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THE EFFECT OF EXERCISE ON RAT BROWN ADIPOSE TISSUE

By

© LOUISE ROBB

Thesis submitted to the School of Graduate Studies in partial fulfillment of the requirements for the degree of Master of Science in Kinanthropology

University of Ottawa

Ottawa, Canada, 1989

Louise Robb, Ottawa, Canada, 1989.

To Mark

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ABSTRACT

The purpose of this study was to determine the effect of chronic endurance exercise on brown adipose tissue size and function in the rat.

Young male Wistar rats were randomly allocated to experimental and control groups. The experimental rats were taught to run on a motorized treadmill and were gradually trained over a period of ten weeks so that they could run continuously for one hour, once a day, 5 days per week, at 25 meters/min on an incline of 10°.

The control rats were (as much as possible) treated the same as the experimental group except that they did not take part in the exercise process. All rats were kept at thermoneutral temperature (27°C) to avoid cold-induced changes in their brown adipose tissue. They were housed in separate cages and fed Lab chow ad lib.

At the end of the training program the rats were sacrificed by decapitation. The brown adipose tissue of each rat was excised and assayed to detect any changes that could have occurred in several biochemical parameters. Protein and DNA content were assayed to assess the size of the brown adipose tissue active mass and the brown adipose tissue cellularity respectively (Himms-Hagen 1983).

Cytochrome oxidase activity was measured to get an indication of the mitochondrial mass and oxidative capacity of the brown adipose tissue (Himms-Hagen 1983). Purine nucleotide binding was measured to determine the specific thermogenic capacity of the brown adipose tissue mitochondria (Himms-Hagen 1983).

All data were expressed as means +/- standard deviations. The statistical analysis used was a two-tailed Student "t"-test. A value of P>0.05 was considered to define the limit where results were not significant.

The results showed a significant increase in brown adipose tissue size in the exercise-trained rats. The interscapular brown adipose tissue protein content and DNA content were found to be significantly increased in the exercised group.

When compared to rat weights, these parameters were also significantly higher in the exercise-trained rats. The total cytochrome oxidase activity to rat weight ratio was also significantly higher in the trained rats.

The total cytochrome oxidase activity, total GDP binding, and total GDP binding capacity to rat weight ratio, were also higher in the trained rats, however these differences were not significant.

The rat weights were significantly lower in the exercise-trained group while the heart weight to rat weight ratios were significantly higher. These results indicate a training effect took place.

The interscapular brown adipose tissue wet weights and the rat food intakes were not found to be significantly different.

These findings are discussed in relation to other studies which have measured some of the same biochemical parameters. Exercise-induced hormonal changes and their possible influences on the results in this and other studies are also discussed.

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CHAPTER I

INTRODUCTION

THE PROBLEM

Obesity is a health problem of epidemic proportions in the Western World. Approximately 30% to 50% of males and 20% to 40% of females over 25 years of age are classified as overweight in Canada, the United States (data for whites only), and Britain, according to the body mass index (Millar & Stephens 1987). Weight control has developed into a several billion dollar ;industry, yet treatment has been woefully ineffective in the long term -- most adults who try to lose weight will regain it. Obesity has thus come to be known as a chronically recurring and self-propagating disorder.

It is axiomatic that obesity results from an energy imbalance, yet the traditional belief that overeating causes this imbalance has been challenged -- at least in some cases (Miller & Parsonage 1975). Studies have found that some overweight people have low energy expenditure due to a low basal metabolic rate (Miller & Parsonage 1975) or a low level of diet-induced thermogenesis (Jequier 1983; Schwartz et al 1983b), thus showing that they suffer from a low energy output rather than an excessive energy input.

Diet-induced thermogenesis is the production of heat that accompanies overeating and is not due to energy used in food storage or waste disposal (Himms-Hagen 1976). In some animals this phenomenon has been shown to occur as a result of brown adipose tissue thermogenesis (Rothwell & Stock 1979).

This link between brown adipose tissue and diet-induced thermogenesis has led to the proposal that brown adipose tissue plays a major role in the regulation of weight (Himms-Hagen 1983, 1983c; Rothwell & Stock 1979).

Brown adipose tissue thermogenesis has also long been known to be responsible for cold-induced non-shivering thermogenesis (Foster & Frydman 1978). Stimulation of brown adipose tissue thermogenesis during both diet-induced thermogenesis and cold-induced non-shivering thermogenesis occurs through the sympathetic nervous system (Jessen 1980; Rothwell & Stock 1979).

The sympathetic nervous system is also implicated in the onset of exercise (Astrand & Rodahl 1977) and studies on exercise have shown that cold tolerance can be improved in humans (Anderson et al 1966; Herberling & Adams 1960) and rats (Chin et al 1978; Stromme & Hammel 1967) by physical activity.

It has also been shown that moderate exercise may potentiate diet-induced thermogenesis in humans (Miller et

al 1967, Miller & Wise 1975b) and rats (Gleeson et al 1979, 1979b, 1982).

The possibility that exercise can affect both cold-induced non-shivering thermogenesis and diet-induced thermogenesis, as well as the role that the sympathetic nervous system plays in both brown adipose tissue thermogenesis and exercise, points to the possibility that exercise may have an effect on brown adipose tissue.

If exercise does affect brown adipose tissue thermogenesis then it must be assumed to have more influence on the energy balance than simply to increase energy output through muscular activity. This implies that exercise could have a more complex role in regulating obesity than is presently known.

AIM OF THE STUDY

This study will attempt to determine whether an aerobic training program of 10 weeks, involving running for 5 days a week on a treadmill for one hour at 25 meters/min at an incline of 10°, will significantly affect brown adipose tissue size or thermogenic capacity in a group of trained rats as compared to a control group.

Brown adipose tissue size and thermogenic capacity will be determined by measuring brown adipose tissue protein

content, DNA content, cytochrome oxidase activity, and mitochondrial GDP binding capacity.

LIMITATIONS

The study will use male Wistar rats. Therefore extrapolation of the results to females, other strains of rats, or other species (such as humans) is limited. Also, financial and temporal constraints limit the size of the test groups to 13 exercise-trained subjects and 10 controls.

The type of exercise used in this study involves running on a motorized treadmill. Therefore comparisons with other forms of exercise (such as swimming) are limited. It should also be noted that due to the machinery available, the exercise-trained rats will regularly be subjected to the stresses of electric shock and noise when on the treadmill. The control rats will not experience such stress.

CHAPTER II

REVIEW OF LITERATURE

PREFACE

Brown adipose tissue (BAT) is an organ that specializes in heat production (Himms-Hagen 1976, 1983, 1983c). It has long been known to play an important role in cold acclimation of animals, such as hibernators, through a process known as cold-induced non-shivering thermogenesis (Himms-Hagen 1983). More recently BAT thermogenesis has been shown to be stimulated by overeating, thus contributing to diet-induced thermogenesis and implicating BAT in weight regulation (Himms-Hagen 1983).

This study deals with the possibility that physical activity may also act as a stimulus for BAT thermogenesis. Most of the work done on brown adipose tissue has involved studies on rodents. Therefore this review will deal primarily with evidence of brown adipose tissue growth and function gathered from animal studies. Work that has involved humans will be stated specifically.

BROWN ADIPOSE TISSUE

BAT is found throughout the body in a number of small deposits (Himms-Hagen 1983, 1983b, 1983d). It is primarily found in the interscapular, subscapular and cervical regions, at the nape of the neck, the axillary and perirenal areas, as well as along the great vessels in the thorax and abdomen, and between the ribs (Himms-Hagen 1983, 1983b).

BAT is richly supplied with sympathetic (Himms-Hagen 1976, 1983, 1983b, 1983d; Nnodim & Lever 1985), vasomotor and parenchymal (Nnodim & Lever 1985) innervations. The vascular supply to BAT is also extensive (Himms-Hagen 1983, 1983b, 1983d). This allows heat to be dissipated rapidly and oxygen and substrates to be supplied rapidly as well (Himms-Hagen 1983).

Rapid communication is also promoted between BAT adipocytes through their many gap junctions (Himms-Hagen 1976). Cells of active BAT are multilocular, unlike white adipose tissue (WAT) cells which are larger and unilocular (Himms-Hagen 1976, 1983, 1983b, 1983c, 1983d). However cells of inactive BAT can have fat droplets so full that they appear to resemble unilocular WAT cells(Himms-Hagen 1983b).

BAT Mitochondria

Also contrasting with WAT is the fact that BAT mitochondria are larger and more plentiful (Himms-Hagen

1983, 1983b; Nnodim & Lever 1985). BAT mitochondria are distinct from those of other tissues in that they have multiple (Astrup 1986), densely packed cristae (Himms-Hagen 1976, 1983, 1983d) and a special proton conductance pathway.

When BAT is thermogenically active, this pathway allows some protons to pass through the inner mitochdrial membrane without being associated with oxidative-phosphorylation (Himms-Hagen 1983, 1983b, 1983d). This uncoupling of oxidative-phosphorylation is what enables BAT to produce heat (Himms-Hagen 1976, 1983d).

At the center of this uncoupling path is a 32 kDa uncoupling protein (UCP) found in the inner membrane of BAT mitochondria (Bukowiecki 1984). This protein is unique to BAT (Bouillaud et al 1985; Cadrin et al 1985; Hansen et al 1984) and immunological studies (Afong et al 1985; Hansen et al 1984; Lean & James 1983), cDNA probing of UCP mRNA's (Bouillaud et al 1985), sequencing of UCP's (Ridley et al 1986), and size evaluation by electrophoresis (Freeman et al 1985) have shown close similarities between UCP's from many species including hamsters and rats (Afong et al 1985; Bouillaud et al 1985; Ridley et al 1986), mice (Afong et al 1985; Bouillaud et al 1985), rabbits (Freeman et al 1985), and even humans (Afong et al 1985; Freeman et al 1985; Lean & James 1983).

The UCP from different species have size variations of about 1.5 to 2.0 kDa (Freeman et al 1985) with the human UCP being about 1 kDa smaller than that of the rat (Afong et al

1985). The size sequence of UCP's is: human UCP < rat <= mouse < hamster <= rabbit (Freeman et al 1985).

Klingenberg & Winkler (1985) found the UCP to be a proton translocator driven by the membrane potential. The passage of protons via this uncoupling pathway is regulated by purine nucleotides which bind and inactivate BAT mitochondrial UCP, and are released from UCP's of thermogenically active BAT (Himms-Hagen 1983, 1983b).

The translocation site, which is weakly dependent on pH, appears to be different from the highly pH-dependent purine nucleotide binding site (Klingenberg & Winkler 1985). However a cysteine residue has been found to be part of the purine nucleotide binding site of BAT UCP (Rial & Nicholls 1986) and an essential sulfhydryl group has been shown to be involved in UCP-mediated H+ transport (Jezek 1987).

It is believed that long chain fatty acids (Bukowiecki 1984, 1985; Cunningham et al 1986; Himms-Hagen 1983, 1983b; LaNoue et al 1986; Rial et al 1983; Strieleman et al 1985) or long chain fatty acyl Coenzyme A esters (Himms-Hagen 1983; Strieleman & Shrago 1985b) are released when BAT is stimulated and are responsible for activating the proton conductance path by reducing the binding of purine nucleotide to the UCP. Thus lipolysis and respiration are functionally coupled in BAT (Bukowiecki 1985). After the fatty acids activate the proton conductance path, they enter

the mitochondria to be used as a substrate for beta-oxidation (Bukowiecki 1984).

When BAT thermogenesis is stimulated the mitochondrial uncoupling path is activated within an hour (Himms-Hagen 1983; Swick & Swick 1986), which has been thought to be too rapid for de novo synthesis of the UCP (Horwitz et al 1985). Furthermore, this rapid activation of the uncoupling path is reversible (Himms-Hagen 1983; Swick & Swick 1986). This has led to the suggestion that the UCP can exist in masked forms (Ashwell et al 1985; Gribskov et al 1986; Himms-Hagen & Triandafillou 1981, Himms-Hagen 1983).

The existence of such masked or unavailable forms of UCP has been disputed (Nedergaard et al 1984, Nedergaard & Cannon 1985; Rial & Nicholls 1984). In fact in stimulated BAT the amount of UCP mRNA has been found to increase very rapidly (Ricquier et al 1984, 1986), implying a rapid onset of de novo synthesis of the UCP. However, changes in purine nucleotide binding without changes in UCP concentration have been shown (Desautels 1985; Himms-Hagen & Triandafillou 1981), thus supporting the existence of masked forms of UCP. Also supporting this is the finding that washing with albumin increases the number of GDP binding sites of BAT mitochondria from cold-adapted as well as non-adapted rats (Gribskov et al 1986).

The rapid increase in GDP binding to BAT mitochondria which occurs when BAT thermogenesis is stimulated can be

measured. This provides a sensitive way to detect the onset of BAT thermogenesis (Himms-Hagen 1983).

BAT Na+/K+ ATPase

It has been suggested that an increase in BAT Na+/K+ ATPase activity also contributes to thermogenesis in BAT cells (Chinet et al 1977; Rothwell et al 1981b). The significance of this contribution has however been questioned (Svoboda et al 1986). As well there is some question as to whether this mechanism can operate at the same time as the uncoupling path, since uncoupling would limit the ATP available for the Na+/K+ ATPase pump (Himms-Hagen 1979).

Nevertheless an increase in Na+/K+ ATPase activity in BAT has been seen in rats that overeat when fed a cafeteria diet. This increase correlates well with the increase in VO2 that is also seen (Rothwell et al 1981b). As well norepinephrine, which stimulates brown adipose tissue thermogenesis (see p.21), causes a beta-mediated, cAMP-dependent increase in Na+ influx in BAT (Connolly et al 1986).

BAT in Humans

The importance of BAT in humans is not yet fully established. It has long been thought to be of little importance in adult humans (Himms-Hagen 1983) although it is

known to produce heat in human infants (Blaza 1983; Dawkin & Hull 1965). There is a growing body of evidence, however, which demonstrates similar metabolic behavior in human and experimental animals in respose to both food and cold (Himms-Hagen 1983). This metabolic behavior in experimental animals has been attributed to BAT (Himms-Hagen 1983). BAT deposits have been found in adult humans (Astrup 1986; Blaza 1983; Himms-Hagen 1983c), but to what degree this BAT is functional is still in question.

It is difficult to assess the functional capacity of human BAT because the best known quantitative methods for doing this involves the use of radioactive microspheres to measure blood flow (Foster & Frydman 1978; Himms-Hagen 1983b), which cannot be done in humans. Qualitative methods of measuring BAT capacity, such as thermography or thermosensitive implants, have demonstrated that after consuming the BAT-stimulating drug ephedrine, adult humans show an increase in heat production in areas where BAT has been found in lab animals (Rothwell & Stock 1979). However it has been argued that this heat production is due simply to an increase in blood flow and not to BAT thermogenesis (Astrup et al 1984).

Astrup (1986) attempted to measure the response of human interscapular brown adipose tissue to ephedrine using the Xenon-133 clearance method which measures the blood flow directly. After verifying that this method was valid by

comparing it to the microsphere technique developed by Foster & Frydman (1978), they measured the blood flow across interscapular areas that had been shown, using thermography, to be warmest when stimulated by ephedrine. These measurements, both from before and after ephedrine stimulation, were found to be similar to blood flow measurements of white adipose tissue from the lumbar region.

This apparent lack of active brown adipose tissue in the interscapular area of humans was confirmed by the absence of brown adipocytes in histologically examined biopsies of the interscapular area. In fact necropsy studies that have involved histological studies have found that few cases had brown adipose tissue deposits in the interscapular area (Astrup 1986). Perirenal brown adipose tissue deposits were more frequently found, though the abundance of brown adipocytes was small and the deposits were not found to be active.

Astrup has suggested that in humans skeletal muscle plays a greater thermogenic role than brown adipose tissue. He argues that, though skeletal muscle contributes only a minor percentage of cold-induced nonshivering thermogenesis in rodents, the more favorable surface area to volume ratio in humans may allow that skeletal muscle thermogenesis is sufficient.

Furthermore, measuring adrenergic stimulation of skeletal muscle oxygen consumption has led him to suggest

that 50% of human thermogenesis could be due to skeletal muscle (Astrup 1986). Skeletal muscle fibers, however, are not thought to receive significant sympathetic nervous system innervation, though indirect stimulation by norepinephrine released nearby could occur. The mechanism by which skeletal muscle could contribute to thermogenesis might involve the Na+/K+ pump (Astrup 1986) or futile cycles (Newsholme 1980).

Evidence pointing to the possibility of functional BAT existing in humans is the finding of the 32k UCP in infants (Hull & Vinter 1984; Lean et al 1986) and, to a lesser extent, in adults (Afong et al 1985; Freeman et al 1985; Lean et al 1986; Nicholls et al 1985). As well, like BAT from other species, human BAT has been found to have the biochemically unique features that give it the potential for thermogenesis (Lean et al 1986b; Lever et al 1986).

Furthermore, the importance of BAT thermogenesis in human metabolism is demonstrated by the fact that humans undergo both diet-induced thermogenesis (Acheson et al 1984; Danforth & Sims 1983; Kasper et al 1973; Miller & Parsonage 1975) and cold-induced non-shivering thermogenesis (Blaza 1983; Jessen 1980).

Although there is no clear evidence that these phenomena are responses to BAT activity, there is circumstantial evidence in the fact that cold-induced non-shivering thermogenesis (Jessen 1980; Jung et al 1979)

and diet-induced thermogenesis (Jung et al 1979) in humans are similar to those in lab animals and both cold-induced non-shivering thermogenesis and diet-induced thermogenesis have been attributed to BAT activity in these animals (Himms-Hagen 1983).

As well, human brown adipose tissue has been shown to undergo adaptive changes typical of thermogenically active brown adipose tissue in patients with conditions resulting in high levels of circulating catecholamines (Astrup 1986).

FACULTATIVE THERMOGENESIS

Facultative thermogenesis, a component of energy expenditure, has two forms, cold-induced non-shivering thermogenesis (CINST) and diet-induced thermogenesis (DIT) (Himms-Hagen 1979). Cold-induced non-shivering thermogenesis has the same function as shivering thermogenesis: the production of heat during exposure to cold; however it does not involve muscle contraction. Diet-induced thermogenesis refers to an increase in metabolic rate that accompanies overeating (Himms-Hagen 1979).

Cold-Induced Non-Shivering Thermogenesis

The degree of CINST varies from one animal species to another (Himms-Hagen 1976). It is greater in smaller animals, hibernators, and cold-acclimated animals

(Himms-Hagen 1976). It is also greater in newborn animals than adults (Himms-Hagen 1976). Generally characteristic of animals that are homeotherms (James & Trayhurn 1981), CINST allows for the maintenance of constant body temperature despite a decrease in the environmental temperature.

Diet-Induced Thermogenesis

DIT is the production of heat accompanying overeating which is not due to the absorption or storage of food or to the disposal of waste (Himms-Hagen 1976). The idea that excess intake of calories can be disposed of by the production of heat originated with work done by Neumann in 1902 (Danforth & Sims 1983; Hervey & Tobin 1983).

The study of DIT, or Luxuskonsumption (as it was called by Neumann), was inspired by the common observation that otherwise similar individuals can have vastly differing levels of food intake without differences in weight (Bradfield & Jourdan 1972; Himms-Hagen 1979; Miller 1982). In fact some individuals of the same age and sex who have similar levels of physical activity can maintain similar builds when one consumes twice as much food as the other (Jequier 1983).

Studies have shown that DIT occurs both in lab animals (Glick et al 1984; Granneman & Campbell 1984; Himms-Hagen 1979; Levin et al 1984; Moss et al 1985; Perkins et ai 1981; Rothwell & Stock 1980, 1982, Rothwell et al 1981, 1981b;

Seydoux et al 1981; Stephens et al 198[illegible]; Stirling & Stock 1968) and humans (Bradfield & Jourdan 1972; Miller & Parsonage 1975, Miller & Wise 1975b, Miller 1979; Rothwell et al 1986b).

The study of DIT has received a considerable amount of attention in view of its implications for the control of obesity. It is thought that a defect in the process of DIT can contribute to obesity (Bouillaud et al 1985; Himms-Hagen 1979; Jequier 1983; Seydoux et al 1981; Stirling & Stock 1968). This implies that there is a strong genetic component in some cases of obesity (Miller 1979).

In some animals the genetic component of obesity is well established. One animal model that has been extensively studied is the ob/ob mouse, which has a recessively inherited genetic component resulting in obesity (James & Trayhurn 1981). This animal has been found to have an unusually efficient metabolism (Himms-Hagen 1979). By lowering their core temperature these mice can reduce their demand for thermogenesis, allowing excess calories to be stored as fat (James & Trayhurn 1981).

Determination of the role played by DIT in human obesity is complicated by a number of factors. One major difficulty is that obese people have widely varying characteristics (Jequier 1983).

One study, by Miller & Parsonage (1975), involved 29 women who claimed to be unable to lose weight. The women

stayed at an isolated country house for three weeks and were kept on a 1500 kcal/day diet. Although most of the women did lose weight, nine women maintained their weight within +/-1kg. Findings such as this indicate that at least in some cases of obesity, though not all, an excess daily intake of calories is not the cause, but rather a decrease in energy expenditure may be more important.

The nine women unable to lose weight in Miller & Parsonage's study were found to have a low basal metabolic rate (BMR) and daily metabolic rate. On the other hand many researchers have found that obese people have a higher BMR than lean people (Jequier 1983), likely because excess body fat is often accompanied by an increase in lean body mass, on which BMR depends (Garrow 1982).

Studies looking specifically at DIT have shown that this component of energy expenditure is reduced in some obese people (Acheson et al 1987; Jequier 1983; Schwartz et al 1983b) but not in others (Nair et al 1983). Some obese humans, like obese animals, have efficient metabolisms and remain obese even without excessive energy intakes (Bradfield & Jourdan 1972; Jequier 1983; Jung et al 1979; Miller & Parsonage 1975).

Also, as with lab animals such as the ob/ob mouse, humans with a tendancy to become obese have been found to have a reduced capacity for CINST and a reduced metabolic response to norepinephrine (Jung et al 1979).

Some studies have found that this reduced response is true for obese people and those who maintain normal weight with strict dieting (Bradfield & Jourdan 1972; Jequier 1983; Jung et al 1979). For these obese people a defect in DIT could be a primary factor in the development of their condition.

Other studies show that the reduction in DIT seen in some obese people returns to normal with a return to normal weight (Acheson et al 1987; Schwartz et al 1983b). This implies that a defect in DIT was not the cause of obesity in these subjects, however such a defect could have contributed to the maintenace of the obese state.

Another similarity between DIT in humans (Himms-Hagen 1983) and animals (Rothwell & Stock 1979) is that the degree of weight gain seen after overeating can vary despite similar energy intakes, and in the case of lab rats, despite animals being the same strain (Rothwell & Stock 1979). This implies that small genetic and/or environmental influences can have a considerable effect on the metabolic response to feeding (Rothwell & Stock 1979; Saxton et al 1984).

The study of DIT is also complicated by the finding that the different components of a meal, i.e. carbohydrates, fats, and proteins, stimulate DIT to different degrees (Glick et al 1984, 1984b; Kevonian et al 1984; Mercer & Trayhurn 1984; Miller et al 1967; Nair et al 1983;

Nedergaard et al 1983; Rothwell et al 1983; Schwartz et al 1983; Tyzbir 1984).

High carbohydrate diets have been shown to increase DIT (Cunningham et al 1983; Rothwell & Stock 1984c). Glucose increases the in vitro rate of BAT respiration significantly, more so than equi-caloric amounts of fructose (Glick et al 1984). Glucose also increases the activity of the sympathetic nerves innervating IBAT (interscapular BAT) whereas other hexoses such as fructose, mannose, and galactose show no effect (Niijima 1986).

Some studies have shown that a high carbohydrate to protein ratio in a meal favours greater DIT (Miller 1982). However excess intake of protein also stimulates DIT (Miller et al 1967). The amount of stimulation of DIT by the fat component of a meal has been found in some cases to be less than that stimulated by carbohydrate (Gleeson et al 1979; Glick et al 1984b; Rothwell et al 1981b), yet in other cases the amount of stimulation of DIT by fat and carbohydrate has been found to be the same (Mercer & Trayhurn 1984; Nedergaard et al 1983; Rothwell et al 1983; Schwartz et al 1983).

Some studies have found that different types of fat, e.g. corn oil as opposed to olive oil, have greater thermogenicity than others (Miller 1982), which could explain some of the conflict above. One study by Nedergaard

et al (1983) found that essential fatty acids had a greater effect on DIT than non-essential fatty acids.

Rothwell and Stock (1987) found that tube-feeding of medium chain triglycerides to young male rats suppressed body weight, energy gain, and energy efficiency, and increased energy expenditure significantly, relative to water-intubated rats that consumed the same total metabolizable energy.

In a study on cold-acclimated mice Mercer and Trayhurn (1984) also found that a high fat diet significantly increased BAT mitochondrial respiration and GDP binding capacity. BAT weight, protein content and cytochrome oxidase activity were, however, unaffected. Thus the proton conductance path of BAT can be directly affected by the triglyceride level in the diet.

It has even been suggested that fat may affect BAT through direct humoral stimulation as opposed to through the sympathetic nervous system (Rothwell et al 1983). However in lab animals high fat diets have been shown to stimulate the sympathetic nervous system; in fact some evidence indicates that fat has a more prolonged effect on the sympathetic nervous system than carbohydrate (Schwartz et al 1983). In human studies, however, it appears that carbohydrate, but not fat, stimulates sympathetic nervous system activity (Schwartz et al 1983).

Alcohol has also been shown to increase energy expenditure in rats, even more than sucrose does (Rothwell & Stock 1984b). The changes seen in BAT were similar in alcohol- and sucrose-treated rats. This indicated that alcohol consumption stimulates BAT thermogenesis as well.

Sodium chloride (NaCl) affects energy expediture in rats as well. Bryant et al (1984) found that, when given 0.9% NaCl instead of water, rats had increased resting VO2's, greater thermogenic responses to either norepinephrine or a meal, and were able to adapt to cafeteria-feeding more rapidly than controls.

The BAT mass and protein content of these rats significantly increased, though their GDP binding capacity was reduced. Norepinephrine-stimulated Na+/K+ ATPase activity increased significantly in the NaCl fed rats compared with controls. This effect of NaCl on BAT thermogenesis seems to indicate that plasma ion concentration or osmolarity may be involved in the thermogenic response of BAT to food (Bryant et al 1984).

Comparison of DIT and CINST

One connection between DIT and CINST is shown by the fact that cold-acclimated animals kept at 4-6°C and animals that exhibit DIT are both hyperphagic (Himms-Hagen 1983). It has also been found that hyperphagic (DIT) rats kept in a warm environment exhibit CINST on acute exposure to cold

(Moore et al 1986b; Rothwell & Stock 1980; Saxton et al 1984).

It has been established that CINST occurs primarily in BAT (Foster & Frydman 1978). This has led to an increased study of DIT in BAT (Gleeson et al 1982; Glick et al 1984; Granneman & Campbell 1984; Hervey & Tobin 1982; Levin et al 1984; Miller 1979; Rothwell & Stock 1980, Rothwell et al 1981b; Stephens et al 1981).

Almost half the caloric intake of cold-acclimated animals kept at 4-6°C is used by BAT to produce heat (Himms-Hagen 1983). This is one demonstration of BAT acting to maintain a metabolic energy balance in hyperphagic animals.

BAT was also shown to be responsible for the DIT seen in cafeteria-fed rats (Himms-Hagen 1983; Rothwell & Stock 1979). The idea of "cafeteria-feeding" was developed by Rothwell and Stock (1979) to enable them to make rats hyperphagic voluntarily. Rats were induced to overeat by supplementing their lab chow diet with a "cafeteria diet".

This diet of palatable food includes four new items per day including sandwiches (cheese, salami, peanut butter and jam), a variety of cookies, candies, and flavoured crackers. The animals on this diet gained much less weight than expected and they lost the excess weight when the cafeteria-feeding was discontinued, even after the palatable

diet was introduced and withdrawn three times (Rothwell & Stock 1979).

In this study detailed measurements on six animals revealed that in spite of an 80% increase in energy consumption only a 27% increase weight was seen in the cafeteria-fed rats compared to chow-fed controls. It was also found that cafeteria-fed rats had a 100% increase in total energy expenditure which could not be attributed either to an increase in activity or in the energy cost of increased fat. The cafeteria-fed rats also had higher VO2's than controls and were more sensitive to norepinephrine throughout a dose range which went from 5-40 ug/100 gms of body weight.

No correlation was found between this increased sensitivity to norepinephrine and body fat content; therefore the increased sensitivity was not due simply to an increase in fat mass. The increase in VO2 caused by norepinephrine was reduced in cafeteria-fed rats but not controls when a beta-blocker was used.

This implies the involvement of the sympathetic nervous system in the control of DIT, which is similar to that which has been found for CINST. Since BAT thermogenesis is stimulated by the sympathetic nervous system (Danforth & Sims 1983; Dawkin & Hull 1965; Rothwell & Stock 1984d), this suggests that DIT and CINST are both a result of BAT thermogenesis.

Further evidence of this is the finding that changes in BAT, similar to those found in cold-adapted rats, were found in cafeteria-fed rats (Rothwell & Stock 1979). Like cold-adapted rats, increased deposits of IBAT were found in the cafeteria-fed rats . These increases were due to active mass, not simply to an increase in lipid content. It was also found that the increase in IBAT mass of cafeteria-fed rats correlated with the increase in VO2 found in these rats, whereas no such correlation was found with control rats.

Another study by Rothwell and Stock (1980), which compared cafeteria-fed and stock-fed rats kept either at thermoneutral (24°C) or cold (4°C) temperatures, further confirmed the similarities between overeating and cold adaptation. Rats that adapted either to cold or overeating showed similar increases in VO2's, as well as similar responses to norepinephrine and similar increases in IBAT deposits. These increases in VO2 were abolished by propranolol, a beta-blocker.

It was also found that rats fed a cafeteria diet and kept in the cold had greater increases in IBAT and VO2 as well as greater responses to norepinephrine than rats that underwent only one treatment.

The importance of BAT thermogenesis in DIT and in the control of obesity is also illustrated by the observation that BAT lipectomy leads to an increased deposition of white

adipose tissue (Moore et al 1985; Stephens et al 1981; Stern et al 1984). BAT lipectomy also significantly reduces the increase in VO2 seen in hyperphagic lean fa/fa Zucker rat pups exposed to acute cold (Moore et al 1986b).

CINST was also shown to be significantly reduced in Djungarian hamsters after BAT lipectomy (Heldmaier & Buchberger 1985). These results further demonstrate the similarities between DIT and CINST and the involvement of BAT in both these processes.

Many studies comparing CINST and DIT, and the involvement of BAT in these processes, have made use of genetically obese animals as experimental models (Himms-Hagen 1983, 1983b, 1983c). These animals are hyperphagic in the dynamic phase of obesity and have an increased metabolic efficiency (Himms-Hagen 1983c). They become obese even if their energy intake is controlled by pair feeding with lean animals (Himms-Hagen 1983c).

The ob/ob mouse is a frequently studied model of an obese animal (Himms-Hagen & Hogan 1981b, Himms-Hagen 1983, 1983b, 1983c; Hogan & Himms-Hagen 1980; Hull & Vinter 1984; Knehans & Romsos 1984; Vander Tuig et al 1984; Zaror-Behrens & Himms-Hagen 1983). This strain of mouse is cold-sensitive as well as obese (Himms-Hagen 1983b; Hull & Vinter 1984) and has a reduced energy expenditure which is quantitatively related to a reduction in BAT function (Himms-Hagen 1983c).

The reduced DIT and the tendency to become obese in the ob/ob mouse occur primarily at temperatures of 17-23°C (Himms-Hagen 1979; James & Trayhurn 1981). At higher temperatures, about 33°C, the thermogenic difference between the ob/ob mice and normal mice is eliminated, and the tendency to put on fat is decreased (James & Trayhurn 1981).

At moderate temperatures the ob/ob mouse may benefit from a more efficient metabolism if food is scarce, but it cannot survive when temperatures go to levels as low as 4°C (Gleeson et al 1982). This was found to be due to an inability to produce enough heat by CINST.

The combination of the inability of the ob/ob mouse to adapt to cold by CINST and its inability to regulate its weight by DIT gives convincing evidence that both these forms of thermogenesis occur by the same mechanism (Gleeson et al 1982; Miller & Wise 1975b).

There is some question as to whether the defect in BAT function of the ob/ob mouse is due to decreased sympathetic nervous system activity (Ashwell & Dunnett 1985b, Ashwell et al 1986; Astrup 1986), or not (Zaror-Behrens & Himms-Hagen 1983). Some researchers have suggested instead that the mitochondria of these mice are abnormal (Hogan & Himms-Hagen 1980; Hull & Vinter 1984). Studies have shown however that the histochemical characteristics of BAT transplanted from obese ob/ob mice into lean mice and vice versa are those of the host and not the donor (Ashwell et al 1986).

As well it has been found that high fat cafeteria-feeding of ob/ob mice increases sympathetic nervous system activity and results in BAT hypertrophy and increased BAT thermogenesis (Himms-Hagen 1976). This indicates that the ob/ob mouse can undergo DIT to some extent under certain conditions, just as it has some capacity for CINST.

Food restriction also increases BAT thermogenesis in the ob/ob mouse (Himms-Hagen 1985). Under these conditions the response of the ob/ob mouse to cold is almost normalized (Himms-Hagen 1985, Himms-Hagen et al 1986). This evidence shows that the intrinsic properties of ob/ob mouse BAT are not the primary cause of the animal's reduced BAT thermogenesis, but that low activity of the sympathetic innervation of this tissue (Ashwell et al 1986) plays a more significant role, and is perhaps the result of a central nervous system defect (Himms-Hagen 1985, Himms-Hagen et al 1986). This central nervous system defect may be related to the inappropriate occurrence of torpor in the fed state (Himms-Hagen 1985).

Whether or not it is a primary or secondary occurrence, the mitochondria of ob/ob mouse BAT have abnormal ultrastructure and reduced purine nucleotide binding capacities, although they have a relatively normal polypeptide composition (Hogan & Himms-Hagen 1980). The 32k protein sites involved in the proton conductance pathway appear to be masked (Hogan & Himms-Hagen 1980).

On exposure to cold (4°C), the GDP binding of these mitochondria does not increase, nor does their ultrastructure change unless the mice are exposed only to a mild drop in temperature (14°C). At 14°C ob/ob mice can survive and, almost like lean mice exposed to greater temperature drops (4°C), their BAT undergoes tissue growth, increases in mitochondrial number and purine binding capacities, as well as changes in mitochondrial ultrastructure (Hogan & Himms-Hagen 1980).

Thus the ob/ob mouse is able to withstand mild drops in temperature, but unable to survive temperatures of 4°C. The defect in ob/ob mouse BAT may also contribute to the animal's obesity (Hogan & Himms-Hagen 1980).

The diabetic obese (db/db) mouse, like the ob/ob mouse, is cold-sensitive with a low capacity to respond to norepinephrine and with an elevated metabolic efficiency (Himms-Hagen 1983b).

Unlike the two mouse strains (ob/ob and db/db), the obese (fa/fa) Zucker rat is not cold-sensitive and BAT from this animal is activated by cold but not by over-feeding (Himms-Hagen 1983b, 1983c; Triandafillou & Himms-Hagen 1983). The defect in fa/fa Zucker rat DIT is thought to originate in the central control of the sympathetic nervous system (Himms-Hagen 1983b; York et al 1984), possibly the hypothalamus (Triandafillou & Himms-Hagen 1983).

Since the BAT of the fa/fa Zucker rat does respond to cold, the tissue itself would appear to be normal (Himms-Hagen 1983b). However there is some evidence that the BAT cells of these rats have a reduced number of beta-adrenoreceptor binding sites (Levin et al 1984b), reduced DNA content (Triandafillou & Himms-Hagen 1983), and reduced mitochondrial GDP binding (Bazin et al 1984; Triandafillou & Himms-Hagen 1983).

This phenomenon, along with the increased lipid content found in fa/fa Zucker BAT cells (Bazin et al 1984; Levin et al 1984b; Triandafillou & Himms-Hagen 1983), could simply be a consequence of inactivity of the tissue.

ADAPTIVE CHANGES THAT OCCUR IN ACTIVATED BAT

BAT growth and development is stimulated by norepinephrine resulting from sympathetic nervous system activity (Bouillaud et al 1984; Himms-Hagen 1983, 1983b; Néchad & Olson 1983; Ricquier et al 1985; Rothwell & Stock 1984d). BAT also grows and increases in thermic capacity during cold exposure (Bukowiecki et al 1982; Himms-Hagen 1983; Ricquier et al 1985; Rothwell & Stock 1984d) and overeating (Bukowiecki et al 1982; Rothwell & Stock 1984d).

The effects of cold exposure and hyperphagia are due to an increase in sympathetic nervous system activity and the

subsequent release of norepinephrine at the level of BAT (Himms-Hagen 1983; Rothwell & Stock 1984d).

The changes that occur as a result of chronic exposure of BAT to these stimuli include true hyperplasia, with: (a) the production of new cells (Bukowiecki 1986; Himms-Hagen 1983b) -- measured by an increase in DNA content (Himms-Hagen & Triandafillou 1981, Himms-Hagen 1983b, 1983c; Schimmel & McCarthy 1985), (b) an increase in BAT size -- seen by an increase in protein content (Himms-Hagen & Triandafillou 1981, Himms-Hagen 1983b, 1983c; Schimmel & McCarthy 1985), and (c) an increase in mitochondrial mass -- shown by an increase in enzymes such as cytochrome oxidase (Himms-Hagen & Triandafillou 1981, Himms-Hagen 1983b, 1983c; Holloway et al 1984; Schimmel & McCarthy 1985; Trayhurn et al 1983). These parameters regress to normal after a certain period of time when the stimulus, such as a cafeteria diet, is removed (Himms-Hagen & Triandafillou 1981).

There is also an increase in the size of gap junctions between the BAT adipocytes, thus increasing cell communication (Schneider-Picard et al 1984). Within the BAT adipocytes structural changes, observable by the electron microscope, occur to the mitochondria (Himms-Hagen 1983).

As well there is an increase in the production of BAT mitochondrial UCP (Falcou et al 1985; Mory et al 1984; Ricquier et al 1984; Trayhurn et al 1983; Young et al 1984) and an increase in GDP binding to BAT (Himms-Hagen &

Triandafillou 1981, Himms-Hagen 1983; Holloway et al 1984; Lupien et al 1985; Schimmel & McCarthy 1985). Other changes, such as a decrease in fatty acid synthesis (Gibbins et al 1985) and an increase in lipoprotein lipase activity (Bertin et al 1985), also occur.

The changes that occur in BAT as a result of chronic stimulation, for example during overeating, increase the animal's capacity for both DIT and CINST -- thus buffering, at least in part, the increase in energy intake (Himms-Hagen 1983).

The ability of an animal's BAT to adapt to an overeating diet varies with the sex and strain of the animal; females seem to be less adaptable than males (Himms-Hagen 1983). The genetic influence on BAT adaptation to overeating can be demonstrated by studies on animal strains that have autosomal recessive genes promoting obesity, such as the ob/ob mouse and the fa/fa Zucker rat (Himms-Hagen 1983).

Many of the changes that occur in thermogenically active BAT are reversed or suppressed under conditions such as fasting (Desautels 1985, Desautels et al 1986; Villarroya et al 1986c), pregnancy (Andrews et al 1986; Trayhurn et al 1982, 1983; Wade et al 1986), and lactation (Isler et al 1984; Moore et al 1986; Trayhurn et al 1982; Villarroya et al 1986, 1986b, 1986c).

BAT thermogenesis (Glick et al 1984; Hayashi & Nagasaka 1983), BAT mass (Higham et al 1984; Rothwell et al 1984; Tulp 1983), tissue protein and mitochondrial protein (Rothwell et al 1984), and adipocyte diameter (Tulp 1983) decrease after short term starvation.

The capacity for BAT mitochondria to bind GDP also decreases with starvation (Rothwell et al 1984). This decrease in GDP binding, as well as a decrease in lipoprotein lipase activity, occurs even if BAT thermogenesis is already reduced by lactation (Villarroya et al 1986c), thus illustrating the role BAT plays in controlling the energy balance.

Hibernation also suppresses BAT thermogenesis (Horwitz et al 1985); yet in arousal from hibernation BAT thermogenesis is a major source of heat, allowing the animal to bring its body temperature up to normal (Nedergaard & Cannon 1987).

Aging of an animal is also related to a decrease in size and thermogenic capacity of BAT (Ashwell et al 1985; Holloway et al 1984; Levin et al 1984; Rothwell & Stock 1983c; Schneider-Picard et al 1984; Stock & Rothwell 1985, 1986). This can however be reversed by sympathetic nervous system stimulation during, for example, cold acclimation (Holloway et al 1984; Schneider-Picard et al 1984).

THE REGULATION OF BAT THERMOGENESIS

Sympathetic Nervous System

BAT thermogenesis is stimulated by the sympathetic nervous system (SNS) (Danforth & Sims 1983; Dawkin & Hull 1965; Rothwell & Stock 1984c) through the action of norepinephrine (Bukowiecki 1984; Danforth & Sims 1983; Foster 1985; Himms-Hagen 1983b, 1983c; Holloway et al 1984;). Norepinephrine acts primarily via beta-adrenergic receptors (Andrews et al 1985; Arch et al 1984; Bukowiecki 1984; Carneheim et al 1984; Fain et al 1984; Himms-Hagen 1983b, 1983c; Horwitz & Hamilton 1984; Mohell et al 1983; Perkins et al 1981; Rothwell et al 1981b, 1985b; Young et al 1984, 1984b, 1985).

It is believed that BAT thermogenesis is activated mainly through agonist interaction with beta-1 type receptors (Bukowiecki 1984; Harris et al 1986; Levin & Sullivan 1986; Mohell et al 1983; Skala 1984), or mixed beta-1 and beta-2 receptors (Rothwell et al 1985b, 1986b).

It has even been suggested that in addition to beta-1 and beta-2 adrenoreceptors, rat BAT adipocytes have a separate beta-adrenoreceptor subtype which selectively stimulates lipolysis (Arch et al 1984). Evidence has also shown that alpha-adrenoreceptors are involved in stimulating BAT thermogenesis (Fain et al 1984; Foster 1985; Harri & Valtola 1975; Horwitz & Hamilton 1984; Ma & Foster 1984;

Mohell et al 1983, 1984, 1984b, 1985; Raasmaja et al 1984, 1984b; Rothwell et al 1981b; Schneider-Picard et al 1985; Skala 1984).

The action of norepinephrine on beta-1 receptors of brown adipocytes results in the activation of adenylate cyclase, which causes an increase in cyclic adenosine monophosphate (cAMP) levels, which results in increased lipolysis and increased respiration (Bukowiecki 1984; Fain et al 1984; Himms-Hagen 1983, 1983b, 1983c; Mohell et al 1983; Skala 1984).

Alpha-adrenergic action of norepinephrine on brown adipocytes is not as clearly established. The alpha-receptor-mediated effect appears to involve Ca++ (Connolly et al 1984; Mohell et al 1983; Nanberg et al 1985, Nanberg & Putney 1986), possibly initiating the mobilization of intracellular Ca++ stores (Connolly et al 1984; Nanberg et al 1985). The Ca++-dependent alpha-adrenergic pathway appears to require extracellular Na+ (Connolly et al 1984), implying that it could be involved in stimulating BAT Na+/K+ ATPase.

Interaction of BAT adipocytes with norepinephrine appears to involve alpha-adrenoreceptors in the first seconds, resulting in an initial depolarization (Horwitz & Hamilton 1984; Schneider-Picard et al 1985) accompanied by a small rapid increase in K+ concentration (Schneider-Picard et al 1985). This is followed by a hyperpolarization (Horwitz & Hamilton 1984) and then a second depolarization

(Horwitz & Hamilton 1984; Schneider-Picard et al 1985) which is accompanied by a slow increase in K+ concentration (Schneider-Picard et al 1985).

The second depolarization is primarily due to agonist interaction with beta-adrenoreceptors (Horwitz & Hamilton 1984; Schneider-Picard et al 1985). This early catecholamine-mediated effect on electrolyte concentration of brown adipocytes implies early stimulation of the Na+/K+ pump (Schneider-Picard et al 1985).

The alpha-adrenoreceptor-mediated events of brown adipocytes are thought to potentiate the cAMP thermogenic response of beta-adrenoreceptors (Foster 1985; Ma & Foster 1984), possibly via alpha-2 type adrenergic activity (Dominguez et al 1986). It has been found that increased cAMP levels in brown adipocytes promote the expression of the gene for the UCP, and that this requires both alpha- and beta-adrenergic stimulation (Jacobsson et al 1986).

As well as increasing BAT mitochondrial respiration, stimulation of alpha-adrenoreceptors on brown adipocytes increases phosphatidyl inositol (PI) turnover (Mohell 1984, 1984b) and de novo synthesis of PI (Fain et al 1984; Nanberg & Putney 1986) and phosphatidic acid (PA) (Fain et al 1984).

In isolated hamster brown adipocytes this breakdown of PI occurs as an early, though short-lived, event during alpha-adrenergic stimulation. This may play an important

role in the subsequent stimulation of BAT respiration (Schimmel et al 1986).

Prolonged catecholamine stimulation of brown adipocytes as a result of cold-acclimation (Mohell 1984; Raasmaja et al 1984; Rothwell et al 1986b) or cafeteria-feeding (Raasmaja et al 1984; Rothwell et al 1986b), increases the number of alpha-1 receptors relative to beta-1 receptors. A decrease in beta-1 receptor density has been shown with chronic catacholamine stimulation (Mohell 1984; Senault et al 1984). This desensitization of beta-adrenoreceptors results in a decreased responsiveness of the cAMP system but lipolysis remains elevated (Schimmel et al 1985b).

<u>The Hypothalamus</u>

It has long been known that the hypothalamus is involved in the regulation of the appetite (Guyton 1981). The ventromedial hypothalamus (VMH) influences the energy balance through both the reduction of food intake (Guyton 1981; Perkins et al 1981; Seydoux et al 1981; Vander Tuig et al 1985) and the promotion of energy output by increased BAT thermogenesis (Fain et al 1984; Himms-Hagen 1984; Hogan et al 1985; Imai-Matsumura & Nakayama 1984; Minokoshi et al 1986; Perkins et al 1981; Saito et al 1985; Seydoux et al 1981; Vander Tuig et al 1985).

The response of BAT to stimulation of the VMH is specific since stimulation of an area 0.5mm dorsal of

lateral to the VMH does not affect BAT (Fain et al 1984). BAT responds to VMH stimulation in a manner similar to that of direct SNS stimulation (Fain et al 1984; Perkins et al 1981). In fact it has been found that VMH stimulation acts by increasing the activity of efferent sympathetic nerves to BAT (Minokoshi et al 1986; Niijima et al 1984).

Animals with hypothalamic obesity, such as gold thioglucose (GTG) obese mice and ventromedial hypothalamic obese rats, have defective DIT but functional CINST (Himms-Hagen 1983b, 1983c; Hogan et al 1982, Hogan & Himms-Hagen 1983; Rohner-Jeanrenaud et al 1982). These animals tend to have more BAT than lean animals (Himms-Hagen 1983b; Hogan & Himms-Hagen 1983) and in the case of GTG mice they have a larger capacity to respond to norepinephrine than lean mice (Hogan & Himms-Hagen 1983).

Exposure to cold in both the static and dynamic phases of obesity of these animals causes stimulation of BAT thermogenesis whereas cafeteria-feeding during the dynamic phase of obesity does not (Himms-Hagen 1983b; Hogan & Himms-Hagen 1983). Thus the VMH does not seem to be an essential central nervous system link for CINST (Hogan et al 1982), but it is involved in DIT. Lesions of the VMH contribute to obesity both by promoting hyperphagia and increases in metabolic efficiency (Perkins et al 1981).

Stimulation of the lateral hypothalamus (LH) induces hunger and thirst and increases the general activity level

of an animal (Guyton 1981). The LH has also been shown to be involved in BAT thermogenesis (Lupien et al 1986; Rothwell & Stock 1982). Lesions of the LH result in an increase in the amount of BAT (Park et al 1986), and increased GDP binding to BAT mitochondria (Lupien et al 1986). LH-lesioned rats, like VMH-lesioned rats, have a normal respose to cold (Park et al 1986).

LH-lesioned rats are also capable of BAT hypertrophy and thermogenesis in response to cafeteria-feeding; in fact this response is greater than that of normal rats of a similar or lower body weight set point (Park et al 1986).

The increase in BAT thermogenesis mediated through the hypothalamus does not appear to depend on the pituitary, as some studies have found hypophysectomy does not inhibit BAT thermogenesis; rather removal of the pituitary increases BAT thermogenesis (Goubern et al 1985; Rothwell & Stock 1985).

The Adrenal Gland

It has been found that adrenalectomy increases BAT thermogenesis (Allars & York 1986; Fukushima et al 1985; Marchington et al 1986; Rothwell & Stock 1984c, 1986; Wickler et al 1986; York et al 1985), likely due to a decrease in levels of corticosterone (Allars & York 1986; Fukushima et al 1985; Rothwell & Stock 1984c; York et al 1985). Adrenalectomy reduces energy intake and increases energy output of ob/ob mice (Vander Tuig et al 1984) and

obese fa/fa Zucker rats (Marchington et al 1983), showing that the adrenal glands affect the energy balance in these rodents.

In the fa/fa Zucker rat, BAT thermogenic response to a single meal is restored to normal by adrenalectomy, and BAT protein content, mitochondrial yield, and GDP binding become similar to lean rats (Marchington et al 1983). The mechanism by which adrenalectomy restores DIT is not clear, although corticosterone treatment of adrenalectomized rats suppresses BAT (Marchington et al 1983). On the other hand, it has been shown that glucocorticoids have a permissive effect on BAT thermogenesis in response to cold and norepinephrine (Marchington et al 1983).

A decrease in the level of male steroid also appears to promote BAT thermogenesis as castration of male rats increases BAT activity (Rothwell & Stock 1986). This tends to confirm the suppressive action of corticosteroids, since sex hormones and corticosteriods are closely related in structure (Lehninger 1975).

The increase in BAT thermogenesis seen with adrenalectomy, and prevented by corticosterone, is believed to be due to changes in SNS activity (Marchington et al 1986; Rothwell & Stock 1984c; York et al 1985).

Thyroid Hormones (T4 and T3)

Like norepinephrine, thyroid hormone is a thermogenic hormone (Danforth & Sims 1983). It does not act solely on BAT, but does play a role in regulating BAT activity, including thermogenesis and growth (Arieli & Chinet 1985; Hayashi & Nagasaka 1983; Himms-Hagen & Hogan 1981b, Himms-Hagen 1983b; Kates & Himms-Hagen 1985; Knehans & Romsos 1984; Puerta et al 1984; Rothwell et al 1983b; Silva & Larsen 1985; Sundin et al 1984; Williams & Ellis 1985).

The effect thyroid hormone has on BAT thermogenesis is illustrated by the fact that, like the ob/ob mouse, thyroidectomised rats cannot survive in the cold (Himms-Hagen & Hogan 1981b). Also improvements of some of the defects in ob/ob mouse BAT can be achieved with thyroxin (T4) treatment (Himms-Hagen & Hogan 1981b; Knehans & Romsos 1984).

Thyroid hormone increases the low GDP binding capacity of ob/ob mouse BAT mitochondria and normalizes the ultrastructure, permitting an improved thermogenic response to cold and norepinephrine (Himms-Hagen & Hogan 1981b). Thyroid hormone increases the amount of Na+/K+ ATPase in both lean and obese mice (Knehans et al 1984), which may also contribute to improved BAT thermogenesis in the ob/ob mouse.

It has also been found that treatment of rat BAT membranes with 3,5,3'-triiodothyronine <T3> increases the number of both beta- and alpha-receptors without affecting the alpha-1 to beta-1 ratio (Rothwell et al 1985c).

An important source of thyroid hormone involved in BAT activity appears to be endogenous T3 (Glick et al 1985; Kates & Himms-Hagen 1985; Silva & Larsen 1986). This endogenous source of T3 has been found to be important in promoting the optimal response of BAT mitochondrial UCP to cold (Bianco & Silva 1987). BAT thyroxin 5'-deiodinase activity has been found to be stimulated by cold exposure (Jones et al 1986; Kopecky et al 1986), norepinephrine (Jones et al 1986; Obregon et al 1987; Silva & Larsen 1986), insulin, and glucagon (Silva & Larsen 1986), and suppressed by pregnancy and lactation (Giralt et al 1986).

Norepinephrine-mediated stimulation of thyroxin 5'-deiodinase activity is blocked by the alpha-1 antagonist prazosin, but the beta-antagonist alprenolol has either no effect or increases the stimulation by norepinephrine (Obregon et al 1987). This implies that thyroxin 5'-deiodinase stimulation is an alpha-mediated event and that beta-agonists could inhibit this activation.

The increases in thyroxin 5'-deiodinase activity caused by cold exposure (Jones et al 1986), norepinephrine (Jones et al 1986; Silva & Larsen 1986), and insulin (Silva & Larsen 1986) were blocked by cycloheximide, an inhibitor of protein synthesis. This shows that the increased enzyme activity was due to de novo synthesis of thyroxin 5'-deiodinase, and not to an increase in activity of enzyme already present. BAT thyroxin 5'-deiodinase has also been

thought to provide a systemic as well as a local source of T3 (Silva & Larsen 1985).

A further indication of the importance of endogenous BAT T3 is the finding of subnormal cold-induced increases in type II 5'-deiodinations in BAT of the genetically obese ob/ob and db/db mice strains (Kaplan & Young 1987). As both these strains have defective CINST (Himms-Hagen 1983b), it is possible that low intracellular BAT T3 could contribute to the impaired thermogenesis experienced by these mice upon exposure to cold (Kaplan & Young 1987).

However obese fa/fa Zucker rats, which do not have defective CINST (Himms-Hagen 1983b, 1983c; Hogan et al 1982, Hogan & Himms-Hagen 1983; Rohner-Jeanrenaud et al 1982), have reduced endogenous BAT T3 production on exposure to cold (Wu et al 1987).

Although there appears to be a relationship between thyroid hormone, cold exposure, and BAT thermogenesis, studies on hamsters and rats have shown that while BAT thyroxin 5'-deiodinase activity increases due to cold exposure, it is not affected by eating a palatable diet (Kopecky et al 1986).

Insulin

Insulin is another hormone that appears to be involved in BAT thermogenesis (Andrews et al 1985; Bartness et al 1986; Begin-Heick & Heick 1984; Habara & Kuroshima 1983;

Mercer & Trayhurn 1984b, 1986; Perkins et al 1981). Supporting this is the demonstration of receptors for insulin in BAT (Begin-Heick & Heick 1984; Peyron et al 1985; Tanti et al 1986), which are decreased in number in BAT from the genetically obese ob/ob mouse as well as from mice rendered obese by GTG injection (Tanti et al 1986).

The effect of insulin on BAT thermogenesis is complex: with decreased insulin, BAT thermogenesis decreases (Bartness et al 1986), however excess insulin also reduces BAT thermogenesis (Bartness et al 1986). Insulin is required for DIT in hyperphagic rats (Perkins et al 1981), and the reduced respiration found in an in vitro preparation of BAT adipocytes from fasted rats can be corrected with the addition of glucose and insulin (Glick et al 1984).

Rats on cafeteria diets who have an impaired glucose tolerance do not experience the decrease in metabolic efficiency associated with overfeeding (Cunningham et al 1983). BAT mass increases in cafeteria-fed rats with or without impaired glucose tolerance, but the DNA content does not increase in the rats with impaired glucose tolerance (Cunningham et al 1983).

Insulin stimulates the SNS (Landsberg 1986). Infusion of insulin in rats for seven days increases resting VO2 by 22%, an effect which is blocked by beta-antagonists (Andrews et al 1985). This insulin treatment also significantly increases BAT mass, protein content, and total thermogenic

capacity as assessed by GDP binding to BAT mitochondria (Andrews et al 1985).

These effects were prevented by prior sympathectomy, confirming the likelihood that insulin effects on BAT are mediated by the SNS (Andrews et al 1985). Furthermore it has been found that in the ob/ob mouse BAT is the main site of glucose utilization when chronic beta-adrenergic treatment is given (Young et al 1984b). Insulin therefore serves to couple dietary intake with SNS-mediated thermogenesis (Landsberg 1986).

Insulin injection also prevents the suppression of BAT thermogenesis caused by subdiaphragmatic vagotomy (Andrews et al 1985), implying that the effects of vagotomy on thermogenesis are a result of a relative insulin deficiency.

Insulin resistance has been found to affect BAT thermogenesis (Danforth & Sims 1983; Jequier 1983; Mercer & Trayhurn 1984b, 1986). The obese ob/ob mouse develops insulin resistance early in life, at which point it also shows a marked reduction in the cold-induced increase in GDP binding to BAT mitochondria (Mercer & Trayhurn 1984b, 1986).

One study also found that obese people with insulin resistance experience a reduced thermic response to an oral dose of glucose (Jequier 1983). The impairment of BAT thermogenesis during acute cold exposure resulting from insulin resistance could be due to a decreased ability of BAT to take up glucose (Mercer & Trayhurn 1984b).

Glucose has been suggested to be an important thermogenic fuel in BAT (McCormack et al 1986). In fact it has been shown that BAT uptake of glucose can play an important role in removal of glucose from the blood (Cooney et al 1985; Young et al 1984b). In vivo glucose utilization by rat BAT can be increased 60-fold to very high rates by insulin (Ferré et al 1986).

It has been suggested that insulin not only acts directly on BAT (Trayhurn & Mercer 1986) but that it also serves as a central signal stimulating BAT thermogenesis (Stock & Rothwell 1986b; Trayhurn & Mercer 1986), possibly through activation of the insulin sensitive VMH (Landsberg 1986; Stock & Rothwell 1986b).

THE EFFECT OF EXERCISE

BAT Thermogenesis

Initial interest in studying the effect of exercise on BAT stems from the finding that exercise enhances cold tolerance in humans (Anderson et al 1966; Herberling & Adams 1960) and rats (Chin et al 1978; Herberling & Adams 1960; Hirata & Nagasaka 1981; Stromme & Hammel 1967) and that exercise may potentiate DIT in humans (Bukowiecki 1985; Miller et al 1967, Miller & Wise 1975b) and rats (Gleeson et al 1979, 1979b, 1982; Hill et al 1983).

Speculation concerning a possible relationship between exercise and BAT thermogenesis is reinforced by the involvement that the SNS has been shown to have in the control of BAT growth and thermogenic response (Himms-Hagen & Triandafillou 1981; Rothwell et al 1981b). As the SNS is also known to be stimulated during exercise (Astrand & Rodahl 1977; LeBlanc et al 1982, 1982b; Wickler et al 1987), it has been suggested that BAT is stimulated as well. This would result in exercise being associated with an increase in BAT growth and thermogenic capacity as seen with cafeteria-feeding and cold exposure.

If exercise can be shown to enhance BAT thermogenesis, then exercise must be assumed to play a more complex and profound role in modifying the energy balance than that of simply increasing energy output through the demand of the

exercise task itself. On the other hand, it is possible that exercise, by providing an alternate form of energy expenditure, could inhibit BAT thermogenesis.

Studies so far completed on the effects of exercise on BAT have yielded conflicting results (Arnold et al 1986, Arnold & Richard 1987, 1987b, 1987c; Bell et al 1984; Dallosso & James 1981; Gohil et al 1984; Harri & Valtola 1975, Harri et al 1982, 1984; Hayashi & Nagasaka 1983; Hirata & Nagasaka 1981, Hirata 1982, 1982b; LeBlanc et al 1982, 1982b, LeBlanc & Diamond 1988; Richard et al 1986; Shibata & Nagasaka 1987; Tokuyama & Okuda 1983; Wickler et al 1987).

In one study comparing physical exercise, cold acclimation, and repeated isoprenaline injections in rats, Harri and Valtola (1975) found that the IBAT hypertrophied in all treatment groups. In later studies however Harri et al (1982, 1984) found that IBAT weight was unchanged by exercise training.

These later studies used running on a treadmill to train the rats, whereas the first study used swimming. Due to the fact that the swimming was done at 30°C, and that evaporation during the drying process would lower the body temperature of the animals, it could be that the effects on IBAT found in this study were due mainly to cold exposure and not to exercise. In the later studies the animals were housed and run at 20°C. This is not a thermoneutral

temperature, and therefore the animals would have been chronically exposed to a certain degree of cold, the effect of which may have masked any effect of exercise on BAT.

Other studies involving both swimming (Bell et al 1984; LeBlanc et al 1982, 1982b, LeBlanc & Diamond 1988) and running on a treadmill (Wickler et al 1987) have also found that exercise did not change BAT weight. Still other studies have found that exercise in the form of treadmill running (Arnold & Richard 1987, 1987c) or swimming (Hirata & Nagasaka 1981, Hirata 1982) actually decreases BAT weight.

Hirata (1982) found that the weight of IBAT of exercise-trained rats was significantly decreased compared to controls unless the rats were not exercised at thermoneutral temperatures, ie. the average BAT weight was significantly lower than controls if the animals swam at 36 or 38°C, but significantly higher if the swimming was done at 32°C.

Arnold and Richard (1987c) showed that training on a treadmill resulted in a decrease in BAT size whether the rats ate a high fat diet or lab chow. Yet in an earlier study using swimming to train male and female rats, LeBlanc et al (1982b) found that, regardless of the animal's gender, BAT weight decreased in exercised rats relative to controls if both were fed a cafeteria diet, but that BAT weight remained unchanged if both treatment groups were fed lab chow.

The differences in the effect of exercise on BAT weight found in the various studies could be due to the fact that BAT weight can vary greatly depending on the amount of lipid present in the tissue (Himms-Hagen 1983c), thus making weight a poor indicator of BAT mass.

Studies that have measured the effect of exercise on BAT protein content have found it to be either unchanged from control values when the training was by running (Arnold & Richard 1987, 1987b; Harri et al 1984) or swimming (LeBlanc & Diamond 1988), or lower than controls after running on a treadmill (Arnold & Richard 1987c; Richard et al 1986). Also, the DNA content of BAT has been shown to be unchanged by exercise training, whether running (Arnold & Richard 1987b) or swimming (LeBlanc & Diamond 1988) was used.

Assessments of BAT mitochondrial mass made by measuring the activity of oxidative enzymes have shown that exercise in the form of treadmill running has either no effect (Harri et al 1984) or decreases BAT mitochondrial mass and oxidative capacity (Gohil et al 1984).

The GDP binding capacity of BAT from rats trained on a treadmill has been shown either to be unaltered regardless of the temperature the rats were housed or trained in (Richard et al 1986), or unaltered if training was at 24°C (Arnold et al 1986, Arnold & Richard 1987, 1987c) or 20°C

(Harri et al 1984), but was found to decrease when the rats were acclimatized to and trained at a temperature of 4°C (Arnold et al 1986).

GDP binding has also been shown to be unaltered, compared to controls, in exercised rats trained on a treadmill, whether both treatment groups are fed lab chow or a high fat diet (Arnold & Richard 1987c).

As well exercise, like caffeine or cold, has been reported to induce an increase in adipocyte proliferaton in brown adipose tissue (Bukowiecki 1985). However relatively few other studies reporting this are available for comparison.

The acute stimulation of BAT thermogenesis can be demonstrated through the administration of norepinephrine (Himms-Hagen 1983c). LeBlanc et al (1982, 1982b) have shown that in rats trained through swimming there was no increase in the catecholamine-mediated thermogenesis typically found in CINST and DIT, if it was assessed by measuring colonic temperature.

This is also found with the results of Stromme & Hammel (1967), who used treadmill running and activity cages to exercise their rats, and Hirata & Nagasaka (1981), who trained their rats through swimming. However these latter authors found that when catecholamine-induced thermogenesis was measured using oxygen consumption (VO2), a significant

increase was seen in exercise-trained rats (Hirata & Nagasaka 1981, Hirata 1982).

On the other hand, the earlier work by Stromme & Hammel (1967) did not show an increase in VO2 after norepinephrine injection in exercise-trained rats. These rats, however, were not anaesthetized so the authors were required to take measurements after about 25 minutes because the rats were initially restless. It is possible that any increase in VO2 of the rats due to norepinephrine could already have decreased to control levels by 25 min.

Hirata has also shown that not only does VO2 increase more in exercise-trained rats than controls during norepinephrine infusion (1981, 1982, 1982b), but that the blood flow to BAT increased more as well (1982). This result is similar to the increase in blood flow to BAT shown in cold-acclimated rats (Foster & Frydman 1978) and it implies that exercise can increase the thermogenic capacity of BAT.

It should be noted however that the studies by Hirata (1981, 1982, 1982b) involved swimming and therefore the possibility exists that the animals were exposed to cold after each swim due to water evaporation, despite the fact that they were quickly wiped dry with towels before being returned to their cages.

Wickler et al (1987) found in their study using treadmill running as exercise that not only was there no increase in VO2 of exercise-trained rats in response to

norepinephrine infusion, but neither was there an increase in blood flow to BAT as a result of norepinephrine infusion in these rats.

Studies of rats by Gleeson et al (1979, 1979b, 1982) and Hill et al (1983) have shown that DIT is potentiated by an exercise task (Gleeson et al 1979b, 1982) as well as by exercise training (Gleeson et al 1979, 1979b; Hill et al 1983), whether the exercise is in the form of swimming (Gleeson et al 1979; Hill et al 1983) or running (Gleeson et al 1979b, 1982). A number of researchers (Bray et al 1974; Miller et al 1967; Samueloff et al 1982; Segal & Gutin 1983) have also found that DIT in humans is augmented by exercise.

An enhancement of DIT by exercise was not seen however in other studies (Arnold & Richard 1987, 1987c; Dallosso & James 1981; LeBlanc et al 1982b, 1984, 1984b). LeBlanc et al found that training of rats through swimming (1982), and training of humans (1984, 1984b), decreased DIT, whereas Dallosso & James (1981) found that acute exercise in humans had no effect on DIT. Arnold & Richard (1987, 1987c) found that exercise training of rats through treadmill running also had no effect on DIT.

Studies on the effect that exercise has on CINST have shown that either there is no effect (Arnold & Richard 1987) or that CINST is inhibited by exercise (Arnold & Richard 1987b; LeBlanc & Diamond 1988; Shibata & Nagasaka 1987).

In a study comparing rats that were: (a) sedentary and kept at 24°C, (b) exercise-trained on a treadmill for 2 hrs daily at 24°C, (c) exposed to cold (-5°C) for 2 hrs daily, or (d) exercised at -5°C for 2 hrs daily, Arnold and Richard (1987b) found that only the rats that were exposed to cold with no exercise had changes in BAT that were indicative of BAT activity (ie. an increase in total BAT protein and DNA content). In this study, therefore, exercising while exposed to cold appeared to prevent BAT thermogenesis and CINST.

Leblanc and Diamond (1988) also found that exercise, in the form of swimming at 37°C, may inhibit CINST in rats in that it accelerates the rate of de-acclimatization to cold as measured by a decrease in IBAT size, protein content, and DNA content. However this same study showed that the thermogenic effect of norepinephrine (as measured by colonic temperature) was significantly higher in rats that exercised during de-acclimatization to cold compared to those who did not exercise during this period.

The finding of LeBlanc and Diamond that a training program involving swimming appears to inhibit CINST brings into question the significance of the cold exposure rats would presumably undergo while drying after each swim.

In LeBlanc and Diamond's study (1988) the rats swam at 37°C and were housed at 24°C. There were no particular precautions described with respect to drying the rats after each swim. It is therefore reasonable to assume that the

rats were at least as vulnerable to post-swim cold exposure as any other rats involved in a swim training program, yet a cold-mediated effect on BAT was not found.

Harri et al (1984) found that rats acclimatized to cold (5 +/- 1°C) and either sedentary or exercise-trained on a treadmill showed no significant differences between their IBAT weight, protein content, or mitochondrial oxidative capacity -- implying that exercise neither inhibits nor enhances BAT thermogenesis during CINST. They concluded that exercise replaced shivering activity and did not affect CINST.

This was the same conclusion made earlier in a study by Hart and Jansky (1963). Arnold et al (1986) also found that exercise totally replaced shivering in rats acclimatized to 24°C, but that it also replaces BAT CINST in cold-acclimatized rats.

As the results from these studies remain contradictory, further investigation of the relationship between exercise and BAT growth is necessary. This study proposes to look at the effect of exercise training on BAT by measuring various biochemical changes in the BAT of male rats after they have undergone an aerobic training program involving running on a treadmill.

Endocrine Systems and the SNS

Exercise is associated with a number of hormonal

changes. Like other forms of stress, it elicits a response from the SNS as well as the hypothalamus, the pituitary and the adrenal gland (Luger et al 1987).

The SNS is stimulated by exercise (Astrand & Rodahl 1977; Fleg et al 1985; Hartley et al 1972, 1972b; LeBlanc et al 1982, 1982b; Luger et al 1987; Pequignot et al 1985; Wickler et al 1987). Plasma concentration of norepinephrine (Hartley et al 1972, 1972b; Kjaer & Galbo 1988; Luger et al 1987; Pequignot et al 1985) and epinephrine (Hartley et al 1972, 1972b; Jobidon et al 1985; Kjaer & Galbo 1988; Luger et al 1987; Pequignot et al 1985) are increased in response to exercise.

This has been found in humans (Hartley et al 1972, 1972b; Kjaer & Galbo 1988; Luger et al 1987), and in animals such as dogs (Pequignot et al 1985), and rats (Jobidon et al 1985).

Exercise also results in an increase in plasma levels of adrenocorticotropic hormone <ACTH> (Luger et al 1987) and cortisol (Askew et al 1975; Buuck & Tharp 1971; Hartley et al 1972, 1972b; Luger et al 1987; MacConnie et al 1986). These increases are more pronounced as the exercise intensity increases (Hartley et al 1972; Luger et al 1987).

Other hormones influenced by exercise include growth hormone <GH> (Hartley et al 1972, 1972b), prolactin and testosterone (MacConnie et al 1986), whose levels increase during exercise. Insulin levels decrease during exercise

(Hartley et al 1972, 1972b; Winder et al 1987) while glucagon levels rise (Winder et al 1987).

Exercise training leads to adaptive changes in both nervous and endocrine systems. In studies on humans it has been shown that aerobic exercise training reduces the response of the SNS (Hartley et al 1972, 1972b; Luger et al 1987) and the hypothalamic-pituitary-adrenal hormone system (Luger et al 1987).

In humans the exercise-induced rise in plasma norepinephrine (seen at the same relative work loads) has been found to either be reduced in aerobicly-trained subjects relative to nontrained subjects (Hartley et al 1972, 1972b), or unchanged (Kjaer & Galbo 1988). However plasma epinephrine concentrations were found to be higher in response to exercise in trained than untrained humans (Kjaer & Galbo 1988).

Trained rats, normal or mildly diabetic, also had higher basal plasma epinephrine concentrations (Jobidon et al 1985). This indicates a training-induced increase in secretion from the adrenal medulla (Jobidon et al 1985; Kjaer & Galbo 1988).

Aerobic training also increases the potential of white adipose tissue cells to release free fatty acids in response to epinephrine stimulation (Askew et al 1975). It has also been found that endurance training increases the oxygen

consumption of skeletal muscle cells stimulated by epinephrine (Richter et al 1984).

Compatible with these results is the suggestion that the decrease in SNS output after training leads to an increased sensitivity of target tissue to catecholamines (Krotkiewski et al 1983).

The response of the adrenal cortex to exercise has been found to be similar to that of other stress factors such as cold and electric shock (Buuck & Tharp 1971). Training leads to a decrease in this stress response to exercise (Luger et al 1987).

Studying rats, Buuck & Tharp (1971) found that chronic exercise in the form of treadmill running led to increased resting levels of corticosterone. These resting levels remained high for four weeks at which point the training program reached its maximum intensity (1 mph, 1 hr/day, 5 day/wk). The resting corticosterone levels were lower by six weeks but still above control levels until after eight weeks of training.

The adrenal cortex was shown to still be functional following this training program because after exhaustive exercise both trained and untrained rats had similarly high corticosterone levels. This implies that the reduction in resting plasma corticosterone seen after training was an

adaptive change. The stress of chronic cold exposure also results in a decrease in plasma corticosterone levels (Buuck & Tharp 1971).

Exercise has an important influence on insulin levels. During exercise plasma insulin concentration decreases in both humans (Hartley et al 1972, 1972b) and rats (Winder et al 1987). Physical training attenuates this decline in insulin levels during exercise (Hartley et al 1972, 1972b). Exercise training also improves glucose tolerance in rats and partially prevents the decrease in glucose tolerance and increase in insulin secretion found in rats fed a cafeteria diet (Richard et al 1982).

Exercise has been known for some time to be useful in treating diabetes mellitus (Jobidon et al 1985). Physical training helps control glucose homeostasis in humans and animals with experimental diabetes (Jobidon et al 1985). This is believed to be a result of the increase in insulin sensitivity seen after training (Jobidon et al 1985; Kjaer & Galbo 1988).

This training-induced increase in insulin sensitivity is thought to be due to the increase in plasma epinephrine seen during exercise (Jobidon et al 1985). In fact healthy rats given repeated injections of epinephrine show increased insulin sensitivity, possibly due to down regulation of adrenal receptors (Jobidon et al 1985). Epinephrine has also been shown to be important in fasted rats for endurance

during exercise, presumably by preventing hypoglycemia (Winder et al 1987).

Exercise has a significant effect on a number of hormones associated with the hypothalamus. ACTH, cortisol, GH, prolactin, and the sex hormones estrogen and testosterone are under the control of the hypothalamus acting through the anterior pituitary gland (Lehninger 1975).

The phenomenon of overtraining is thought to involve a hypothalamic dysfunction (Barron et al 1985). Barron et al (1985) studied this condition and found that overtrained runners have a number of symptoms, including: impaired training; reduced racing time; apathy; and a "heavy-legged" feeling. Male runners with these symptoms were shown to have reduced cortisol, ACTH, GH, and prolactin levels in response to the stress of insulin-induced hypoglycemia.

Asymptomatic runners had hormone levels indicative of normal responses to insulin-induced hypoglycemia whether they were tested one month before, or 24 hours after a 42 km race or 48 hrs after a 92 km race. This shows that a reduced hormonal response was not due to the stress of a race itself as measurements soon after did not affect the results. After four weeks of rest the previously overtrained athletes had test results similar to those of the asymptomatic runners (Barron et al 1985).

Excessive training is also associated with hypothalamic dysfunction causing reduced hypothalamic gonadotropin releasing hormone (Barron et al 1985; MacConnie et al 1986). This is common in female athletes (Barron et al 1985; MacConnie et al 1986) and ballet dancers and results in low estrogen levels (MacConnie et al 1986) and amenorrhea (Barron et al 1985; MacConnie et al 1986).

Highly trained male athletes have also been found to have low levels of hypothalamic gonadotropin releasing hormone but plasma testosterone levels were not affected, perhaps due to a reduction in steroid clearance (MacConnie et al 1986).

Aging is associated with a reduced response to environmental stimuli such as exercise (Mazzeo et al 1986). Mazzeo's group (1986) found that in female rats cardiac catecholamine, especially norepinephrine, decreased with age, both at rest and in response to exercise. This trend is counteracted though to some degree by endurance training (Mazzeo et al 1986). Also, adrenal catecholamine levels increase with age in female rats (Mazzeo et al 1986).

Plasma catecholamines are increased in healthy elderly men, relative to young men, in response to exercise (Fleg et al 1985). A study by Fleg et al (1985) found that plasma norepinephrine levels also increased with age in response to other stresses such as cold pressor testing.

As well, aging is associated with a reduction in the maximal heart rate. This age-related difference in exercise cardiovascular performance may be due to a decreased target organ sensitivity to adrenergic stimulation (Fleg et al 1985).

TABLE 1: EXERCISE AND BAT SUMMARY

All studies used rats, except where noted.
* = acute exercise, all other studies used chronic exercise.

S = exercise by swimming. T = exercise by treadmill running.
e = exercising temperature (°C). h = housing temperature (°C).
F = females used, FM = females & males used (all others used males only).

	Studies that show exercise stimulates BAT thermogenesis	Studies that show exercise has no effect on BAT	Studies that show exercise inhibits BAT thermogenesis
Size measured by wet weight	Harri & Valtola 1975: S,30e	Harri et al 1982:T,20eh 1984:T,20eh Bell et al (mice) 1984:S,33/36e LeBlanc et al 1982:S,35e,26h,F 1982b:S,36e,27h,FM 1988:S,37e,24h Wickler et al 1987:T,24eh Shibata et al 1987:T,24eh	Arnold et al 1987a:T,24eh 1987c:T,24eh Hirata et al 1981:S,36e,24h 1982a:S,27h Tokuyama et al 1983
Protein content		Arnold et al 1987a:T,24eh 1987b:T,24eh Harri et al 1984:T,20eh LeBlanc et al 1988:S,37e,24h	Arnold et al 1987c:T,24eh Richard et al 1986:T,24/4eh
DNA content		Arnold et al 1987b:T,24eh LeBlanc et al 1988:S,37e,24h	
Mitochondrial mass		Harri et al 1984:T,20eh	Gohil et al 1984:T

TABLE 1 Continued

	Studies that show exercise stimulates BAT thermogenesis	Studies that show exercise has no effect on BAT	Studies that show exercise inhibits BAT thermogenesis
GDP binding capacity		Arnold et al 1986:*,T,24eh 1987a:T,24eh Richard et al 1986:T,24/4eh Harri et al 1984:T,20eh	Arnold et al 1986:*,T,4eh
Response to NE measured by colonic temperature		LeBlanc et al 1982:S,35e,26h,F 1982b:S,36e,27h,FM Hirata et al 1981:S,36e,24h Stromme et al 1967:T,26eh Shibata et al 1987:T,24eh	
Response to NE measured by VO2	Hirata et al 1981:S,36e,24h 1982a:S,32/36/38e,27h 1982b:S,36e,24h	Stromme et al 1967:T,26eh Wickler et al 1987:T,24eh Shibata et al 1987:T,24eh	
Response to NE measured by blood flow to BAT	Hirata et al 1982a:S,32/36/38e,27h	Wickler et al 1987:T,24eh	

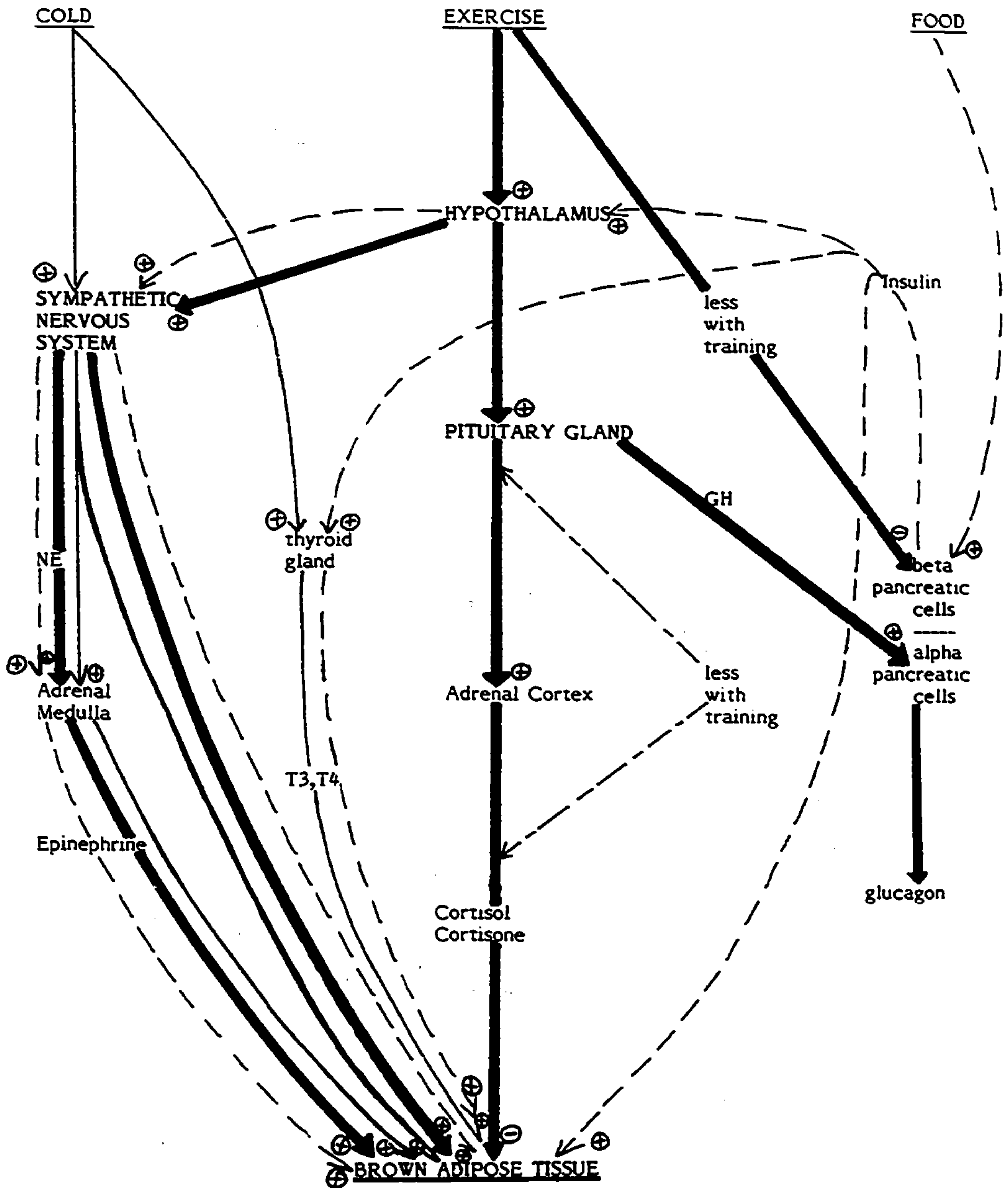
NE = norepinephrine.

SUMMARY

A clear picture of the effect that exercise has on brown adipose tissue has not been developed by the studies that have been done so far. A wide variety of exercise protocols have been used (various regimens of swimming or running) and conflicting results have been found even in studies done by the same research groups (Arnold & Richard 1987a, 1987b, 1987c; LeBlanc & Diamond 1988; Richard et al 1986; Harri & Valtola 1975, Harri et al 1982, 1984).

It is necessary that this situation be clarified. This study will attempt to improve the understanding of the effect that treadmill running has on rat IBAT through the use of a simple aerobic training program and an analysis of several of the critical biochemical factors that define IBAT size and thermogenic capacity.

REGULATION OF BAT THERMOGENESIS: SUMMARY



CHAPTER III

METHODOLOGY

INTRODUCTION

The purpose of the study was to determine the effect of aerobic exercise training on the thermogenic capacity of brown adipose tissue in the rat. The exercise training program involved a duration run on a treadmill. The thermogenic capacity of brown adipose tissue was assessed by measuring the total protein, total DNA, cytochrome oxidase activity, and the level of purine nucleotide binding to brown adipose tissue mitochondria. These measurements give an indication of the active tissue mass, cellularity, mitochondrial mass (oxidative capacity), and the availability of uncoupling protein (UCP) respectively.

THE TRAINING PROGRAM

Young male wistar rats (195 to 220 grams at the start of the program) were randomly divided into an exercise group of thirteen rats and a control group of ten rats.

All the rats were weighed once a week. They had no restrictions on water consumption and were fed ad lib with Purina rat chow. The weight of food consumed was recorded

once a week. All the animals were kept on a regular light cycle. The dark cycle began at 17h and ended at 5h. All training and handling was done during the dark cycle. The rats were all housed in separate cages. The cages were approximately 45 x 27 x 20 cm and were made of transparent polycarbonate and covered with stainless steel grids. The animals were housed in a room kept at 26-25°C.

The exercised rats were trained by running once a day, 5 days a week, on a motor-driven Quinton rodent treadmill adjusted to an incline of 10° The initial run of 5 minutes at 9.0 meters/min increased gradually, reaching 20 min at 19.4 meters/min by the end of week #1, and 40 min at 24.4 meters/min by the end of week #2. The maximum speed of 25 meters/min was reached by the end of week #4, and the maximum time of the run (1 hour) was reached by week #7.

The maximum intensity of the run was therefore 1 hour at 25 m/min, once a day, 5 days a week. This intensity was reached by week #7 and maintained for another 3 weeks -- a total program of 10 weeks. This exercise regime was expected to result in training as a similar program gave increased oxidative capacity in the rat gastrocnemius as measured by succinic dehydrogenase activity (Williams et al 1984).

The running of the rats was done at room temperature (20°C). The rats were run in two groups, the first group consisting of seven rats (E1 to E7), the second group consisting of six rats (E8 to E13). The runs were begun between 17h and 19h, which was the beginning of the dark

cycle. During the time that the rats were being run the lights were turned on in both the housing and the running room. This was for a maximum of 2 hrs 15 min. per session.

While the first group of rats was running, the second group of runners and the controls remained in the housing room. After running, the first group of rats was immediately returned to the housing room before the second group of runners was taken out to run. The time required to bring one group of rats from the housing room to maximum speed on the treadmill was 106 +/- 9 sec.(see appendix) The time between the end of the run and the return of the rats to the housing room was 188 +/- 36 sec.(see appendix)

The control rats received the same treatment as the exercised rats with regards to everything except the actual exercise training. This is to say that the control rats did not run on the treadmill, nor were they subjected to the mild electric shock occasionally necessary to induce some of the exercising rats to keep running. The use of electric shock to encourage the exercise-trained rats to run varied from rat to rat and from day to day.

Some rats received few or no shocks throughout the entire training program. Various other rats would usually receive shocks only at the beginning of each run, or throughout, or only at the end of each run. Still other rats would require a number of electric shocks one day, and receive few or none another day.

The control rats were not subjected to the sound of the treadmill motor, nor to the 20°C temperature of the training room as regularly as the exercised rats. They were however frequently brought into the training room when the treadmill was going, while being weighed or being made accustomed to handling.

SACRIFICE OF THE ANIMALS

The animals were sacrificed in groups of five or six between 6h15 and 7h. The time of sacrifice was therefore just after the end of the dark cycle, at which time the GDP binding levels are highest (Rothwell et al 1983d). The trained group of rats were rested 36 hours before sacrifice in order to measure only the chronic training effect on brown adipose tissue and not the acute effect of the exercise.

PREPARATION OF THE CRUDE HOMOGENATE

Immediately after each rat was sacrificed and desanguinated the interscapular brown adipose tissue was removed and kept at 4°C for all subsequent steps. Any adherent muscle, white adipose tissue, or connective tissue was dissected away from the brown adipose tissue and the tissue was weighed. The interscapular brown adipose tissue

was then minced and homogenized with a teflon/glass homogenizer.

The isolation buffer was: 0.25 M Sucrose; 0.2 mM EDTA (free acid); 1.0 mM Hepes pH 7.2 (adjusted with KOH) (Saito et al 1985). 1.0 ml of this homogenate, from the total of 5.0 mls, was saved for protein, DNA, and cytochrome oxidase assays, and the remainder was used for isolation of mitochondria.

ISOLATION OF BAT MITOCHONDRIA

The mitochondrial isolation method used was based on the method of Slinde et al (1975). The crude homogenate was centrifuged at 755 g (2500 RPM) for 10 minutes. The supernatant was removed from between the pellet and a superficial layer of lipid.

The lipid and pellet were resuspended in a fresh 5 mls of isolation medium and this was spun again at 755 g for 10 minutes. The supernatant from this spin was removed in the same manner as the first.

The two supernatants were combined and centrifuged at 17,000 g (11,700 RPM) for 14 minutes. The supernatant from this high speed spin was discarded and the mitochondrial pellet was gently resuspended in a minimum volume of isolation buffer (150 to 250 ul).

All centrifugation was done on a Sorvall RC2-B centrifuge at 4°C using an SS-34 rotor. The brake was left off for these spins. The method used a 'low-speed' spin that was lower than the 3020 g (5000 RPM) recommended by Slinde because this caused a significant amount of mitochondria to pellet out.

The final 'high-speed' spin (17,000 g x 14 min) was shown by Slinde to be the best method of sedimenting mitochondria from both thermically active and inactive brown adipose tissue. No washing of the pellet was done after the 'high-speed' spin because this caused too great a loss of mitochondria. The initial homogenization volume of 5 mls (not the 42 mls described by Slinde) also was used in order to increase the mitochondrial yield.

These modifications of the method of Slinde et al were made because of the limited number of rats used. The brown adipose tissue from each rat was isolated and analysed separately with no pooling of rat brown adipose tissue from similar treatment groups.

PROTEIN ANALYSIS

Protein was assayed according to a modified Lowry procedure devised by Schacterle & Pollack (1973). The samples were prepared by taking 10 ul of every homogenate and mitochondrial suspension and adding each to a (separate)

tube containing 5 mls of cold 10% TCA. The 10 ul volume was measured with a Hamilton syringe to ensure accuracy.

The samples were allowed to precipitate in the 10% TCA on ice for at least half an hour. The tubes were then spun down at 4080 g (5700 RPM on an HB40) for 15 minutes. The supernatants were then poured off and the test tubes were allowed to drain.

The precipitates were dissolved in 1.0 ml of 0.5 N NaOH, and then 1.0 ml of alkaline copper reagent was added. The samples were then incubated at room temperature for 10 minutes. The alkaline copper reagent consisted of: 10% Sodium Carbonate; 0.1% Na,K-Tartrate; 0.05% Copper Sulfate. Na,K-Tartrate was used rather than the K-Tartrate recommended in the original procedure due to availability.

Following the 10 minute alkaline copper incubation, 4.0 mls of a freshly prepared 1.0 N solution of Folin-Ciocalteu phenol reagent were added to each sample. The addition of the phenol reagent was done forcibly and rapidly with immediate vortexing. The samples were then incubated in a 55°C water bath for 10 minutes. After this the samples were cooled rapidly in ice water and read on an LKB Biochrome spectrophometer at 650 nm.

A standard curve was prepared using different concentrations of Bovine Serum Albumin (Sigma). The standard curve consists of a graph of absorbance vs mg protein from

which the amount of protein in each sample can be determined.

MEASUREMENT OF PURINE NUCLEOTIDE BINDING TO MITOCHONDRIA

The method used to measure purine nucleotide binding was based on the method of Nicholls (1976) as modified by Desautels et al (1978). The purine binding sites were determined from the binding of <3H>-labelled GDP in the presence of atractyloside. Each mitochondrial sample was measured for total binding and non-specific binding.

Tubes containing 300 ul of incubation medium were prepared at room temperature. The incubation medium contained: 20 mM TES; 10 mM Choline Chloride; 1.0 mM di-Sodium EDTA; 5.0 uM Rotenone; 100 uM Potassium Atractyloside; 120,000 dpm <14C>-Sucrose. The tubes measuring non-specific binding contained in addition: 100 uM ADP (non-radioactive).

Each measurement (both specific and non-specific) was done in triplicate or quadruplicate. To each tube 20 or 25 ul of isolated mitochondria (approx. 8-13 ug/ul) was added. One minute after this addition the binding reaction was initiated by rapidly adding 5 ul <3H>-GDP (10 uM, 450,000 dpm final assay concentration). After 30 seconds the mitochondria were pelleted by a 2 minute spin in an IEC micro-MB centrifuge. The supernatants were aspirated and

discarded. The pellets were dissolved in NCS Tissue Solubilizer (Amersham) overnight at 55°C then counted (liquid scintillation counting in the presence of PPO).

The binding of the nucleotide was calculated from the amount of tritiated nucleotide present after correction for the amount trapped in the water in the pellet, which is determined from the values for <14C>-Sucrose present (Desautels et al 1978).

CYTOCHROME OXIDASE ASSAY

The cytochrome oxidase assay used was that of the Himms-Hagen laboratory (Behrens & Himms-Hagen 1977; Desautels & Himms-Hagen 1979). This assay makes use of the Clark oxygen electrode which measures the rate at which oxygen is removed from the reaction medium by the action of cytochrome oxidase.

The reaction medium consisted of: 0.2 mM reduced Cytochrome c; 0.02 M Ascorbic Acid in 0.1 M Potassium Phosphate pH 6.6. The samples used were aliquots of brown adipose tissue homogenate activated by solubilization in Lubrol WX and frozen overnight.

The final protein concentration of the samples was 0.5 to 0.8 mg/ml. The reactions were carried out in a closed reaction vessel at 37°C containing 3.0 mls of reaction medium, to which 30 ul of sample was added.

The cytochrome c solution was prepared according to the method of Wharton & Tzagaloff (1967) though dialysis to remove excess ascorbate was not done. The concentration of the total and reduced cytochrome c was determined using the extinction coefficients of Yonetani (1967).

DNA ASSAY

This method of measuring DNA uses the fluorescence enhancing dye Bisbenzimidazol (Hoechst 33258) which binds to DNA (Downs & Wilfinger 1983). Samples of 1.0 ml were prepared from brown adipose tissue homogenates in a buffer containing: 100 mM NaCl; 10 mM EDTA; 10 mM Tris; 0.025 N Ammonium Hydroxide; 0.005% Triton X-100.

This buffer was used because it maintains the double stranded structure of DNA, inhibits endogenous DNAse activity, and prevents the interference of chromosomal protein in the binding of the DNA to the dye. This allows an accurate determination of DNA content by fluorometry.

The Hoechst 33258 dye stock of 200 ug/ml water was diluted before each assay to a final concentration of 1.5 ug/ml with assay buffer. Just before reading, 1.0 ml of dilute dye solution was added to the 1.0 ml samples. The DNA fluorescence was measured on a Turner 430 fluorometer set at an excitation wavelength of 350 nm and an emission of 455 nm with a 10 nm slit width.

The readings were done at room temperature and with each set of samples a standard curve of 5 to 150 ng DNA was prepared using calf thymus DNA (Sigma). This stock DNA solution was prepared by dissolving 9 mg DNA in 25 mls of 5.0 mM NaOH. The final concentration of this DNA was verified spectrophotometrically.

STATISTICS

Results are expressed as means +/- standard deviations. The statistical analysis used to determine significant differences between the test groups was the two-tailed Student "t"-test. Differences with $P>0.05$ are not considered to be significant.

CHAPTER IV

RESULTS

RAT WEIGHTS AND FOOD INTAKE

The exercise-trained rats had significantly lower final weights compared to the sedentary controls (P<0.0005, Table 2). The average weight gain was also significantly lower in the trained group (P<0.0005, Table 2) Lower body weights and weight gains are commonly-found effects of training (Harpur 1980). Despite the difference in body weight and weight gain, no significant difference was found in the food intake or food intake per rat weight of the two groups (Table 2).

HEART WEIGHTS

Heart weights were not found to be significantly different. However the heart weight to body weight ratio was significantly larger in the trained rats (P<0.005, Table 2). Similar training effects in treadmill run rats were found by Buuck & Tharp (1971) and these were accompanied by a reduction in resting heart rates in the trained rats. A decrease in resting heart rate as well as cardiac hypertrophy are considered to indicate that training has taken place (Buuck & Tharp 1971).

IBAT APPEARANCE

The IBAT from the control rats was pale and small, one organ being so pale and small that it was hard to locate. The IBAT from the trained rats was dark, well-defined, and not as fatty as the tissue from the control rats.

IBAT SIZE

A comparison of the IBAT wet weights showed no significant difference.(Table 3) The IBAT wet weight to body weight ratio was not different either.(Table 4) The protein content of the IBAT from the trained rats was significantly higher.($P<0.001$, Table 3) As well the IBAT protein to rat weight ratio was significantly greater in the trained group.($P<0.0005$, Table 4)

IBAT CELLULARITY: DNA CONTENT

The DNA content ($P<0.001$, Table 3), and DNA content to rat weight ratio ($P<0.0005$, Table 4), were both significantly higher in the trained rats.

IBAT MITOCHONDRIAL MASS AND OXIDATIVE CAPACITY

The ratio of total cytochrome oxidase activity to rat weight (Table 5) was significantly higher in the IBAT of the trained rats.($P<0.05$) There was however no significant difference between the specific or total cytochrome oxidase activities of the two groups, though the total cytochrome oxidase activity was greater in the IBAT of the exercise-trained rats.(Table 5) The protein yield of mitochondria isolated from the IBAT was significantly larger in the exercise-trained group.($P<0.01$, Table 6)

GDP BINDING CAPACITY

A comparison of the GDP binding capacity of the IBAT mitochondria from the exercise-trained rats and the sedentary controls showed no significant difference.

The specific binding (pmol/mg) was slightly higher in the control group (Table 6), however this difference was not significant. The total binding (pmol) and the total binding per rat weight (pmol/kg) were both higher in the IBAT mitochondria of the exercise-trained rats (Table 6), but neither of these differences was significant.

TABLE 2: RAT WEIGHTS AND FOOD INTAKE

	Final weight (gm)	Total weight gain (gm)	Food intake (gm/day)
Exercise-trained rats	441.7 +/- 25.2	236.5 +/- 20.5	23.05 +/- 1.29
Sedentary control rats	508.9 +/- 46.9	299.3 +/- 41.6	23.89 +/- 1.58
	P<0.0005	P<0.0005	NSD

	Food intake/ rat weight (gm/day/gm)	Heart weight (gm)	Heart weight/ rat weight (mg/gm)
Exercise-trained rats	6.9 +/- 2.1	1.383 +/- 0.187	3.13 +/- 0.36
Sedentary control rats	6.6 +/- 1.9	1.375 +/- 0.156	2.70 +/- 0.20
	NSD	NSD	P<0.005

NSD = no significant difference (ie. P>0.05)

TABLE 3: CHARACTERISTICS OF IBAT

	IBAT wet weight (mg)	Total protein in IBAT (mg)	Total DNA in IBAT (ug)
Exercise-trained rats	309.8 +/- 83.1	11.02 +/- 3.20	167.8 +/- 37.6
Sedentary control rats	340.2 +/- 68.9	6.79 +/- 1.95	112.8 +/- 27.7
	NSD	P<0.001	P<0.001

TABLE 4: IBAT PARAMETERS RELATIVE TO RAT WEIGHT

	mg IBAT wet weight /gm rat	ug IBAT protein /gm rat	ug IBAT DNA /gm rat
Exercise-trained rats	0.70 +/- 0.19	25.1 +/- 7.9	0.381 +/- 0.092
Sedentary control rats	0.67 +/- 0.12	13.5 +/- 4.0	0.224 +/- 0.062
	NSD	P<0.0005	P<0.0005

NSD = no significant difference (ie. P>0.05)

TABLE 5: MITOCHONDRIAL CYTOCHROME OXIDASE VALUES

	Specific Activity (ug O/mg/min)	Total Activity (ug O/min)	Total Activity /rat weight (ug O/min/gm)
Exercise-trained rats	5.86 +/- 1.87	67.70 +/- 34.98	0.155 +/- 0.084
Sedentary control rats	5.88 +/- 1.99	42.79 +/- 24.77	0.085 +/- 0.047
	NSD	NSD	P<0.05

TABLE 6: MITOCHONDRIAL GDP BINDING CAPACITY

	Mitochondrial total protein (mg)	Specific binding (pmol/mg)	Total binding (pmol)	Total binding /rat wt (pmol/kg)
Exercise-trained rats	2.12 +/- 0.81	11.7 +/- 5.9	26.6 +/- 19.7	61.1 +/- 47.0
Sedentary control rats	1.23 +/- 0.58	15.5 +/- 8.0	20.9 +/- 14.8	41.3 +/- 27.7
	P<0.01	NSD	NSD	NSD

O = oxygen atoms; wt = weight;
NSD = no significant difference (i.e. P>0.05)

CHAPTER V

DISCUSSION

In this study aerobic exercise training was shown to promote some of the adaptive changes typically seen in thermogenically active brown adipose tissue. The protein and DNA contents of the interscapular brown adipose tissue from the exercised rats were significantly higher than those of the sedentary controls. The cytochrome oxidase activity and GDP binding capacity of the interscapular brown adipose tissue mitochondria were not, however, significantly different in the two treatment groups.

These results imply that, though there was interscapular brown adipose tissue growth in the exercise-trained rats, there was no increase in the brown adipose tissue thermogenic capacity. It should be noted however that both the interscapular brown adipose tissue total tissue cytochrome oxidase activity and the total tissue cytochrome oxidase activity/rat weight ratio were greater in the exercise-trained rats, though only the latter was significantly greater.

This shows that the exercise program in this study favors an increase in interscapular brown adipose tissue mitochondrial mass and therefore an increase in oxidative capacity. Likewise the total GDP binding capacity and the

total GDP binding capacity per rat weight were higher in the exercise-trained rats, though these increases were not statistically significant.

It appears therefore that the exercise program in this study promoted interscapular brown adipose tissue growth and a mild improvement in interscapular brown adipose tissue thermogenic capacity.

The average weight as well as the average weight gain of the exercise-trained rats was found to be significantly lower than that of the control group. This occurred despite there being a very small and insignificant difference in the average daily food intake of the two groups.

It is reasonable to attribute this lower weight level of the trained group to the increased energy output resulting from regular exercise. However some contribution from brown adipose tissue thermogenesis to this increase in evergy output seems possible in view of the adaptive changes seen in the interscapular brown adipose tissue of the trained rats.

Neither the interscapular brown adipose tissue wet weight nor the interscapular brown adipose tissue wet weight to body weight ratio were found to be significantly different between the two groups. This is likely due to differences in lipid content, and is not an accurate reflection of any differences in active tissue mass between the interscapular brown adipose tissues (Himms-Hagen 1983c).

A better indication of active tissue mass is the total protein content, which was 11.02 +/- 3.20 mg for the trained rats compared to 6.79 +/- 1.95 mg for the controls. This shows a significant increase in the interscapular brown adipose tissue mass in the exercise-trained rats relative to the sedentary controls. Confirming this is the significantly higher DNA content found in the tissue from trained rats compared to the controls. This shows an increased number of cells in the interscapular brown adipose tissue of the trained rats.

Also, the protein content to rat weight ratio and the DNA content to rat weight ratio were both significantly greater in the trained rats, which further demonstrates an increased interscapular brown adipose tissue growth in the rats that were exercise trained, compared to the sedentary control group.

The specific activities of the interscapular brown adipose tissue cytochrome oxidase from the trained and untrained rats were 5.86 +/- 1.87 ug O/mg/min and 5.88 +/- 1.99 ug O/mg/min respectively. These activities are very similar, indicating that the oxidative capacity of individual mitochondria from the interscapular brown adipose tissue of exercise-trained rats was the same as that from sedentary rats. However the total tissue cytochrome oxidase activities were 67.70 +/- 34.98 ug O/min from the

interscapular brown adipose tissue of the trained rats and 42.79 +/- 24.77 ug O/min from the sedentary rats.

This shows an increase in total tissue cytochrome oxidase activity in the interscapular brown adipose tissue from the exercise-trained rats and though this increase was not significant it shows that these rats tended to have more mitochondria and therefore a higher total tissue oxidative capacity. Further confirming this is the significantly higher total tissue cytochrome oxidase activity to rat weight ratio found in the interscapular brown adipose tissue of the exercise-trained rats. A significantly higher protein yield was also found from mitochondria isolated from the interscapular brown adipose tissue of the exercise-trained rats relative to the sedentary controls.

This gives a further indication of an increase in mitochondrial mass in the exercise-trained rats, although the possibility exists that the protein yield of the mitochondria isolated from the controls could have been adversely affected by the higher white fat content that appeared to be present. It was clearly observed, however, that the interscapular brown adipose tissues from the exercise-trained rats were a much darker brown than those from the control rats.

Since it is the large number of mitochondria in brown adipose tissue that give it its typical brown color, one would think that the darker brown color of the interscapular

brown adipose tissue of the exercise-trained rats indicates a larger amount of mitochondria in these tissues compared to the very pale, almost white, interscapular brown adipose tissue found in the controls.

The specific and total GDP binding capacities of the interscapular brown adipose tissue mitochondria were both found to be not significantly altered by exercise. Also, though the total GDP binding to rat weight ratio was higher in the exercise-trained rats, 61.1 +/- 47.0 pmol/kg versus 41.3 +/- 27.7 pmol/kg for the controls, this difference was also not significant. These results are in agreement with other studies (Arnold 1986, Arnold & Richard 1987, 1987c; Harri et al 1984; Richard et al 1986).

However the finding that exercise did not increase the interscapular brown adipose tissue GDP binding capacity is not consistent with the finding of an increased interscapular brown adipose tissue growth and oxidative capacity, as indicated by an increased protein and DNA content and an increase in total cytochrome oxidase to rat weight ratio.

The explanation for this could be that the last exercise bout was at least 36 hours before sacrifice. This means that the last stimulus was at least 36 hours before the GDP binding was measured in the exercised rats. In this time it is possible that some of the UCP's in the interscapular brown adipose tissue mitochondria of the

exercise-trained rats assumed masked forms and thus were unavailable for GDP binding.

Even though there was no increase in the GDP binding capacity of the interscapular brown adipose tissue mitochondria of the exercise-trained rats, the thermogenic capacity of the interscapular brown adipose tissue from these rats was still greater than controls in view of the small increase in mitochondrial mass.

This slightly greater mitochondrial mass in the interscapular brown adipose tissue of the exercise-trained rats would give the tissue from these rats an increased ability to produce heat despite the fact that the individual mitochondria had a no greater capacity to produce heat than the mitochondria from the control rat tissue.

The finding of an increased brown adipose tissue size with only a small increase in oxidative capacity and no increase in GDP binding capacity may be because the exercise program in this study resulted in a small stimulus to brown adipose tissue thermogenesis, one much less effective than cold or overeating. The training program may have provided sufficient stimulation only to cause an increase in tissue growth and favor an increased mitochondrial mass, but without creating an increase in UCP.

The results of this study are in partial disagreement with a number of other studies (Arnold & Richard 1987, 1987b, 1987c; Gohil et al 1984; Harri et al 1984; LeBlanc &

Diamond 1988; Richard et al 1986). The explanation for this may be found in the differences in the exercise programs used, in the ambient temperatures that the animals were exposed to, or to a difference in experimental procedure.

One critical aspect of the experimental procedure involves the dissection of the interscapular brown adipose tissue and the removal of all other adherent tissue. While removal of white fat and muscle is relatively easy, the removal of blood vessels and in particular connective tissue is difficult. Extreme care was taken during this step of the procedure because if this dissection is not done well the exercise-induced changes (in protein content particularly) could be masked by the excessive contribution from other tissues.

As well, great care was taken not to dissect away too much of the white adipose tissue in close proximity to the brown adipose tissue. This was done in order to avoid removing the brown adipocytes interspersed with the white fat at the periphery of the brown adipose tissue.

Another factor that could mask the effect of exercise training on brown adipose tissue is cold exposure. Cold clearly stimulates brown adipose tissue thermogenesis (Foster & Frydman 1978), therefore exposure of one or both treatment groups to cold could result in the measurement of the cold-induced effect on brown adipose tissue rather than an exercise effect.

To minimize any effect of cold, strict regulation of the ambient temperature was maintained in the room where the rats were housed. This temperature was kept at 27 +/- 2°C. Under these circumstances the interscapular brown adipose tissue of all the rats -- trained or untrained -- should not have undergone any adaptive changes due to cold-induced non-shivering thermogenesis.

The treadmill runs were done at cooler temperatures: 20.8 +/- 1.9^{o}C. This was done because the animals produce heat while running, thereby risking excessive heat stress if the exercise room temperature had been kept at 27°C. Furthermore the exercise-induced increase in body temperature would presumably compensate for the cooler room temperature.

When the rats were brought into the cooler exercise room, they began their run immediately -- generally reaching maximum speed approximately two minutes after leaving the warm housing room.(see Appendix) When the run was finished the rats were quickly returned to the 27°C housing room. This generally took under four minutes.(see Appendix) Despite the fact that this transfer time was very brief, a slight cold effect on the interscapular brown adipose tissue of the trained rats may have been possible.

However this effect should have been minimal, as during the run, and for some time after the run, the body temperature of the animals was elevated. Although this

increased body temperature was not measured, it was clearly evident when the animals were removed from the treadmill and returned to their cages.

It is therefore only the time span between leaving the housing room and reaching an elevated body temperature at the beginning of the run that actually posed a significant risk of causing a cold-induced effect on the interscapular brown adipose tissue of the exercise-trained rats. This time may have been slightly more than two minutes as it may have taken a short while after reaching maximum speed on the treadmill for the rise in body temperature of the rats to compensate for their exposure to a temperature about 7°C lower than their housing room.

It should be noted, however, that though the training room was cooler than the room where the rats were housed, the temperature difference was very small -- no more than 10°C and usually only 7°C. In a study by Arnold & Richard (1987b), rats housed at 24°C were subjected to either: two hours of daily exercise on a treadmill at 24°C, or two hours of daily exposure to -5°C, or two hours of daily exercise on a treadmill at -5°C. They found that only the sedentary rats exposed to cold had increases in interscapular brown adipose tissue protein and DNA content.

Thus, this experiment indicated that not only did exercise not affect interscapular brown adipose tissue, but that it prevented the effects of cold exposure when the

treadill running was done at $-5^{o}C$. This study states that "Upon entering the cold room, trained cold rats immediately started exercising and upon completion they were quickly moved back to their $24^{o}C$ environment." Clearly Arnold & Richard faced the same problem of a brief cold exposure before and after exercise that was present in this study, however the temperature difference in the Arnold & Richard protocol was approximately $29^{o}C$.

In spite of a far greater temperature difference, no effect was found from the brief cold exposure incurred before and after the rats' exercise period. This casts serious doubt on the possibility that the brief exposure to a much less significant temperature drop of only about $7^{o}C$ could have played a major role in causing any changes in the interscapular brown adipose tissue of the exercise-trained rats in this study.

Furthermore an attempt was made to balance the effect of the brief exposure to slightly cooler temperatures that the trained rats were subjected to, by occasionally exposing the control rats to the same 20.8 +/- $1.9^{o}C$ environment for brief periods. The exposure of the control rats to the cooler temperature of the training room was not as regular as that of the exercised rats. This was largely due to the difficulty in determining exactly what length of exposure time would be equivalent to the exposure time of the training rats.

It would have been inappropriate to keep the control rats at 20.8 +/- 1.9°C during the entire time of the run as the exercising rats had an exercise-induced rise in body temperature that the sedentary controls did not experience. For this same reason, it would not have been appropriate to include the time between the end of the run and the return of the rats to the housing room (about four minutes) in any calculation of the daily exposure to the running room to which one might subject the controls. This leaves only the brief time between the moment the rats left the housing room and the point at the beginning of the run when they achieved a sufficiently elevated body temperature.

The time to reach a body temperature that would counter the drop in temperature of the training room relative to the housing room was not measured, as it would have been hard to define and also there was a lack of the necessary equipment for such a task. This time span was also hard to estimate and may have varied from rat to rat or changed with the progression of the training program. Some rats may have experienced an anticipatory rise in body temperature before even getting on the treadmill.

These concerns, plus the mild and brief nature of the cold exposure endured by the exercise-trained rats, made the assignment of a particular daily cold exposure for the control rats unjustified. Thus, the control rats were exposed to the cooler exercise room only when time

permitted, such as when they were being accustomed to being handled.

It seems likely that the explanation for the increase in interscapular brown adipose tissue protein and DNA content found in the exercise-trained rats of this study must be found in some aspect of their treatment other than this brief and mild exposure to a cool (approximately 20°C) temperature before and after each run.

It is also important to note that in this study no significant difference was found between the food intake or food intake per rat weight of the trained and sedentary rats. Any significant difference in food intake between the treatment groups could introduce an effect on interscapular brown adipose tissue due not to exercise but to overeating or undereating. Overeating stimulates BAT thermogenesis (Rothwell & Stock 1979) whereas undereating suppresses it (Desautels 1985, Desautels et al 1986; Villarroya et al 1986c), thereby interfering with an attempt to isolate any effect due specifically to exercise.

It is perhaps in the variety of exercise protocols used to study the effect of exercise on brown adipose tissue that one may find the most interesting explanation for the differences in the results of the different studies.

The studies that have used biochemical assays to analyse the relationship between exercise and brown adipose tissue in rats have used a number of exercise programs.

These programs have included acute exercise bouts (Arnold et al 1986; Gohil et al 1984), and long term training (Arnold & Richard 1987, 1987b, 1987c; Gohil et al 1984; Harri et al 1984; LeBlanc & Diamond 1988; Richard et al 1986).

The training programs have been of different intensities and have included treadmill running (Arnold et al 1986, Arnold & Richard 1987, 1987b, 1987c; Gohil et al 1984; Harri et al 1984; Richard et al 1986) and swimming (LeBlanc & Diamond 1988). Most of the studies have used male rats but one used females (Gohil et al 1984).

These variables have led to different results even in studies done by the same research groups (Arnold & Richard 1987, 1987b, 1987c; Harri & Valtola 1975, Harri et al 1982, 1984; LeBlanc & Diamond 1988; Richard et al 1986). The differences in results obtained with different exercise protocols could be due to different stress levels. The protocol used in this study was a straightforward gradual aerobic training program which emphasized minimizing stress.

Stress and exercise are associated with increased sympathetic nervous system activity (Luger et al 1987) and with an increase in plasma cortisol levels (Luger et al 1987; Buuck & Tharp 1971). Sympathetic nervous system activity stimulates brown adipose tissue thermogenesis (Danforth & Sims 1983; Dawkin & Hull 1965; Rothwell & Stock 1984d), however cortisol has been shown to suppress brown adipose tissue thermogenesis (Allars & York 1986; Fukushima

et al 1985; Marchington et al 1983; Rothwell & Stock 1984d; York et al 1985).

The effect, therefore, that stress and exercise have on brown adipose tissue must depend on the balance achieved between hormone levels resulting from the degree to which secretion from the sympathetic nervous system and the adrenal cortex are stimulated.

The exercise-induced increase in circulating cortisol increases further with higher levels of exercise intensity (Hartley et al 1972; Luger et al 1987). Training however has been shown to reduce the response of the adrenal cortex to exercise (Buuck & Tharp 1971; Luger et al 1987).

In a study involving rats trained by a treadmill running program which by four weeks reached 1 mph (approx. 27 m/min) for 1 hr/day, 5 days/week, Buuck & Tharp (1971) found that resting plasma corticosterone levels were high during the first four weeks and lower by six weeks but still above control levels until the eighth week.

This shows that while the exercise intensity is increasing to reach the maximum speed and duration of the training program, the resting plama corticosterone levels are high. They also remain high for some time after the maximum intensity has been reached. At which point in a training program the resting plasma corticosterone level would return to normal may well vary with different maximum

intensities and rates at which the maximum intensity is reached.

In the present study the maximum intensity of the training program was treadmill running at 25 m/min, 1 hr/day, 5 days/week. By the fifth week the rats were running at 25 m/min for 50 min, and were therefore close to the maximum intensity. The full intensity was reached by the seventh week of the ten week program.

With this gradual approach to a moderate intensity training program it seems likely that the resting plasma corticosterone levels of the exercise-trained rats should not have reached high levels and should have returned to normal by the time of sacrifice. This, however, was not verified.

Although plasma cortisol levels are predicted to have been low in the exercise-trained rats in this study, this is not a sufficient explanation for the finding of brown adipose tissue growth. It is also important to consider the effect of training on the sympathetic nervous system.

Some evidence has shown that exercise training reduces the response of the sympathetic nervous system to exercise (Hartley et al 1972, 1972b; Luger et al 1987). However other studies have found an increase in plasma epinephrine after exercise training, possibly due to increased secretion from the adrenal medulla (Jobidon 1985; Kjaer & Galbo 1988).

It has also been suggested that the reduction in firing of the adrenergic nervous system results in increased sensitivity of target tissues (Krotkiewski et al 1983). Richter et al (1984) have found that endurance training increases the ability of epinephrine to stimulate oxygen consumption in perfused skeletal muscle.

Aerobic exercise training has also been found to increase the epinephrine-mediated release of free fatty acids from white adipose tissue (Askew et al 1975). This evidence is in agreement with the possibility that exercise training results in an increase in the sensitivity of some tissues to catecholamine stimulation.

If brown adipose tissue sensitivity to catecholamine stimulation is augmented by aerobic training and adrenal secretion of epinephrine is increased as well, then a decrease in firing from sympathetic nerves after training would not decrease the sympathetic nervous system stimulation of brown adipose tissue.

This would mean that the choice of a training program which allows plasma cortisol levels to return to normal may be critical in order to show an exercise-induced increase in brown adipose tissue growth and thermogenic capacity. This therefore could explain the difference between the results found in this study and the results of others.

To show this conclusively more study must be done to compare training programs at different intensities with

plasma cortisol levels and changes in brown adipose tissue thermogenic capacity. Demonstration of an increase in brown adipose tissue sensitivity to catecholamine after training is also important.

It may be that in order to increase brown adipose tissue thermogenesis the intensity, or stress level, of a training program must not only be low enough to keep cortisol levels down but high enough to get a sufficient stimulatory effect from catecholamines.

Another factor that would be useful to investigate is the role played by insulin in influencing the results in this and other studies. Insulin levels decrease with exercise (Hartley et al 1972, 1972b; Winder et al 1987) though this decrease is less severe after training (Hartley et al 1972, 1972b). Since insulin stimulates the sympathetic nervous system (Landsberg 1986) and therefore brown adipose tissue thermogenesis, the exercise-induced decrease in plasma insulin levels could act to suppress brown adipose tissue thermogenesis.

On the other hand, exercise training increases insulin sensitivity (Jobidon et al 1985). Therefore the decrease in plasma insulin levels during exercise in a trained animal might not act to suppress brown adipose tissue thermogenesis. This remains to be investigated. In fact, since insulin resistance has been associated with possible suppression of brown adipose tissue thermogenesis (Jequier

1983; Mercer & Trayhurn 1984b, 1986), it may be found that the influence of exercise training on insulin could lead to the stimulation of brown adipose tissue thermogenesis.

Also, different exercise protocols may produce different changes in plasma insulin levels and insulin sensitivity. This factor may further explain the variation in results found in the studies done thus far on the interaction between exercise and brown adipose tissue thermogenesis.

It is therefore important to do more detailed studies which will examine the influence of exercise on hormones such as insulin, cortisol, and catecholamines, and compare these hormone changes with changes in brown adipose tissue thermogenesis.

CHAPTER VI

CONCLUSION AND RECOMMENDATIONS

The fact that training would increase brown adipose tissue size and lead to a mild improvement in the thermogenic capacity is in some ways perplexing as brown adipose tissue thermogenesis and exercise are processes that would compete for calories. Also both processes produce heat.

The finding that brown adipose tissue thermogenic capacity can be increased with aerobic exercise training may be due to the fact that physical activity may act to 'tune' the body generally. The advantage of the attendant increase in the thermogenic capacity of a trained animal may be to have its brown adipose tissue primed and ready to increase heat production quickly.

This may be very useful if the animal is suddenly exposed to cold. Sudden drops in temperature are common in the wild, particularly in the spring and fall in temperate climates when night-time temperatures are much lower than those during the day.

An ability to increase brown adipose tissue thermogenesis in the cold would reduce the need for shivering and preserve intramuscular fuel stores for physical activity such as flight from a predator.

An increased capacity for brown adipose tissue thermogenesis would also be useful for an aerobically trained animal in allowing it to maintain a lower body weight. Excessive weight would interfere with physical performance. Thus an animal that needs to run frequently and for significant lengths of time would have an advantage if it were relatively lean.

It is also true that exercise results in the mobilization of fuel such as fatty acids (Astrand & Rodahl 1977). When exercise stops it is likely that the fuel remaining in transport in the blood would be used to replenish muscular stores and be returned to white adipose tissue for storage. However it is possible that brown adipose tissue thermogenesis would be useful to use up some of this fuel, thus returning the body to homeostasis and resting blood fuel levels more quickly.

If the exercise stress that an animal is subjected to is too great, the benefits of an increased brown adipose tissue thermogenic capacity may be counteracted by other factors. Higher levels of exercise intensity require greater use of fuel, therefore fuel conservation may outweigh the need to maintain the animal's body in a state of readiness for cold exposure.

At the end of an intense exercise bout the body may need to use all extra fuel mobilized for replenishing intramuscular stores. This would negate any benefit of brown

adipose tissue thermogenesis in returning the body to resting blood fuel levels. Brown adipose tissue thermogenesis would also be unnecessary for the maintenance of a relatively low body mass if the intensity of the physical activity that the animal is subjected to remains sufficiently elevated.

A great deal of work is yet required to give a fully clarified picture of the effect that exercise has on BAT thermogenic capacity. More detailed work relating exercise-induced hormonal levels to changes in brown adipose tissue thermogenesis are needed.

It would be useful to do a series of studies using exercise programs of graded intensities, measuring changes in blood hormonal levels, and comparing these changes with any changes in BAT biochemical characteristics such as protein and DNA content, mitochondrial mass, oxidative capacity, and mitochondrial GDP binding capacity.

Further investigation of the effect of exercise on brown adipose tissue thermogenesis would increase the understanding of the influence of exercise on mammalian physiology. It would also add to the understanding of the mechanisms by which brown adipose tissue is activated.

A better comprehension of brown adipose tissue thermogenesis and exercise and the role they play in human physiology is of great importance. Since brown adipose tissue may be found to play an important role in the control

of human obesity, knowledge of the influence that specific exercise regimens have on human brown adipose tissue thermogenesis could provide valuable information for the establishment of improved methods of treating obesity.

As obesity leads to a variety of health problems, this study and others on the effect of exercise on brown adipose tissue could provide a significant benefit to the human community by facilitating a more effective system of health care.

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APPENDIX

1. RAW DATA: INDIVIDUAL SUBJECT VALUES

WEIGHTS AND FOOD INTAKE

Rat	Final weight (gm)	Weight gain (gm)	Food intake (gm/day)	Heart weight (gm)	IBAT weight (gm)
Control 1	445.5	232.5	21.77	1.252	0.312
2	565.5	345.5	25.34	1.477	0.355
3	452.5	252.5	22.56	1.081	0.340
4	519.5	309.5	24.34	1.387	0.363
5	453.0	251.0	21.08	1.341	0.327
6	540.5	332.5	25.28	1.503	0.219
7	564.5	345.5	25.37	1.392	0.438
8	535.5	318.5	23.47	1.620	0.452
9	532.5	321.5	25.02	1.460	0.317
10	480.0	284.0	24.64	1.232	0.279
Exercise 1	419.5	223.5	21.08	1.253	0.244
2	446.0	237.0	23.29	1.734	0.346
3	456.5	249.5	22.72	1.305	0.362
4	442.5	236.5	22.30	1.198	0.234
5	438.5	235.5	22.25	1.139	0.320
6	417.0	213.0	21.90	1.272	0.254
7	444.0	242.0	21.64	1.314	0.393
8	442.0	236.0	25.92	1.299	0.480
9	412.5	209.5	23.61	1.318	0.354
10	422.5	215.5	23.58	1.512	0.243
11	425.0	230.0	22.78	1.379	0.191
12	473.5	263.5	24.57	1.530	0.370
13	503.0	283.0	24.24	1.722	0.236

IBAT & MITOCHONDRIAL PARAMETERS

Rat	IBAT protein (mg)	IBAT DNA (mg)	Mito. protein (mg)	Cyt.Ox. spec.act. (ug O/mg/m)	GDP spec.bd. (pmol/mg)
Control 1	7.20	0.141	1.12	7.32	12.6
2	3.38	0.076	0.33	2.82	0
3	5.63	0.100	0.83	4.90	27.2
4	5.63	0.108	0.87	4.75	13.4
5	7.35	0.121	1.10	4.57	18.8
6	6.90	0.105	1.68	7.64	15.6
7	5.33	0.088	0.74	4.93	12.3
8	10.35	0.171	2.14	8.72	14.2
9	8.85	0.123	1.94	8.47	28.1
10	7.25	0.095	1.52	4.63	12.6
Exercise 1	11.48	0.155	2.25	7.85	11.0
2	9.08	0.153	1.64	6.23	1.3
3	15.75	0.198	3.23	6.59	10.0
4	6.38	0.100	1.06	5.08	12.4
5	9.30	0.165	2.28	6.16	5.1
6	14.40	0.195	2.65	5.53	19.1
7	11.70	0.207	2.33	5.81	8.5
8	11.33	0.202	2.45	7.93	22.3
9	17.10	0.225	3.81	8.36	17.4
10	10.73	0.150	1.77	5.12	12.1
11	6.83	0.108	1.16	3.52	10.3
12	8.78	0.175	1.18	1.44	6.0
13	10.43	0.148	1.82	6.51	16.3

Mito. = Mitochondrial; Cyt.Ox. = Cytochrome oxidase;
spec. = specific; act. = activity; bd. = binding
O = oxygen atoms; m = minute.

2. TIME ESTIMATES

An Estimation of the Time Required to Bring Six Exercise-Trained Rats from the Housing Room to Maximum Speed on the Treadmill at the Beginning of their Run:

Trial	Time (min & sec)	Time in sec
1	1' 45"	105
2	1' 34"	94
3	1' 48"	108
4	2' 02"	122
5	1' 40"	100
6	1' 44"	104
Average	1' 46"	106 +/- 9

An Estimation of the Time Required to Remove Six Rats from the Treadmill and Return them to the Housing Room after the End of their Run:

Trial	Time (min & sec)	Time in sec
1	3' 08"	188
2	2' 30"	150
3	2' 58"	178
4	3' 55"	235
5	4' 03"	243
6	3' 18"	198
7	2' 33"	153
8	2' 41"	161
Average	3' 08"	188 +/- 36