Alpha-Noradrenergic Regulation of Nutrient Intake in Genetically Obese (ob/ob) and Lean (+/?) Mice

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BY

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Abstract

Noradrenergic mechanisms in the hypothalamic paraventricular nucleus, principally of the alpha-2 subtype, are implicated in the regulation of food intake (Leibowitz et al., 1985). Given the increased levels of norepinephrine in the paraventricular nucleus of the obese (ob/ob) mouse, a genetically determined abnormality in the alpha-2 noradrenergic system may contribute to the hyperphagia of the ob/ob. Therefore, alpha-noradrenergic regulation of nutrient intake was examined in genetically obese and lean (+/?) mice. Experiment 1 determined the time required for ob/ob and +/? mice to adapt to a 6-h restricted feeding schedule. In Experiment 2 ob/ob and +/? mice were adapted to 6-h access to carbohydrate, fat, and protein before receiving vehicle injections ip 30 min before diet access, for 2 days, with intakes recorded at 1, 3, and 6 h. Then, separate groups (n=14) of ob/ob and +/? mice received 0.1 mg/kg or 0.5 mg/kg clonidine, an alpha-2 agonist, or vehicle 30 min prior to diet access. Clonidine decreased total caloric intake and intake from carbohydrate, fat, and protein in ob/ob and +/? mice (ps<.005). However, at 1 h, 0.5 mg/kg clonidine increased the proportion of energy from carbohydrate. Experiment 3 examined the specificity of the alpha-2 receptor mechanism in feeding. Yohimbine, an alpha-2 antagonist, was administered at doses of 3 mg/kg and 5 mg/kg ip, alone or prior to clonidine (0.5 mg/kg) or

vehicle. Mice administered either vehicle, 3 mg/kg, or 5 mg/kg ip yohimbine 30 min prior to clonidine and mice administered either dose of yohimbine prior to vehicle (n=7), decreased total food intake (p<.001), although the proportion of carbohydrate ingested was greater when 3 mg/kg or 5 mg/kg yohimbine preceded clonidine, or 5 mg/kg yohimbine preceded vehicle, than when either 3 mg/kg yohimbine or vehicle preceded vehicle, or vehicle preceded clonidine (p<.001). Although clonidine and yohimbine alone had similar effects, possibly implicating more than one receptor mechanism, joint administration tended to reverse the effect either drug had on its own. Given that previous research has suggested that clonidine can increase food intake in the rat (Leibowitz et al., 1985), the apparent difference in the effect of clonidine may be dose-dependent, or attributed to interspecies differences with respect to clonidine's mechanism of action. Despite suggestions to the contrary in the literature, there were no Phenotype x Drug interactions.

Alpha-Noradrenergic Regulation of Nutrient Intake in Genetically Obese (ob/ob) and Lean (+/?) Mice

The genetically obese mouse (ob/ob) is characterised by abnormal behavioural, physiological, and hormonal states, including hyperphagia, increased adiposity, and hyperinsulinemia (Mrosovsky & Melnyk, 1982; Sclafani, 1984; Storlien, 1984). Although the primary defect responsible for the syndrome has not been determined, an abnormality in central nervous system (CNS) function may contribute to the hyperphagia of the ob/ob mouse (Callahan, Beales, & Oltmans, 1984; Oltmans, Lorden, Callahan, Beales, & Fields, 1981). Evidence of CNS abnormalities in the ob/ob include decreased neuronal size in several brain regions (Bereiter & Jeanrenaud, 1979), altered dendritic orientation in lateral and ventromedial hypothalamic nuclei (Bereiter & Jeanrenaud, 1980), decreased levels of cholecystokinin in the cerebral cortex (Straus & Yalow, 1979), and increased hypothalamic norepinephrine (NE) levels (Feldman, Blalock, & Zern, 1979). This latter finding is of particular interest in terms of the hyperphagia exhibited by the ob/ob mutant, as hypothalamic NE has been proposed to play an important role in the regulation of feeding (Kuprys & Oltmans, 1982; Leibowitz, Brown, Tretter, & Kirschgessner, 1985).

Although hypothalamic catecholamines are known to modulate total energy intake (Hoebel, 1984; Morley & Levine, 1985) and have been implicated in specific macronutrient selection (Mauron, Wurtman, & Wurtman, 1980; Sclafani & Aravich, 1983; Shor-Posner, Azar, Insinga, & Leibowitz, 1985), research has yet to examine neurochemical regulation of macronutrient intake in the obese mouse. The current study, therefore, examined the impact of pharmacological manipulations of the noradrenergic system on macronutrient selection in the ob/ob mouse.

The Genetically Obese Mouse

The genetic aberration in the ob/ob is an autosomal recessive trait, a single point mutation in the genetic code leading to the production of a defective peptide (Connolly & Carnie 1984; Storlien, 1984). The homozygous recessive ob/ob displays marked obesity, involving increases in both size and number of adipocytes (Faust, Johnson, Stern, & Hirsch, 1978; Johnson & Hirsch, 1972). The rate of lipogenesis is increased in the liver and fat (Assimacopoulos-Jeannet, Singh, Le Marchand, Loten, & Jeanrenaud, 1974). In contrast, the homozygous dominant (+/+) wild type and the heterozygous +/ob are phenotypically lean. Procreation is confined to the mating of +/ob heterozygous mice because the ob/ob is infertile.

The ob/ob is hyperphagic, which is manifested in an increased caloric intake (Bray & York, 1971; Sclafani, 1984; Stricker, 1978). Obese mice maintain an elevated energy intake regardless of their pattern of self-selected nutrient intake (Romsos & Ferguson, 1982), and adult obese mice prefer to self-select a higher proportion of energy from fat than from protein or carbohydrate (Castonguay, Rowland, & Stern, 1985; Romsos, Chee, & Bergen, 1982). Obese mice allowed to self-select from diets varying in protein and carbohydrate consume as much protein as lean mice (Chee, Romsos, & Bergen, 1981; Chee, Romsos, Bergen, & Leveille, 1981; Romsos, Chee, & Bergen, 1982). When given access to diets varying in protein and fat, both obese and lean mice reduce their intake of protein (Romsos, Chee, & Bergen, 1982). The reduction in protein intake appears to be secondary to a greater preference for a high-fat diet rather than a high-carbohydrate diet and suggests that a nonprotein energy source can affect self-selected protein intake.

Romsos & Ferguson (1982) have found that energy derived from carbohydrate, fat, and protein averaged 12%, 68%, and 20%, respectively, for obese mice and 25%, 53%, and 22%, respectively, for lean mice. Therefore, both obese and lean mice self-select more energy from fat, although the ob/ob appears to maximise fat intake resulting in a greater increase in body weight gain. However, increased adiposity

has not been attributed to overeating alone because the ob/ob remains obese even when pair-fed with lean (+/?) littermates (Stock & Rothwell, 1982).

The ob/ob is also hyperinsulinemic (Dubuc, 1977; Storlien, 1984). Elevated plasma insulin concentrations are associated with an increase in the number and size of insulin-secreting beta cells of the pancreas (Storlien, 1984). The ob/ob exhibits glucose intolerance. Marked insulin resistance is associated with the progressive loss of insulin receptors (Herberg & Coleman, 1977). As a result, the hypoglycemic effect, normally associated with elevated levels of circulating insulin, does not characterise the ob/ob mutant. Also, enzymes involved in gluconeogenesis, found to be decreased in the hyperinsulinemic state, remain elevated in the ob/ob, an abnormailty which occurs early in development and may be associated with the loss of insulin receptor sites in the liver (Coleman, 1978).

Another major component of the ob/ob syndrome is the thermogenic defect (Seydoux, Rochner-Jeanrenaud, Assimacopoulos-Jeannet, & Jeanrenaud-Girardier, 1981). A chronically lower colonic temperature and resting metabolic rate at ambient temperatures below thermoneutrality suggest that the ob/ob is hypothermic and hypometabolic, as

indicated by a lowered rate of oxygen consumption (Boisonneault, Hornshuh, Simons, Romsos, & Leveille, 1978; Carlisle & Dubuc, 1984; Thurlby & Trayhurn, 1978).

Preweanling obese mice exhibit defective thermogenesis as well as differences in energy metabolism, which are evident as early as 5 days postpartum (Boisonneault et al., 1978), suggesting that abnormalities in both thermoregulation and metabolism are not secondary to obesity. That is, defective physiological thermoregulation precedes the divergence in body mass of lean and obese mice. Hypothermia, therefore, may be a primary contributor in the development of obesity, as a greater percentage of the diet is diverted to fat rather than to energy for thermogenesis. By expending less energy on thermoregulatory thermogenesis, obese mice store intake as white fat, resulting in gross adiposity (Thurlby & Trayhurn, 1979; Trayhurn & James, 1980). The lowered expenditure of energy on thermoregulatory heat production leads to an increased metabolic efficiency which appears to underlie the development and maintenance of obesity.

Hogan & Himms-Hagen (1980) have identified a mitochondrial defect in brown adipose tissue of reduced binding of purine nucleotides and a failure of the ob/ob to respond to cold stress with an increase in purine binding. Reduced NE turnover in brown adipose tissue is also

characteristic of the ob/ob (Knelans & Romsos, 1982). Therefore, an important mechanism in heat production, nonshivering thermogenesis, involving extra-muscular sources or areas of brown adipose tissue, is defective in the ob/ob mouse (Trayhurn, Goodbody, & James, 1982). As a result, the ob/ob displays an impairment in increasing heat production. The reduction in nonshivering thermogenesis, in turn, is manifested in a decreased metabolic rate and lowered colonic temperature (Batt & Hambi, 1982; Trayhurn & James 1980).

In addition to these symptoms, biochemical and neuroanatomical abnormalities have been cited as evidence of a CNS defect in the ob/ob mouse. Altered neuroanatomical organisation in the CNS includes reduced brain mass and cortical brain volume and significantly reduced soma cross-sectional areas in the ventromedial hypothalamus (VMH) of ob/obs when compared to lean controls (Bereiter & Jeanrenaud, 1979).

Elevated levels of NE (Feldman et al., 1979; Oltmans, 1983), pituitary dopamine (DA) (Lorden & Oltmans, 1977; Oltmans, 1983), 5-hydroxytryptamine (5-HT) (Garthwaite, Martinson, Tseng, Hagan, & Menahan, 1980), and beta-endorphin (Govoni & Yang, 1981) have also been detected in the ob/ob mouse. Endogenous hypothalamic NE levels are increased in the paraventricular nucleus (PVN) and the VMH

(Oltmans, 1983), areas which have been implicated in the regulation of feeding (Hoebel, 1984; Leibowitz et al., 1985). Following reserpine treatment, which acts to deplete catecholamines by affecting the ability of the adrenergic vesicle to store transmitter, hypothalamic NE levels of the ob/ob are not depleted to the same levels as in similarly treated lean mice (Oltmans, Olsauskas, & Comaty, 1980). Thus, increased NE levels and altered sensitivity to pharmacological manipulation in the ob/ob could involve a number of neurochemical mechanisms, including abnormal release mechanisms of the presynaptic neuron, modified storage properties, or altered reuptake mechanisms.

An increase in alpha-1 adrenergic receptor density is found in the ob/ob, although no differences in alpha-2 receptor density and affinity are known to exist between obese and lean mice (Callahan et al., 1984; Oltmans et al., 1981). No significant changes between lean and obese mice are found in either receptor number or affinity for alpha-adrenergic receptors in the cortex, or for dopaminergic or muscarinic receptors in the cortex or corpus striatum (Oltmans et al., 1981). Receptor abnormalities in the ob/ob, therefore, do not extend to all receptors, as the increase in alpha-adrenergic receptors in the hypothalamus appears relatively unique. However, a decrease in cholecystokinin (Straus & Yalow, 1979) and an increase in

cholecystokinin receptors (Hays & Paul, 1981) have been found in the cerebral cortex of obese mice, suggesting that multiple abnormalities in neural systems involved in the control of feeding exist in the ob/ob mutant.

Hypothalamic Control of Food Intake

Noradrenergic mechanisms located in the hypothalamic PVN have a physiological role in the control of feeding. Norepinephrine injected directly into this nucleus stimulates food intake in the satiated animal and potentiates food ingestion in the hungry rodent, and thus reflects the function of a physiological control mechanism (Hoebel, 1984; Lichtenstein, Marinescu, & Leibowitz, 1984; Morley & Levine, 1985). That is, the paraventricular and medial hypothalamic regions have been implicated in satiety control involving alpha-adrenergic and serotonergic (5-HT) systems (Blundell, 1979; Hoebel, 1984; Leibowitz, 1982). Norepinephrine acts to inhibit satiety; whereas 5-HT facilitates satiety. The PVN, therefore, is characterised by alpha-adrenergic inhibition and serotonergic excitation of a proposed satiety system.

In contrast, beta-adrenergic and dopaminergic systems in the perifornical lateral hypothalamic (LH) region inhibit feeding (Leibowitz, 1980; Leibowitz & Rossakis, 1978; Morley & Levine, 1985). Norepinephrine, therefore, injected into

the LH inhibits feeding through beta-adrenergic stimulation. Amphetamine treatment has been found to produce a dose-dependent decrease in food intake in both lean and obese mice, suggesting that amphetamine-induced release of NE contributes to the inhibition of feeding (Kuprys & Oltmans, 1982). However, when alpha-methyl-para-tyrosine is administered to ob/ob mice, the NE content, and presumably the synaptic availability of NE, is reduced and hyperphagia is potentiated (Batt, Wilson, & Topping, 1978).

Acute injection of NE is most effective in initiating a feeding response in rats when it is injected directly into the PVN region (Leibowitz, 1978). At this site, injection of NE produces the strongest feeding response at the lowest threshold dose. The ingestive response is similar to normal feeding in terms of magnitude and duration. In addition, chronic injection of NE into the PVN results in hyperphagia and increased body weight gain (Leibowitz et al., 1984).

Similar results are found following electrolytic lesions to the PVN. Lesions to the caudal aspect of the PVN are most effective in eliciting hyperphagia and body weight gain (Leibowitz, Hammer, & Chang, 1981). However, feeding behaviour induced by central NE injection is attenuated following discrete lesions to the PVN (Leibowitz, Hammer, & Chang, 1983). It appears that the integrity of the PVN is

essential for a normal feeding response to be elicited by central NE administration. Given that destruction of PVN noradrenergic innervation following local administration of 6-hydroxydopamine (6-OHDA) results in hypophagia, this would suggest that hyperphagia resulting from PVN electrolytic lesions cannot be attributed to the destruction of noradrenergic afferents to the nucleus, but reflects damage to PVN efferent projections through which NE may act to control satiety (Leibowitz & Brown, 1980; Shor-Posner, Azar, Jhanwar-Uniyal, Filart, & Leibowitz, 1986).

Norepinephrine injected into the PVN at near physiological doses stimulates food intake in the satiated animal, an effect which is blocked by selective alpha-2 adrenergic receptor antagonists, including yohimbine and rauwolscine, but not by beta-adrenergic receptor blockers, nor by blockers of serotonergic, cholinergic, or dopaminergic receptors (Callahan et al., 1984; Goldman, Marino, & Leibowitz, 1985; Leibowitz, 1980). Clonidine, an alpha-2 adrenergic agonist, increases total food intake in hungry and sated mice and rats (Callahan et al., 1984; Leibowitz et al., 1985, Marino, De Bellis, & Leibowitz, 1983). These effects are seen when these drugs are administered either centrally or peripherally (McCabe, De Bellis, & Leibowitz, 1984). Callahan et al. (1984) have found that peripherally administered yohimbine and

rauwolscine significantly reduced 3- and 6-h food intake in both ob/ob and lean mice; however, ob/ob mice were more sensitive to the anorectic effect than were lean mice. Anorectic doses of yohimbine did not affect water intake in water-deprived mice, suggesting a specific effect of the drug on food intake. Furthermore, clonidine (0.1 mg/kg) increased 1-h food intake in obese mice at doses which did not affect food intake in lean mice, suggesting that obese mice were also more sensitive to the hyperphagic effect of clonidine. However, higher doses of clonidine resulted in an apparent suppression of intake in both obese and lean mice. Given that the ob/ob has increased levels of NE in the PVN, the hyperphagia exhibited by this mutant may be attributed, at least in part, to an impaired satiety control mechanism.

Tricyclic antidepressants have also been found to enhance total food intake, specifically following injection into the PVN (Leibowitz et al., 1985). The response to such drugs as desimpramine, protryptyline, and amitriptyline is selectively blocked by alpha-noradrenergic antagonists and is also antagonised by drugs which inhibit the synthesis of endogenous NE (Leibowitz, Arcomano, & Hammer, 1978; Leibowitz et al., 1985). Antidepressant agents, therefore, known to block the neuronal reuptake of NE, elicit feeding by enhancing synaptic availability of endogenous NE specifically in the PVN.

The feeding response resulting from administration of tricyclic antidepressants is prevented by local administration of CA synthesis inhibitors, suggesting that antidepressant-induced feeding is mediated by presynaptic NE stores (Leibowitz, 1978). In contrast, feeding elicited by exogenous NE is unaffected by the same synthesis inhibitors, and is consistent with a postsynaptic mechanism for NE (Goldman et al., 1985). Electrolytic and neurotoxic lesions of the specific ascending noradrenergic fibres that innervate the PVN abolish the antidepressant feeding response, but leave intact or potentiate the postsynaptic NE feeding response (Leibowitz & Brown, 1980).

Given that NE may act via alpha-2 receptors located on the postsynaptic membrane (Langer, 1981), that is, alpha-2 receptors may exist post- as well as presynaptically in the brain, it is possible that clonidine, like NE, may affect food intake via a postsynaptic alpha-2 receptor mechanism. Clonidine-induced feeding is suppressed by injections of yohimbine but not prazosin or corynanthine, alpha-1 adrenergic antagonists (Goldman et al., 1985; Schlemmer, Elder, Casper, & Davis, 1981), implicating alpha-2 receptors rather than postsynaptic alpha-1 receptors in the regulation of feeding.

Postsynaptic alpha-2 receptors are known to mediate a number of physiological effects resulting from clonidine administration, including cardiovascular and sedative effects (Hamilton & Longman, 1982; Nasif, Kempf, Cardo, & Velley, 1983; Spyraki & Fibiger, 1982), which suggests that alpha-2 postsynaptic involvement in feeding is not improbable. Feeding induced by central injection of clonidine, like NE, is unaffected by local administration of alpha-methyl-para-tyrosine (Goldman et al., 1985). Injection of 6-OHDA into the PVN, in contrast to electrolytic lesions, leaves the feeding response evoked by peripheral injection of clonidine intact (Shor-Posner, Azar, & Leibowitz, 1984). As well, biochemical analyses examining the effect of clonidine on NE turnover have indicated that noradrenergic terminals in the PVN respond uniquely to peripheral clonidine administration, in a manner inconsistent with a presynaptic site of action (Jhanwar-Uniyal, Levin, & Leibowitz, 1985). Norepinephrine, clonidine, and tricyclic antidepressants, therefore, may act to stimulate food intake through the same noradrenergic system but at different parts of the synapse.

Statement of the Problem

Until recently, most studies examining the neuropharmacology of feeding have utilised a single, nutritionally complete diet with little concern for the

nutritional composition of the diet (Blundell & Hill, 1986). However, central and peripheral pharmacological manipulations may alter macronutrient selection, suggesting that specific brain neurotransmitters may have a function in balancing the proportion of carbohydrate, protein, and fat consumed by a rat (Kanarek, Marks-Kaufman, Ruthazer, & Gualtieri, 1983; Leibowitz, Roosin, & Rosenn, 1984). For example, Leibowitz et al. (1985) have shown that endogenous NE, acting on alpha-adrenoceptors in the PVN, regulates carbohydrate ingestion in the rat.

Similarly, clonidine and tricyclic antidepressants selectively stimulate carbohydrate ingestion in the rat. Although clonidine has also been found to affect the ingestion of protein and fat, this effect is considerably less than the potentiation of carbohydrate intake, resulting in a significant decline in the proportion of fat and protein consumed (Leibowitz, 1985). That is, both fat and protein intake are increased following clonidine administration; however, when proportion of total energy intake is considered, a reduction in percentage concentration of protein and fat is found. Thus, while NE, clonidine, and tricyclic antidepressants all increase total food intake, these drugs also selectively potentiate carbohydrate ingestion. Therefore, noradrenergic neurons innervating the PVN in the rat have been implicated in the

regulation of carbohydrate selection, and this neurochemical system mediates the stimulating action of clonidine and antidepressants on carbohydrate ingestion.

However, although hypothalamic catecholamines are known to modulate total energy intake and have been implicated in specific macronutrient selection, research has yet to examine neurochemical regulation of macronutrient intake in an obese pathological model, such as the ob/ob mutant, which exhibits hyperphagia. Given the elevated NE levels in the ob/ob, it is possible that a genetically determined abnormality in the PVN-noradrenergic system may lead to the hyperphagia exhibited by the ob/ob mutant. Altered neurochemical mechanisms in this region may allow an increase in the amount of neurotransmitter reaching the postsynaptic membrane. It follows that increased PVN-NE would result in increased postsynaptic alpha-2 receptor stimulation which, in turn, may lead to the hyperphagia exhibited by this mutant.

The present research, therefore, examined the impact of pharmacological manipulations on macronutrient selection in the ob/ob, using a self-selection feeding paradigm, in which mice were given free access to separate sources of each of the three macronutrients, carbohydrate, protein, and fat. The alpha-2 adrenergic agonist clonidine, known to alter

total food intake, was administered to assess its effects on specific macronutrient selection in both ob/ob and +/? mice. Although clonidine was known to alter total food intake and especially carbohydrate ingestion in the rat, its effects on macronutrient intake in the mouse, and in particular the ob/ob, were unknown. The impact of yohimbine, an alpha-2 adrenergic receptor blocker, was also examined to assess its effects on total food intake and nutrient selection in lean and obese mice treated with clonidine. It was hypothesised that total food intake in ob/ob and +/? mice would be altered following clonidine administration. Clonidine was also expected to increase the proportion of carbohydrate ingested by mice. In contrast it was expected that yohimbine would antagonise the effect of clonidine on food intake in obese and lean mice.

Experiment 1

Nutrient selection can be altered by a number of external environmental factors including a restricted feeding regimen (Blundell, 1983; Li & Anderson, 1984), suggesting that drug effects may differ depending on the extent of food deprivation. However, Leibowitz et al. (1985) have found that a 6-h restricted feeding schedule yields a relatively stable baseline nutrient selection pattern and total diet intake scores similar to 24-h food intake scores under ad-lib food conditions in the rat. Therefore, 24-h food

intake measures may also be accurately measured within a 6-h feeding paradigm in the rat. However, previous research monitoring feeding behaviour has shown that obese mice adapt less readily to restricted feeding schedules or meal-feeding paradigms (Callahan et al., 1984; Jagot, Dickerson, & Webb, 1982; Kuprys & Oltmans, 1982), although diet adaptation has only been assessed in research examining macronutrient intake, in lean and obese mice, where nutrient intake was not restricted (Romsos, Chee, & Bergen, 1982; Romsos & Ferguson, 1982). The purpose of Experiment 1 was to determine the time required for ob/ob and +/? mice to adapt to a 6-h feeding regime, implemented in Experiments 2 and 3 where the effects of clonidine and yohimbine were examined, and to assess the comparability of macronutrient self-selection in free-feeding and meal-feeding mice.

Method

Subjects

Obese (C57Bl/6J, ob/ob, n=16) and lean (C57Bl/6J, +/?, n=16) adult male mice, 10 weeks of age at the start of the experiment, were obtained from Jackson Laboratories, Bar Harbor, ME, USA.

Heterozygote crosses between mice carrying the obese (ob) gene lead to animals of three genotypes: the homozygous, dominant (+/+) wild type, the heterozygote (+/ob), and the

homozygous recessive type (ob/ob). Both the +/+ and +/ob are phenotypically lean, while the ob/ob is obese. However, the +/ob mouse is not identifiable from its +/+ littermate, and for this reason, lean littermate controls were identified as +/?.

Mice were individually housed in hanging wire cages (24.5 cm x 18 cm x 18 cm), suspended over wood-chip bedding, with a nest of bedding in each cage. All mice were maintained at 23°C on a 12-h light-dark cycle (lights on 0730 h). Colony room humidity ranged from 30-40%.

Apparatus

Diets were presented individually in circular aluminum containers with a stainless steel cover with four 1-cm holes, to allow access to the macronutrient but minimise food spillage. Water was available in calibrated 100-ml Wahmann drinking tubes. Body weights (g) and food intake (g) were measured using a Mettler Digital Balance (Model No. PB 300) to the nearest .01 g.

Diets

Three single-energy-source diets (cf. Leibowitz et al., 1985) were presented simultaneously to each animal. The carbohydrate ration was composed of 43.9% dextrin (R. Wine Baril), 43.9% corn starch (St. Lawrence Starch Ltd.), 4% minerals (United States Biochemicals), 3% vitamins (United

States Biochemicals), 5% fibre (ICN Nutritional Biochemicals), and 0.2% choline (United States Biochemicals). The protein component consisted of 86.3% vitamin-free casein (United States Biochemicals), 4% minerals, 3% vitamins, 5% fibre, 1.5% methionine (United States Biochemicals), and 0.2% choline. The fat diet was composed of 70.5% lard (Tenderflake), 10% essential fatty acids (Mazola Corn Oil), 8% minerals, 6% vitamins, 5% fibre, and 0.5% choline. Diets were formulated according to American Institute of Nutrition guidelines. Due to caloric dilution with non-nutritive bulk, calculation of caloric density was based on caloric coefficients of 3.7 kcal/g for carbohydrate and protein and 7.7 kcal/g for fat. Standard laboratory stock diet, Wayne F-6 Rodent Blox, consisted of 24% protein, 6.5% fat, 45.4% carbohydrate (nitrogren free extract), 3.7% crude fibre, 7.9% ash, and 12.5% moisture, yielding a calculated metabolizable energy of 3.1 kcal/g.

Design and Procedure

Mice were initially adapted to the colony room for three days with ad-lib access to standard laboratory stock diet (Wayne Rodent Blox) and water. Following lab adaptation, all mice were adapted to the separate nutrient diets for two days before being randomly assigned to one of two experimental conditions with matching for body weight. Eight ob/ob and 8 +/? mice (n=16) were adapted to a 6-h

restricted feeding schedule (18-h food deprivation) with ad-lib water access. Individual food cups of carbohydrate, protein, and fat, with preweighed amounts of the macronutrient, were presented between 0900 h and 1500 h, with measures of macronutrient intake following the 6-h nutrient presentation. Food spillage was added to the unconsumed total. The placement of the food cups within the cage was changed daily to prevent the occurrence of position preferences. Fresh diet was provided daily.

A control group of 8 ob/ob and 8 +/? mice (n=16) continued to receive 24-h free access to individual sources of carbohydrate, protein, and fat. Macronutrient intake was recorded for each 24-h period. Water was available ad lib.

Total food intake and individual macronutrient intakes, measured to the nearest 0.01 g, expressed in kilocalories and kilocalories of energy/gram body weight, were calculated on each test day. Water intake and body weights were also monitored. This procedure was repeated daily until macronutrient intake for restricted and unrestricted obese mice had stabilised, indicating that food-restricted mice had adapted to the feeding schedule. The procedure was also repeated for restricted and unrestricted lean mice. Body weight and nutrient intake measures were used to determine whether the mice were exhibiting normal growth patterns and

balanced nutrient selection. Mice on the 6-h restricted feeding schedule were given 24-h access to the diets if body weights decreased below 80% of original body weight. If body weights did not increase following 24 h access to the diets, one pellet of standard laboratory stock diet was given to any animal experiencing difficulty in adaptation. Mice on the 24-h free access schedule also received one lab chow pellet if similar problems in adaptation to the diets were experienced.

Independent variables included phenotype (ob/ob, +/?), regimen (restricted, unrestricted), and weeks on the regimen, with dependent measures of total energy intake, carbohydrate, protein, and fat intake in kcals, and body weights. The proportion of each nutrient contributing to total energy intake was also assessed.

Results

A 2 x 2 x 4 (Phenotype x Regimen x Weeks) analysis of variance (ANOVA), with repeated measures on the third variable, was performed on measures of total energy intake in kilocalories, kilocalories/ gram body weight, the proportion of each nutrient contributing to total energy intake, and body weights. A Type 1 error rate of .05 was maintained. Post hoc Tukey tests (Hays, 1981) were performed on all group mean differences.

The ANOVA examining energy intake in kilocalories/ gram body weight indicated significant main effects for phenotype, $F(1, 28)=312.65$, $p<.0001$, regimen, $F(1, 28)=38.26$, $p<.0001$, and weeks, $F(3, 84)=6.99$, $p<.0003$ (Table A-1). Although measures of energy intake did not differ between Week 1 ($M=0.40$), Week 2 ($M=0.42$), and Week 3 ($M=0.42$), mice consumed fewer kilocalories/ gram body weight in Week 4 ($M=0.38$). As indicated in Figure 1, obese mice consumed fewer kilocalories/ gram body weight ($M=0.24$) than lean controls ($M=0.58$) across weeks, $F(3, 84)=7.96$, $p<.0001$. Although obese mice maintained stable energy intakes across all four weeks ($Ms=0.23, 0.22, 0.25, 0.23$), lean mice showed relatively stable intakes for Week 1 ($M=0.57$), Week 2 ($M=0.62$), and Week 3 ($M=0.60$), with a marked decline in Week 4 ($M=0.52$). Furthermore, Figure 2 illustrates that mice on the 6-h restricted feeding schedule consumed fewer kilocalories/ gram body weight ($M=0.35$) than mice on 24-h free access to the diets ($M=0.47$), across weeks, $F(3, 84)=3.99$, $p<.01$. While mice on the 6-h schedule maintained stable energy intakes across weeks ($Ms=0.33, 0.38, 0.35, 0.34$), mice on the 24-h free access schedule maintained stable intakes across the initial three weeks of testing ($Ms=0.47, 0.48, 0.50$) followed by a significant decrease in energy intake in Week 4 ($M=0.42$).

See Figure 1 and 2 page 113 and 114

When intake measures were analysed in terms of total kilocalories, the ANOVA indicated significant main effects for phenotype, $F(1, 28)=22.85$, $p<.0001$, regimen, $F(1, 28)=46.06$, $p<.0001$, and weeks, $F(3, 84)=3.99$, $p<.01$, but no significant interactions (Table A-2), indicating that obese mice consumed fewer kilocalories ($M=10.64$) than lean mice ($M=14.36$), and that mice on the 6-h restricted schedule consumed fewer kilocalories ($M=9.85$) than mice on the 24-h free access schedule ($M=15.14$). Although total intake in kilocalories did not differ significantly between Week 1 ($M=12.74$), Week 2 ($M=12.85$), and Week 3 ($M=12.83$), intake declined in Week 4 ($M=11.57$).

A number of obese and lean mice experienced difficulty in adaptation to the restricted feeding schedule and to the diets and required food supplements. Of the eight obese mice on the 6-h restricted access schedule, six required 24-h access to diets during the third week of the experiment. Four of these six mice subsequently required one lab chow pellet to increase body weights to an acceptable level. In contrast, two of the eight lean mice on the 6-h schedule required 24-h access to diets during

Week 3. Two obese mice and one lean mouse on the 24-h schedule experienced difficulty in adaptation to the diets during Week 3 and were given a lab chow pellet in order to increase body weights. The ANOVA on intake measures, which included supplements, was similar to the ANOVA examining intake in kilocalories, which did not include supplements.

Proportion of nutrient intake, relative to total energy intake, is outlined in Table 1. In terms of the proportion of carbohydrate ingested, the ANOVA indicated a significant main effect for weeks, $F(3, 84)=5.01$, $p<.003$, and a significant Phenotype x Weeks interaction, $F(3, 84)=4.57$, $p<.005$ (Table A-3). The proportion of carbohydrate ingested across weeks was similar for obese and lean mice except in Week 4, where the proportion of carbohydrate was greater for leans ($M=0.25$) than for obese mice ($M=0.19$).

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The ANOVA examining the proportion of fat intake indicated a similar main effect for weeks, $F(3, 84)=4.66$, $p<.005$, and a significant Phenotype x Weeks interaction, $F(3, 84)=3.34$, $p<.02$ (Table A-4). Although obese and lean mice did not differ significantly in proportion of fat ingested in Week 1, Week 2, or Week 3, obese mice did

consume a higher proportion of fat (M=0.71) than lean mice (M=0.63) in Week 4.

In terms of the proportion of protein ingested relative to total energy intake, mice on the 6-h regimen consumed significantly less protein (M=0.11) than mice on the 24-h regimen (M=0.15), F(1, 28)=7.75, p<.009 (Table A-5). Furthermore, protein intake did not differ significantly in Week 1 (M=0.12), Week 2 (M=0.14), or Week 3 (M=0.14), although protein intake decreased significantly in Week 4 (M=0.11) when compared to either Week 2 or Week 3.

An ANOVA on body weight indicated that all main effects and interactions were significant (Table A-6). Obese mice (M=44.02) weighed more than lean mice (M=24.61), F(1, 28)=480.91, p<.0001. Mice on the 6-h feeding regimen weighed less (M=32.93) than mice on the 24-h regimen (M=35.71), F(1, 28)=9.87, p<.004. Obese mice on the 6-h schedule weighed less (M=41.54) than ob/ob mice on 24-h free access (M=46.51), whereas body weights were similar for +/? mice regardless of the feeding regimen (6-h M=24.32, 24-h M=24.91), F(1, 28)=6.12, p<.02. In particular, a significant Phenotype x Regimen x Weeks interaction, F(3, 84)=6.41, p<.0006, showed that lean mice on the 6-h regimen weighed the same as lean mice on the 24-h regimen throughout the 4-week test. Obese mice on the 6-h regimen weighed the

same as obese mice on the 24-h regimen during Week 1. However, by Week 2, obese mice on the 6-h schedule had significantly lower body weights than obese mice on the 24-h free access schedule, a difference that was maintained in Week 3 and Week 4. Although the Phenotype x Regimen interaction was not significant in the ANOVA examining intake in kilocalories/ gram body weight, it would appear that obese mice on the 6-h schedule did not ingest enough energy to maintain body weights comparable to those of obese mice on the 24-h feed; whereas, lean mice on the 6-h schedule did eat enough to maintain body weights.

Discussion

Although mice on the 6-h restricted feeding schedule consumed fewer kilocalories than mice on 24-h free access to diets, ob/ob and +/? mice on the 6-h regimen did maintain stable energy intake across weeks, suggesting that total energy intake had stabilised one week after mice had been introduced to the 6-h regimen.

Proportion of nutrient intake in restricted-feeding and free-feeding mice appeared comparable. Mice on the 6-h and 24-h regimens self-selected a greater proportion of energy from fat, a finding consistent with previous research (Romsos & Ferguson, 1982). Although proportion of protein ingested was reduced in mice on the 6-h regimen, proportion

of carbohydrate and fat did not differ between mice on the 6-h and 24-h regimens. Furthermore, proportion measures were similar for lean and obese mice until the fourth week on the diets.

Previous research examining feeding behaviour has indicated that obese mice adapt less readily to a restricted feeding schedule in which the source of energy is a standard laboratory chow diet (Callahan et al., 1984; Jagot, Dickerson, & Webb, 1982; Kuprys & Oltmans, 1982). In the present study adaptation differences were assessed in a paradigm in which lean and obese mice were allowed restricted access to three single-energy-source diets. Although obese mice did experience greater difficulty in adapting to a 6-h regimen than lean mice, problems in adaptation were not evident until the third week on the regimen.

Furthermore, although the ob/ob is hyperphagic when given ad-lib access to standard laboratory chow (Sclafani, 1984), Romsos & Ferguson (1982) have found that caloric intake did not differ significantly between lean and obese mice following ad-lib feeding of carbohydrate, fat, and protein. In the present study, obese mice on the 6-h and 24-h regimen consumed fewer kilocalories than lean mice. Given that total energy intake was also reduced in mice on the 6-h

regimen in comparison to mice on the 24-h regimen, and that obese mice on the 6-h regimen were unable to maintain body weights comparable to obese mice on the 24-h free feed after one week on the restricted regimen, adaptation differences to the single-energy-source diets and the restricted regimen may have resulted in the reduced intake.

Experiment 2

The present study examined the effects of clonidine, an alpha-2 adrenergic agonist, on feeding in obese and lean mice. A self-selection, 6-h restricted feeding paradigm, as in Experiment 1, was adopted to selectively monitor the ingestion of the three macronutrients, carbohydrate, protein, and fat, simultaneously available.

Method

Subjects

Obese (C57Bl/6J, ob/ob, n=42) and lean (C57Bl/6J, +/?, n=42) adult male mice, 14 weeks at the start of the experiment, included animals from the previous experiment as well as additional obese and lean mice. All mice not yet adapted to 6-h restricted feeding were adapted to the feeding schedule prior to drug treatment. Mice were individually housed as in Experiment 1 and tested in home cages.

Apparatus

Diets were presented in circular aluminum containers and water was available in calibrated Wahmann drinking tubes as in Experment 1. Carbohydrate, protein, and fat intake and body weights were measured using a Mettler Digital Balance (Model No. PB 300).

Diets and Drug

Composition of the three single-energy-source diets, carbohydrate, protein and fat, is described in Experiment 1. Caloric density was calculated using caloric coefficients of 3.7 kcal/g for carbohydrate and protein and 7.7 kcal/g for fat.

Clonidine-HCl (Sigma), a relatively specific alpha-2 agonist, was administered at the following doses: 0.1 mg/kg and 0.5 mg/kg intraperitoneal (ip). Clonidine (CLON) doses were selected from published reports indicating an effect on food intake in obese mice and in rats (Callahan et al., 1984; Mauron, Wurtman & Wurtman, 1980) but produced no overt signs of locomotor depression or illness (Callahan et al., 1984). The drug was administered in isotonic saline vehicle (VEH) at 0.5 ml/100 g body weight.

Design and Procedure

All mice were adapted, upon arrival to the laboratory, on stock diet. Mice were then adapted to a 6-h restricted feeding schedule with access to carbohydrate, protein, and fat for the remainder of the experiment. Water was available ad lib. The minimum time of one week, required for adaptation, was determined in Experiment 1. Obese and lean mice were assigned to one of three treatment groups to assess the effects of CLON on feeding: a VEH control, a low CLON, and high CLON treatment condition. Groups were matched for body weight within a phenotype and across treatment condition.

Following baseline measures of macronutrient intake, all mice, regardless of their drug condition, received a VEH injection of saline for two consecutive days. On the third and final day of injections, mice were administered an appropriate body weight dose of either VEH or CLON according to group assignment. The low CLON group included a total of 14 ob/ob and 14 +/? mice and received 0.1 mg/kg ip of CLON. An equal number of obese and lean mice were assigned to the high CLON group and received an injection of 0.5 mg/kg CLON ip. The control group of 14 ob/ob and 14 +/? mice received a VEH injection.

Preweighed amounts of carbohydrate, fat, and protein were given to each animal in each treatment condition. Diets were presented between 0900 h and 1500 h. Total intake and individual macronutrient intakes, measured to the nearest 0.01 g, expressed in grams and kilocalories, were taken 1, 3, and 6 h (Callahan et al., 1984; Leibowitz et al., 1985), following initial food presentation, to assess the effect of CLON on macronutrient intake. Clonidine doses, prepared daily, and VEH were coded blind to the experimenter, and were administered 30 min prior to food presentation.

Independent variables included phenotype (ob/ob, +/?), drug treatment (mg/kg), and sampling time (1, 3, 6 h), with dependent measures of total intake and carbohydrate, fat, and protein intake in kilocalories. Body weights were recorded daily to determine that no animal was below 80% of its free-feeding pretest weight. Body weights were not analysed.

Results

A 2 x 3 x 3 (Phenotype x Treatment x Sampling Time) ANOVA with repeated measures on the third variable was performed on measures of total energy intake, carbohydrate, fat, and protein intake, and the proportion of each nutrient to total energy intake. As well, ANOVA examined intake measures in terms of percentage VEH intake. That is, intake measures

were assessed in comparison to energy intake on the day prior to drug treatment, where VEH was administered to all mice regardless of treatment condition. A Type 1 error rate of .05 was maintained. Post hoc Tukey tests (Hays, 1981) were performed on all group mean differences.

The ANOVA examining energy intake in kilocalories indicated a significant main effect for phenotype, $F(1, 78)=32.42$, $p<.0001$, treatment, $F(2, 78)=20.61$, $p<.0001$, and sampling time, $F(2, 156)=529.46$, $p<.0001$ (Table A-7). Mice administered 0.5 mg/kg and 0.1 mg/kg CLON consumed significantly fewer kilocalories (*M*s=4.70, 5.82) than mice treated with VEH (*M*=8.16). Although *ob*/*ob* mice consumed fewer kilocalories (*M*=4.94) than +/? mice (*M*=7.50), all mice increased caloric intake across sampling time. A significant Phenotype x Sampling Time interaction suggested that obese mice, across treatment conditions, consumed significantly fewer kilocalories in comparison to +/? mice, at 1, 3, and 6 h following diet presentation, $F(2, 156)=10.23$, $p<.0001$. However, obese and lean mice administered 0.5 mg/kg CLON did consume similar amounts of energy (*M*s=4.11, 5.29), although *ob*/*ob* mice administered 0.1 mg/kg CLON or VEH consumed fewer kilocalories (*M*s=4.75, 5.96) than similarly treated +/? mice (*M*s=6.86, 10.36), $F(2, 78)=4.58$, $p<.01$.

Given that the results of Experiment 1 indicated that obese mice consumed less energy on a 6-h restricted feeding schedule when compared to lean controls, a more accurate assessment of the effect of CLON on energy intake would include a comparison of intake measures to the day prior to drug administration, indicating the extent to which energy intake is suppressed. Energy intake, therefore, was also assessed in terms of percentage VEH intake because of the apparent difference in baseline intake measures between ob/ob and +/? mice.

The ANOVA indicated significant main effects for phenotype, $F(1, 78)=4.99$, $p<.02$, treatment, $F(2, 78)=32.72$, $p<.0001$, and sampling time, $F(2, 156)=16.66$, $p<.0001$, but no significant Phenotype x Treatment interaction (Table A-8). Percentage of VEH intake was lower for obese mice ($M=73.33$) than lean mice ($M=85.22$), suggesting that ob/ob mice showed a greater suppression of caloric intake relative to VEH baseline. Ob/ob mice administered 0.5 mg/kg and 0.1 mg/kg of CLON did not differ significantly in percentage VEH intake, although both groups demonstrated a significant suppression of intake when compared to ob/ob mice treated with VEH. Lean mice in all treatment conditions differed significantly in percentage VEH intake. A significant Treatment X Sampling Time interaction, $F(4, 156)=12.75$, $p<.0001$, indicated that percentage VEH intake was

significantly lower on 1-h intake measures for obese and lean mice administered 0.5 mg/kg CLON (*M*=23.0) than for mice administered 0.1 mg/kg CLON (*M*=65.25), which differed significantly from VEH-treated mice (*M*=114.65). However, by the end of the test day, the only significant difference in percentage VEH intake rested between mice administered 0.5 mg/kg CLON and VEH. Obese mice in all treatment groups differed significantly 1 h following initial diet presentation. Three h following diet presentation, only *ob*/*ob* mice administered 0.5 mg/kg CLON and VEH differed significantly. Similar results were apparent for lean mice except that +/? mice administered 0.5 mg/kg CLON continued to show a significant difference from VEH controls throughout the remainder of the test day.

Total energy intake and carbohydrate, fat, and protein intake are outlined in Table 2. Obese mice ingested fewer kilocalories of carbohydrate (*M*=0.95) than lean mice (*M*=1.62), $F(1, 78)=30.55$, $p<.0001$ (Table A-9). Mice administered 0.5 mg/kg and 0.1 mg/kg CLON ingested similar amounts of carbohydrate (*M*s=0.93, 1.19) but significantly less than mice administered VEH (*M*=1.73), $F(2, 78)=14.94$, $p<.0001$. Obese mice administered 0.5 mg/kg CLON consumed significantly fewer kilocalories of carbohydrate (*M*=0.72) than *ob*/*ob* mice administered VEH (*M*=1.26), although *ob*/*ob* mice administered 0.1 mg/kg CLON did not differ

significantly from either group (M=0.87). In contrast, +/? mice administered 0.5 mg/kg and 0.1 mg/kg CLON ingested less carbohydrate (Ms=1.15, 1.51) than +/? mice treated with VEH (M=2.21). Although all mice increased carbohydrate ingestion across sampling time, $F(2, 156)=301.43$, $p<.0001$, a significant Phenotype x Sampling Time interaction indicated that ob/ob mice consumed less carbohydrate than +/? mice 1, 3, and 6 h following diet presentation, $F(2, 156)=20.51$, $p<.0001$.

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Proportion of carbohydrate ingested was greater 1 h following diet presentation (M=0.25) than 3 or 6 h following diet presentation (Ms=0.21, 0.21), $F(2, 156)=5.57$, $p<.005$, and significantly greater for mice administered 0.5 mg/kg CLON 1 h following diet presentation when compared to mice administered 0.1 mg/kg CLON or VEH, $F(4, 156)=7.05$, $p<.0001$ (Table A-10). However, by the third hour, proportion of carbohydrate ingested was similar across groups.

Measures of percentage VEH intake of carbohydrate indicated significant main effects for phenotype, $F(1, 78)=10.11$, $p<.002$, treatment, $F(2, 78)=11.55$, $p<.0001$, and sampling time, $F(2, 156)=4.31$, $p<.02$ (Table A-11).

Percentage VEH intake was lower for obese mice (M=70.90) than lean mice (M=97.87), suggesting that carbohydrate ingestion was suppressed more in obese than lean mice. Also, percentage VEH intake was lower for mice administered 0.5 mg/kg CLON (M=63.28) compared to mice administered 0.1 mg/kg CLON (M=77.93) or VEH (M=111.94), although mice administered 0.1 mg/kg CLON also differed significantly from controls. A significant Treatment x Sampling Time interaction indicated that percentage VEH intake was lower for obese and lean mice administered 0.5 mg/kg CLON (M=47.22) when compared to controls (M=123.83) 1 and 3 h following diet presentation, $F(4, 156)=4.18$, $p<.003$. Percentage VEH intake was lower for obese mice administered 0.5 mg/kg and 0.1 mg/kg CLON (Ms=35.87, 55.47) in comparison to ob/ob mice administered VEH (M=116.99), 1 h following diet presentation, although this difference was not maintained on subsequent measures. Percentage intake for +/? mice administered 0.5 mg/kg and 0.1 mg/kg CLON (Ms=58.58, 78.06) was lower than +/? VEH controls (M=130.67) 1 h following diet presentation, and this difference was maintained 3 h following diet presentation.

The ANOVA examining fat intake in kilocalories indicated that obese mice consumed fewer kilocalories of fat (M=3.25) than lean mice (M=5.03), $F(1, 78)=27.15$, $p<.0001$, and that mice admininistered 0.5 mg/kg and 0.1 mg/kg of CLON consumed

fewer kilocalories (Ms=3.14, 3.94) than VEH controls (M=5.33), F(2, 78)=13.90, p<.0001 (Table A-12). Although fat intake in obese mice administered 0.5 mg/kg CLON did not differ from obese mice administered VEH, fat intake in +/? mice administered 0.5 mg/kg CLON did differ significantly from +/? mice administered VEH. A significant Phenotype x Treatment interaction indicated that fat intake did not differ between ob/ob and +/? mice administered 0.5 mg/kg and 0.1 mg/kg CLON, although fat intake was greater for +/? mice administered VEH compared to ob/obs administered vehicle, F(2, 78)=4.42, p<.02. Although fat intake increased across sampling time, F(2, 156)=362.18, p<.0001, ob/ob mice consumed less fat than +/?, F(2, 156)=5.36, p<.006.

Proportion of fat ingested was significantly reduced in mice administered 0.5 mg/kg CLON (M=0.53) compared to mice administered 0.1 mg/kg CLON or VEH (Ms=0.62, 0.65), F(1, 78)=5.22, p<.007 (Table A-13). Although proportion of fat ingested was significantly lower 1 h following diet presentation (M=0.52) when compared to measures 3 and 6 h following initial access to the diets (Ms=0.63, 0.65), proportion measures increased 3 h following diet presentation and did not differ from proportion measures 6 h following diet presentation, F(2, 156)=19.35, p<.0001. A significant Phenotype x Sampling Time interaction indicated that obese mice consumed less fat than lean mice 1 h

following initial access to the diets, although no differences in intake were found 3 and 6 h following diet presentation, $F(2, 156)=5.48$, $p<.005$. As well, proportion of fat ingested, although significantly lower for obese and lean mice administered 0.5 mg/kg clonidine compared to mice administered 0.1 mg/kg CLON or VEH, 1 h following initial diet presentation, did not differ between treatment groups on subsequent intake measures, $F(4, 156)=10.96$, $p<.0001$.

Fat intake measures analysed as a percentage of VEH intake indicated a significant main effect for phenotype, $F(1, 78)=3.98$, $p<.04$, treatment, $F(2, 78)=15.23$, $p<.0001$, and sampling time, $F(2, 156)=11.22$, $p<.0001$ (Table A-14). Percentage VEH intake was lower for *ob*/*ob* mice ($M=75.89$) than +/? mice ($M=95.04$), and for obese and lean mice administered 0.5 mg/kg and 0.1 mg/kg CLON ($Ms=55.90$, 80.35) compared to mice treated with VEH ($M=120.15$). Percentage VEH intake was also lower 1 h following diet presentation ($M=71.32$) compared to 6-h intake measures ($M=95.41$). A significant Treatment x Sampling Time interaction indicated that mice administered 0.5 mg/kg and 0.1 mg/kg CLON differed significantly from VEH, demonstrating lower percentage VEH intake measures across sampling time, but only differed significantly from each other 1 h following diet presentation, $F(4, 156)=11.82$, $p<.0001$. Percentage VEH intake measures were lower for obese mice administered 0.5

mg/kg CLON (M=13.13) in comparison to obese mice administered 0.1 mg/kg CLON (M=61.43), which differed significantly from obese controls (M=103.57), 1 h following diet presentation. Three h following diet presentation obese mice administered 0.5 mg/kg CLON (M=55.64) differed significantly from controls (M= 97.75), although this difference was not maintained on the 6-h measure. Percentage VEH intake measures for lean mice administered 0.5 mg/kg CLON were lower (M=19.33) in comparison to +/? mice administered 0.1 mg/kg CLON (M=72.50) and VEH (M=157.98), which also differed significantly, on 1 h intake measures. Percentage VEH intake measures 3 h following diet presentation indicated that +/? mice administered 0.5 mg/kg and 0.1 mg/kg CLON (Ms=78.65, 89.38) differed significantly from +/? VEH controls (M=134.02). Six h following diet presentation, only +/? mice administered 0.5 mg/kg CLON (M=87.83) and VEH (M=123.35) differed significanlty.

The ANOVA examining protein intake indicated that obese and lean mice administered 0.5 mg/kg and 0.1 mg/kg CLON consumed significantly fewer kilocalories of protein (Ms=0.62, 0.69) than mice administered VEH (M=1.09), $F(2, 78)=5.63$, $p<.005$ (Table A-15). Although protein intake in obese mice administered 0.5 mg/kg and 0.1 mg/kg CLON did not differ from obese mice administered VEH, protein intake in +/? mice administered 0.5 mg/kg CLON did differ from +/?

administered VEH. A main effect for sampling time indicated that protein intake increased in all groups across sampling time, $F(2, 156)$, $p<.0001$.

Proportion of protein ingested differed significantly in ob/ob mice ($M=0.19$) and +/? mice ($M=0.13$), $F(1, 78)=8.47$, $p<.005$, and in mice administered 0.5 mg/kg CLON ($M=0.20$) and VEH ($M=0.13$), $F(2, 78)=4.17$, $p<.02$ (Table A-16). Proportion of protein intake 1 h following diet presentation was significantly greater ($M=0.21$) than proportion measures 3 and 6 h following diet presentation ($Ms=0.15, 0.12$), $F(2, 156)=18.93$, p.>0001. A significant Phenotype X Sampling Time interaction indicated that proportion of protein intake was greater for obese mice 1 and 3 h following diet presentation but was equal to that of +/? mice 6 h following diet presentation, $F(2, 156)=4.61$, $p<.03$. As well, a significant Treatment x Sampling Time interaction indicated that proportion measures were greater in mice administered 0.5 mg/kg CLON in comparison to mice administered 0.1 mg/kg CLON and VEH, which also differed significantly, 1 h following diet presentation, although this difference was not maintained on subsequent measures, $F(4, 156)=6.50$, $p<.0001$. Protein intake, analysed as a percentage of VEH intake, failed to indicate any significant main effects or interactions (Table A-17).

Discussion

The results of the current study indicate that the alpha-2 agonist, clonidine, can reduce energy intake in obese and lean mice. These results are partially consistent with those of Callahan et al. (1984), in which 0.5 mg/kg CLON decreased food intake in ob/ob and +/? mice. However, Callahan et al. (1984) also reported that 0.1 mg/kg CLON increased 1-h food intake in obese mice, a dose that in the present study, reduced intake in both ob/ob and +/? mice. Given that Callahan et al. (1984) examined clonidine's effects on the intake of standard laboratory chow while the current study assessed macronutrient intake, it is possible that the differential effect of clonidine can be attributed, in part, to the differences in the test diets, as ob/ob mice are hyperphagic on a diet consisting of lab chow in comparison to +/? mice, but show a tendency to be hypophagic on a diet consisting of separate macronutrient sources in a restricted feeding regimen.

Furthermore, reports of the effects of clonidine on food intake in mice and rats appear inconsistent. Although some researchers have found clonidine to be anorectic in mice (Callahan et al., 1984), others have reported either no effect (Mauron et al., 1980) or an increase in food intake in the rat (Leibowitz et al., 1985) following clonidine

administration. However, these apparent differences in the effects of clonidine may, in fact, depend on the dose of clonidine administered, on interspecies differences in clonidine's mechanism of action, on the frequency of drug administration, or on the interval following clonidine administration at which feeding behaviour is measured, or on differences in test diets.

The most consistent finding in the previous research literature is an increase in food intake following clonidine administration (Callahan et al., 1984; Fahrbach, Tretter, Aravich, McCabe, & Leibowitz, 1980; Leibowitz et al., 1985; Schlemmer et al., 1981). Given that chronic injection of NE into the PVN results in hyperphagia in the rat (Leibowitz et al., 1984), the alpha-2 receptor agonist, clonidine would also be expected to stimulate food ingestion through a postsynaptic receptor mechanism. However, given that some studies have found no effect or an anorectic effect of clonidine following its administration, these results suggest that clonidine can produce a dose-dependent biphasic effect on food intake (Callahan et al., 1984). At lower doses, clonidine may produce either no effect or an increase in food intake, while at higher doses (as in the current study), administration of clonidine may result in a suppression of intake. Mauron et al. (1980) found that lower doses of clonidine (0.025-0.05 mg/kg ip) increased

total food intake in the rat. A dose of 0.1 mg/kg was less effective in increasing intake, while 0.2 mg/kg CLON did not alter food intake compared to controls. Mauron et al. (1980) believed that the 0.2 mg/kg dose had a marked sedative effect, thereby suggesting a narrow dose range in which clonidine may function to increase food intake. This finding with food intake is consistent with the narrow therapeutic range that characterises clonidine's antihypertensive effect (Christersson, Frisk-Kolmberg, & Paalzow, 1979). Therefore, the effects of clonidine on food intake appear to be dose-dependent.

Contrary to the speculation that clonidine acts to affect food intake via a postsynaptic alpha-2 receptor mechanism, Callahan et al. (1984) have reported that at high doses clonidine reduced food intake in lean and obese mice by acting on alpha-2 presynaptic receptors. However, this reasoning is inconsistent with current research that suggests that the alpha-2 receptor within the PVN is postsynaptic (Golman et al., 1985; Jhanwar-Uniyal et al., 1985). Furthermore, clonidine, at high doses, stimulates not only alpha-2 presynaptic receptors but also postsynaptic alpha-2 and alpha-1 receptors (U'Prichard, 1981; U'Prichard et al., 1977). Therefore limited speculation about the presynaptic or postsynaptic locus of alpha-adrenergic influence on food intake can follow from the results of the present study.

The paraventricular and medial hypothalamic regions have been implicated in satiety control, involving alpha-adrenergic and serotonergic systems (Hoebel, 1984; Leibowitz, 1982), where NE inhibits satiety and 5-HT facilitates satiety. If clonidine acts only on the alpha-2 adrenergic receptor, then it would be expected to increase food intake. However, given the reduction in total food intake and macronutrient intake in the present study, it is possible that clonidine acted to facilitate satiety by acting on 5-HT. Although the clonidine doses used in the present study were selected for their ability to affect the alpha-2 receptor (Callahan et al., 1984), Anden et al. (1970) have shown that clonidine decreases 5-HT turnover. Therefore, it is possible that the doses of clonidine in the present study did not have a selective effect on the alpha-2 receptor.

Previous research has suggested that obese mice are more sensitive to clonidine's effects on food intake (Callahan et al., 1984). If clonidine's effects are indeed biphasic, the fact that obese mice increased intake at a dose where lean mice did not, does not necessarily suggest that +/? are less sensitive to clonidine. In fact, it is possible that lean mice were more sensitive to the anorectic effects of clonidine and therefore displayed a suppression of intake at doses where obese mice were still unaffected. This is

consistent with the present study in which +/? mice tended to display hypophagia at a lower dose of clonidine where obese mice did not.

Although the anorectic effects of clonidine treatment resulted in an apparent suppression of carbohydrate, fat and protein, the proportion of dietary calories derived from carbohydrate was increased on 1-h intake measures in mice administered the higher dose of clonidine. The proportion of calories ingested as protein was also increased on the 1-h intake measure. However, because measures of percentage of vehicle intake of protein were not significant and because ob/ob mice treated with clonidine did not differ significantly from vehicle, the effects of clonidine on protein ingestion may be less important. It is possible that proportion of protein ingested was increased simply because of the extent of suppression of total energy intake. Although fat intake was also suppressed, it is interesting to note that caloric intake of fat in obese mice treated with clonidine did not differ from vehicle. However, in terms of proportion of fat ingested and as a percentage of vehicle intake, fat intake appeared suppressed. Therefore, the macronutrient most consistently affected by clonidine is carbohydrate, a finding consistent with previous reports examining clonidine's effects on nutrient intake. Although clonidine, at low doses, selectively potentiates

carbohydrate ingestion in the rat (Leibowitz et al., 1985), in the present study, clonidine also principally affected carbohydrate ingestion in ob/ob and +/? mice, although the effect was anorectic.

In summary, the results of the current study suggest that clonidine can affect total energy intake and nutrient intake in obese and lean mice. Given that the doses of clonidine in the present study appeared to be anorectic, whether lower doses of clonidine may increase food intake and possibly carbohydrate ingestion in ob/ob and +/? mice, or have similar anorectic effects as the doses used here, remains to be investigated.

Experiment 3

Increased food intake following clonidine administration has been shown to be blocked by the selective alpha-2 adrenergic antagonist, yohimbine (Leibowitz, 1980), and when yohimbine is administered alone, a reduction in total food intake of lean and obese mice has been found (Callahan et al., 1984). Yohimbine has also been shown to differentially affect total food intake in +/? and ob/ob mice, that is, obese mice appear to be more sensitive to the anorectic effects of yohimbine than lean mice (Callahan et al., 1984). The present study examined the effect of yohimbine on macronutrient intake in lean and obese mice. The effect of

yohimbine administered prior to clonidine treatment was also assessed in an attempt to examine the specificity of clonidine's effect on macronutrient intake.

Method

Subjects

Obese (C57Bl/6J, ob/ob, n=42) and lean (C57Bl/6J, +/?, n=42) adult male mice, 15 weeks at the start of the experiment, included animals from the previous experiment following a drug-free period of 3 days, although mice continued on the 6-h feeding regimen. Mice were individually housed as in the previous two experiments and tested in home cages.

Apparatus

Diets were presented in food containers and water in calibrated drinking tubes as in the previous experiments. Nutrient intake and body weights were measured using a Mettler Digital Balance (Model No. PB 300).

Diets and Drugs

Composition of the test diets is described in Experiment 1. Caloric coefficients of 3.7 kcal/g for carbohydrate and protein and 7.7 kcal for fat were used.

Yohimbine-HCl (YOH) (Sigma), a relatively specific alpha-2 receptor antagonist, was administered at 3 mg/kg and

5 mg/kg ip. Clonidine-HCl (Sigma), a relatively specific alpha-2 agonist, was administered at 0.5 mg/kg ip. Yohimbine doses were selected on published reports indicating an effect on food intake (Callahan et al., 1984), and the clonidine dose was selected from Experiment 2. Drugs were dissolved in saline (VEH) at 0.5 ml/100 g body weight.

Design and Procedure

Following Experiment 2 mice received VEH injections of saline for three consecutive drug-free days, which allowed animals to remain adapted to the injection schedule used in the present study (Oltmans, personal communication, 24 August 1987). At this time mice were assigned to one of six treatment conditions. Each group included 7 ob/ob and 7 +/? mice. On the fourth day of injections, mice in the low YOH-CLON group received 3 mg/kg ip of YOH 30 min prior to 0.5 mg/kg ip of CLON. Mice in the high YOH-CLON group received 5 mg/kg ip of YOH 30 min prior to 0.5 mg/kg ip CLON. A third group received a VEH injection with CLON 0.5 mg/kg ip. Mice in the low YOH-VEH group received 3 mg/kg ip of YOH paired with a VEH injection. Mice in the high YOH-VEH group received 5 mg/kg ip of YOH preceding a VEH injection. A control group received two concurrent injections of VEH. Drug doses of YOH and CLON used in this experiment are known to elicit no overt signs of locomotor depression or malaise in mice (Callahan et al., 1984).

Preweighed amounts of carbohydrate, protein, and fat were given to each animal in all treatment conditions between 0900 h and 1500 h. Total intake and individual nutrient intakes, measured to the nearest 0.01 g, expressed in kilocalories, were taken at 1, 3, and 6 h (Callahan et al., 1984; Leibowitz et al., 1985), following inital food presentation. CLON was administered 30 min prior to food presentation, and both drugs were coded blind to the experimenter. In addition, body weights and water intakes were monitored daily.

Independent variables included phenotype (ob/ob, +/?), treatment (drug dose), and sampling time (1, 3, 6 h), with dependent measures of total food intake, carbohydrate, protein, and fat intake in kcals. Body weights, although not analysed, were used to determine that mice were not below 80% of pretest body weights.

Results

A 2 x 6 x 3 (Phenotype x Treatment x Sampling Time) ANOVA with repeated measures on the third variable, was performed on measures of total energy intake, carbohydrate, fat, and protein intake in kilocalories, and the proportion of each nutrient to total energy intake. Intake measures were also assessed as a percentage of VEH intake, that is, in comparison to energy intake on the day immediately prior to

drug treatment, where VEH was administered to all mice. A Type 1 error rate of .05 was maintained. Post hoc Tukey tests (Hays, 1981) were performed on all group mean differences.

Total energy intake is outlined in Table 3. The ANOVA examining energy intake in kilocalories indicated a significant main effect for phenotype, $F(1, 72)=16.21$, $p<.0001$, treatment, $F(5, 72)=6.10$, $p<.0001$, and sampling time, $F(2, 144)=509.79$, $p<.0001$ (Table A-18). Obese mice consumed fewer kilocalories ($M=4.63$) than lean mice ($M=6.47$). Mice administered VEH-CLON, 5 mg/kg YOH-CLON, 3 mg/kg YOH-CLON, and 5 mg/kg YOH-VEH consumed significantly fewer kilocalories than mice treated with VEH. Although all mice increased caloric intake across sampling time, a significant Phenotype x Sampling Time interaction suggested that obese mice consumed significantly fewer kilocalories in comparison to +/? mice at 1, 3, and 6 h following diet presentation, $F(2, 144)=11.68$, $p<.0001$. Obese and lean mice administered 5 mg/kg YOH-CLON, 3 mg/kg YOH-CLON, 5 mg/kg YOH-VEH, and VEH-CLON, all consumed significantly fewer kilocalories of energy than VEH controls, 1 h following initial access to diets, $F(10, 144)=2.56$, $p<.007$. Three h following diet presentation all treatment groups differed significantly from controls, and this difference was maintained on the 6-h intake measure.

See Table 3 page 108

Energy intake assessed in terms of percentage VEH intake indicated a significant main effect for treatment, $F(5, 72)=8.70$, $p<.001$, and sampling time, $F(2, 144)=38.05$, $p<.0001$ (Table A-19). Percentage of VEH intake was significantly lower for mice administered VEH-CLON ($M=46.35$) compared to mice administered 5 mg/kg YOH-CLON ($M=70.46$), 3 mg/kg YOH-VEH ($M=78.24$), or VEH ($M=92.64$), but similar to mice administered 3 mg/kg YOH-CLON ($M=62.24$) or 5 mg/kg YOH-VEH ($M=61.58$), both of which also differed significantly from VEH. A significant Treatment x Sampling Time interaction indicated that all treatment groups with the exception of mice administered 3 mg/kg YOH-VEH differed significantly from VEH on 1-h intake measures, $F(10, 144)=2.51$, $p<.01$. Also, mice administered VEH-CLON differed from all treatment groups with the exception of mice administered 5 mg/kg YOH-VEH. Mice administered 5 mg/kg YOH-VEH also showed a greater suppression in intake when compared to the 3 mg/kg YOH-VEH treatment group. On the 3-h intake measure, only mice treated with 3 mg/kg YOH-CLON, VEH-CLON, and 5 mg/kg YOH-VEH differed significantly from VEH, and on the 6-h measure, only the 3 mg/kg YOH-CLON and VEH-CLON treatment groups differed significantly from VEH controls.

Carbohydrate intake is outlined in Table 4. Obese mice ingested fewer kilocalories of carbohydrate (M=1.19) than lean mice (M=1.91), F(1, 72)=27.71, p<.0001 (Table A-20). Mice administered VEH-CLON ingested significantly less carbohydrate (M=0.98) than mice administered 5 mg/kg YOH-VEH (M=1.71) or 3 mg/kg YOH-VEH (M=1.84), F(5, 72)=3.07, p<.01. Although all mice increased carbohydrate intake across sampling time, F(2, 144)=326.79, p<.0001, a significant Phenotype x Sampling Time interaction indicated that ob/ob mice consumed significantly fewer kilocalories of carbohydrate than +/? mice 1, 3, and 6 h following diet presentation, F(2, 144)=15.08, p<.0001.

See Table 4 page 109

Proportion of carbohydrate intake was greater 1 h following diet presentation (M=0.37) than 3 or 6 h following diet presentation (Ms=0.31, 0.28), F(2, 144)=16.83, p<.0001, and significantly greater for mice administered 5 mg/kg YOH-CLON (M=0.41), 3mg/kg YOH-CLON (M=0.34), and 5 mg/kg YOH-VEH (M=0.39), compared to mice administered 3 mg/kg YOH-VEH (M=0.32), VEH-CLON (M=0.25), or VEH (M=0.20), F(5, 72)=6.05, p<.0001 (Table A-21). Mice administered 5 mg/kg YOH-CLON and 5 mg/kg YOH-VEH also differed significantly from mice administered VEH-CLON. Lean mice ingested,

proportionally, more carbohydrate than ob/ob mice 1 h following diet presentation, although this difference was not evident on subsequent intake measures, $F(2, 144)=3.70$, $p<.03$. A significant Treatment x Sampling Time interaction indicated that all treatment groups, with the exception of mice administered VEH-CLON, differed significantly from VEH, 1 h following diet presentation, $F(10, 144)=2.27$, $p<.02$, and the proportion of carbohydrate ingested was greatest for mice administered 5 mg/kg YOH-CLON and 5 mg/kg YOH-VEH. On the 3-h intake measure only the VEH-CLON treatment group did not differ significantly from VEH and on the 6-h measure, only the VEH-CLON and the 3 mg/kg YOH-VEH treatment groups did not differ significantly from VEH controls.

Measures of percentage VEH intake of carbohydrate indicated a significant main effect for treatment, $F(5, 72)=2.91$, $p<.02$, but no other significant main effects or interactions (Table A-22). Percentage intake of carbohydrate was reduced in mice administered VEH-CLON ($M=52.26$), compared to mice treated with 5 mg/kg YOH-VEH ($M=136.81$) and 3 mg/kg YOH-VEH ($M=134.96$) although no treatment group differed significantly from VEH.

Fat intake is outlined in Table 5. The ANOVA examining fat intake indicated that obese mice consumed fewer kilocalories of fat ($M=2.83$) than lean mice ($M=3.74$), $F(1,$

72)=7.29, $p<.009$ (Table A-23). All treatment groups consumed significantly fewer kilocalories of fat than VEH controls, $F(5, 72)=7.38$, $p<.0001$. Fat intake increased across sampling time, $F(2, 144)=289.91$, $p<.0001$, and although fat intake was similar for ob/ob and +/? mice on 1-h intake measures, ingestion of fat was greater for +/? mice on 3 and 6-h intake measures, $F(2, 144)=4.13$, $p<.02$. A significant Treatment x Sampling Time interaction indicated that mice in all treatment groups consumed significantly fewer kilocalories of fat than VEH controls, 1, 3, and 6 h following diet presentation, $F(10, 144)=1.91$, $p<.05$.

See Table 5 page 110

Proportion of fat ingested was significantly reduced in all treatment groups compared to VEH controls, $F(5, 72)=6.28$, $p<.0001$, and increased across sampling time $F(2, 144)=26.88$, $p<.0001$ (Table A-24). A significant Treatment x Sampling Time interaction indicated that proportion of fat ingested was lower for all treatment groups, compared to VEH controls, 1 h following initial diet presentation, $F(10, 144)=2.71$, $p<.005$. Proportion measures were also lower for mice administered 5 mg/kg YOH-VEH compared to mice treated with 3 mg/kg YOH-VEH, VEH-CLON, or 3 mg/kg YOH-CLON, and similar to mice administered 5 mg/kg YOH-CLON, which was

also significantly lower than the 3 mg/kg YOH-CLON treatment group. Although all groups differed significantly from VEH 3 h following diet presentation, only the 5 mg/kg YOH-VEH treatment group differed significantly from VEH on the 6-h intake measure.

Fat intake measures analysed as a percentage of VEH intake indicated a significant main effect for treatment, $F(5, 72)=6.09$, $p<.0001$, and sampling time, $F(2, 144)=41.48$, $p<.0001$ (Table A-25). Percentage VEH intake was lower for all treatment groups compared to mice administered VEH, and lowest for mice administered 5 mg/kg YOH-VEH, when compared to mice administered 3 mg/kg YOH-VEH or 5 mg/kg YOH-CLON, but not significantly different from mice treated with VEH-CLON or 3 mg/kg YOH-CLON. A significant Phenotype x Treatment interaction indicated that percentage of VEH intake was similar for obese and lean mice in all treatment groups with the exception of obese mice administered VEH-CLON ($M=29.88$) and lean mice administered VEH-CLON ($M=69.35$), $F(5, 72)=2.41$, $p<.04$. Percentage VEH intake was significantly lower for mice in all treatment groups compared to VEH, 1 h following diet presentation, $F(10, 144)=8.94$, $p<.01$, and was also significantly lower in mice administered 5 mg/kg YOH-VEH compared to mice administered 3 mg/kg YOH-VEH, 5 mg/kg YOH-CLON and 3 mg/kg YOH-CLON, but not significantly different from mice administered VEH-CLON.

On the 3-h intake measure all groups differed significantly from VEH and on the 6-h measure, all groups were significantly different from VEH with the exception of the 3 mg/kg YOH-VEH treatment group.

Protein intake is outlined in Table 6. The ANOVA examining protein intake in kilocalories indicated a significant main effect for sampling time, $F(2, 144)=110.45$, $p<.0001$, and a significant Phenotype x Sampling Time interaction, $F(2, 144)=3.96$, $p<.02$ (Table A-26). Although protein intake increased across sampling time, ob/ob mice consumed signicantly less protein than +/? mice 1, 3, and 6 h following initial diet presentation.

See Table 6 page 111

Proportion of protein ingested was greater in all treatment groups compared to VEH controls, $F(5, 72)=2.45$, $p<.05$ (Table A-27). Mice administered VEH-CLON ingested, proportionally, more protein ($M=0.20$), than any other treatment group, followed by mice administered 5 mg/kg YOH-VEH ($M=0.17$) and 3 mg/kg YOH-VEH ($M=0.14$) which also differed significantly from each other. Mice treated with 5 mg/kg YOH-CLON ($M=0.12$) did not differ significantly from the 3 mg/kg YOH-CLON treatment group ($M=0.12$), although both

treatment groups differed significantly from all other treatment groups. Proportion of protein intake 1 h following diet presentation was significantly greater (M=0.16) than proportion measures 3 and 6 h following diet presentation (Ms=0.13, 0.12), $F(2, 144)=6.06$, $p<.003$. A significant Phenotype x Sampling Time interaction indicated that proportion of protein intake was greater for obese mice 1 h following diet presentation, but was equal to that of +/? mice 3 and 6 h following diet presentation, $F(2, 144)=4.98$, $p<.008$. As well, a significant Treatment x Sampling Time interaction indicated that proportion measures were greater for mice administered VEH-CLON and 5 mg/kg YOH-VEH compared to VEH controls, 1 h following diet presentation, although this difference was not maintained on subsequent intake measures, $F(10, 144)=4.33$, $p<.0001$. A significant Phenotype x Treatment x Sampling Time interaction indicated that proportion of protein ingested was significantly greater for obese mice administered VEH-CLON compared to +/? mice administered VEH-CLON, 1 h following diet presentation, but not evident 3 or 6 h following diet presentation, $F(10, 144)=3.91$, $p<.0001$. Protein intake, analysed as a percentage of VEH intake, failed to indicate any significant main effects or interactions (Table A-28).

Discussion

The results of the present study indicate that the alpha-2 antagonist, yohimbine, as well as clonidine, an alpha-2 agonist, can reduce energy intake in obese and lean mice. Again, these results are consistent with previous reports in which yohimbine has been shown to reduce food intake (Callahan et al., 1984; Mauron et al., 1980). Although yohimbine suppressed intake in a dose-dependent manner, when the drug was administered prior to treatment with clonidine, there was a tendency for the higher dose of yohimbine to antagonise the anorectic effects of clonidine. That is, although mice administered either clonidine or the higher dose of yohimbine (5 mg/kg) both supressed intake and did not differ significantly on percentage of vehicle intake measures, energy intake for mice treated with clonidine was significantly less than mice administered 5 mg/kg yohimbine prior to clonidine treatment. The fact that mice treated with clonidine and 3 mg/kg yohimbine paired with clonidine did not differ significantly, suggests that the lower dose of yohimbine was not effective in antagonising clonidine's effect on food intake.

The anorectic effect of yohimbine is not likely attributed to a drug-induced general malaise. Callahan et al. (1984) using the same doses as in the present study

found no obvious signs of distress or locomotor suppression following yohimbine treatment. In addition, Callahan et al. (1984) also reported that effective anorectic doses of yohimbine did not affect water intake in water-deprived mice, which again supports the contention that the anorectic effect of yohimbine was a specific drug action.

While clonidine's effect on food intake appears to be biphasic, it is also possible that at high doses the effect of clonidine treatment may simply reflect a non-specific effect with no physiological significance. However, dose levels of yohimbine as employed in the present study are considered to be relatively low (Callahan et al., 1984), a condition which would favour selective interaction with the alpha-2 receptor (Anden, Puaksens, & Svensson, 1982).

Although it would appear that yohimbine can reduce energy intake at relatively lower doses (<6 mg/kg ip), a number of reports have sugggested that yohimbine, administered at higher doses, can actually increase food intake in the rat (Goldman et al., 1985; Mauron et al., 1980). One possible explanation for the apparent biphasic effect of yohimbine may relate to the drug's specificity for the alpha-2 receptor. Although yohimbine was used in the present study because of its specificity to the alpha-2 adrenergic receptor, it is not absolutely specific to this receptor and

may affect dopaminergic systems (Papeschi & Theiss, 1975; Scatton, Zivokovic, & Dedek, 1980). Given that the lateral perifornical hypothalamus, lateral to the paraventricular and ventromedial nuclei, is the most responsive brain region in which dopamine can suppress feeding behaviour (Leibowitz & Rossakis, 1979), it is possible that yohimbine at higher doses, acts to facilitate feeding via its antidopaminergic properties (Goldman et al., 1985; Scatton, Dedek, & Zivokovic, 1983). Consistent with the hypothesis that yohimbine can act via a blockade of dopaminergic feeding-inhibitory receptors is the additional evidence that peripheral yohimbine-induced feeding is unaffected by PVN electrolytic lesions (Shor-Posner, Azar, & Leibowitz, 1984), and that the general alpha-adrenergic receptor blocker, phentolamine, which has little or no antidopaminergic properties (Scatton et al., 1980), is ineffective as a feeding stimulant, but significantly attenuates the feeding response induced by subsequent administration of norepinephrine or clonidine (Goldman et al., 1985). However, the results of the present study indicate that yohimbine can reduce total energy intake, as well as antagonise the anorectic effect of clonidine, implicating an alpha-2 receptor mechanism in feeding.

Macronutrient intake appeared to be differentially affected by drug treatment. Proportion of protein ingested

was increased in all treatment groups compared to controls. However, the fact that measures of percentage of vehicle intake and protein intake in kilocalories were not significant suggests that clonidine and yohimbine did not directly affect protein intake. Again, it is possible that the proportion of protein ingested was increased simply because of the extent of suppression of total energy intake. Fat intake was also suppressed in all treatment groups, as was proportion of fat ingested and measures of percentage of vehicle intake, with a tendency for mice administered 5 mg/kg YOH-VEH to demonstrate the greatest suppression of fat.

Furthermore, measures of percentage of vehicle intake indicated that carbohydrate intake was suppressed more in mice administered clonidine than yohimbine. However, mice administered yohimbine, or yohimbine prior to clonidine treatment demonstrated an increase in the proportion of carbohydrate ingested, with mice in the 5 mg/kg YOH-CLON and the 5 mg/kg YOH-VEH treatment groups ingesting, proportionally, more carbohydrate. Although the effects of clonidine and yohimbine do not appear to be specific to carbohydrate, given that fat intake is also suppressed, the ingestion of carbohydrate is uniquely affected following yohimbine and clonidine treatment.

General Discussion

In summary, the results of Experiments 2 and 3 indicate an anorectic effect of clonidine, an alpha-2 agonist, and yohimbine, an alpha-2 antagonist. At doses employed in the current studies clonidine and yohimbine reduced food intake while increasing the proportion of carbohydrate ingested. The increased proportion of energy derived from carbohydrate occurred principally at 1 h into the 6-h feed (Experiment 2) for clonidine-treated mice and at 1-h measures for all yohimbine-treated groups compared to vehicle-treated mice (Experiment 3). The proportion of carbohydrate was also increased in clonidine-injected, vehicle-pretreated mice in Experiment 3, although this effect was not significant. Examination of the mean proportion of carbohydrate intake in the total 6-h intake in comparable clonidine-treated groups in Experiment 2 and Experiment 3 revealed a similar change from vehicle-injected mice. However, the reduction in sample size from Experiment 2 (n=14 per group) to Experiment 3 (n=7 per group) may have been sufficient to increase the error term in the post hoc tests, resulting in an effect that did not reach the designated alpha level. However, given the reported biphasic nature of clonidine's effect on food intake in the ob/ob mouse (Callahan et al., 1984), it remains possible that lower doses of clonidine may in fact increase caloric intake in mice.

Although yohimbine suppressed intake in a dose-dependent manner, pretreatment with yohimbine prior to clonidine administration antagonised the anorectic effects of clonidine. This result suggests that the alpha-noradrenergic receptor mechanism, implicated in the regulation of food intake in ob/ob and lean mice, is of the alpha-2 subtype. Current research suggests that the alpha-2 postsynaptic receptor within the PVN is involved in feeding regulation (Goldman et al., 1985; Jhanwar-Uniyal et al., 1985). Although this nucleus is one of a complex of neural mechanisms, which may regulate ingestive behaviour, its effects on carbohydrate intake may be unique. Contrary to Callahan et al.'s (1984) demonstration of ob/ob's greater sensitivity to clonidine, yohimbine, and rauwolscine (another alpha-2 blocker), neither Experiment 2 nor 3 yielded any Drug x Phenotype interactions. Obese mice responded in a similar direction as lean mice to clonidine alone, yohimbine alone, and clonidine with yohimbine pretreatment.

However, given the increase in hypothalamic alpha-1 receptor density in the ob/ob (Callahan et al., 1984; Oltmans et al., 1981), it is possible that an alpha-1, as well as an alpha-2 noradrenergic receptor mechanism, may contribute to a defect in the hypothalamic NE system which regulates food intake in the ob/ob. No research has yet

addressed this possibility pharmacologically. Although the precise nature of the noradrenergic abnormality remains unknown, an increase in endogenous hypothalamic NE has been found in ob/ob mice, predominately in the paraventricular and ventromedial nuclei (Oltmans, 1983), areas which have been shown to be involved in the regulation of feeding (Hobel, 1984; Leibowitz et al., 1985).

In addition to an aberrant hypothalamic NE system, other biochemical and neuroanatomical abnormalities have been cited as evidence of a CNS defect in the ob/ob mouse (Bereiter & Jeanrenaud, 1979 Garthwaite et al., 1980; Govoni & Lang, 1981; Hays & Paul, 1981; Oltman, 1983), and suggest that multiple abnormalities in neural systems involved in the control of feeding exist in the ob/ob mutant.

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APPENDIX A-1

ANOVA Summary Table of Total Energy

Intake/ Gram Body Weight

Source	SS/MS	F	df	p
Phenotype	SS= 3.8121 MS= 3.8121	312.65	1, 28	0.0001
Regimen	SS= 0.4665 MS= 0.4665	38.26	1, 28	0.0001
Phenotype x Regimen	SS= 0.00001 MS= 0.00001	0.01	1, 28	0.9341
Error	SS= 0.3414 MS= 0.0121			
Weeks	SS= 0.0471 MS= 0.0157	6.99	3, 84	0.0003
Phenotype x Weeks	SS= 0.0536 MS= 0.0179	7.96	3, 84	0.0001
Regimen x Weeks	SS= 0.0269 MS= 0.0089	3.99	3, 84	0.0104
Phenotype x Regimen x Weeks	SS= 0.0053 MS= 0.0018	0.78	3, 84	0.5073
Error	SS= 0.1886 MS= 0.0022			

APPENDIX A-2

ANOVA Summary Table of Total Energy Intake (kcal)

Source	SS/MS	F	df	p
Phenotype	SS= 444.52 MS= 444.52	22.85	1, 28	0.0001
Regimen	SS= 896.06 MS= 896.06	46.06	1, 28	0.0001
Phenotype x Regimen	SS= 81.246 MS= 81.246	4.18	1, 28	0.0505
Error	SS= 544.76 MS= 19.456			
Weeks	SS= 36.709 MS= 12.237	3.99	3, 84	0.0105
Phenotype x Weeks	SS= 16.833 MS= 5.6109	1.83	3, 84	0.1484
Regimen x Weeks	SS= 23.183 MS= 7.7277	2.52	3, 84	0.0637
Phenotype x Regimen x Weeks	SS= 0.5374 MS= 0.1791	0.06	3, 84	0.9814
Error	SS= 257.89 MS= 3.0701			

APPENDIX A-3

ANOVA Summary Table of Proportion of

Carbohydrate Ingested

Source	SS/MS	F	df	p
Phenotype	SS= 0.0042 MS= 0.0042	0.10	1, 28	0.7509
Regimen	SS= 0.0002 MS= 0.0002	0.01	1, 28	0.9440
Phenotype x Regimen	SS= 0.0830 MS= 0.0830	2.02	1, 28	0.1665
Error	SS= 1.1525 MS= 0.0412			
Weeks	SS= 0.0536 MS= 0.0179	5.01	3, 84	0.0030
Phenotype x Weeks	SS= 0.0489 MS= 0.0163	4.57	3, 84	0.0052
Regimen x Weeks	SS= 0.0027 MS= 0.0009	0.26	3, 84	0.8562
Phenotype x Regimen x Weeks	SS= 0.0025 MS= 0.0008	0.23	3, 84	0.8726
Error	SS= 0.2996 MS= 0.0036			

APPENDIX A-4

ANOVA Summary Table of Proportion of

Fat Ingested

Source	SS/MS	F	df	p
Phenotype	SS= 0.0352 MS= 0.0352	0.69	1, 28	0.4116
Regimen	SS= 0.0466 MS= 0.0466	0.92	1, 28	0.3455
Phenotype x Regimen	SS= 0.0557 MS= 0.0557	1.10	1, 28	0.3031
Error	SS= 1.4179 MS= 0.0506			
Weeks	SS= 0.0819 MS= 0.0273	4.66	3, 84	0.0046
Phenotype x Weeks	SS= 0.0588 MS= 0.0196	3.34	3, 84	0.0230
Regimen x Weeks	SS= 0.0127 MS= 0.0042	0.72	3, 84	0.5431
Phenotype x Regimen x Weeks	SS= 0.0084 MS= 0.0028	0.48	3, 84	0.7004
Error	SS= 0.4924 MS= 0.0059			

APPENDIX A-5

ANOVA Summary Table of Proportion of

Protein Ingested

Source	SS/MS	F	df	p
Phenotype	SS= 0.0169 MS= 0.0169	2.87	1, 28	0.1013
Regimen	SS= 0.0458 MS= 0.0458	7.75	1, 28	0.0095
Phenotype x Regimen	SS= 0.0024 MS= 0.0024	0.41	1, 28	0.5266
Error	SS= 0.1654 MS= 0.0059			
Weeks	SS= 0.0205 MS= 0.0068	4.74	3, 84	0.0042
Phenotype x Weeks	SS= 0.0004 MS= 0.0001	0.08	3, 84	0.9691
Regimen x Weeks	SS= 0.0054 MS= 0.0018	1.25	3, 84	0.2984
Phenotype x Regimen x Weeks	SS= 0.0044 MS= 0.0014	1.03	3, 84	0.3857
Error	SS= 0.1201 MS= 0.0014			

APPENDIX A-6

ANOVA Summary Table of Body Weights

Source	SS/MS	F	df	p
Phenotype	SS= 12054.84 MS= 12054.84	480.91	1, 28	0.0001
Regimen	SS= 247.46 MS= 247.46	9.87	1, 28	0.0039
Phenotype x Regimen	SS= 153.38 MS= 153.38	6.12	1, 28	0.0197
Error	SS= 701.85 MS= 25.066			
Weeks	SS= 271.05 MS= 90.349	21.15	3, 84	0.0001
Phenotype x Weeks	SS= 215.14 MS= 71.714	16.79	3, 84	0.0001
Regimen x Weeks	SS= 64.734 MS= 21.578	5.05	3, 84	0.0029
Phenotype x Regimen x Weeks	SS= 82.113 MS= 27.371	6.41	3, 84	0.0006
Error	SS= 358.83 MS= 4.2718			

APPENDIX A-7

ANOVA Summary Table of Total Energy Intake (kcal)

Source	SS/MS	F	df	p
Phenotype	SS= 411.73 MS= 411.73	32.42	1, 78	0.0001
Treatment	SS= 523.48 MS= 261.74	20.61	2, 78	0.0001
Phenotype x Treatment	SS= 116.20 MS= 58.104	4.58	2, 78	0.0132
Error	SS= 990.54 MS= 12.699			
Sampling Time	SS= 1978.48 MS= 989.242	529.46	2, 156	0.0001
Phenotype x Sampling Time	SS= 38.227 MS= 19.113	10.23	2, 156	0.0001
Treatment x Sampling Time	SS= 8.2170 MS= 2.0542	1.10	4, 156	0.3589
Phenotype x Treatment x Sampling Time	SS= 3.8632 MS= 0.9658	0.52	4, 156	0.7234
Error	SS= 291.47 MS= 1.8684			

APPENDIX A-8

ANOVA Summary Table of Percent Vehicle Intake

Source	SS/MS	F	df	p
Phenotype	SS= 8910.92 MS= 8910.92	4.99	1, 78	0.0283
Treatment	SS= 116778.63 MS= 58389.319	32.72	2, 78	0.0001
Phenotype x Treatment	SS= 1979.59 MS= 989.796	0.55	2, 78	0.5765
Error	SS= 139173.05 MS= 1784.2699			
Sampling Time	SS= 21481.93 MS= 10740.96	16.66	2, 156	0.0001
Phenotype x Sampling Time	SS= 105.45 MS= 52.728	0.08	2, 156	0.9251
Treatment x Sampling Time	SS= 32876.81 MS= 8219.204	12.75	4, 156	0.0001
Phenotype x Treatment x Sampling Time	SS= 1651.59 MS= 412.899	0.64	4, 156	0.6344
Error	SS= 100561.93 MS= 644.62780			

APPENDIX A-9

ANOVA Summary Table of Carbohydrate Intake (kcal)

Source	SS/MS	F	df	p
Phenotype	SS= 28.5194 MS= 28.5194	30.55	1, 78	0.0001
Treatment	SS= 27.897360 MS= 13.948680	14.94	2, 78	0.0001
Phenotype x Treatment	SS= 2.864728 MS= 1.432364	1.53	2, 78	0.2220
Error	SS= 72.807447 MS= 0.9334288			
Sampling Time	SS= 94.49101 MS= 47.24550	301.43	2, 156	0.0001
Phenotype x Sampling Time	SS= 6.428778 MS= 3.214389	20.51	2, 156	0.0001
Treatment x Sampling Time	SS= 0.477668 MS= 0.119417	0.76	4, 156	0.5516
Phenotype x Treatment x Sampling Time	SS= 0.235959 MS= 0.058990	0.38	4, 156	0.8253
Error	SS= 24.45142 MS= 0.156739			

APPENDIX A-10

ANOVA Summary Table of Proportion of Carbohydrate

Source	SS/MS	F	df	p
Phenotype	SS= 0.006057 MS= 0.006057	0.22	1, 78	0.6389
Treatment	SS= 0.077078 MS= 0.038539	1.41	2, 78	0.2498
Phenotype x Treatment	SS= 0.015378 MS= 0.007689	0.28	2, 78	0.7552
Error	SS= 2.128821 MS= 0.027295			
Sampling Time	SS= 0.089442 MS= 0.044721	5.57	2, 156	0.0046
Phenotype x Sampling Time	SS= 0.034218 MS= 0.017109	2.13	2, 156	0.1221
Treatment x Sampling Time	SS= 0.226303 MS= 0.056576	7.05	4, 156	0.0005
Phenotype x Treatment x Sampling Time	SS= 0.030052 MS= 0.007513	0.94	4, 156	0.4447
Error	SS= 1.252045 MS= 0.008025			

APPENDIX A-11

ANOVA Summary Table of Percent Vehicle

Intake of Carbohydrate

Source	SS/MS	F	df	p
Phenotype	SS= 45819.27 MS= 45819.27	10.11	1, 78	0.0021
Treatment	SS= 104713.63 MS= 52356.815	11.55	2, 78	0.0001
Phenotype x Treatment	SS= 1213.179 MS= 606.5898	0.13	2, 78	0.8750
Error	SS= 353648.02 MS= 4533.9490			
Sampling Time	SS= 11502.11 MS= 5751.056	4.31	2, 156	0.0151
Phenotype x Sampling Time	SS= 1728.782 MS= 864.3913	0.65	2, 156	0.5246
Treatment x Sampling Time	SS= 22330.35 MS= 5582.587	4.18	4, 156	0.0030
Phenotype x Treatment x Sampling Time	SS= 4507.390 MS= 1126.847	0.84	4, 156	0.4989
Error	SS= 208146.13 MS= 1334.2700			

APPENDIX A-12

ANOVA Summary Table of Fat Intake (kcal)

Source	SS/MS	F	df	p
Phenotype	SS= 201.0271 MS= 201.0271	582.74	1, 78	0.0001
Treatment	SS= 205.8078 MS= 102.9039	13.90	2, 78	0.0001
Phenotype x Treatment	SS= 65.43683 MS= 32.71841	4.42	2, 78	0.0152
Error	SS= 577.6216 MS= 7.405452			
Sampling Time	SS= 852.5899 MS= 426.2949	362.18	2, 156	0.0001
Phenotype x Sampling Time	SS= 12.60679 MS= 6.303398	5.36	2, 156	0.0056
Treatment x Sampling Time	SS= 11.16598 MS= 2.791495	2.37	4, 156	0.0547
Phenotype x Treatment x Sampling Time	SS= 3.194635 MS= 0.798659	0.68	4, 156	0.6078
Error	SS= 183.6175 MS= 1.177035			

APPENDIX A-13

ANOVA Summary Table of Proportion of Fat

Source	SS/MS	F	df	p
Phenotype	SS= 0.173906 MS= 0.173906	3.10	1, 78	0.0821
Treatment	SS= 0.585640 MS= 0.292820	5.22	2, 78	0.0074
Phenotype x Treatment	SS= 0.003747 MS= 0.001874	0.03	2, 78	0.9671
Error	SS= 4.371770 MS= 0.056048			
Sampling Time	SS= 0.774718 MS= 0.387359	19.35	2, 156	0.0001
Phenotype x Sampling Time	SS= 0.219272 MS= 0.387359	5.48	2, 156	0.0050
Treatment x Sampling Time	SS= 0.877435 MS= 0.219359	10.96	4, 156	0.0001
Phenotype x Treatment x Sampling Time	SS= 0.076486 MS= 0.019121	0.96	4, 156	0.4338
Error	SS= 3.122112 MS= 0.020013			

APPENDIX A-14

ANOVA Summary Table of Percent Vehicle

Intake of Fat

Source	SS/MS	F	df	p
Phenotype	SS= 23113.15 MS= 23113.15	3.98	1, 78	0.0494
Treatment	SS= 176701.86 MS= 88350.931	15.23	2, 78	0.0001
Phenotype x Treatment	SS= 9692.812 MS= 4846.406	0.84	2, 78	0.4376
Error	SS= 452596.62 MS= 5802.5208			
Sampling Time	SS= 26598.62 MS= 13299.31	11.22	2, 156	0.0001
Phenotype x Sampling Time	SS= 1864.687 MS= 932.3451	0.79	2, 156	0.4317
Treatment x Sampling Time	SS= 56032.30 MS= 14008.07	11.82	4, 156	0.0001
Phenotype x Treatment x Sampling Time	SS= 3828.051 MS= 1126.847	0.81	4, 156	0.5222
Error	SS= 184923.38 MS= 1185.4063			

APPENDIX A-15

ANOVA Summary Table of Protein Intake (kcal)

Source	SS/MS	F	df	p
Phenotype	SS= 0.597120 MS= 0.597120	0.61	1, 78	0.4386
Treatment	SS= 11.09638 MS= 5.548190	5.63	2, 78	0.0052
Phenotype x Treatment	SS= 1.139886 MS= 0.569943	0.58	2, 78	0.5631
Error	SS= 76.82989 MS= 0.984986			
Sampling Time	SS= 30.98240 MS= 15.49120	170.60	2, 156	0.0001
Phenotype x Sampling Time	SS= 0.159700 MS= 0.079850	0.88	2, 156	0.4171
Treatment x Sampling Time	SS= 0.199837 MS= 0.049959	0.55	4, 156	0.6992
Phenotype x Treatment x Sampling Time	SS= 0.128731 MS= 0.032183	0.35	4, 156	0.8407
Error	SS= 14.16532 MS= 0.090803			

APPENDIX A-16

ANOVA Summary Table of Proportion of Protein

Source	SS/MS	F	df	p
Phenotype	SS= 0.244872 MS= 0.244872	8.47	1, 78	0.0047
Treatment	SS= 0.246856 MS= 0.123428	4.27	2, 78	0.0174
Phenotype x Treatment	SS= 0.029409 MS= 0.014705	0.51	2, 78	0.6034
Error	SS= 2.256073 MS= 0.028924			
Sampling Time	SS= 0.346923 MS= 0.173461	18.93	2, 156	0.0001
Phenotype x Sampling Time	SS= 0.084522 MS= 0.208360	4.61	2, 156	0.0113
Treatment x Sampling Time	SS= 0.238254 MS= 0.059564	6.50	4, 156	0.0001
Phenotype x Treatment x Sampling Time	SS= 0.037614 MS= 0.009404	1.03	4, 156	0.3957
Error	SS= 1.429659 MS= 0.009164			

APPENDIX A-17

ANOVA Summary Table of Percent Vehicle

Intake of Protein

Source	SS/MS	F	df	p
Phenotype	SS= 9962.676 MS= 9962.676	0.83	1, 78	0.3663
Treatment	SS= 26207.929 MS= 13103.964	1.09	2, 78	0.3425
Phenotype x Treatment	SS= 23635.13 MS= 11817.56	0.98	2, 78	0.3800
Error	SS= 940860.62 MS= 12062.315			
Sampling Time	SS= 1512.895 MS= 756.4478	0.39	2, 156	0.6749
Phenotype x Sampling Time	SS= 1592.012 MS= 796.0061	0.41	2, 156	0.6612
Treatment x Sampling Time	SS= 18881.62 MS= 4720.405	2.46	4, 156	0.0510
Phenotype x Treatment x Sampling Time	SS= 3403.955 MS= 850.9889	0.44	4, 156	0.7771
Error	SS= 299394.13 MS= 1919.1931			

APPENDIX A-18

ANOVA Summary Table of Total Energy Intake (kcal)

Source	SS/MS	F	df	p
Phenotype	SS= 213.49 MS= 213.49	16.21	1, 72	0.0001
Treatment	SS= 401.81 MS= 80.363	6.10	5, 72	0.0001
Phenotype x Treatment	SS= 19.242 MS= 3.8485	0.29	5, 72	0.9158
Error	SS= 948.08 MS= 13.167			
Sampling Time	SS= 1699.58 MS= 849.790	509.79	2, 144	0.0001
Phenotype x Sampling Time	SS= 38.930 MS= 19.465	11.68	2, 144	0.0001
Treatment x Sampling Time	SS= 42.590 MS= 4.2590	2.56	10, 144	0.0072
Phenotype x Treatment x Sampling Time	SS= 11.011 MS= 1.1011	0.66	10, 144	0.7593
Error	SS= 240.03 MS= 1.6669			

APPENDIX A-19

ANOVA Summary Table of Percent Vehicle Intake

Source	SS/MS	F	df	p
Phenotype	SS= 68.8624 MS= 68.8624	0.06	1, 72	0.8125
Treatment	SS= 52879.484 MS= 10575.896	8.70	5, 72	0.0001
Phenotype x Treatment	SS= 11566.99 MS= 2313.399	1.90	5, 72	0.1042
Error	SS= 87488.427 MS= 1215.1170			
Sampling Time	SS= 22440.26 MS= 11220.13	34.40	2, 144	0.0001
Phenotype x Sampling Time	SS= 943.17 MS= 471.58	1.60	2, 144	0.2056
Treatment x Sampling Time	SS= 7387.729 MS= 738.7729	2.51	10, 144	0.0084
Phenotype x Treatment x Sampling Time	SS= 4556.90 MS= 455.690	1.55	10, 144	0.1292
Error	SS= 42467.327 MS= 294.91199			

APPENDIX A-20

ANOVA Summary Table of Carbohydrate Intake (kcal)

Source	SS/MS	F	df	p
Phenotype	SS= 32.8347 MS= 32.8347	27.71	1, 72	0.0001
Treatment	SS= 18.181803 MS= 3.6323610	3.07	5, 72	0.0145
Phenotype x Treatment	SS= 6.669447 MS= 1.333889	1.13	5, 72	0.3545
Error	SS= 85.304353 MS= 1.1847826			
Sampling Time	SS= 98.72085 MS= 49.36042	326.79	2, 144	0.0001
Phenotype x Sampling Time	SS= 4.556188 MS= 2.278094	15.08	2, 144	0.0001
Treatment x Sampling Time	SS= 1.395737 MS= 0.139574	0.88	10, 144	0.5130
Phenotype x Treatment x Sampling Time	SS= 2.672850 MS= 0.267285	1.77	10, 144	0.0712
Error	SS= 21.75038 MS= 0.151044			

APPENDIX A-21

ANOVA Summary Table of Proportion of Carbohydrate

Source	SS/MS	F	df	p
Phenotype	SS= 0.071092 MS= 0.071092	1.50	1, 72	0.2253
Treatment	SS= 1.437905 MS= 0.287581	6.05	5, 72	0.0001
Phenotype x Treatment	SS= 0.028637 MS= 0.041727	0.88	5, 72	0.5003
Error	SS= 3.421426 MS= 0.047519			
Sampling Time	SS= 0.382925 MS= 0.191463	16.83	2, 144	0.0001
Phenotype x Sampling Time	SS= 0.084074 MS= 0.042037	3.70	2, 144	0.0272
Treatment x Sampling Time	SS= 0.258281 MS= 0.025828	2.27	10, 144	0.0169
Phenotype x Treatment x Sampling Time	SS= 0.108961 MS= 0.010896	0.96	10, 144	0.4826
Error	SS= 1.637706 MS= 0.011372			

APPENDIX A-22

ANOVA Summary Table of Percent Vehicle

Intake of Carbohydrate

Source	SS/MS	F	df	p
Phenotype	SS= 12057.15 MS= 12057.15	0.85	1, 72	0.3601
Treatment	SS= 207004.82 MS= 41400.965	2.91	5, 72	0.0189
Phenotype x Treatment	SS= 41675.26 MS= 8335.053	0.59	5, 72	0.7103
Error	SS= 1.0234887 MS= 14215.122			
Sampling Time	SS= 5015.259 MS= 2507.629	0.70	2, 144	0.4975
Phenotype x Sampling Time	SS= 10289.05 MS= 5144.526	1.44	2, 144	0.2405
Treatment x Sampling Time	SS= 55796.88 MS= 5579.688	1.56	10, 144	0.1240
Phenotype x Treatment x Sampling Time	SS= 43811.36 MS= 4381.136	1.23	10, 144	0.2793
Error	SS= 514681.51 MS= 3574.1771			

APPENDIX A-23

ANOVA Summary Table of Fat Intake (kcal)

Source	SS/MS	F	df	p
Phenotype	SS= 51.84806 MS= 51.84806	7.29	1, 72	0.0086
Treatment	SS= 262.5653 MS= 52.51306	7.38	5, 72	0.0001
Phenotype x Treatment	SS= 23.77387 MS= 4.754775	0.67	5, 72	0.6487
Error	SS= 512.2030 MS= 7.113930			
Sampling Time	SS= 672.0963 MS= 336.0481	289.91	2, 144	0.0001
Phenotype x Sampling Time	SS= 9.585007 MS= 4.792504	4.13	2, 144	0.0180
Treatment x Sampling Time	SS= 22.14379 MS= 2.214379	1.19	10, 144	0.0482
Phenotype x Treatment x Sampling Time	SS= 6.026352 MS= 0.602635	0.52	10, 144	0.8740
Error	SS= 166.9175 MS= 1.159149			

APPENDIX A-24

ANOVA Summary Table of Proportion of Fat

Source	SS/MS	F	df	p
Phenotype	SS= 0.000544 MS= 0.000544	0.01	1, 72	0.9240
Treatment	SS= 1.886994 MS= 0.377399	6.28	5, 72	0.0001
Phenotype x Treatment	SS= 0.445540 MS= 0.089108	1.48	5, 72	0.2060
Error	SS= 4.3257894 MS= 0.0600804			
Sampling Time	SS= 0.783595 MS= 0.391798	26.88	2, 144	0.0001
Phenotype x Sampling Time	SS= 0.014691 MS= 0.007346	0.50	2, 144	0.6052
Treatment x Sampling Time	SS= 0.395493 MS= 0.039549	2.71	10, 144	0.0044
Phenotype x Treatment x Sampling Time	SS= 0.205354 MS= 0.020535	1.41	10, 144	0.1818
Error	SS= 2.0987787 MS= 0.0145748			

APPENDIX A-25

ANOVA Summary Table of Percent Vehicle

Intake of Fat

Source	SS/MS	F	df	p
Phenotype	SS= 2322.709 MS= 2322.709	1.01	1, 72	0.3173
Treatment	SS= 69713.61 MS= 13942.72	6.09	5, 72	0.0001
Phenotype x Treatment	SS= 27620.62 MS= 5524.124	2.41	5, 72	0.0444
Error	SS= 164904.74 MS= 2290.3437			
Sampling Time	SS= 32034.56 MS= 16017.28	41.48	2, 144	0.0001
Phenotype x Sampling Time	SS= 621.7427 MS= 310.8713	0.81	2, 144	0.4490
Treatment x Sampling Time	SS= 9806.718 MS= 980.6718	2.54	10, 144	0.0075
Phenotype x Treatment x Sampling Time	SS= 6362.144 MS= 636.2144	1.65	10, 144	0.0989
Error	SS= 55601.66 MS= 386.1226			

APPENDIX A-26

ANOVA Summary Table of Protein Intake (kcal)

Source	SS/MS	F	df	p
Phenotype	SS= 2.824943 MS= 2.824943	2.93	1, 72	0.0910
Treatment	SS= 7.987697 MS= 1.597539	1.66	5, 72	0.1554
Phenotype x Treatment	SS= 0.520231 MS= 0.104046	0.11	5, 72	0.9902
Error	SS= 69.30301 MS= 0.962541			
Sampling Time	SS= 29.06896 MS= 14.53448	110.45	2, 144	0.0001
Phenotype x Sampling Time	SS= 1.041807 MS= 0.520903	3.96	2, 144	0.0212
Treatment x Sampling Time	SS= 1.059641 MS= 0.105964	0.81	10, 144	0.6239
Phenotype x Treatment x Sampling Time	SS= 0.557293 MS= 0.055729	0.42	10, 144	0.9334
Error	SS= 18.95017 MS= 0.013159			

APPENDIX A-27

ANOVA Summary Table of Proportion of Protein

Source	SS/MS	F	df	p
Phenotype	SS= 0.059195 MS= 0.059195	2.27	1, 72	0.1362
Treatment	SS= 0.319731 MS= 0.063946	2.45	5, 72	0.0413
Phenotype x Treatment	SS= 0.220815 MS= 0.044163	1.69	5, 72	0.1469
Error	SS= 1.876377 MS= 0.026060			
Sampling Time	SS= 0.072213 MS= 0.036106	6.06	2, 144	0.0030
Phenotype x Sampling Time	SS= 0.059375 MS= 0.029688	4.98	2, 144	0.0081
Treatment x Sampling Time	SS= 0.258194 MS= 0.025819	4.33	10, 144	0.0001
Phenotype x Treatment x Sampling Time	SS= 0.232930 MS= 0.023293	3.91	10, 144	0.0001
Error	SS= 85856677 MS= 0.005962			

APPENDIX A-28

ANOVA Summary Table of Percent Vehicle

Intake of Protein

Source	SS/MS	F	df	p
Phenotype	SS= 518.9400 MS= 518.9400	0.09	1, 72	0.7678
Treatment	SS= 48916.499 MS= 9783.2999	1.66	5, 72	0.1564
Phenotype x Treatment	SS= 26921.92 MS= 5384.385	0.91	5, 72	0.4787
Error	SS= 425433.11 MS= 5908.7932			
Sampling Time	SS= 11034.19 MS= 5517.096	2.59	2, 144	0.0788
Phenotype x Sampling Time	SS= 4202.259 MS= 2101.129	0.98	2, 144	0.3760
Treatment x Sampling Time	SS= 29809.80 MS= 2980.980	1.40	10, 144	0.1870
Phenotype x Treatment x Sampling Time	SS= 6590.176 MS= 659.0176	0.31	10, 144	0.9779
Error	SS= 307185.53 MS= 2133.2329			

Table 1

Mean (+SE) Proportion of Total Energy Intake from Carbohydrate, Fat, and Protein as a Function of Phenotype, Regimen, and Weeks on Diet

	Phenotype			
Week	Obese[a]		Lean[b]	
	Carbohydrate			
	6 h	24 h	6 h	24 h
Week 1	0.21 (0.03)	0.15 (0.03)	0.13 (0.03)	0.18 (0.05)
Week 2	0.22 (0.06)	0.17 (0.03)	0.16 (0.02)	0.20 (0.04)
Week 3	0.22 (0.04)	0.17 (0.02)	0.22 (0.03)	0.25 (0.06)
Week 4	0.21 (0.06)	0.16 (0.01)	0.20 (0.04)	0.28 (0.06)
	Fat			
Week 1	0.68 (0.04)	0.73 (0.04)	0.76 (0.04)	0.68 (0.05)
Week 2	0.68 (0.06)	0.65 (0.04)	0.71 (0.03)	0.63 (0.04)
Week 3	0.67 (0.05)	0.68 (0.03)	0.63 (0.05)	0.59 (0.05)
Week 4	0.72 (0.06)	0.70 (0.03)	0.68 (0.05)	0.57 (0.06)
	Protein			
Week 1	0.11 (0.01)	0.11 (0.01)	0.11 (0.01)	0.14 (0.02)
Week 2	0.10 (0.01)	0.16 (0.02)	0.13 (0.01)	0.17 (0.02)
Week 3	0.11 (0.02)	0.15 (0.03)	0.14 (0.02)	0.17 (0.02)
Week 4	0.07 (0.01)	0.13 (0.02)	0.11 (0.02)	0.14 (0.02)

[a] *n*=16.

[b] *n*=16.

Table 2

Mean (+SE) Energy Intake and Nutrient Intake as a Function of Phenotype and Treatment

		Carbohydrate Intake (kcal)		Proportion		% Vehicle	
Obese[a]	0.5 mg/kg CLON	0.72	(0.09)	0.24	(0.02)	52.53	(4.92)
	0.1 mg/kg CLON	0.87	(0.10)	0.20	(0.02)	64.32	(6.01)
	Vehicle[b]	1.26	(0.12)	0.22	(0.01)	95.82	(9.70)
Lean	0.5 mg/kg CLON	1.15	(0.16)	0.25	(0.03)	74.03	(8.41)
	0.1 mg/kg CLON	1.51	(0.16)	0.23	(0.02)	91.51	(5.71)
	Vehicle	2.21	(0.18)	0.21	(0.01)	128.07	(9.57)
		Fat					
Obese	0.5 mg/kg CLON	2.73	(0.40)	0.51	(0.04)	49.87	(6.48)
	0.1 mg/kg CLON	3.27	(0.37)	0.59	(0.04)	75.95	(7.67)
	Vehicle	3.73	(0.34)	0.62	(0.02)	101.86	(7.05)
Lean	0.5 mg/kg CLON	3.55	(0.48)	0.56	(0.04)	61.94	(7.77)
	0.1 mg/kg CLON	4.61	(0.37)	0.65	(0.02)	84.75	(6.34)
	Vehicle	6.93	(0.42)	0.67	(0.01)	138.43	(13.1)
		Protein					
Obese	0.5 mg/kg CLON	0.66	(0.09)	0.23	(0.02)	92.14	(9.74)
	0.1 mg/kg CLON	0.64	(0.08)	0.20	(0.03)	113.48	(10.6)
	Vehicle	0.97	(0.17)	0.15	(0.02)	109.47	(7.75)
Lean	0.5 mg/kg CLON	0.59	(0.07)	0.18	(0.02)	86.53	(19.2)
	0.1 mg/kg CLON	0.74	(0.08)	0.11	(0.01)	74.48	(6.06)
	Vehicle	1.23	(0.14)	0.11	(0.01)	116.34	(7.80)

		Total Energy Intake Intake (kcal)		% Vehicle	
Obese	0.5 mg/kg CLON	4.11	(0.55)	50.83	(4.82)
	0.1 mg/kg CLON	4.78	(0.51)	72.23	(6.03)
	Vehicle	5.96	(0.51)	96.94	(4.89)
Lean	0.5 mg/kg CLON	5.29	(0.66)	57.65	(5.39)
	0.1 mg/kg CLON	6.86	(0.53)	81.38	(4.38)
	Vehicle	10.36	(0.63)	116.65	(6.33)

[a] n=42. [b] n=42.

Table 3

Mean (+SE) Energy Intake as a Function of Phenotype and Treatment

	Energy Intake (kcal)	% Vehicle
Obese[a]		
5 mg/kg YOH-CLON	3.74 (0.62)	76.08 (4.99)
3 mg/kg YOH-CLON	4.76 (0.63)	68.67 (5.09)
VEH-CLON	2.49 (0.49)	34.91 (6.03)
5 mg/kg YOH-VEH	4.74 (0.83)	59.87 (4.91)
3 mg/kg YOH-VEH	5.30 (0.62)	83.06 (4.68)
VEH	6.75 (0.82)	85.78 (3.12)
Lean[b]		
5 mg/kg YOH-CLON	5.53 (0.77)	64.84 (5.54)
3 mg/kg YOH-CLON	5.82 (0.69)	55.80 (8.08)
VEH-CLON	5.26 (0.80)	57.79 (7.19)
5 mg/kg YOH-VEH	6.24 (1.01)	63.30 (7.07)
3 mg/kg YOH-VEH	6.94 (0.80)	73.42 (4.68)
VEH	9.05 (0.99)	99.50 (5.56)

[a] n=42.

[b] n=42.

Table 4

Mean (+SE) Carbohydrate Intake as a Function of Phenotype and Treatment

	Intake (kcal)	Proportion	% Vehicle
Obese[a]			
5 mg/kg YOH-CLON	1.30 (0.17)	0.40 (0.03)	110.60 (8.17)
3 mg/kg YOH-CLON	1.47 (0.17)	0.37 (0.04)	107.44 (8.69)
VEH-CLON	0.65 (0.16)	0.24 (0.04)	46.12 (8.37)
5 mg/kg YOH-VEH	1.25 (0.12)	0.38 (0.05)	168.13 (47.5)
3 mg/kg YOH-VEH	1.20 (0.10)	0.25 (0.02)	145.99 (26.5)
VEH	1.25 (0.16)	0.19 (0.18)	99.27 (7.82)
Lean[b]			
5 mg/kg YOH-CLON	1.91 (0.23)	0.43 (0.05)	122.77 (17.8)
3 mg/kg YOH-CLON	1.68 (0.20)	0.31 (0.03)	87.45 (10.9)
VEH-CLON	1.32 (0.23)	0.27 (0.03)	58.40 (6.36)
5 mg/kg YOH-VEH	2.16 (0.28)	0.41 (0.03)	105.48 (7.78)
3 mg/kg YOH-VEH	2.48 (0.24)	0.39 (0.03)	123.92 (8.53)
VEH	1.90 (0.29)	0.28 (0.03)	96.50 (9.68)

[a] n=42.

[b] n=42.

Table 5

Mean (+SE) Fat Intake as a Function of Phenotype and Treatment

	Intake (kcal)	Proportion	% Vehicle
Obese[a]			
5 mg/kg YOH-CLON	2.08 (0.45)	0.45 (0.04)	65.81 (6.92)
3 mg/kg YOH-CLON	2.63 (0.42)	0.50 (0.04)	54.59 (6.30)
VEH-CLON	1.49 (0.33)	0.47 (0.06)	29.88 (5.84)
5 mg/kg YOH-VEH	2.74 (0.62)	0.43 (0.06)	42.06 (7.44)
3 mg/kg YOH-VEH	3.29 (0.43)	0.60 (0.02)	76.92 (4.56)
VEH	4.68 (0.57)	0.69 (0.01)	81.58 (4.30)
Lean[b]			
5 mg/kg YOH-CLON	2.87 (0.45)	0.44 (0.04)	53.89 (7.25)
3 mg/kg YOH-CLON	3.41 (0.42)	0.55 (0.04)	51.88 (9.12)
VEH-CLON	3.42 (0.33)	0.60 (0.04)	69.35 (11.3)
5 mg/kg YOH-VEH	3.08 (0.62)	0.41 (0.04)	46.69 (8.65)
3 mg/kg YOH-VEH	3.46 (0.43)	0.45 (0.03)	56.96 (5.47)
VEH	6.17 (0.57)	0.69 (0.03)	108.49 (7.54)

[a] n=42.

[b] n=42.

Table 6

Mean (+SE) Protein Intake as a Function of Phenotype and Treatment

	Intake (kcal)	Proportion	% Vehicle
[a] Obese			
5 mg/kg YOH-CLON	0.36 (0.04)	0.13 (0.03)	103.44 (15.8)
3 mg/kg YOH-CLON	0.64 (0.16)	0.11 (0.02)	92.14 (9.18)
VEH-CLON	0.35 (0.06)	0.28 (0.06)	67.47 (10.8)
5 mg/kg YOH-VEH	0.75 (0.15)	0.17 (0.02)	76.73 (9.76)
3 mg/kg YOH-VEH	0.80 (0.21)	0.13 (0.02)	73.69 (6.35)
VEH	0.77 (0.17)	0.10 (0.01)	112.92 (11.5)
[b] Lean			
5 mg/kg YOH-CLON	0.74 (0.16)	0.11 (0.02)	65.10 (9.26)
3 mg/kg YOH-CLON	0.73 (0.12)	0.12 (0.01)	95.62 (20.8)
VEH-CLON	0.51 (0.09)	0.12 (0.02)	54.97 (7.30)
5 mg/kg YOH-VEH	0.99 (0.20)	0.17 (0.02)	100.63 (13.1)
3 mg/kg YOH-VEH	0.99 (0.15)	0.14 (0.02)	90.68 (13.6)
VEH	0.97 (0.22)	0.09 (0.01)	102.17 (14.5)

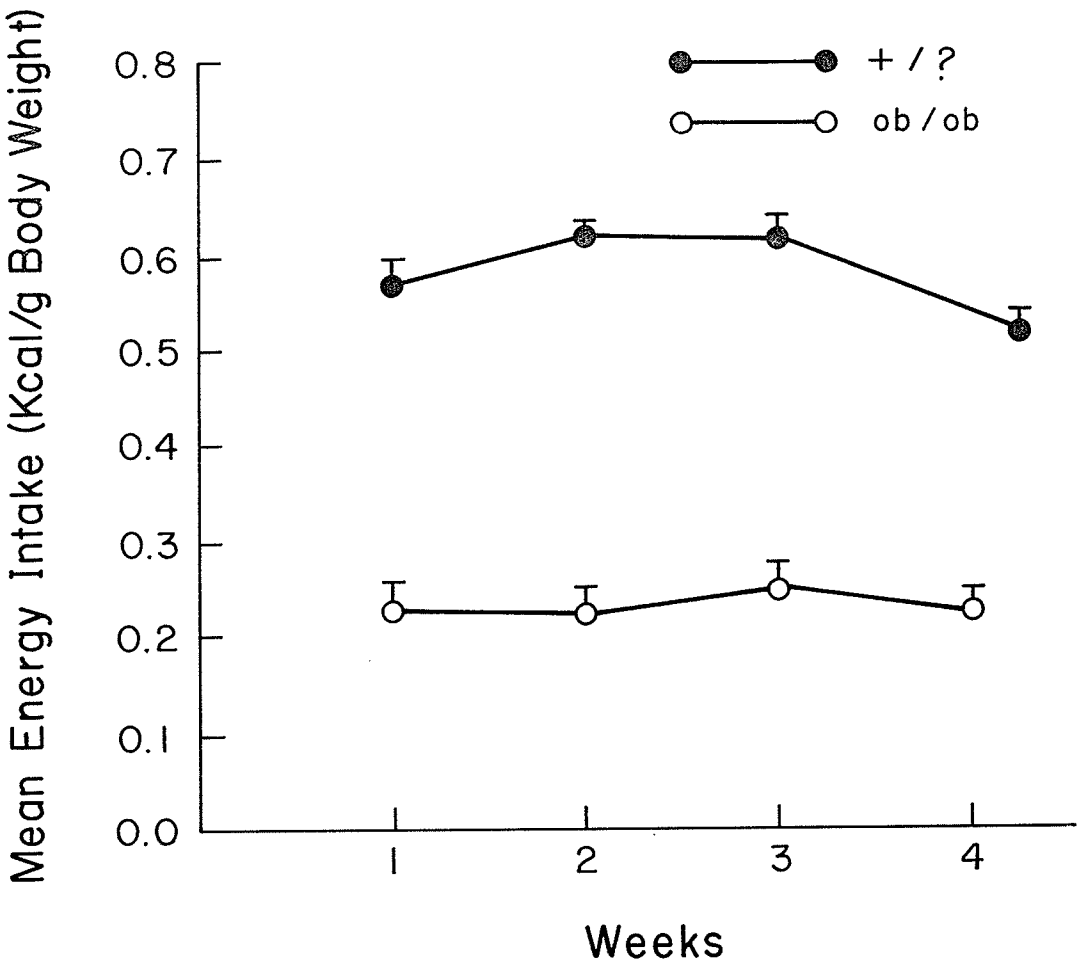
[a] n=42.

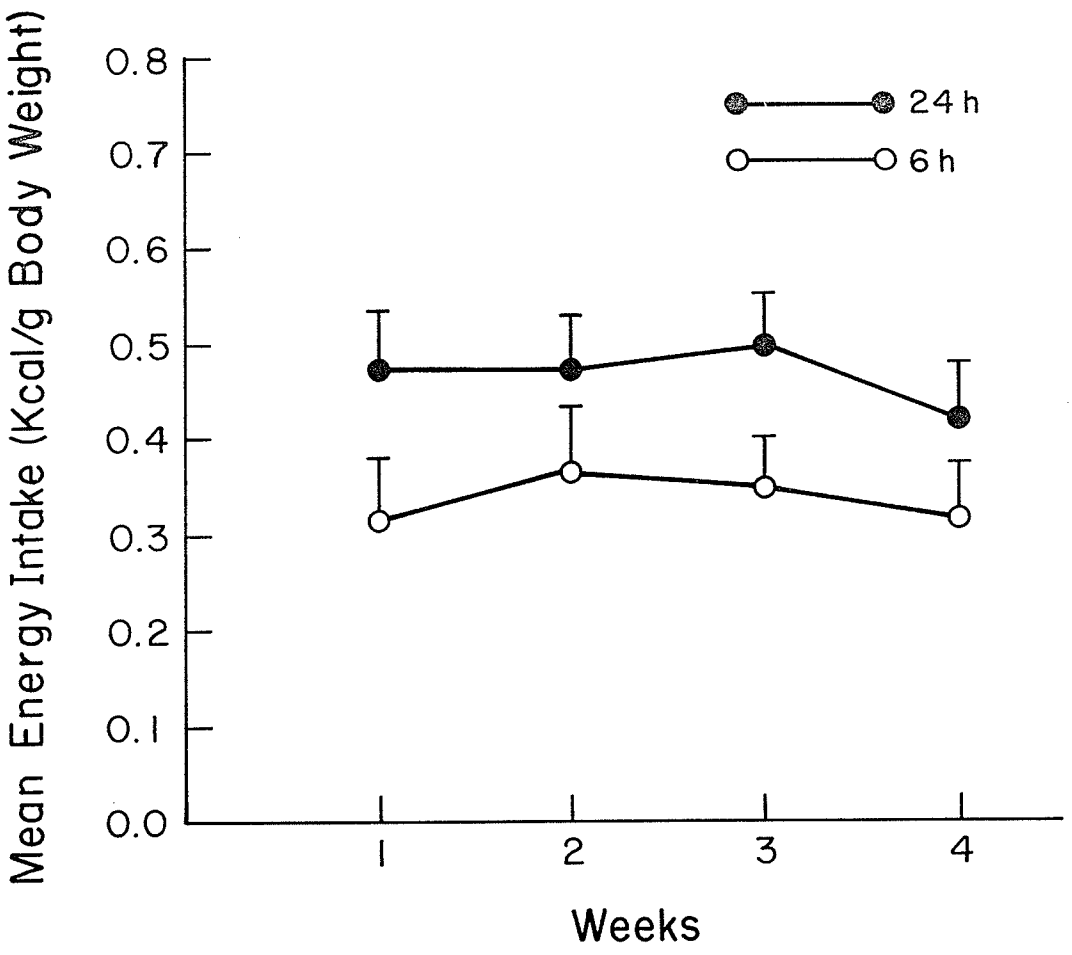
[b] n=42.

Figure Captions

Figure 1. Mean (+SE) energy intake as a function of phentoype (ob/ob, +/?) and weeks of testing.

Figure 2. Mean (+SE) energy intake as a function of dietary regimen (6-h access, 24-h access) and weeks of testing.


Mean Energy Intake (Kcal/g Body Weight)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
+ / ?
ob / ob
1
2
3
4
Weeks


Mean Energy Intake (Kcal/g Body Weight)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
24 h
6 h
1
2
3
4
Weeks