

subjects with family history of obesity, the impaired thermogenic capacity is suggested to be constitutive.

Research by Morgan et al (1982), a direct extension of the York et al (1980) study (reviewed in the preceding chapter, Section 3.2.1), also supports the view that a defective sympathetically activated mechanism underpins diminished thermogenic ability. In the Morgan et al (1982) study, high and low energy intake subjects used by York et al (1980) were orally administered ephedrine at two dosage levels. However, because of presentation differences in the physical characteristics data of both studies, the two low energy intake groups used by Morgan et al (1982) are on the verge of obesity rather than being classifiable as obese (i.e. 22.0% and 21.82% mean body fat respectively). Results of ephedrine administration show a similar trend to DIT responses recorded by York et al (1980). To dosage levels of 0.50 and 0.25 mg/Kg body weight, high energy intake subjects evidenced mean increases over RMR of 11.2% and 15.7% respectively, whereas low energy intake counterparts demonstrated increases of 10.3% and 5.2% respectively. In continuity with the stratification of results observed by York et al (1980), these findings indicate that sensitivity to a sympatheticomimetic is increased in energetically inefficient individuals. Further, as with thermic response to a small meal (i.e. 500 Kcal), energetically efficient individuals show a significantly reduced response to the lesser ephedrine dose. The results also suggest that a dosage of 0.5 mg/Kg body weight is sufficient to produce maximal thermogenesis in most individuals. In contrast to the results of Jung, Shetty, James, Barrand, & Callingham (1979), plasma free fatty acid concentrations, although

increased in all subject groups, were significantly higher in high energy intake subjects, particularly after the large ephedrine dose. It is unclear whether this finding relates to differential effects of ephedrine and norephinephrine dosage differences between studies, or subject differences.

4.3 RESEARCH QUESTIONS

Although conclusions reached in the animal laboratory may not be applicable to man, the occurrence of subnormal thermogenesis in obese humans and mutant obese animals suggests that a common metabolic malfunction mediates both conditions. Animal research clearly identifies BAT as the prime metabolic operative for energy balance in genetically normal rodents and the prime metabolic liability in part producing obesity in mutants. Histological and sympathetic stimulation evidence suggests that BAT is present and physiologically active in man. Further, drug administration studies suggest that a defective sympathetic mechanism underpins the impoverished thermogenic ability of the obese. With respect to these findings it is reasonable to speculate that (i) BAT may mediate thermogenesis in humans, and (ii) that a failure of this function is in part responsible for obesity.

This thesis examines the first speculation in two experiments:

Experiment 1

Research Question Number 3

Is BAT involved in mediating the DIT response during post-prandial conditions?

Experiment 2

Research Question Number 1

Can the postprandial DIT response to overfeeding be blocked by a beta-adrenergic antagonist?

CHAPTER 5

EXPERIMENT 1.

5.1 METHOD

5.1.1 Subjects

Seven male university students, ranging from lean individuals to borderline obese in terms of percentage body fat (see Table 1), were selected for testing. No subject was employing dietary restraint at the time of the experiment. All subjects were declared to be in good health following a medical examination prior to experimentation. In addition, every subject was fully briefed on the nature of each phase of the experiment and signed an informed voluntary consent form to this effect (see Appendix A).

5.1.2 Design

Subjects were tested in response to two independent stimuli; overfeeding and ephedrine. Each subject acted as his own control and the running of experimental/control trials were counterbalanced. To minimize circadian influences on responding each respective block of trials were conducted during the same daily time period.

In the overfeeding phase five consecutive days of energy overload were compared to a similar period during which subjects maintained normal dietary intake. Dependent variable measurement was undertaken in two trials in each 24 hour feeding cycle, one trial following lunch (postprandial) and the other before breakfast (resting state). Every trial period was sixty minutes in duration and data werecollected over one minute periods every ten minutes during the

TABLE 1: Physical Characteristics of Subjects

Subject	Weight (Kgm)	Height (cm)	% Body Fat*	Normal daily energy intake (Kcal)
MA	58.5	173	10.5	2,421
PB	66.3	171.5	10.5	4,018
MJ	71.3	179	10.0	2,481
GF	83.3	185	15.75	3,442
MS	91.1	197	22.2	3,810
AG	74.4	181	20.1	2,341
KD	68.6	174	14.0	1,340

* Percentage body fat determined from measurement of skinfold thickness, following Womersley and Durnin (1974).

final thirty minutes of a trial. The design employed therefore consisted of two conditions (overfeeding and normal intake), five testing days per condition, two trials per day (postprandial and resting state) and three scoring periods within each trial, yielding a 2 x 5 x 2 x 3 factorial design with repeated measures for each dependent variable.

One trial was conducted for each condition, drug and placebo, in the ephedrine phase. Both trials were two hours in duration and data was collected over one minute periods every ten minutes during the final ninety minutes of each trial. A 2 x 9 factorial design with repeated measures for each dependent variable was thus created.

Oxygen consumption (see Section 7.3.1), skin temperature at probable BAT sites (see Figures 2A and 2B), and heart rate (HR) were measured during postprandial trials of the overfeeding phase and in the ephedrine phase. Only O_2 consumption was measured in the resting state trials of the overfeeding phase. HR was recorded for two reasons, primarily as a secondary assessment of MR (see Garrow, 1978), and also to provide immediate feedback on a subject's reaction to drug administration. In addition to the above dependent variables, shoulder EMG was recorded during all trials to ensure that later scoring of O_2 and HR data points did not correspond with periods of muscle activity. External air temperature and humidity were recorded prior to the commencement of all trials to enable determination of O_2 content in a subjects air supply (see section 5.3.1).

5.2 PROCEDURE

5.2.1 Overfeeding Phase

All subjects were required to record their daily nutritional intake for seven days immediately prior to testing. This 'diet diary' (Garrow, 1978; see Table 2 and Appendix B) provided an

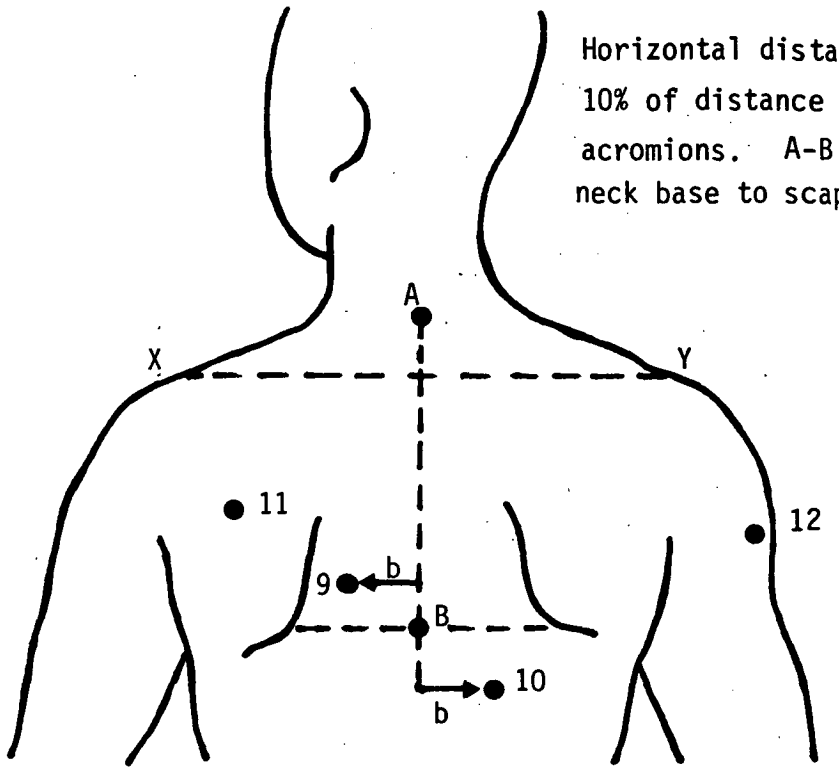

Figure 2A: Thermocouple placement on lower back and right arm.

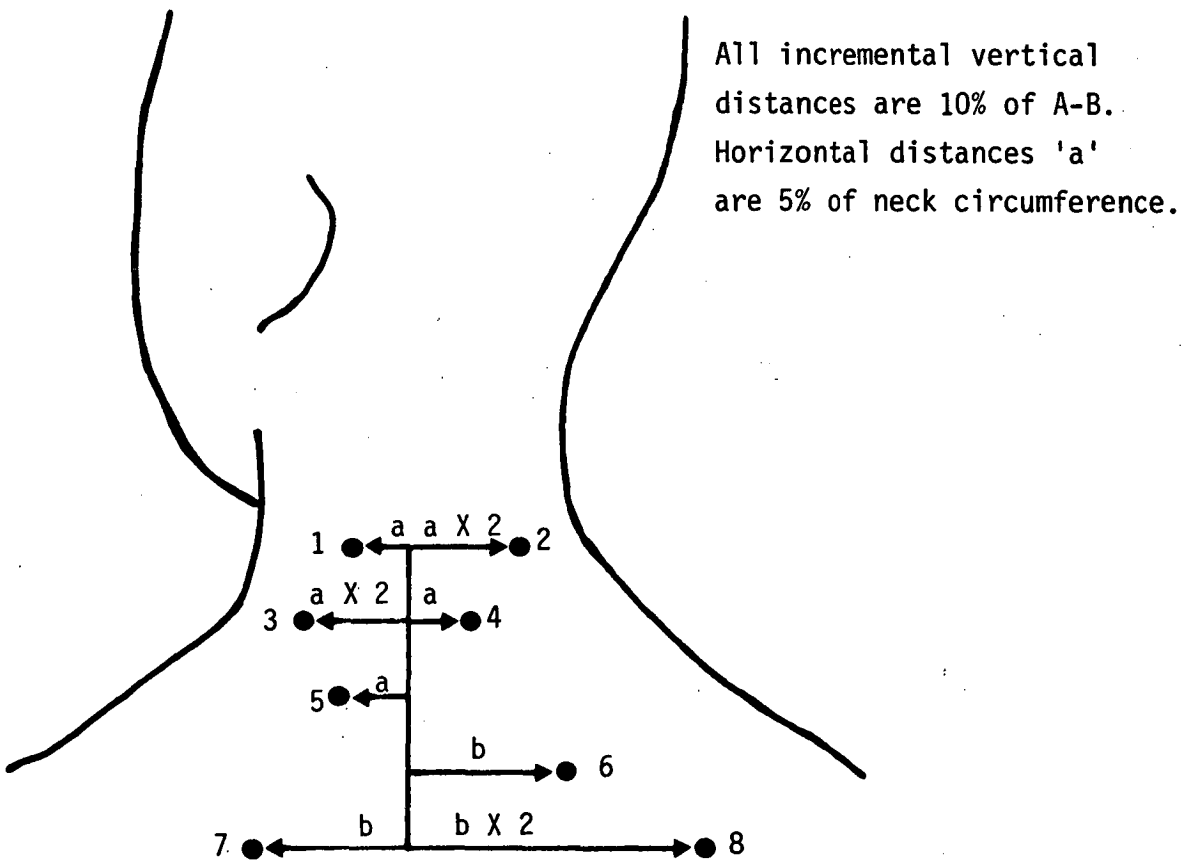

Figure 2B: Thermocouple placement on neck and shoulder. The twelve thermocouple points are arranged in four groups: Neck (1-4), Shoulder (5-8), Sub-scapular (9-10) and Reference (11-12).

estimation of a subject's average daily energy consumption. Recording of intake was continued during experimental and control conditions to ensure that normal energy consumption was being maintained. During the overfeeding period subjects were required to augment their normal intake by at least 60% via consumption of the nutritionally balanced liquid food 'Ensure Plus" supplied in 355 Kcal/8Fl oz cans from a Government Medical Store. One subject, AG, found excessive consumption of the liquid food unpalatable and met his excess energy quota through eating meat pies. Subjects in the overfeeding condition were required to consume approximately 50% of their excess energy quota during the morning of a 24 hour feeding cycle (each 24 hour feeding cycle was considered to run from the commencement of breakfast).

5.2.1.1 Postprandial Trials

Following lunch a subject reported to the laboratory, stripped to the waist and removed his shoes. Body weight was recorded and HR/EMG electrodes attached to the chest and right shoulder respectively. The subject then lay face down on a bed while skin temperature thermocouples were attached. It should be appreciated that the experimental room was maintained at thermoneutrality ($26^{o}C \pm 2^{o}C$) during all trials. An airtight multiharness face mask which incorporated a non return valve was then snugly fitted. After insuring that the subject was comfortable, the experimenter proceeded to the monitoring room and the trial commenced. Subjects were instructed to rest quietly throughout each trial, the first thirty minutes of which was designated an adaption period. A subject could call the

TABLE 2: Nutritional Breakdown of Diet and Daily Energy Intake for Control and Overfeeding Conditions, Experiment 1.

Subject	Condition	% Protein	% Fat	% Carbohydrate	Kcal/day Day 1	Day 2	Day 3	Day 4	Day 5
MA	Control	17.11	21.41	61.48	2329	1955	3074	2359	2389
	Overfeeding	17.82	20.45	61.73	4085	4137	4110	4095	3804
PB	Control	15.31	23.09	61.60	3376	2999	2651	2687	2117
	Overfeeding	18.23	20.12	61.65	4066	5500	4145	4606	4877
MJ	Control	17.31	20.32	62.36	2793	2632	2359	2115	2399
	Overfeeding	16.89	20.45	62.66	4244	4873	3391	4840	4814
GF	Control	15.25	23.95	60.80	3289	3337	3555	3430	4594
	Overfeeding	17.21	20.92	61.07	5624	5203	5914	4848	4715
MS	Control	17.25	20.14	62.61	3664	3517	3660	3295	4054
	Overfeeding	17.10	21.12	62.78	5193	5571	6215	5387	5420
AG	Control	18.03	20.38	61.78	1606	1593	2102	1648	2673
	Overfeeding	17.87	20.38	61.75	4384	2702	4932	4371	3856
KD	Control	28.02	10.69	61.29	1365	1309	1379	1340	1340
	Overfeeding	22.00	13.40	64.60	2785	2729	2799	2760	2760

experimenter by means of an electronic 'beeper' if a problem arose (e.g. if the face mask developed a leak, etc.) and the subject was required to give a short beep every ten minutes to assure the experimenter that he was not asleep (a clock was provided in the experimental room for this purpose).

5.2.1.2 Resting State Trials

Identical preparation procedure and running conditions were adhered to during resting state testing with the exception that skin temperature, HR and EMG were not recorded.

5.2.2 Ephedrine Phase

An identical preparation procedure to that described for the postprandial trials in the overfeeding phase was followed. The first thirty minutes of every two hour trial was designated an adaption period. Following this period the experimenter re-entered the experimental room and removed the subject's mask. The subject immediately swallowed the designated medication (ephedrine or placebo) with the aid of a small drink of water and the mask was re-fitted. The ninety minute experimental period was then monitored during which the subject lay undisturbed save for the requirement to beep every ten minutes.

Ephedrine was administered according to body weight, 1mg/kg in tablets of 15 mg. During all trials appropriate medication was on hand to reverse excessive effects of the ephedrine. Commercially available

saccrine tablets which were similar in size and colour to the ephedrine were employed as placebos. Subjects were ignorant regarding the placebo administered although they were informed prior to experimentation that one dosage of tablets may taste bitter when swallowed while the other may taste sweet. To minimize the likelihood that subjects would intentionally attempt to discriminate the trial condition through tablet mastication they were instructed to throw their dosage to the back of their throat and immediately swallow.

5.3 DEPENDENT VARIABLE MEASUREMENT

Recording of HR and EMG was undertaken with a Beckman Polygraph using appropriate couplers and routine operating procedure.

5.3.1 Determination of Metabolic Rate

As a CO_2 analyzer was not available, MR was estimated from O_2 consumption alone rather than both O_2 consumption and CO_2 production. This procedure was considered satisfactory since subjects consumed a standard supplement during overfeeding (i.e Ensure Plus) and as such could be expected to be metabolizing somewhat similar proportions of nutrient in this condition (see Table 2). Consumption of O_2 was determined through assessment of O_2 present in inspired air, measurement of inspired air volume and measurement of O_2 present in expired air. Figure 3 provides a schematic representation of the apparatus layout for dependent variable measurement.

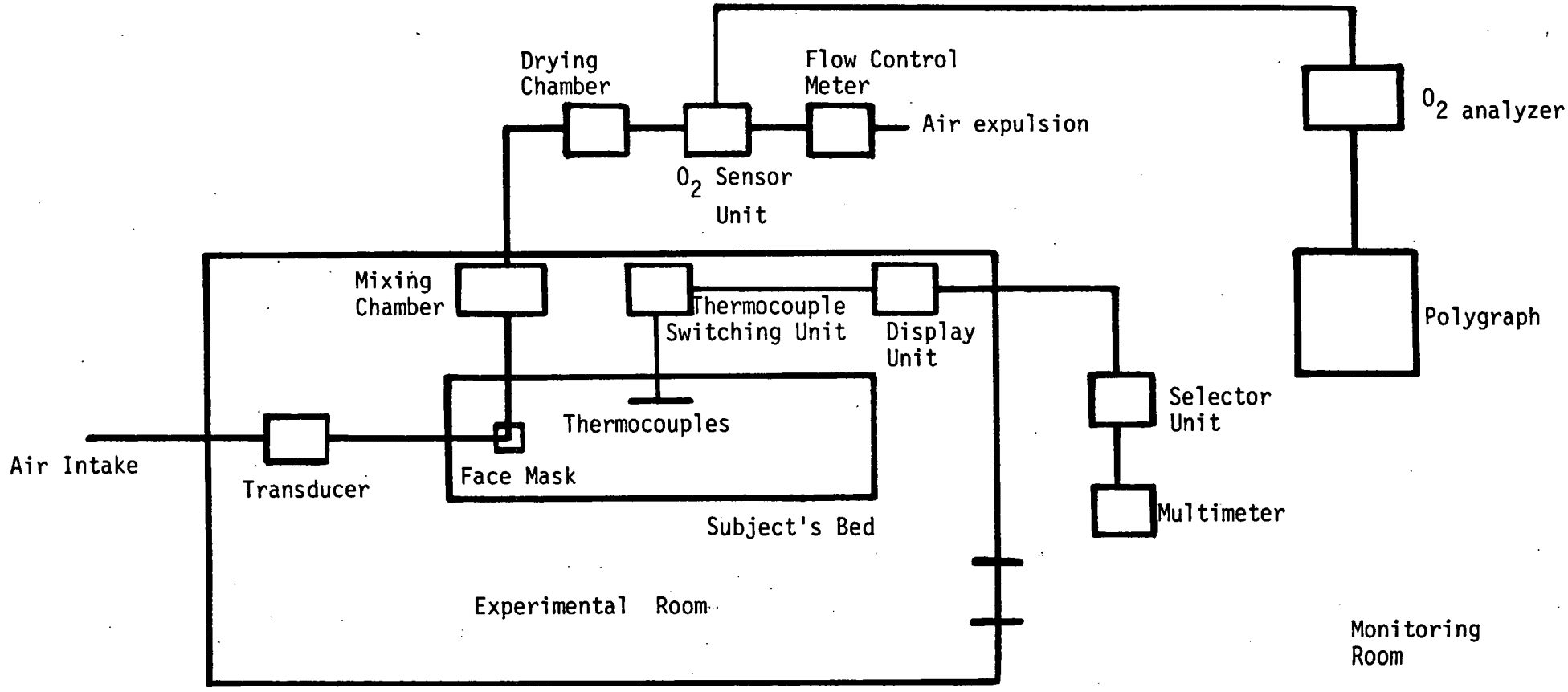

Figure 3: Schematic representation of experimental apparatus, Experiment 1.

The percentage of O_2 in inspired air was assessed through reference to a temperature/humidity chart presented in the Operating and Service Manual for the Applied Electrochemistry Inc. S-3A Oxygen Analyzer. External air temperature was measured via a thermocouple permanently attached to the extraction end of the air intake tube. This thermocouple was connected to the sensor unit of the skin temperature apparatus (see following section) and provided a sensitivity of $0.1^{o}C$. Humidity was measured with an E.T.I. Humidity Meter, Project No. 256, which yielded graduated readings to 10.0% relative humidity. This unit was located in the inlet portal of the air intake tube.

Intake air volume was assessed with a Washington M-400 transducer fitted into the air intake tube line. The signal from this transducer was recorded on the polygraph via a FC112 coupler calibrated to provide litres of air inspired per twenty second time period (sensitivity 0.1 l/20 sec).

Expired air O_2 concentration was measured with an Applied Electrochemistry Inc. S-3A Oxygen Analyzer and an N-22M Sensor Unit, this system affording a sensitivity of 0.01% O_2. A subject's expired air was initially collected in a ten litre mixing chamber from which a continual air sample was extracted by an Applied Electrochemistry R-1 Flow Control Meter and passed, via a calcium chloride drying chamber, to the N-22M Sensor Unit at a flow rate of 150ml/min. The percentage of O_2 in the sample air stream being simultaneously displayed on the S-3A Oxygen Analyzer and recorded on the polygraph.

It was assumed that a subject's volume of inspired air ($\dot{V}_I$) was

equal to the volume of expired air (V_E). O_2 consumption was calculated using the formula:

$$\text{Volume of } O_2 \text{ (ml/min)} = (V_I \times F_IO_2) - (V_E \times F_EO_2)$$

where F_IO_2 = percentage of O_2 in inspired air

F_EO_2 = percentage of O_2 in expired air

5.3.2 Measurement of Skin Temperature.

As indicated in Figures 2A and 2B, twelve thermocouples arranged in four groups were employed in temperature measurement. Thermocouple Nos 1-10 were attached to a subject's upper back and neck at sites of probable BAT deposits (i.e. following Heaton, 1972; Rothwell & Stock, 1979). Thermocouple Nos 11 and 12 were reference probes attached at non-BAT sites.

All thermocouples (type K with PTFE sleeving) were integrated into a specially constructed remote relay switching unit which in turn was linked to a Digitron Instrumentation Ltd Display Unit, Model No 2751-K. Individual thermocouple signals were fed to the monitoring room from this unit by manual manipulation of a Digitron Selector Unit Model No PT100 (see Figure 3). An Escourt Digital Multimeter, Model No EDM-101, linked to the selector unit, provided direct display of temperature at any placement site (sensitivity 0.1^oC).

5.4 RESULTS

Subjects increased their daily energy intake by a mean of 1,927 Kcal during overfeeding, respresenting a mean percentage increase across the experimental condition of 76.6% (range 44.5% to 110.5%). Six of the seven subjects gained more weight while overfeeding than during the control condition. The failure of subject PB to increase weight gain with energy overload may reflect a decrease in his body water content during the experimental period. Strong et al (1967) attribute wide variation in subject weight gain following four days overfeeding to changes in body water content (a retention range from -750 g to + 1540 g). Individual mean daily energy intake and weight change data is presented in Table 3.

It should be appreciated that O_2 consumption values obtained in Experiment 1 were higher than usual for postprandial and resting state conditions (see McArdle, Katch & Katch, 1981) as subjects were monitored while lying on their stomach rather than their backs, to facilitate skin temperature measurement.

5.4.1 The Occurrence of Diet-Induced Thermogenesis with Overfeeding

A 2 x 5 x 2 x 3 ANOVA with repeated measures was performed on both O_2 consumption and HR data. This analysis consisted of two conditions (normal dietary intake and overfeeding), five days, two assessment periods in each 24 hour feeding cycle (postprandial and resting state), and three scoring times within each period (at 40, 50 and 60 minutes respectively). Summary analyses of variance tables are presented in

Appendix C and D respectively.

In respect to differences between conditions, O_2 consumption rose 9.8% from a mean of 812.90 ml/min during control trials to a mean of 892.22 ml/min in the experimental condition. The main effect of conditions approached significance ($F(1,6) = 4.59$, $p=.076$). HR increased 6.3% from 65.5 BPM to 69.6 BPM. This rise was significant ($F(1,6) = 48.69$, $p < .001$).

Although the magnitude of the O_2 consumption increase was greater during the postprandial period than the resting state, 12.03% as opposed to 7.14% respectively, the interaction between conditions and periods was not significant ($F(1,6) = .68$, $p > .40$). As would be expected, O_2 intake was higher during the postprandial than resting state periods ($F(1,6) = 18.20$, $p < .01$). In general, this evidence suggests that MR rose during overfeeding and that the increase was independent of time of testing. Nevertheless, a notable feature of the data is considerable individual differences in response to overfeeding (see Table 4). These differences are analysed further in a later section.

5.4.2 The Time Course of Diet-Induced Thermogenesis with Overfeeding

No main effect for days was observed for O_2 consumption ($F(4,24) = 1.06$, $p > .39$), although HR showed a mean positive drift across days of 10.9 BPM ($F(4,24) = 11.99$, $p < .001$). Further, an interaction did not occur between conditions and days for either O_2 consumption ($F(4,24) = .58$, $p > .68$), or HR ($F(4,24) = 1.31$, $p > .29$), or between conditions, days and periods ($F(4,24) = .77$, $p > .55$). Taken together, these results

Table 3. Mean Daily Intake and Weight Change for Control and Overfeeding Conditions, Experiment 1.

Subject	Condition	Mean Daily Intake (Kcal)	Percentage Increase in Kcal consumed during Experimental Conditions	Weight Change per Condition (g)
MA	Control	2421	66.0	800
	Overfeeding	4018		1,100
PB	Control	2766	76.9	250
	Overfeeding	4893		100
MJ	Control	2460	80.2	650
	Overfeeding	4432		2,050
GF	Control	3641	44.5	850
	Overfeeding	5261		1,250
MS	Control	3638	52.8	-450
	Overfeeding	5557		750
AG	Control	1924	110.5	-750
	Overfeeding	4049		750
KD	Control	1347	105.4	-5
	Overfeeding	2767		350

suggest an immediate increase in MR following the first day of overfeeding which was maintained until day five (see Figures 4 and 5). The positive drift in HR across days, which occurred in both conditions, may reflect increasing subject discomfort with the experimental procedure.

5.4.3 Brown Adipose Tissue Activity During Overfeeding

Skin temperature data were analysed using a 2 x 5 x 4 x 3 ANOVA, with

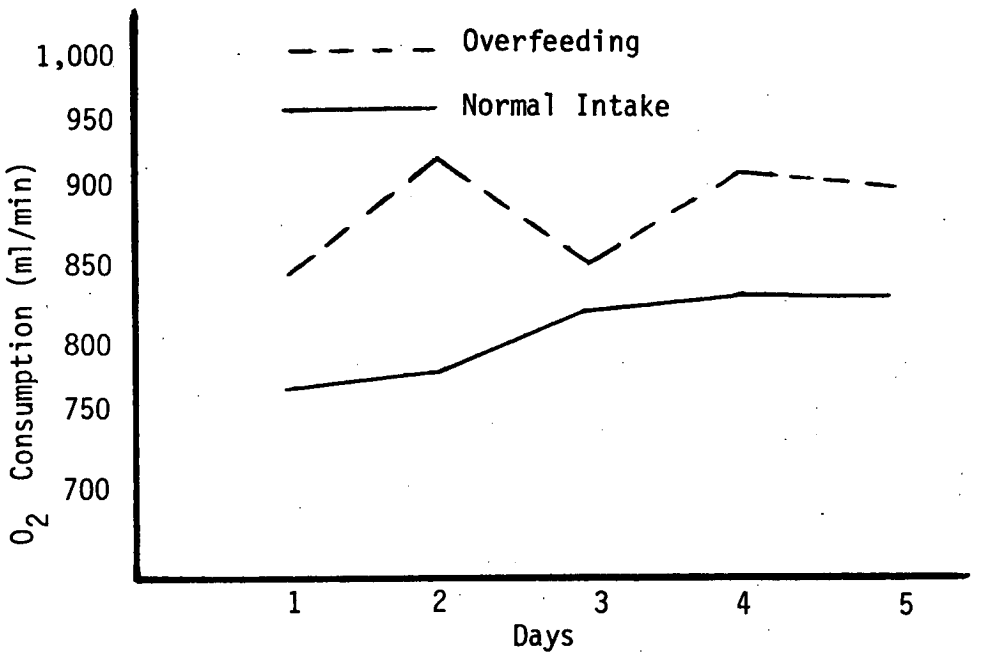

Figure 4: Mean O_2 Consumption for normal intake and overfeeding conditions on each day of testing, Exp. 1.

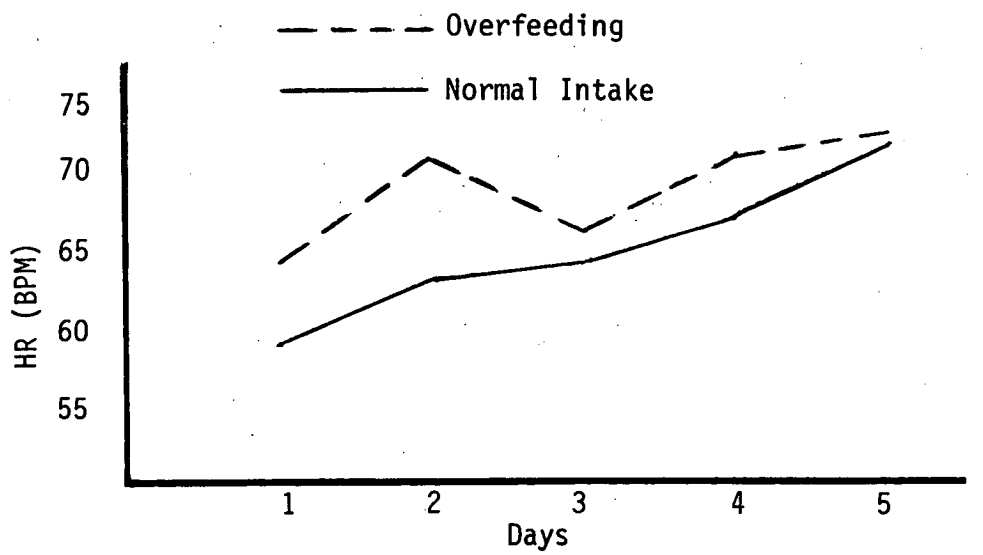

Figure 5: Mean HR increase for normal intake and overfeeding conditions on each day of testing, Exp. 1.

repeated measures, comprising two conditions (normal dietary intake and overfeeding), five days, four thermocouple groupings (see Figures 2A and 2B), and three scoring times within each assessment period (at 40, 50 and 60 minutes respectively). A summary analysis of variance table is presented in Appendix E.

A mean of 34.31°C. and 34.37°C. was recorded during normal and excess energy intake conditions respectively. This temperature difference was not significant, ($F(1,6) = 0.06$, $p > .80$). Further, an interaction between condition and thermocouple group was not evident ($F(3,15) = 0.02$, $p > .99$). Inspection of temperature change data at individual thermocouple placement sites yielded no indication of discrete thermic activity. These findings suggest that BAT thermogenesis does not mediate DIT.

5.4.4 Oxygen Consumption and Heart Rate Change Following Ephedrine Administration

A 2 x 9 ANOVA with repeated measures was performed on both O_2 consumption and HR data, comprising two conditions (normal dietary intake and overfeeding) and nine scoring times within each assessment period (40, 50, 60, 70, 80, 90, 100, 110 and 120 minutes respectively). Summary analysis of variance tables are presented in Appendix F and G respectively.

Administration of ephedrine significantly increased O_2 consumption from 856.47 ml/min in the placebo condition to 959.05 ml/min in the experimental condition ($F(1,6) = 16.84$, $p < .01$). HR increased from

Table 4: Percentage Change in O_2 Consumption and HR from Baseline to Overfeeding Conditions for Postprandial and Resting State Trials in the Overfeeding Phase, and Percentage Change in O_2 Consumption and HR Across Conditions in the Ephedrine Phase, Experiment 1. (Note: The ephedrine data has been adjusted for differences in initial values (first 70 min) between conditions, see section 7.4.4 for discussion.)

Subject	Overfeeding Phase				Ephedrine Phase	
	Postprandial		Resting State	Mean Change in O_2 Consumption		
	O_2	HR	O_2		O_2	HR
MA	2.01	11.15	13.47	7.74	17.46	2.13
PB	1.99	4.63	7.97	4.98	16.11	48.84
MJ	-9.17	2.62	3.95	-2.61	-12.25	12.75
GF	15.31	6.22	19.82	17.57	39.83	14.94
MS	40.44	8.93	11.23	25.84	12.95	0.08
AG	26.59	5.51	-0.27	13.16	24.46	15.56
KD	6.11	6.28	22.07	14.09	9.49	0.22

65.2 BPM to 69.1 BPM across conditions, a marginal rise approaching significance ($F(1,6) = 5.61$, $p = .056$). Inspection of Figures 6 and 7 indicates that part of this effect was due to an initial difference in MR which could not have been due to sympathetic stimulation. The most likely explanation for this occurrence is that some subjects discriminated the ephedrine taste and showed anticipatory responses. This view is reinforced by the observation that the initial rise in MR at the beginning of the session was restricted to three subjects.

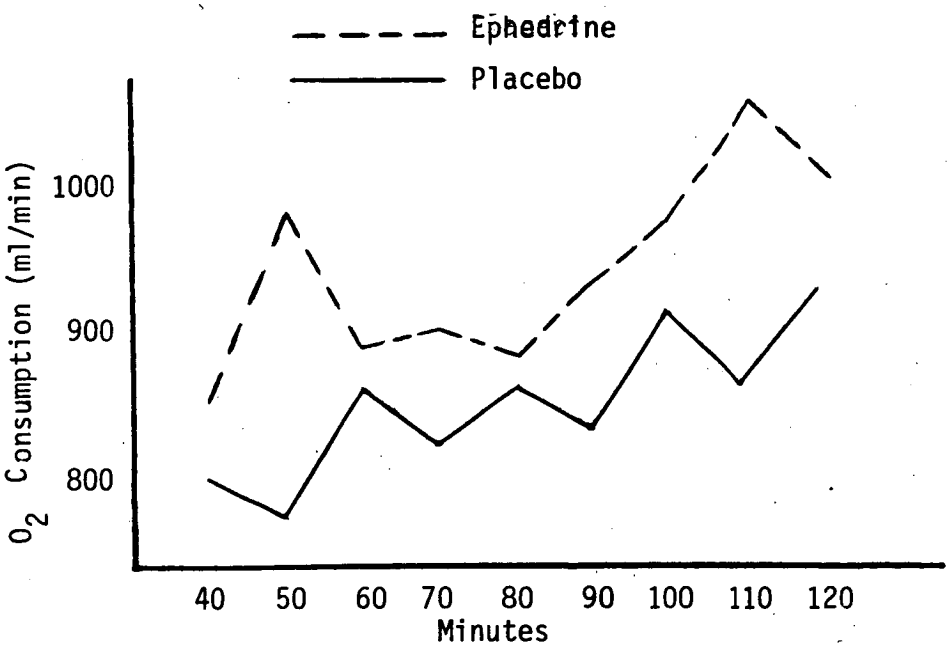

Figure 6: Mean O_2 Consumption for placebo and ephedrine conditions during total scoring period, Experiment 1.

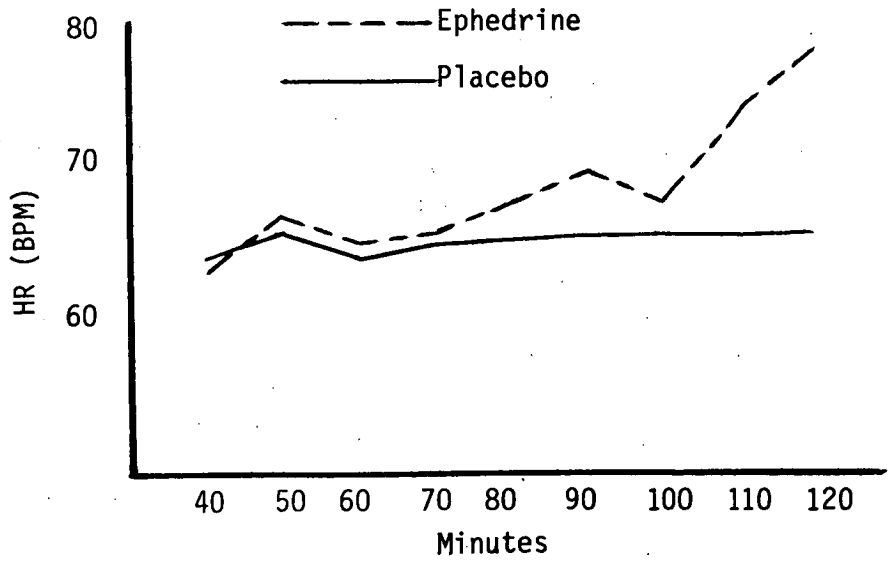

Figure 7: Mean HR for placebo and ephedrine conditions during total scoring period, Experiment 1.

A significant main effect for time was evident for both O_2 consumption ($F(8,48) = 3.17$, $p < .01$), and HR ($F(8,48) = 3.26$, $p < .01$). Further, a specific effect for ephedrine is indicated in the significant interaction between condition and time for O_2 consumption ($F(8,48) = 3.13$, $p < .01$). The interaction almost reached significance for HR ($F(8,48) = 2.00$, $p = .066$). Examination of condition by time means (see Figures 6 and 7) reveals that the interaction resulted from a steep increase in MR over the last 20 minutes of the experimental condition.

In order to further examine the strength of the ephedrine effect, O_2 consumption and HR values for the last 20 minutes of testing were adjusted for any anticipatory rise in MR occurring during the first 70 minutes of testing. This division of data was selected because of the inflection in the curves evident in Figures 6 and 7. The correction consisted of subtracting the percentage difference obtained over the first 70 minutes from that derived from the final two scoring times. Analysis of these data showed a significant increase for O_2 consumption ($t(6) = 2.59$, $p < .05$) and an increase of marginal significance for HR ($t(6) = 2.10$, $p > .05, < .10$). This analysis is in actuality a conservative estimate of the ephedrine effect as it assumes that any anticipatory rise in MR and the ephedrine effect itself were additive. Thus, the effect of ephedrine on MR is clear despite the apparent failure to successfully conceal conditions from all subjects.

5.4.5 Brown Adipose Tissue Activity Following Ephedrine Administration

Temperature data was analysed using a 2 x 4 x 9 ANOVA with repeated measures: 2 conditions (placebo and ephedrine), 4 thermocouple groupings (Figure 2A and 2B), and 9 scoring times within each assessment period

(at 40, 50, 60, 70, 80, 90, 100, 110 and 120 minutes respectively). A summary analysis of variance table is presented in Appendix H.

Skin temperature varied from a mean of 34.37 ^{o}C. in the placebo condition to a mean of 34.41^{o}C. during the ephedrine trial, a non-significant difference ($F(1,6) = 0.06$, $p > .80$). No interaction was found between condition and thermocouple group ($F(3,15) = .33$, $p > .80$), or between condition and time ($F(8,40) = 1.24$, $p > .3$). Inspection of individual placement temperature change yielded no indication of discrete thermic activity. As MR increased over the last 20 minutes of the ephedrine trial, a separate ANOVA was performed on this segment of the temperature data. No main effect for temperature was found ($F(1,6) = 1.31$, $p > 0.30$), and a significant interaction did not occur between condition and thermocouple placement ($F(3,15) = 0.40$, $p > .70$). These results suggest that BAT thermogenesis did not occur following ephedrine administration.

5.4.6 Comparison of Individual Oxygen Consumption and Heart Rate Increases Resulting from Overfeeding and Ephedrine Administration

Table 4 provides the percentage difference between conditions for both O_2 consumption and HR during the overfeeding and ephedrine trials. As is readily discernable, considerable individual differences characterize the overfeeding and ephedrine data. One prediction arising from the hypothesis that DIT and NST are mediated by the same mechanism is that individual MR responses to both overfeeding and ephedrine are related. The results from analysis of O_2 consumption data are in the correct direction though not significant. Correlation coefficient values for

postprandial responding in respect to ephedrine, resting state responding in respect to ephedrine, and the average overfeeding effect in respect to ephedrine are r = .46, .32, and .56 respectively (see Figure 8). An inverse relationship is obtained for postprandial HR in respect to ephedrine stimulated HR, the correlation coefficient being r = -.52. In view of the small number of subjects tested, and the level of the correlation coefficients, the relationship between overfeeding and ephedrine stimulation remains equivocal.

In order to further explore individual differences, DIT and ephedrine responses were compared to the anthropometric characteristics of subjects. DIT response (averaged over days and periods) and percentage body fat yielded a correlation coefficient of r =.85 ((df, 6) $p < .05$, see Figure 9). A similar analysis with ephedrine responses produced a correlation coefficient of r = .39. The relationship between DIT response and (i), body weight and (ii), average daily energy intake were not significant (r = .70 and .36 respectively). A similar analysis in respect to ephedrine responses produced correlation coefficients of r = .25 and .32 respectively. While the number of subjects tested in the two experiments are insufficient to allow conclusions in relation to individual differences, the substantial relationship between body fat and DIT suggests that body composition and DIT may be interdependent.

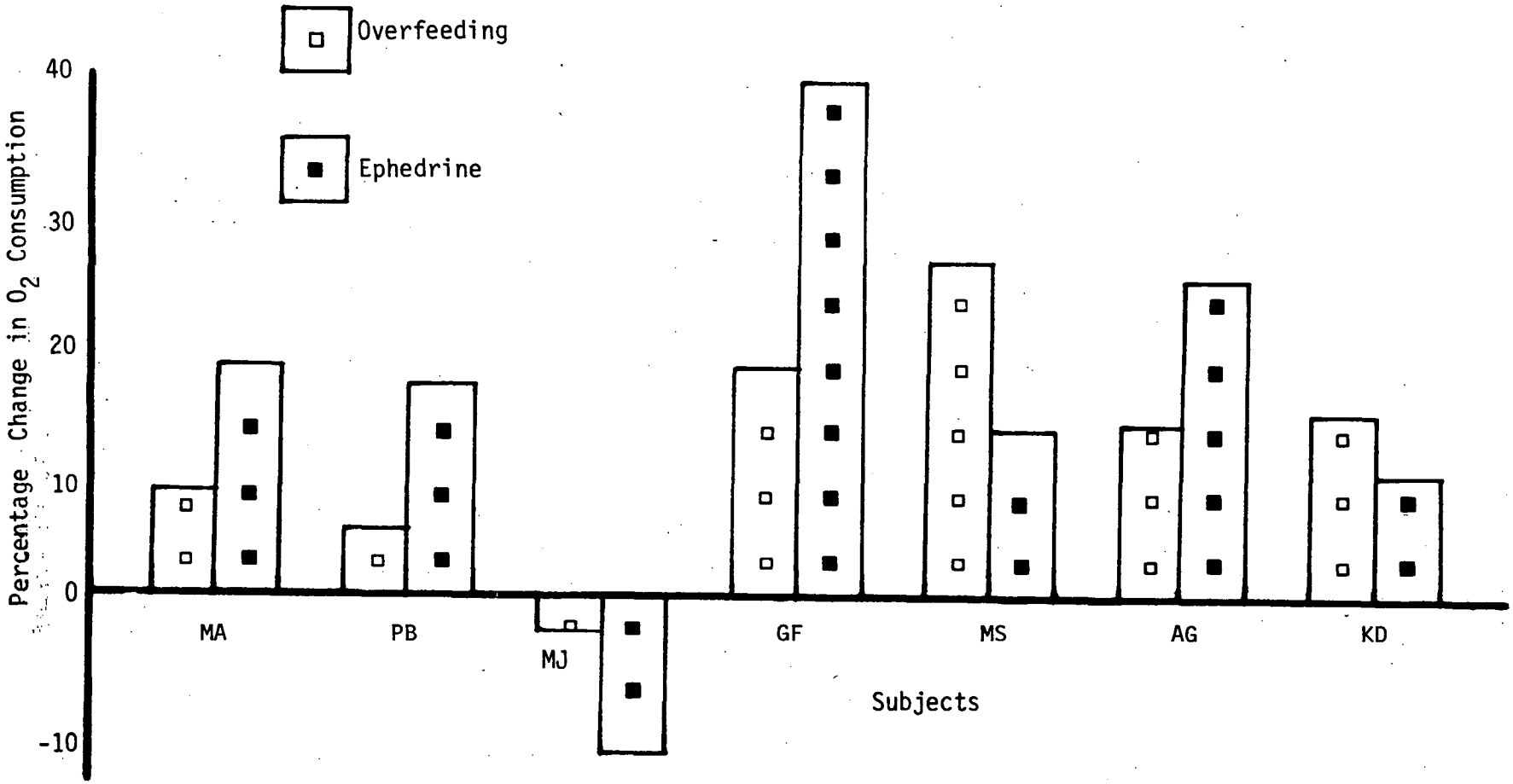

Figure 8: Individual percentage change in O_2 Consumption averaged across periods for Overfeeding trials and during ephedrine trials, Experiment 1.

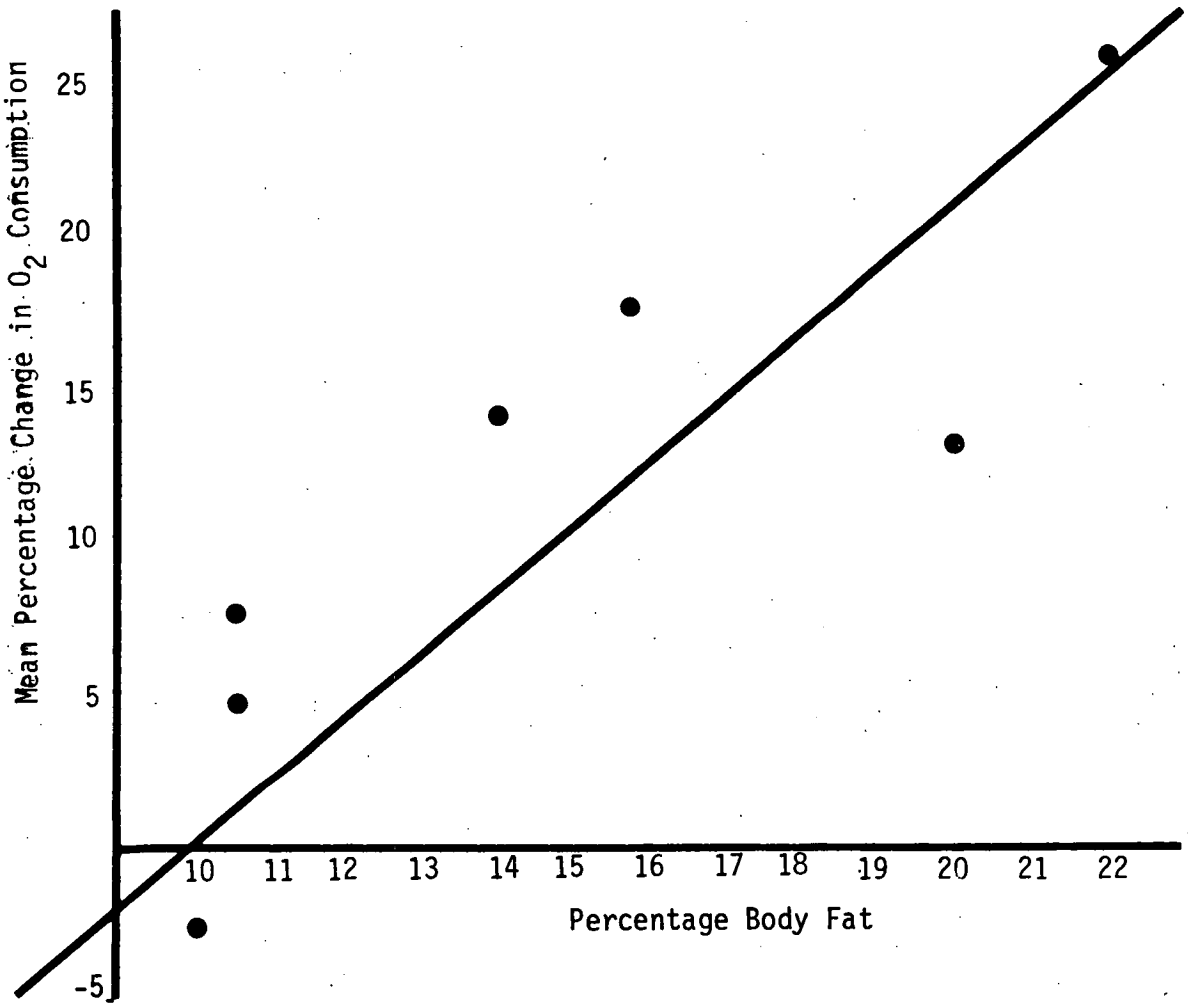

Figure 9: Comparison of mean percentage change in O_2 Consumption across normal intake and overfeeding conditions with percentage body fat, Experiment 1.

CHAPTER 6

EXPERIMENT 2

6.1 SUBJECTS

Three subjects from Experiment 1 who each demonstrated a strong thermic response to both ephedrine administration and overfeeding were tested in Experiment 2 (subjects GF, MS and AG). Each subject was fully briefed on the nature of the experiment and signed an informed voluntary consent form prior to experimentation (see Appendix I).

6.2 DESIGN

The effect of propranolol on postprandial DIT was tested in a 2 x 2 factorial design. Two levels of food consumption, normal dietary intake and overfeeding, comprised one category of the design, propranolol and a placebo condition comprised the second category. Subjects were tested twice in each respective cell of the design and the normal dietary intake and overfeeding conditions were conducted in separate blocks. As such, each subject was run for four days in each of these conditions, the propranolol and placebo being administered in an ABBA sequence respectively. The design and counterbalancing for each subject is given in Table 5. To minimize circadian influences all trials were conducted during the same daily time period. One minute periods were scored every five minutes during the second thirty minutes of each hour trial period. The final design therefore consisted of two conditions (normal intake and overfeeding) interposed with two other conditions (drug and placebo), and six scoring periods within each trial, thus creating a 2 x 2 x 6 factorial design with repeated measures for each independent variable. Metabolic rate, HR and shoulder EMG were measured during all trials.

Table 5: Design and Counter Balancing Across Subjects, Experiment 2.

Subject		Dietary Condition							
		Normal Intake				Overfeeding			
		Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
GF	DRUG CONDITION	Placebo	Propran-olol	Propran-olol	Placebo	Placebo	Propran-olol	Propran-olol	Placebo
MS		Placebo	Propran-olol	Propran-olol	Placebo	Placebo	Propran-olol	Propran-olol	Placebo
AG		Propran-olol	Placebo	Placebo	Propran-olol	Propran-olol	Placebo	Placebo	Propran-olol

6.3 PROCEDURE

Dietary records were kept for a week prior to and throughout testing (see Table 6). As with Experiment 1, overfeeding was accomplished via consumption of Ensure Plus, with the same consumption regimen being followed during experimentation. Subject AG again met his excess energy quota through eating meat pies. All testing was conducted after lunch, i.e. during the postprandial period. Subjects ingested their designated dosage of propranolol (1.0 mg/kg, in 40 mg tablets) or a placebo two hours prior to the commencement of a trial. During all trials appropriate medication was on hand to reverse excessive effects of the propanolol. The placebo utilized was specially made up by the pharmacy of a local hospital to closely resemble the propranolol in size and colour. Propranolol and placebo tablets could not be discriminated by taste when swallowed. Subject preparation and running conditions were the same as adopted in the Experiment 1 overfeeding phase resting state trials with the exception that subjects were monitored while laying on their backs.

6.4 DEPENDENT VARIABLE MEASUREMENT

The same apparatus used in Experiment 1 was employed in this experiment with the exception that air intake volume was assessed with a Fleisch Pneumotachograph linked to a Gould Inc Pressure Transducer, Model No PM 15E. A continual breath by breath inspiration volume was charted from the transducer signal using a Resetting Intergrating Coupler, No. 9873B (sensitivity 0.05 l/respiration cycle).

Table 6: Nutritional Breakdown of Diet and Daily Energy Intake for Control and Overfeeding Conditions, Experiment 2.

Subject	Condition	%Protein	%Fat	%Carbohydrate	Kcal/day Day 1	Day 2	Day 3	Day 4
GF	Control	16.27	38.47	45.26	2855	3225	2788	2971
	Overfeeding	15.91	34.68	49.41	5174	4862	4594	2414
MS	Control	16.89	31.79	51.32	3316	2878	2990	3218
	Overfeeding	16.37	36.52	47.11	5470	5038	5150	5378
AG	Control	18.33	24.07	57.60	1587	2376	2002	2403
	Overfeeding	16.88	27.61	55.51	3747	4536	4162	4563

6.5 RESULTS

Daily energy intake increased by a mean of 1,873 Kcal with overfeeding, representing a mean percentage increase across conditions of 59.2% (range 49.2% to 69.46%). All subjects gained more weight while overfeeding than during the normal intake condition. Individual mean daily energy intake and weight change data are presented in Table 7.

As is evident in Figures 10 and 11, and Table 8, subjects replicated their Experiment 1 overfeeding response patterns by demonstrating an immediate and sustained increase in O_2 consumption and HR with energy overload. As no sequencing effects were evident between days and dietary condition, the data were averaged over replications and conditions.

As only three subjects were tested in this experiment, inferential analysis of the data was considered inappropriate (following Keppel, 1973). Overfeeding during placebo trails produced a mean increase in O_2

Table 7: Mean Daily Intake and Weight Change for Control and Overfeeding Conditions, Experiment 2.

Subject	Condition	Mean Daily Intake (Kcal)	Percentage Increase in Kcal consumed during Experimental Condition	Weight Change per Condition (gm)
GF	Control	2959		-560
	Overfeeding	4261	69.4	872
MS	Control	3100		250
	Overfeeding	5259	58.96	700
AG	Control	2092		100
	Overfeeding	4252	49.2	620

Table 8: Individual Mean HR and O_2 Consumption for Control/Overfeeding and Drug/Placebo Condition, Experiment 2.

Subject	Normal Intake				Overfeeding			
	Placebo		Propranolol		Placebo		Propranolol	
	HR (BPM)	O_2 (ml/min)	HR (BPM)	O_2 (ml/min)	HR (BPM)	O_2 (ml/min)	HR (BPM)	O_2 (ml/min)
GF	51.5	265.10	42.8	287.20	62.2	307.90	59.7	298.45
MS	56.3	383.17	53.2	389.20	62.8	447.17	60.1	505.40
AG	54.0	415.65	50.3	365.55	57.3	477.30	52.7	452.87

consumption and HR of 15.8% and 12.6% respectively. In propranolol trials, overfeeding resulted in a mean increase in these dependent variables of 20.6% and 17.9% respectively. As is apparent from these results, beta-adrenergic blockage did not reduce heightened MR arising

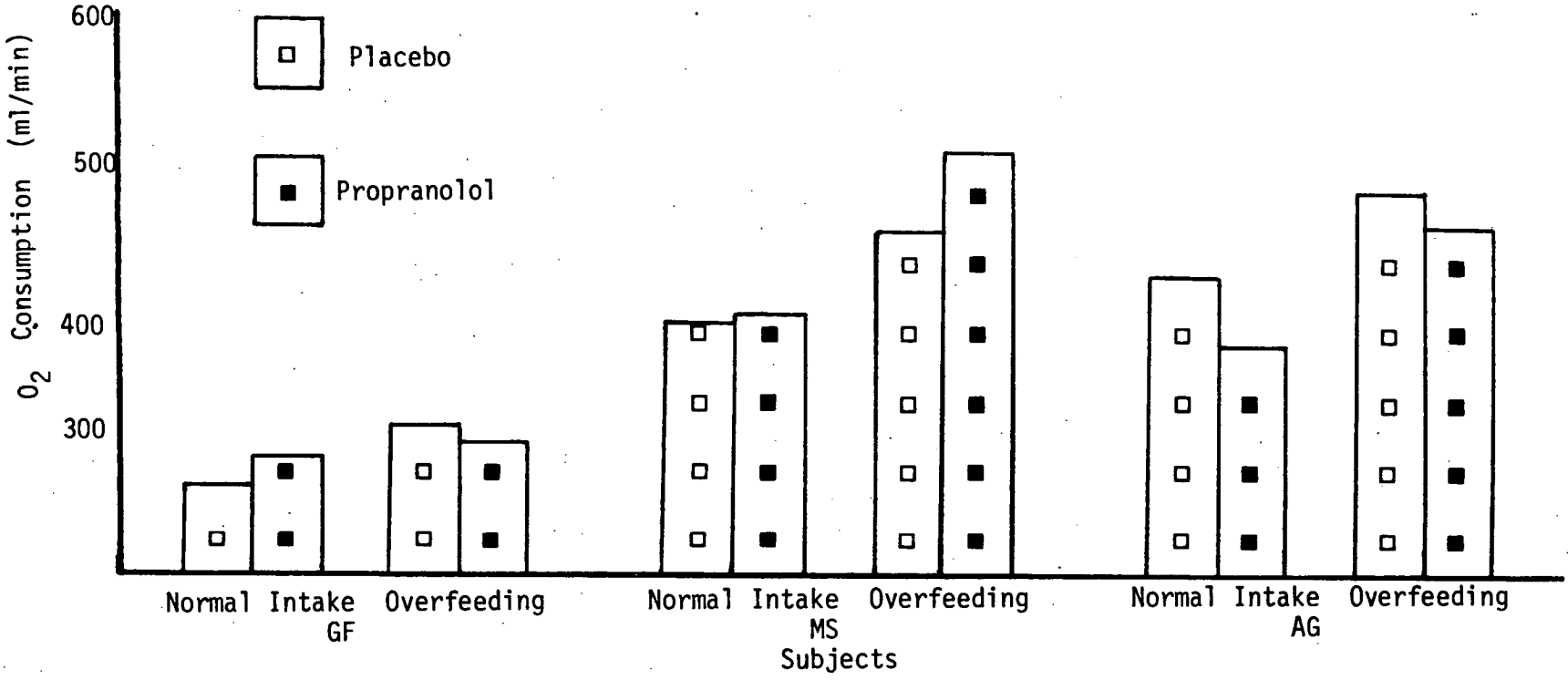

Figure 10: Individual mean O_2 Consumption during control/overfeeding and drug/placebo conditions, Experiment 2.

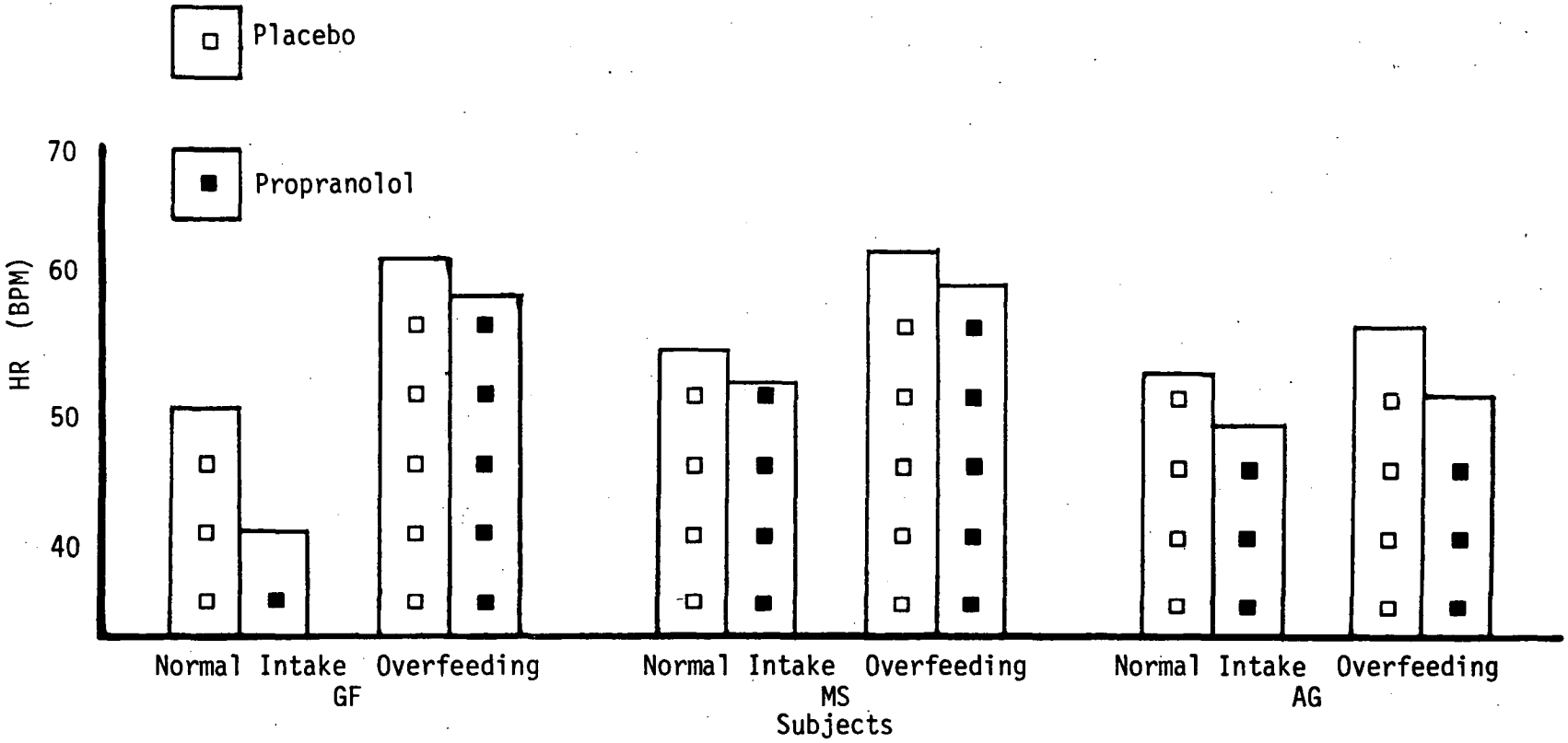

Figure 11: Individual mean HR during control/overfeeding and drug/placebo conditions, Experiment 2.

from overfeeding. Propranolol produced a similar mean decrease in HR in each dietary condition; -9.6% and -5.4% in normal intake and overfeeding trials respectively. O_2 consumption appeared unaffected by the antagonist presence, displaying a 2.1% decrease with normal intake and an increase of 2.0% with energy overload. Only one subject, GF, showed a marginally larger propranolol effect for O_2 consumption when overfed. As such the results offer no support for speculation regarding an inhibitory effect of beta-adrenergic blockers on DIT. postprandial DIT.

CHAPTER 7

DISCUSSION

In respect to the four research questions addressed by this thesis the following conclusions can be drawn. Firstly, although the increase in O_2 consumption just failed to reach significance in Experiment 1, the general pattern of both O_2 and HR results from the two studies support the conclusion that overfeeding produced an immediate and sustained metabolic response during both postprandial and resting state conditions in some individuals. In respect to idiosyncratic responding, however, DIT does not appear to be accurately reflected by stimulation with ephedrine. BAT activity, as determined by skin temperature increase, is not evident in postprandial thermogenesis or following ephedrine administration. In addition, the contribution of BAT to DIT is further called into question by the failure of propranolol to specifically block postprandial responding.

7.1 OVERFEEDING AND DIET-INDUCED THERMOGENESIS

The occurrence of an immediate increase in postprandial DIT with overfeeding, as shown in Experiment 1 and Experiment 2, does not in itself indicate the presence of a homeostatic mechanism for maintaining energy balance, since the effect may be entirely due to the metabolization of excess nutrient. Of greater significance to proposals concerning an adaptive component in DIT (i.e. Rothwell & Stock, 1981; James et al, 1979) is the indication of an immediately evident 7.14% mean rise in resting state O_2 consumption with energy overload. This observation supports the data obtained by Dauncey (1980) and stands contrary to Garrows (1978) proposal of a threshold for the response in the order of 23 Mcal.

As the increase in RMR is beneath 10% the possibility must be considered

that the result is artifactual and arises simply from subject arousal due to unpleasant proprioceptive stimulation caused by nutrient overload (see Garrow, 1974; 1978). This contention has some credence since pilot trials showed that normally fed individuals experienced mild discomfort laying quietly on their stomach for an hour while wearing a snugly fitting mask, an experience possibly exacerbated with nutrient overload. Notwithstanding this contention, two factors argue that DIT was demonstrated during the resting state. Firstly, the mean increase in MR approximates the mean rise of 10% - 12% observed by researchers using non-obtrusive metabolic room analysis (i.e. Apfelbaum et al, 1971; Dauncey, 1980), thereby suggesting that the result is not artefactual. Secondly, and more importantly, as in other studies only a proportion of subjects demonstrated considerable RMR increase with overfeeding; Table 4 shows that two subjects evidenced a rise in O_2 consumption of approximately 20% in the experimental condition. This increase strongly suggests that certain individuals possess the ability to dissipate excess energy during the resting state. It should also be appreciated that two other subjects showed a negligible change in RMR following five days overfeeding (i.e. a mean change of 3.95% and -0.27% respectively). In respect to these findings, a realistic conclusion regarding the time course of resting state DIT is that some individuals immediately show the response in varying degrees following an excess daily intake of approximately 1,900 Kcals, while other individuals do not manifest the response during a short term overfeeding regimen.

It is apparent that the two counter opinions regarding the overfeeding stimulus necessary to initiate resting state DIT, as found in Garrow (1978) and Dauncey (1980), may in fact be reconcilable. This suggestion

of conciliation arises from the unexpected finding of a high correlation between a subjects mean change in O_2 consumption across testing periods with overfeeding, and his percentage body fat. The relationship suggests that DIT is a function of dietary history. Thus, prolonged excessive energy intake may have the dual effect of increasing fat stores and stimulating DIT response ability, while dietary restraint may deplete fat stores and weaken a homeostatic response capacity. As such, individuals with appreciable fat stores would be expected to demonstrate a resting DIT response following a single days overfeeding and lean individuals would be expected to show the response only after considerable energy overload, possibly in the order of 23 Mcal (i.e. as proposed by Garrow, 1978).

This suggestion of DIT responsiveness vis a vis dietary history gains some support from both the animal and human literature. Rothwell and Stock (1979) found that cafeteria fed rats relative to control littermates evidence both the greatest percentage body fat and sensitivity to norephinephrine infusion. The conclusion that thermogenic responsiveness can be stimulated is also drawn by Davis (1961) following demonstration of previously non-existant NST in repeatedly cold exposed individuals. Further, the prediction that dietary restraint prevents DIT development obtains support from Dauncey's (1980) observation that the only subject in her study to show no increase in RMR with overfeeding was an individual who had maintained his body-weight constant for six months on an intake of approximately 1,453 Kcal/day. The data presented by York et al (1980) and Morgan et al (1982) can likewise be interpreted as supporting the dietary prediction since low energy intake subjects demonstrated a diminished MR increase following both food

consumption and ephedrine administration when compared to the response of high energy intake subjects.

It should be appreciated that the relationship observed between O_2 consumption and percentage body fat is in conflict with Dauncey's (1980) absorbative phase data, recalculated and presented by James and Trayhurn (1981b), which indicate a strong inverse relationship between DIT and body fat. The implication drawn by these researchers is that diminished DIT response ability, a suggested constitutional disposition, orients an individual toward obesity. In respect to the above discussion, it may be the case that the Cambridge focus on genetic predisposition is too simplistic, and that while it may determine efficiency of nutrient metabolism in some individuals, others may develop the response by challenging their energy balance. However, as only seven subjects were run in Experiment 1, and Dauncey's (1980) research employed only eight subjects, it should be borne in mind that conclusions drawn from these populations can at best be regarded as tentative.

7.2 DETERMINATION OF DIET-INDUCED THERMOGENESIS RESPONSE ABILITY THROUGH EPHEDRINE STIMULATION

Considered from the standpoint of speculation in the current literature that both DIT and NST are mediated by a common beta-adrenergic mechanism (e.g. Rothwell & Stock, 1981b; James & Trayhurn, 1981b), the stratified findings presented by Morgan et al (1982) concerning thermic responses in high and low energy intake subjects to both food consumption and ephedrine administration can be seen as suggesting concomitance between an individuals DIT and his/her response to a sympatheticomimetic

drug. Support for this suggestion was not found in Experiment 1.

The negative result does not appear attributable to inadequate sympathetic stimulation as Morgan et al's (1982) data suggests that the ephedrine dosage employed (1mg/kg body weight) is sufficient to produce NST. It may be the case however, following the Cambridge group's speculation of a relationship between dietary fat and DIT, that greater unanimity between DIT and ephedrine stimulation will occur with a particular diet and/or caloric intake. Further, the observation that one subject failed to show both an overfeeding and an ephedrine response suggests that ephedrine administration may identify non-DIT responders in subject populations.

The inverse relationship observed between mean increase in postprandial HR with overfeeding and ephedrine stimulated HR most probably reflects Garrow's (1978) point that while HR is an adequate second order index of MR, it is also a prime index of arousal. It appears that levels of individual discomfort during testing disallowed observance of a positive relationship between overfeeding and ephedrine trials.

7.3 BROWN ADIPOSE TISSUE ACTIVITY

The data obtained in Experiment 1 and 2 fails to provide evidence for BAT mediated DIT. This result conforms with the finding that ephedrine stimulated MR does not predict DIT.

In respect to Experiment 1, the dosage level of ephedrine administered could be expected to stimulate BAT responding (following Rothwell & Stock, 1979). Further, it appears unlikely that a grid comprising

ten thermocouple points placed over probable BAT sites would have been insufficient to detect localized temperature change in at least one subject. Thus, the result obtained must be viewed as challenging the BAT hypothesis.

A propranolol dosage level of 1 mg/kg body weight, employed in Experiment 2, falls approximately at the mean of the usual medical dosage range (Bennett, 1982) and as such could be expected to block beta-adrenergic receptor sites. According to Bennett(1982), however, effective beta-adrenergic blockage reduces resting cardiac output by 25% and exercise-induced tachycardia by about one third. In Experiment 2, mean HR decreased by 5-7% in the overfeeding/propranolol condition. Thus, the possibility can not be excluded that approximately 80 mg of propranolol per subject was insufficient to counteract agonist presence at receptor sites.

Notwithstanding this possibility, it should also be appreciated that the BAT hypothesis has recently been disputed by Hervey and Tobin (1983) on the grounds that propranolol fails to inhibit DIT. These authors report that 15 mg/kg body weight of propranolol did not affect O_2 consumption in rats following tube-fed normal and oversized meals, and cite research indicating that 80 mg of propranolol had no influence on human MR following ingestion of 250 g of glucose. The result obtained in Experiment 2 is consistent with the findings presented by Hervey and Tobin (1983).

7.4 CONCLUSIONS AND RECOMMENDATIONS

In agreement with the results of Dauncey (1980), it appears that

resting state DIT can occur following a single days energy overload. An individuals capacity for DIT, however, may in some cases depend upon development of the response through overfeeding. It is therefore suggested that research be directed to examining the influence of dietary history on DIT propensity. This proposal is a considerable departure from the present orientation of the literature which for the most part has singularly focused upon a genetic basis for thermogenic responsiveness.

In line with this proposal, it also seems reasonable to suggest a less impoverished acknowledgement of factors that may effect obesity. At present, subject classification is 'monochromatic', involving either an obese/lean distinction (e.g. Shetty et al, 1979; Jung, Shetty, James, Barrand & Callingham, 1979) or comparison of high versus low energy intake individuals (e.g. Morgan et al, 1982). This energy status approach detracts from data replication since it overlooks other potential influences on energy balance; e.g. physical exercise incurred either during employment or through sport (see Thompson, Jamie, Lahey & Cureton, 1982) and psychological influences on diet and metabolism (see Bludnell, 1980; Rodin, 1981). Indeed, it is surprising that the considerable individual differences in DIT repeatedly presented in the literature has not prompted researchers to more stringently appraise subject characteristics.

Although a strong relationship between DIT and ephedrine stimulated MR was not found, further work in this area is recommended. The impetus for research arises from two sources: (a) studies which suggest that a beta-adrenergic mechanism underlies DIT (e.g. Jung, Shetty, James, Barrand & Callingham, 1979; Morgan et al, 1982), and (b) the Cambridge Group's proposal regarding a link between dietary fat and

DIT. In respect to this literature, investigation should be directed to examining the relationship between sympathetic stimulation and DIT resulting from a particular diet.

Although the ability of moderate propranolol doses to inhibit DIT may be called into question, the result obtained in both Experiments does not auger well for the BAT hypothesis in man. At present, the proposal is supported only by drug stimulation research and an inability to replicate this data (i.e. in Experiment 1) bolsters Hervey and Tobin's (1983) thesis that there is no strong human evidence for the organ's thermogenic output. As the BAT hypothesis is strongly advocated in the current literature there is an imperative need for invasive research into BAT occurrence and activity in adults. For example, microcalorimetry investigation of agonist/antagonist effects on the heat production of brown adipocytes obtained by percutaneous biopsy. Such research, already being conducted in respect to metabolic activity of white adipocytes (Sorbris, Monti, Nilsson-Ehle & Wadso, 1982), will answer the fundamental question of BAT's capacity for thermogenesis. There is also a pressing need to continue the line of research undertaken in Experiment 1 to determine the role of BAT in DIT. Ideally, future studies will employ a combination of thermogramic survey and biopsy assay to evaluate BAT responding.

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APPENDICES

APPENDIX A: Informed Consent Form for participation in Experiment 1.

INFORMED CONSENT TO PARTICIPATE IN AN EXPERIMENT

I agree to participate in an experiment, conducted in the Psychology Department of the University of Tasmania by the Departments of Psychology and Anatomy, on the effects of the sympatheticomimetic drug Ephedrin and overfeeding on metabolic rate and skin temperature. I understand that the dose of the drug to be given is 1 mg/kg body weight, and that on a separate occasion I will be required to consume approximately 2,000 kcal excess food daily for five days in the form of ENSURE PLUS, a high calorie liquid nutrition. I have had the nature of the possible side effects of the ephedrin explained to me by either Dr. Wallace or Dr. Trinder. I understand that I should not be taking any medication during the course of the experiment (unless reported to Dr. Wallace or Dr. Trinder) and that I should not exercise on the days on which the drug is given. I do not have, to the best of my knowledge, any medical problem which would be affected by a stimulant such as ephedrine. I agree to volunteer to participate in the experiment.

Signed_____ Date_____

Witness_____ Date_____

APPENDIX B: Daily Nutrient Intake Recording Sheet.

Subject.....

Date.....

Condition.....

TIME	TYPE OF FOOD	AMOUNT	PROTEIN 9.	FAT 9.	CARBO-HYDRATE 9.	kcal
WAKE TO END OF BREAKFAST						
BREAKFAST TO LUNCH						
LUNCH						
LUNCH TO DINNER						
DINNER						
DINNER TO BED						
DURING NIGHT						
TOTAL						

APPENDIX C: Summary of the ANOVA conducted on the Overfeeding O_2 Consumption Data.

Source	DF	MS	F	P
S	6	38787.56021	206.2846	<.001
C	1	6605.69029	4.5928	NS
D	4	575.70856	1.0579	NS
P	1	20010.41653	18.2017	<.01
T	2	179.98970	1.2704	NS
SC	6	1438.26750	7.6492	<.001
SD	24	544.22237	2.8293	<.001
SP	6	1099.37193	5.8468	<.001
ST	12	141.67876	0.7535	NS
CD	4	345.90955	0.5781	NS
CP	1	670.80482	0.6754	NS
CT	2	47.45023	0.2100	NS
DP	4	308.15623	0.9048	NS
DT	8	186.79862	1.4076	NS
PT	2	159.30737	0.7377	NS
SCD	24	598.39935	3.1825	<.001
SCP	6	993.19521	5.2821	<.001
SCT	12	225.99458	1.2019	NS
SDP	24	340.59258	1.8114	<.05
SDT	48	132.71036	0.7058	NS
SPT	12	215.94716	1.1485	NS
CDP	4	321.09735	0.7717	NS
CDT	8	279.48021	1.6676	NS
CPT	2	759.38120	5.7494	<.05
DPT	8	740.05832	0.4950	NS
SCDP	24	416.10404	2.2130	<.01
SCDT	48	167.83342	0.8926	NS
SCPT	12	132.07948	0.7024	NS
SDPT	48	149.62507	0.7958	NS
CDPT	8	157.56076	0.8380	NS
SCDPT	48	188.02938	-	-

S=Subjects
D=Days
C=Conditions (Overfeeding and Normal Intake)
P=Periods (Postprandial and Resting State)
T=Times (40, 50, and 60 minutes, respectively)

APPENDIX D: Summary of the ANOVA conducted on the Overfeeding HR Data.

Source	DF	MS	F	P
S	6	1935.9984127	90.3274	<.001
C	1	829.8047619	48.6858	<.001
D	4	714.6833333	11.9846	<.001
T	2	267.3190476	11.7033	<.01
SC	6	18.3380952	0.8556	NS
SD	24	59.6333333	2.7823	<.01
ST	12	22.8412698	1.0657	NS
CD	4	83.8642857	1.3102	NS
CT	2	20.4619048	1.0887	NS
DT	8	27.2119048	1.1625	NS
SCD	24	64.0087302	2.9864	<.001
SCT	12	18.7952381	0.8769	NS
SDT	48	23.4077381	1.0921	NS
CDT	8	35.5928572	1.6606	NS
SCDT	48	21.4331349	-	-

S = Subjects
C = Conditions (overfeeding and normal intake)
D = Days
T = Times (40, 50, 60 minutes respectively)

APPENDIX E: Summary of the ANOVA conducted on the Overfeeding Skin Temperature Data.

Source	DF	MS	F	P
S	5	4.01	12.10	<.001
C	1	0.71	0.06	NS
D	4	10.73	2.88	<.05
P	3	25.30	19.08	<.001
T	2	0.89	5.73	<.01
SC	5	12.01	36.30	<.001
SD	20	3.72	11.25	<.001
SP	15	1.33	4.00	<.001
ST	10	0.16	0.47	NS
CD	4	2.13	0.47	NS
CP	3	0.03	0.02	NS
CT	2	1.08	1.61	NS
DP	12	0.95	1.34	NS
DT	8	0.34	0.40	NS
PT	6	0.18	0.75	NS
SCD	20	4.57	13.82	<.001
SCP	15	1.24	3.75	<.001
SCT	10	0.67	2.02	<.04
SDP	60	0.71	2.14	<.001
SDT	40	0.86	2.61	<.001
SPT	30	0.25	0.74	NS
CDP	12	0.56	0.78	NS
CDT	8	0.74	0.81	NS
CPT	6	0.16	0.42	NS
DPT	24	0.29	0.82	NS
SCDP	60	0.72	2.19	<.001
SCDT	40	0.92	2.78	<.001
SCPT	30	0.38	1.14	NS
SDPT	120	0.35	1.06	NS
CDPT	24	0.57	1.73	<.01
SCDPT	120	0.33	-	-

S = Subjects
C = Conditions
D = Days
P = Temperature Probe Placement
T = Time within sessions

APPENDIX F: Summary of the ANOVA conducted on the Ephedrine O_2 Consumption Data.

Source	DF	MS	F	P
S	6	5054.004219	56.0413	<.001
C	1	3314.800896	16.8378	<.01
T	8	531.075877	3.1702	<.01
SC	6	196.867183	2.1830	NS
ST	48	167.522677	1.8576	<.05
CT	8	282.291592	3.1302	<.01
SCT	48	90.183638	-	-

S = Subjects

C = Conditions (Ephedrine and Placebo)

T = Times (40 - 120 minutes respectively)

APPENDIX G: Summary of the ANOVA conducted on the Ephedrine HR Data.

Source	DF	MS	F	P
S	6	288.2354498	97.6209	<.001
C	1	276.3888889	5.6131	NS
T	8	94.6448413	3.2596	<.01
SC	6	84.8703704	2.8765	<.05
ST	48	29.0360450	0.9841	NS
CT	8	59.0853175	2.0026	NS
SCT	48	29.5042989	-	-

S = Subjects

C = Conditions (Ephedrine and Placebo)

T = Times (40 - 120 minutes respectively)

APPENDIX H: Summary of the ANOVA conducted on Ephedrine Skin Temperature Data.

Source	DF	MS	F	P
S	5	9.21	168.48	< .001
C	1	0.14	0.06	NS
P	3	15.22	5.58	< .009
T	8	1.02	1.08	NS
SC	5	2.29	41.92	< .001
SP	15	2.73	49.89	< .001
ST	40	0.95	17.41	< .001
CP	3	0.37	0.33	NS
CT	8	0.97	1.24	NS
PT	24	0.53	0.99	NS
SCP	15	1.12	20.52	< .001
SCT	40	0.78	14.34	< .001
SPT	120	0.05	0.99	NS
CPT	24	0.06	1.05	NS
SCPT	120	0.05	-	-

S = Subjects

C = Conditions

P = Temperature probe placement

T = Times within sessions

APPENDIX I: Informed Consent Form for participation in Experiment 2.

INFORMED CONSENT TO PARTICIPATE IN AN EXPERIMENT

I agree to participate in an experiment, conducted in the Psychology Department of the University of Tasmania by the Departments of Psychology and Anatomy, on the effects of the sympathetic antagonist drug Propranolol vis a vis overfeeding. I understand that the doses of the drug to be given are 80 mg per trial, and that I will be required to consume approximately 2,000 kcal excess food daily for five days in the form of Ensure Plus, a high calorie liquid nutrition. I have had the nature of the possible side effects of the propranolol explained to me by either Dr. Wallace or Dr. Trinder. I understand that I should not be taking any medication during the course of the experiment (unless reported to Dr. Wallace or Dr. Trinder) and that I should not exercise on the days on which the drug is given. I do not have, to the best of my knowledge, any medical problem which would be affected by a sympathetic antagonist such as propranolol. I agree to volunteer to participate in the experiment.

Signed_____Date_____

Witness_____Date_____