High Fat and High Carbohydrate Diets: Their Psychobiological Impacts on Obese (ob/ob) and Lean (ob/+, +/+) Mice

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Alan B. Slusky

Department of Psychology

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ALAN B. SLUSKY

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Abstract

Although much research has examined the ob/ob mouse as an animal model of human genetic obesity (Bray & York, 1971), less attention has been paid to its homozygous lean littermate (ob/+ mouse). A few studies (Coleman, 1979; Joosten & Van der Kroon, 1974b; Yen, Lowry, & Steinmetz, 1968) have demonstrated that, like the lean human with one lean and one obese parent, the ob/+ demonstrates a predisposition to obesity-related characteristics. This study examined the responses (body temperature (Tc), body weight (BW), food intake (FI), water intake (WI), temperature response to a cold challenge, epididymal fat pad weights, plasma glucose levels and plasma insulin levels) of 21 ob/ob, 41 ob/+, and 21 +/+ mice to either a high carbohydrate (HC), control, or high fat (HF) diet. In light of previous research it was hypothesized that ob/+s would display responses to the diets, which would be intermediate to those of the ob/obs and +/+s. Mice were lab-adapted for 7 days (feeding on standard lab chow) and then assigned (matching procedure) to one of the three diets, on which they fed for the next 21 days (testing phase). During testing, FI, WI, Tc and BW were assessed daily. On Day 29 all mice were cold stressed for 1 h (5°C) and had their Tcs assessed at 15 min intervals. After being fasted on Day 31, Day 32 saw mice sacrificed,

blood collected for glucose and insulin analyses, and fat pads removed and weighed. The 3 X 3 ANOVAs revealed ob/+s to be intermediate to ob/obs and +/+s on measures of fat pad weight, pad/g BW and plasma glucose, thus demonstrating the ob gene's effect on the physiology of the ob/+. Difficulties in measuring FI and problems with the high fat diet (all mice lost weight on this diet) preclude a firm statement on the influence of dietary intake on the ob gene-induced expression of obesity in the ob/+. Nevertheless, the study succeeded in highlighting diet's effect on the ob/+ mouse and reinforced the concept of the existence of a genetic predisposition to obesity in animals.

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General Background

According to the Select Committee on Nutrition and Human Needs of the U.S. Senate, obesity is a "killer disease" that in 1977 alone affected at least 30 million people (as cited in Margules, 1979). In other western industrialized countries, estimates of its prevalence range from 10-50% (Bray, 1985). Whatever the number of affected, obesity is a potentially life-threatening condition. Its occurrence has been correlated with reduced life expectancy; increased incidence of diabetes mellitus, gall bladder problems, cardiovascular disease, and thin skin which tears easily; greater likelihood to die from anesthesia; greater susceptibility to die from infection, and greater chances of developing phlebitis and gout (as cited in Margules, 1979). Death from coronary heart disease is elevated by about 40% in the obese, while death from cardiovascular and renal diseases and from diabetes is increased by 50% and 80-130%, respectively (Stock & Rothwell, 1982). In fact, Bray (1979) estimated that if everyone were at optimal weight, there would be 25% less coronary heart disease and 35% less congestive heart failure and brain infarction.

Obesity may be due to either an increased intake of energy, a decreased expenditure of energy, or a combination of these two

factors (Stock & Rothwell, 1982). Beyond this simple dichotomy, however, the etiology of this disorder is lacking. Even if a consensus could be achieved, several theories would still conflict on the causes of the increased intake, or the decreased expenditure of energy, or both. An additional problem involves assessing energy intake and expenditure and fat content in humans. One accepted definition of obesity describes it as an excess accumulation of body fat, with obese individuals possessing 10-20% more body fat than lean persons (Stock & Rothwell, 1982). To increase experimental control in this area, research has looked to animal models of human obesity (Stock & Rothwell, 1982).

One model involves the transmission of obesity, as a single autosomal recessive mutation, with the obese mouse (ob/ob) and the Zucker fatty rat (fa/fa) being the most studied species (Bray & York, 1971; Storlien, 1984). Several researchers have suggested the ob/ob as a viable model of human obesity, but less attention has been paid to the ob/ob's lean (ob/+, +/+) littermates, in spite of recent evidence, which points to the ob/+ sharing many of the obesity-associated physiological abnormalities of the ob/ob (Connolly & Carnie, 1984). The purpose of this study is to (a) further examine biobehavioral differences among heterozygous (ob/+) lean, homozygous (+/+) lean, and ob/ob mice and (b) to examine the role the ob/+ mouse may play as an animal model of a human predisposition to obesity.

The Genetically-Obese Mouse

The ob/ob inherits its obesity recessively on Chromosome Number 6 (Ingalls, Dickie, & Snell, 1950). The predominant phenotypic attribute of the ob/ob is its obesity, which becomes visually apparent 26 days postpartum (Hellman, 1966; Westman, 1968), although an analysis of fat distribution permits identification at 21 days (Chlouverakis & White, 1969). Besides the mouse's gross body size, other, more subtle indications of its genotype exist. The ob/ob is also hyperphagic, which is manifested as a larger than normal food intake (Fuller & Jacoby, 1955; Mayer, Dickie, Bates, & Vitale, 1951; McClintock & Lifson, 1958). In all cases, ob/obs ate more of a standard lab chow diet than their lean counterparts. When ob/obs were pair-fed with lean mice, obesity still developed (Stock & Rothwell, 1982), indicating that the ob/ob gains fat due to a reduced energy expenditure (Dickerson & Gowan, 1947). Subsequent research has confirmed the ob/ob's lowered energy expenditure in the form of lowered metabolic rate (MR) and colonic temperature (Tc) (Boissoneault, Hornshuh, Simons, Romsos, & Leveille, 1978; Bray & York, 1971; Thurlby & Trayhurn, 1979) when compared to lean littermates. The fact that from the second week of life, ob/obs are far less active than their lean littermates (Joosten & van der Kroon, 1974a; Yen & Acton, 1972) may contribute to their decreased energy expenditure.

The ob/ob is hyperglycemic (Mayer, Bates & Dickie, 1951) and hyperinsulinemic (Genuth, 1969; Stauffacher, Lambert, Vecchio, &

Renold, 1967), but the hyperglycemia doesn't reach its peak (at 12 weeks of age, Mayer, Russel, Bates, & Dickie, 1952) until after the onset of obesity and hyperinsulinemia (Westman, 1968). The elevated insulin levels are present by 1 month of age and peak at 6 months (Westman, 1968). The ob/ob also demonstrated insulin resistance (Dubuc, 1976). The insulin resistance, likely a result of receptor (and postreceptor) defects in response to hyperinsulinemia, may account for the elevated blood glucose (Bonora, Coscelli, & Butturini, 1985).

The ob/ob has poor physiological thermoregulation. Specifically, the ob/ob displays impaired nonshivering thermogenesis (Sclafani, 1984; Thurlby & Trayhurn, 1979; Trayhurn & James, 1978; Trayhurn & James, 1980), which manifests itself as a depressed MR and Tc (viz., Tc is 1-2°C lower in ob/obs than in lean mice at 23°C). However, the ob/ob can thermoregulate behaviorally to compensate for this metabolic defect (Dauncey, 1984; Slusky & Wilson, 1986; Wilson & Sinha, 1985).

With regard to brain biogenic amines and neuropeptides, levels of norepinephrine (NE), dopamine (DA) (Feldman, Blalock, & Zern, 1979; Lorden, Oltmans, & Margules, 1975; Oltmans, 1983), beta-endorphin (Bovoni & Yang, 1981; Margules, Moisset, Lewis, Shibuya, & Pert, 1978), and serotonin (Garthwaite, Martinson, Tseng, Hagen, & Menahan, 1980) are elevated in the ob/ob when compared to its lean littermates. Lorden et al. (1975) reported elevated hypothalamic NE levels in the ob/ob, 50% greater than in leans (Lorden & Oltmans,

1977). A related finding by Oltmans, Olsauskas, and Comaty (1980) revealed that ob/obs' elevated NE levels were less sensitive to reserpine (a drug that decreases NE concentration by affecting the ability of the adrenergic vesicles to store the NE) than were +/?s. Specifically, the +/?s' NE levels decreased significantly faster than those of the ob/obs, and the NE levels of the ob/obs were always greater than those of the +/?s. Furthermore, Oltmans, Lorden, Callahan, Beales, and Fields (1981) reported a significantly greater number of alpha-adrenergic receptors in the hypothalamus of the ob/obs as compared to leans (although recent work by Callahan, Beales and Oltmans (1984) disputes this finding). The areas of greatest NE increase have been localized to the paraventricular nucleus (PVN) of the hypothalamus and the lateral hypothalamus, two areas associated with the regulation of feeding (Oltmans, 1983). Leibowitz (1978) and Leibowitz, Brown, Tretter, & Kirsehgessner (1985) have confirmed the PVN as a primary brain site involved in the alpha-adrenergic regulation of feeding and drinking in the rat. Norepinephrine, alpha-adrenergic agonists (e.g., clonidine), and tricyclic antidepressants (which increase available NE by decreasing presynaptic reuptake) all elicited feeding in sated rats, when injected into the PVN. Clonidine and the tricyclic antidepressants exerted their effects primarily on carbohydrate ingestion and were effective when injected either centrally or peripherally. Furthermore, Leibowitz, Weiss, Yee, & Tretter (1985) showed that NE, injected into the PVN, caused a selective increase in carbohydrate

ingestion. Thus, if an increase in NE has primary alpha-adrenergic potency in the PVN, and if ob/obs have increased PVN-NE, then their overeating may be partly related to a chronically impaired or maladjusted satiety mechanism. Areas other than the PVN, which are less associated with feeding, had less elevated NE levels. In another animal model of genetic obesity, Cruce, Thoa, & Jacobowitz (1976, 1978) found higher PVN-NE levels in fa/fa rats than in lean controls, thereby demonstrating generality of this finding between the ob/ob mouse and fatty rat.

Lorden et al. (1975), Oltmans (1983), and Feldman et al. (1979) reported no differences in hypothalamic levels of DA between ob/obs and leans. When individual hypothalamic nuclei were examined, however, Oltmans (1983) showed decreased DA levels in the arcuate-infundibulum region of the ob/ob hypothalamus. Conversely, Lorden and Oltmans (1977) reported elevated pituitary DA levels in ob/obs. Thus, while it is clear that ob/obs have elevated NE levels in areas known to be associated with feeding, diet selection, and energy expenditure, the results are not as clear for DA.

The ob/ob has elevated beta-endorphin levels (Garthwaite et al., 1980; Margules at al., 1978). Garthwaite et al. (1980) reported that these elevated beta-endorphin levels persist until 62 weeks of age (the normal life span of the ob/ob) when a sharp decline occurs. Pituitary beta-endorphin levels have been examined in other nongenetic obesities (e.g., ventromedial hypothalamic lesions) and have no etiological significance (Gunion & Peters, 1981). Grandison &

Guidotti (1977) have shown that central injection of beta-endorphin increased food intake in rats, and therefore the elevated beta-endorphin levels of the ob/ob have been suggested as one cause of its obesity (Margules et al., 1978). The ob/ob also has elevated brain serotonin levels (Garthwaite et al., 1980), which could play a significant role in the feeding and adiposity of the ob/ob. In addition, ICV injection of serotonin produces hypothermia in mice (Ritzmann & Tabakoff, 1975), which can implicate serotonin in the lowered Tc of the ob/ob.

Not only do specific transmitters influence diet selection, but diets composed of different proportions of macronutrients can influence central and peripheral transmitter synthesis (e.g., Sved, 1983, pp. 256-261). For example, Knehans and Romsos (1984) demonstrated the effect of diet on NE levels by either pair-feeding lean and obese mice or by feeding them a sucrose- or fat-supplemented diet and by measuring NE turnover in brown adipose tissue. Brown adipose tissue, activated by sympathetic preganglionics (viz., NE), is the primary thermogenic organ in mammals, responsible for both non-shivering thermogenesis (NST) and dietary-induced thermogenesis (DIT) (Landsberg & Young, 1984; Morgan & Goldberg, 1981; Rothwell & Stock, 1984). Trayhurn, Jones, McGuckin, and Goodbody (1982) have linked decreased BAT response to diet changes with enhanced adiposity in the ob/ob. Knehans and Romsos's (1984) study revealed that although pair-feeding caused a 41% drop in food intake for the ob/obs, it caused no drop in BAT-NE turnover. When the ob/obs were

fed the sucrose- or fat-supplemented diets, BAT-NE turnover increased by 40%, but O_2 consumption remained unchanged, indicating a decreased capacity for DIT in the ob/ob. Thus, overfeeding fat or carbohydrate increases NE turnover rates in the ob/ob, but with limited effect on energy expenditure via BAT.

Similarly, serotonin synthesis is diet-dependent. Serotonin is a neurotramsmitter that is synthesized from the amino acid precursor tryptophan, which is available in protein-containing foods (Wurtman, 1982). The conversion of brain tryptophan to serotonin is proportional to the amount of carbohydrate in the diet (Fernstrom & Wurtman, 1971) and reflects a high ratio of tryptophan to loose neutral amino acids (LNAA) (viz., leucine, isoleucine, valine, tyrosine, and phenylalanine) (Wutrman, 1982). The only way for these amino acids to cross the blood-brain barrier is via carrier molecules in the endothelial cells lining the brain capillaries (Wurtman, 1982). All amino acids compete for the same carrier molecules. When a high carbohydrate, low protein diet is ingested, the insulin that is secreted in response to the carbohydrate causes the LNAA to move from the plasma into the tissue (e.g., skeletal muscle). However, tryptophan can't go into tissue as readily because it is bound to albumin, thus causing the tryptophan/LNAA ratio to rise. Tryptophan now has less competition for the blood-brain barrier carrier molecules (Wurtman & Wurtman, 1984) and moves into the brain causing (a) a rise in brain serotonin levels, (b) a decrease in subsequent carbohydrate intake (Wurtman, Moses, & Wurtman, 1983; Wurtman &

Wurtman, 1979), and (c) an increase in subsequent protein intake (Ashley & Anderson, 1975; Li & Anderson, 1984).

Conversely, a decrease in brain serotonin increases carbohydrate intake, decreases protein intake (Ashley, Coscina, & Anderson, 1979), and increases fat intake (Waldbillig, Bartness, & Stanley, 1981). This effect has been demonstrated in both rats (Wurtman & Wurtman, 1979) and humans (Ashley, Fleury, Golay, Maeder, & Leathwood, 1985; Wurtman, Wurtman, Growdon, Henry, Lipscomb, & Zeisel, 1981) and suggests that serotonin may inhibit food intake (Waldbillig, Bartness, & Stanley, 1981). Wurtman et al. (1981) showed that doses of l-tryptophan or d-l fenfluramine (a drug that transiently increases the release of serotonin into brain synapses) decreased carbohydrate ingestion in humans. Moreover, Ashley (1985) demonstrated that the tryptophan/LNAA ratio of obese humans decreases with increased glucose intolerance and increased chronicity of the obese state. Accordingly, the increased preference for carbohydrate appears to reflect a decreased level of brain serotonin, and carbohydrate ingestion is an attempt to counter the neurotransmitter deficit (Ashley, 1985).

Another possible cause of the ob/ob's aberrant feeding behavior is a defect in this serotonin-controlled feedback system, where the last meal affects the choice of the next meal. For example, the ob/ob's blood-brain barrier transport system may be defective and therefore not respond appropriately to changes in the plasma tryptophan/LNAA ratio. On the other hand, the ob/ob's brain may not

have enough serotonin neurons to affect subsequent behavior. The serotonergic neurons that do exist may in some way be defective. Whatever the situation, serotonin may play a role in the adiposity of the obese mouse.

Finally, brain weights of ob/obs were significantly less than those of lean mice. As well, the cortical brain volume of the ob/obs was less than the leans', as were the soma cross-sectional areas in the ventromedial hypothalamus (Bereiter & Jeanrenaud, 1979).

The ob/ob as an Animal Model of Human Obesity

To invoke the ob/ob as a viable model of human genetic obesity, it should mirror the metabolic abnormalities of the obese human. While this relationship is not perfect, several metabolic anomalies are shared.

Hyperphagia in obese humans is not as clear as that seen for the ob/ob. Fullerton, Getto, Swift, and Carlson, (1985) have suggested that obese humans do overeat at one time or another during the development of their obesity. Furthermore, this overeating often takes the form of binge eating, with carbohydrate being the primary constituent of the diet (Abraham & Beaumont, 1982). Thus, obese humans may be hyperphagic, but it may be time- and nutrient-specific. Although Stunkard & Kaplan (1977) observed that obese humans eat more food, faster than lean humans, Bellisle & LeMagnen (1981) have shown that obese persons do not overeat relative to their body size. That is, obese humans may eat more than lean humans, but in reality, they are maintaining an increased food intake to defend an increased body

weight set point (Keesey & Powley, 1986).

The hyperglycemia of the obese human is well documented (Felber, Mayer, Curchod, Maeder, Pahud, & Jequier, 1981; Felber, Mayer, Curchod, Iselin, Rouselle, Maeder, Pahud, & Jequier, 1981; Hansen, Jen, Pek, & Wolfe, 1982; Sato, Watanabe, Suzuki, Negishi, Kawazu, Wakabayashi, & Ishii, 1983). Felber et al. (1981) pointed out that the level of glucose intolerance is a positive function of the obesity of the subject. However, as Hansen et al. (1982) observed, insulin and glucose levels in man can fluctuate widely.

Several authors have found obese humans to be hyperinsulinemic relative to lean subjects (Beck, Koumans, Winterling, Stein, Daughaday, & Kipnis, 1964; Grey & Kipnis, 1971; Kreisberg, Boshell, DiPlacido, & Roddam, 1967; Nair, Halliday, & Garrow, 1983). In other words, in response to an elevated blood glucose level, the obese human secretes more insulin than the lean human. Beck et al. (1964) reported that obese subjects had post-oral glucose load insulin levels (before fasting) 3.5 times greater than leans'. Kreisberg et al. (1967) have postulated that this hyperinsulinemia is equivalent to insulin resistance. This interpretation is shared for the ob/ob (Storlien, 1984). Thus, a chronic elevated intake of carbohydrates may lead to elevated glucose levels, and insulin resistance may produce the paradoxically concomitant hyperinsulinemia.

It is known that both obese men and genetically obese mice have difficulty maintaining their body temperatures in the face of a cold challenge. This difficulty is likely due to a decreased thermogenic

capacity (Stock & Rothwell, 1982). Thus like the ob/ob, the obese human has impaired nonshivering thermogenesis (Stock & Rothwell, 1982).

The role of neurotransmitter levels in human obesity is unclear. Endorphin levels of obese humans appear to be no different from those of lean family members (Cohen, Pickar, Cohen, Wise, & Cooper, 1984), but there is an indication of lowered brain serotonin (Ashley, 1985; Wurtman et al., 1981). Thus, the neurotransmitter levels of obese humans do not conform to those of the ob/ob. In view of the majority of preceding obesity-associated biobehavioral abnormalities, however, the ob/ob may be a good model of human genetic obesity.

Aside from metabolic abnormalities, sometimes mice become obese simply because they overeat. When mice are fed a "cafeteria" diet (Rothwell, Saville, & Stock, 1982) consisting of several types of highly palatable foods, excessive weight gain results. Not only do they overeat, but they show marked food preferences. In fact, they can distinguish between different brands of the same food (Sclafani & Springer, 1976; Stock & Rothwell, 1982). Similarly, when humans are presented with diets consisting of various highly palatable foods, weight gain occurs; whereas in cultures with a bland, monotonous diet, obesity is rarely seen (Stock & Rothwell, 1982). The primary nutrients in cafeteria diets are fats and carbohydrates, and much research has been done on the effects of high fat and high carbohydrate diets on the ob/ob.

In general, when presented with several diets differing in

nutritional composition, obese mice will maximize fat intake (Hamilton, 1964; Romsos & Ferguson, 1982) and thereby maximize weight gain (Genuth, 1976; Herberg & Kley, 1975). Castonguay, Rowland, and Stern (1985) showed that this preference is due to factors associated with the animal's obesity and not to its dietary history. This increased ingestion of fat (or a similiar elevated ingestion of carbohydrates) induces several metabolic alterations. York (1975) demonstrated that high carbohydrate diets raised serum glucose and insulin levels in lean mice, but had no such effect on obese mice. However, high fat diets lowered insulin levels and raised serum free fatty acid (FFA) levels in both obese and lean mice. Genuth (1976) and Herberg and Kley (1975) confirmed these results and pointed out that the high fat diet lowered blood glucose levels in the ob/ob, relative to their lean controls. While the ob/obs always had slightly higher glucose levels than their lean littermates, this hyperglycemia was significant only on a 0% fat, 66% carbohydrate diet (Genuth, 1976). Romsos and Ferguson (1982) noted that ob/obs self-select the same proportion of fat, carbohydrate, and protein as leans, at 4 weeks of age. However, as hyperinsulinemia sets in, ob/obs begin to differentially select fat, which acts to lower insulin levels (albeit to a higher than normal level). Increased dietary fat intake is not essential for weight gain in the ob/ob, but the gain is faster when fat is present in the diet (Genuth, 1976). High carbohydrate diets also decrease O_2 consumption in both lean and obese mice, while high fat diets increase O_2 consumption (Fanelli & Kaplan, 1978),

thus indicating a differential effect of diet on metabolism.

The ob/+ Mouse in Obesity Research

A major problem in evaluating the results of previous research on biobehavioral profiles of genetically-obese and lean rodents has been the absence of genetic specification of the lean sample. Because many experimenters have bred their own mice from heterozygous lean (ob/+) pairings, they have compared obese mice, which are genotypically and phenotypically obese, with lean mice, which consist of both homozygous and heterozygous littermates. Genotypic identification of the lean littermates can be accomplished only by breeding to known ob/+ mice. Furthermore, little consideration has been given to the potential for a differential impact of environmental factors, such as dietary constituents, on these two genetic subpopulations. Dubuc (1976), however, explained that several studies had shown a gene-dosage effect in ob/+ lean mice, with heterozygotes displaying intermediate values, on several reliable metabolic, hormonal, and morphologic indicators of obesity, between homozygous lean and obese mice. For example, Yen, Lowry, and Steinmetz (1968) examined the glucose oxidation rate in lean and obese mice. They found a lower rate in ob/obs than in +/+s, with an intermediate rate for ob/+s. Joosten and Van der Kroon (1974b) demonstrated a similiar effect by observing a difference between the fat cell sizes of ob/+s and +/+s at 13 days of age. Connolly and Carnie (1984) found differences in GDP (guanosine di-phosphate) binding to mitochondria in BAT between +/+s and ob/+s, and Coleman (1979) has found that both ob/+s and

db/+s (heterozygotic lean diabetic mouse) survived longer on a total fast than their respective homozygotic lean counterparts. Lastly, Flatt and Bailey (1981) demonstrated that ob/+s have higher circulating concentrations of insulin and plasma glucose than do +/+s. Thus, several physiological parameters have differentiated the ob/+ from the +/+ mouse. Researchers, however, have not yet compared the responses of these genotypically different lean mice to high fat and high carbohydrate diets.

The genotypic distinction is especially important for human obesity, because most populations of obese humans are heterogenous (Stock & Rothwell, 1982), although a strong genetic, possibly polygenic (Saxton, Eisen, & Leatherwood, 1984), component exists in human obesity. For example, the incidence of obesity in children is 9% when both parents are lean, 40% when one parent is obese, and 80% when both parents are obese. Furthermore, identical twins tend to have similiar body weights, even when they are brought up in different environments (Stock & Rothwell, 1982). This genetic contribution to obesity, however, may be modified, by environmental influences, such as diet. Thus, distinguishing between how ob/+s and +/+s react to different diets has direct implications for those humans, who are genetically predisposed to obesity, but who need an environmental event (i.e., altered macronutrient composition) to trigger their latent adiposity.

Statement of the Problem

To the extent that the ob/+ mouse may be influenced in its response to different diets by the ob gene, changes in biobehavioral characteristics of ob/+s on different diets could shed light on which macronutrients support the expression of the ob gene. Thus, this study investigated the differential responses of ob/obs, ob/+s, and +/+s to high fat, high carbohydrate, and control diets and evaluated the contribution of diet composition to augmenting the moderate gene-dose effect of the ob gene under standard dietary regimens. Adult mice of each genotype fed on either a high fat, a high carbohydrate, or a control diet for 3 weeks. During this time, Tc, body weight, food intake (joules/g body weight), and water intake were monitored daily to assess the onset of diet-dependent changes in these measures. At the end of the 3 weeks, the mice's responses to a cold stressor (1 h at 5°C) were assessed, followed several days later by postmortem analysis of adiposity (epididymal white fat pad weight), plasma glucose levels and plasma insulin levels.

In accordance with previous research, it was expected that the ob/obs would eat more of all diets in both total daily intake in grams and total daily energy intake (joules/g body weight) and would, therefore, demonstrate a greater percentage change in body weight over the dietary regimen than either the ob/+ or +/+ mice. Using postweanling animals, Lin, Romsos, and Leveille (1977) demonstrated that +/?s ate more (total gram intake) and ingested more kilojoules of energy on a high fat or stock diet (high carbohydrate) than leans.

Young leans did not eat significantly more of either high fat or stock diets. Young ob/obs gained more weight faster on high fat diets than leans, and by 9 weeks postpartum the obese mice had attained significantly heavier body weights than the ob/obs on stock diet. In contrast, young leans (+/?) gained to the same level on either diet. These results were obtained in young mice comparable in age to mice in the present study; thus, several speculations can be drawn: (a) The ob/ob mice on the high fat regimen should ingest more food and more metabolizable energy/gram body weight than ob/ob mice on either the high carbohydrate or control diets. (b) This differential intake should result in the greatest weight gain in adult ob/obs on the high fat diet. (c) To the extent that the ob gene in the heterozygous lean mouse predisposes this subject towards obese-like symptoms under optimal environmental conditions, ob/+ mice should show the greatest intakes and weight gains on the diet which potentiates adiposity in the ob/ob, namely the high fat diet. (d) Homozygous lean mice should show little or no difference in intake among the diet types. If +/+ mice also show enhanced feeding to the high fat diet compared to the other two regimens, they still are unlikely to demonstrate exaggerated body weight gains to the extent that their ability to compensate metabolically for the enhanced energy intake is unimpaired. Although ob/obs show enhanced NE synthesis to fat- or sucrose-laden diets (Knehans & Romsos, 1984), this enhanced neurotransmitter activity per se does not increase

brown fat metabolism. Therefore, augmented energy intake should not be matched by augmented energy expenditure, thereby improving the chances for greater body weight gain on high fat and high carbohydrate diets than homozygous leans. Since the ob gene has been demonstrated to depress glucose oxidation on heterozygous leans, ob/+s should also gain more weight than homozygotes by a less efficient metabolic compensation for increased energy intake.

To the extent that BAT activation is highly correlated with body temperature (Landsberg & Young, 1984), ob/obs on all diets should display the lowest Tcs of all mice. Ob/+s, due to the presence of the ob gene, should show Tcs intermediate to those of the ob/obs and the +/+s. In particular, ob/+s on the high fat diet should show the lowest Tc of all heterozygous leans due to the enhanced intake of fat commonly seen in rats and mice and the presumed BAT defect due to the ob gene (Connolly & Carnie, 1984). No difference in Tc was anticipated between ob/+s on the high carbohydrate and control diets. Furthermore, the ob/obs should have the most difficulty maintaining their Tcs in response to the cold challenge and should, therefore, show the steepest drop in Tc following the challenge.

It follows that if the ob gene depresses glucose oxidation, the ob/obs should display the highest glucose levels, the +/+s the lowest, and the ob/+s an intermediate level. As well, within each genotype, the animals on the high-carbohydrate diet should show glucose levels higher than their same-genotype counterparts. Because high fat diets depress glucose levels in the ob/ob, the ob/obs on the

high fat diet should show the lowest plasma glucose levels. Because glucose levels and insulin levels are so highly correlated, the same speculations are made regarding insulin levels.

Finally, those mice that weigh the most at the end of the study should have the heaviest fat pads due to hypertrophy and hyperplasia. Thus, ob/obs on the high fat diet should have the heaviest fat pads overall, followed by ob/+s on the high fat diet. The +/+ mice should show negligible alterations in fat pad mass across carbohydrate and control diets, because higher energy intake should be matched more closely by energy expenditure, thereby leaving less energy to be stored as fat.

Method

Subjects

Young male obese (C57Bl/6J ob/ob; n=21) and lean (C57Bl/6J ob/+; n=42; C57Bl/6J +/+; n=21) mice were purchased from Jackson Laboratories, Bar Harbor, ME, USA. Males were used because female lean mice have 4-day estrus cycles, which might have confounded the dependent measures. Twice as many ob/+s, as ob/obs and +/+s, were sampled because ob/+s are twice as prevalent in the population. A total sample size of 83 (one mouse was sacrificed during testing), combined with a Cohen's size effect index (Cohen, 1977) of .32, gave this study a power of approximately .76. All mice were housed one per cage in hanging wire cages over wood-chip bedding, with food (Wayne Mouse Chow Blox), a cup with wood-chip bedding, and fresh water continuously available, for the first week the mice were in the lab.

All mice were housed in the same mouse colony room, which was maintained at 24.4±.1°C, 40-50% relative humidity, on a 14-h light, 10-h dark cycle (lights on at 0730h). All mice were 41.5±.4 days of age upon arrival in the lab.

Apparatus

All mice were housed in hanging wire cages (24.5 cm X 18 cm X 18 cm). Mouse Tcs were measured via a ball-tipped thermocouple (Model No.RET-3, diameter = 0.0625 cm, Bailey Instruments) and read from a Bailey Instruments Digital Telethermometer (Model No. BAT-12). Mouse and food weights were read (to the nearest .01g) from a Mettler Digital Balance (Model No. PB 300). Fat pads and drugs (sodium pentobarbitone) were weighed on a Sartorius analytical balance (Model No. 125646). The test diets were fed in semi-circular, plastic, bird food cups (diameter = 6.5 cm, depth = 3.4 cm), which clipped onto the front of each cage via two metal hooks. The tops of these cups were covered with stainless steel lids with three 1-cm holes drilled in them (to minimize spillage). Cold tolerance testing took place in a walk-in cooler (Model No. W.I.D.C., Coldstream Products of Canada) at 5°C. All instruments were calibrated at the start of each day.

Diets

The high carbohydrate (HC) diet contained (in g per 100g) casein, 20.0; D-L methionine, 0.3; mineral mix, 3.5; vitamin mix, 1.0; choline bitartrate, 0.2; cellulose, 4.0; corn oil, 5.0; and glucose, 66.0. The high fat (HF) diet was formulated on a similiar basis by replacing 40.7 g of glucose with 27.1 g of tallow. The

control diet consisted of casein, 24.0; D-L methionine, 0.35; mineral mix, 4.0; vitamin mix, 1.2; choline bitartrate, 0.24; cellulose, 5.0; corn oil, 6.0; glucose, 45.21; tallow, 14.0. The stock diet (Wayne Mouse Chow Blox) consisted of starch, 45.4; protein, 24.0; moisture, 12.2; ash, 7.9; fat, 6.0; and fiber, 4.5 (Dr. J. Drews, Personal communication, May, 1986; see Appendix E for composition of diets). The energy content of the high carbohydrate, control, and high fat diets (in kilojoules/g) were 17.10, 20.06, and 22.91, respectively. In order to give the diets a liquid-mash consistency, 29% H_2O was added to the high carbohydrate diet and 35% H_2O was added to the control diet. However, due to dehydration, the high carbohydrate and control diets became granular, while the high fat diet took on a paste form (without the addition of any water).

Procedure

Upon arrival in the lab, each mouse was lightly anesthetized with ether and ear-punched for identification. The lean mice were genotyped by someone other than the experimenter. Thus, the experimenter was blind to the genotype of the lean mice (ob/+ or +/+). The mice were then randomly assigned to the three diet conditions to yield the following treatment groups: (a) High-fat obese (b) High-carbohydrate obese, (c) Control obese, (d) High-fat heterozygous lean, (e) High-carbohydrate heterozygous lean, (f) Control heterozygous lean, (g) High-fat homozygous lean, (h) High-carbohydrate homozygous lean, and (i) Control homozygous lean. Mice arrived in the lab in six different shipments. Within each

shipment, assignment to diets was made randomly, with the provision that the mean group weight across diets, within each genotype was equal. At the same time each day, for the next 7 days, each mouse was weighed, and its Tc was measured by inserting the thermocouple (lubricated with mineral oil) 2.0 cm beyond the anal orifice (Dauncey, 1984). Each day's run was begun no earlier than 1000 h to avoid the hypothermic effect of spontaneous torpor (Webb, Jagot, & Jakobson, 1981). This first week constituted a general adaptation period, and during this time, all mice were fed the stock diet.

On Day 8, all mice were given the diet appropriate for their experimental condition. In each case, more than sufficient food was given. Fresh water was continuously available in 100-ml calibrated drinking tubes (Wahmann). On Days 9-29, once per day, each mouse's Tc, food intake, body weight, and water intake were recorded. The mice and the measures were taken in the same order each day to avoid any unnecessary disorientation of the mice. Following the 21 days on the diet program, on Day 30, each mouse was placed (along with its cage, with food and water removed) into a walk-in cooler (5^{o}C) for 1 h (cold tolerance testing). At 15-min intervals, the mouse was removed from its cage, and its Tc was recorded to give an indirect indication of its autonomic thermoregulatory capacity. On the day following cold-tolerance testing (Day 31), mice were fasted, and on the following day (Day 32) sacrificed with an intraperitoneal injection of Sodium Pentobarbitone (110mg/Kg body weight dose in an injectable volume of 1 ml/100g body weight) and then decapitated.

Core blood samples were taken immediately into heparinized vacutainers, rimmed, and centrifuged for 5 min at 4970 RPM, to obtain plasma for glucose and insulin determinations. The plasma supernatant was pipetted from each sample into plastic autocontainers, stoppered, labeled, and stored at -20°C until all mice had been sacrificed, at which time all the glucose and insulin assays were run. Following the removal of the blood, each carcass was dissected, and the epididymal fat pads were removed and weighed.

Glucose Analysis

Glucose content was analyzed via the 16-UV hexokinase reagent kit supplied by Sigma Chemical Co. (St. Louis, MO, USA). Determination of the glucose content was made in duplicate (in most instances) with correction of final values for hemolysis, following a standard hexokinase reaction. Absorbance of each sample was read at 340nm with the aid of a Bausch & Lomb Spectronic 70 UV Spectrophotometer. The procedure followed is highlighted in Appendix A.

Insulin Analysis

Insulin content was assessed via a double antibody radioimmunoassay procedure, as outlined in Appendix B. It was carried out in the Animal Science lab of Dr. A. Kennedy, of the Faculty of Animal Science, University of Manitoba. Appropriate radioactive insulin and rat insulin standards were used to yield a heterologous assay.

Adipose Tissue Analysis

After blood had been collected for glucose and insulin analysis, the carcasses were dissected and the epididymal fat pads removed. The method employed was similiar to that of Johnson and Hirsch (1972). The epididymal pads were exposed by an abdominal incision and retraction of the overlapping posterior alimentary canal and were removed just distal to the internal spermatic artery in the base of each of the pads. The pads were immediately weighed upon removal.

Results

Statistical Analyses

Absolute body weight (BW) and body temperature (Tc) data were analyzed via 3 X 3 X 4 X 7 (Genotype X Diet X Week X Day) analyses of variance (ANOVA) with repeated measures on the third and fourth variables. In addition, two derived scores for both dependent variables were considered. For both, change scores (seven scores--each representing the mean of 3 successive test days - the mean of the last 3 adaptation days) were analyzed in 3 X 3 (Genotype X Diet) ANOVAs. Percentage change scores (((Day 28 BW or Tc - Day 7 BW or Tc)/Day 7 BW or Tc) X 100) were also examined in 3 X 3 (Genotype X Diet) ANOVAs. These derived scores were calculated to account for intergenotype differences on baseline BWs and Tcs.

Cold challenge Tcs were analyzed in two ways. First, all raw data were examined in a 3 X 3 X 5 (Genotype X Diet X Sampling Time) repeated measures ANOVA, with repeated measures on the third variable. In addition, change scores (viz., Posttest Tc - Pretest Tc)

were analyzed in a 3 X 3 (Genotype X Diet) ANOVA.

Absolute fat pad weights were analyzed in a 3 X 3 (Genotype X Diet) ANOVA; subsequently, a derived score (fat pad weight divided by BW at time of sacrifice) was examined via a 3 X 3 (Genotype X Diet) ANOVA.

Repeated measures analyses (viz., cold challenge Tcs, BW over adaptation and diet phases, and Tc over adaptation and diet phases) were also subjected to orthogonal trend analyses. Appendixes C1-C10 contain summary tables for all statistical analyses.

During the running of the study, an attempt was made to obtain enough blood plasma from each animal to yield two samples each of blood glucose and insulin. Where two samples were obtained, a mean value was calculated and used in the analysis. If only one sample could be obtained, that value was used. If no sample was obtained (as occurred with some of the lighter mice; see p. 39 and p. 41), the group mean was used as the measure for that animal. This mean score substitution occurred in seven instances: five times in the glucose analysis and twice in the insulin analysis. Both glucose and insulin data were analyzed twice in two 3 X 3 (Genotype X Diet) ANOVAs: one ANOVA on the data with the missing values and one for the data with the added group means.

Food intake data were not available for analysis due to excessive spillage. Several attempts were made to minimize this spillage (e.g., placing screen at the bottom of the cage, placing newsprint under the cage, putting perforated lids on the food cups); but in most

instances the spillage, when combined with the accumulated fecal boli, made quantification impossible. Because food intake was measured for 21 days, if a particular mouse's spillage was quantifiable for 11 or more days, that mouse was considered a nonspiller. Appendix D presents a tabular account of the number of nonspillers in each group. Examination of this table highlights the difficulties in making intergenotype and interdiet comparisons.

For all analyses, a Type I error rate (α) of .05 was maintained. Analyses of Variance were run under version 5.16 of SAS using Proc GLM, emphasizing sums of squares option number 3 (SS3). The SS3 option was used because the hypotheses tested using SS3 are not a function of the cell counts and hence allow for unequal cell sizes. Trend analyses were run using BMDP statistical software. Any post hoc probing of significant effects used Tukey's Studentized Range (HSD) Test (α=.05). Finally, linear contrasts were used to probe for individual mean differences within significant interactions. A Tukey's critical *t* value was used to assess contrast significance in order to maintain an overall experiment-wise error rate of .05.

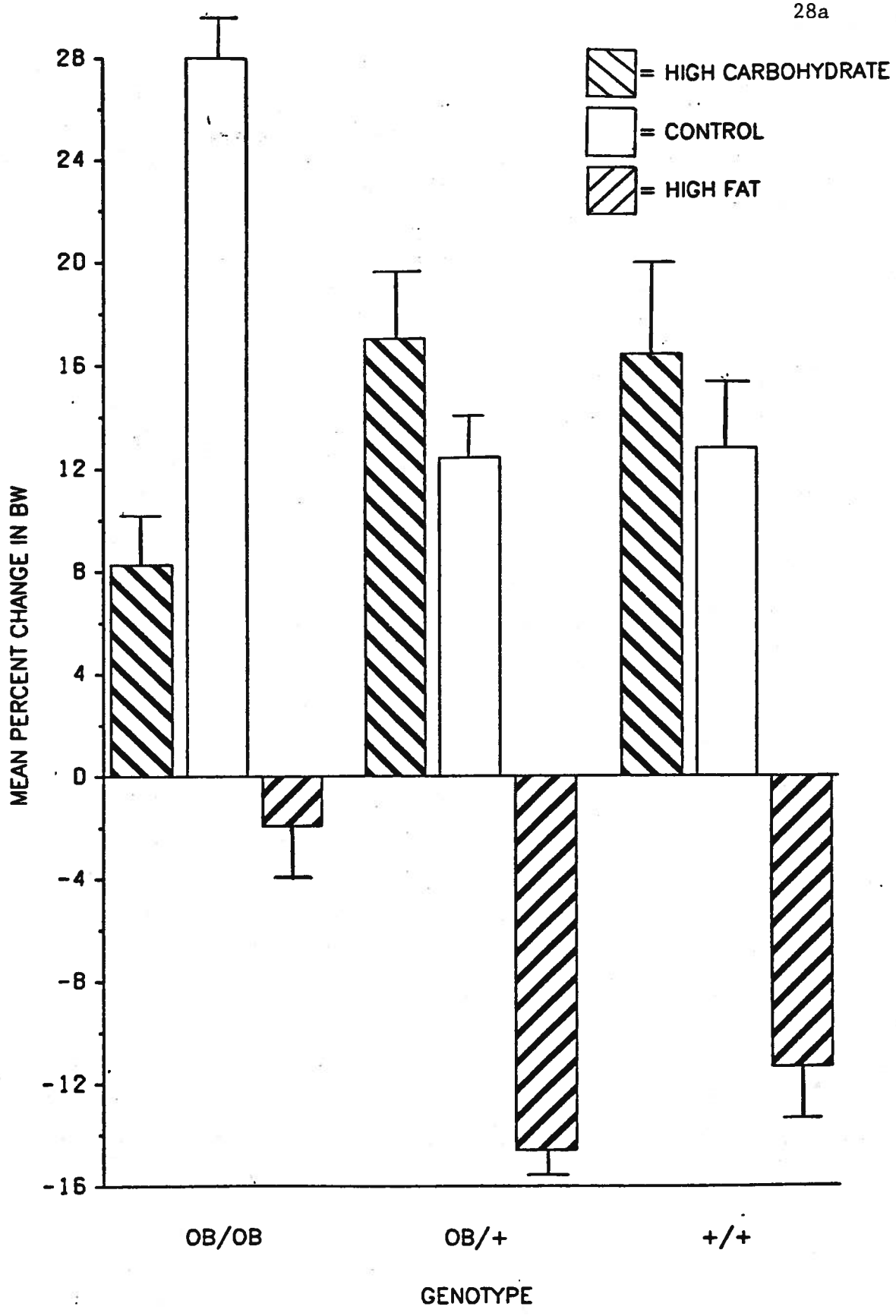
Body Weight

Because the three genotypes sampled had different baseline body weights, most results will be from analyses performed on the percentage change in body weight scores--seven scores, each representing the percentage change in body weight from pre-diet adaptation weights to each of seven 3-day intervals during the administration of the test diets. The analysis indicated

significance at the .01 level for all Genotype and Diet effects and Genotype X Diet interactions. Overall, in accordance with my hypothesis, the *ob*/*ob*s gained more weight than the leans, $F(2, 74) = 5.78$, $p<.005$. However, percentage body weight change did not differ between *ob*/+s and +/+s. A diet effect was present, $F(2, 74) = 94.36$, $p<.001$, with animals gaining more weight on both the HC diet and control diet than on the high fat diet. In addition, a Tukey's analysis revealed that *ob*/*ob*s gained the most weight on the control diet, whereas the leans gained the most on the HC diet, Genotype X Diet: $F(4, 74) = 8.18$, $p<.001$ (see Fig. 1). Although no mice showed an overall percentage body weight loss (*i.e.*, a loss in weight from baseline), all mice on the high fat diet did lose weight during the testing phase of the study.

One could speculate that had these mice not been allowed a week of ad-lib feeding on a stock diet (with the concomitant increase in BW), they would have displayed a negative change. An analysis of the raw body weight data was done to examine trends across the 4 weeks of testing. This analysis revealed significant Genotype X Week, $F(6, 222) = 38.47$, $p<.001$, and Diet X Week, $F(6, 222) = 67.37$, $p<.001$, interactions. An orthogonal trend analysis on these data revealed significant linear, $F(2, 74) = 41.13$, $p<.001$, and quadratic, $F(2, 74) = 37.32$, $p<.001$, trends for the Genotype X Week interaction. This trend was manifested in a linear increase in body weight across the 4 weeks, for all genotypes on the control and HC diets. The quadratic trend reflects the initial increase and subsequent decrease

Figure 1. Mean (±SEM) percentage change in body weight (BW) for obese (ob/ob) and lean (ob/+, +/+) mice across the three diet conditions.


= HIGH CARBOHYDRATE
= CONTROL
= HIGH FAT
28
24
20
16
12
8
4
0
-4
-8
-12
-16
MEAN PERCENT CHANGE IN BW
OB/OB
OB/+
+/+
GENOTYPE

in body weight seen in the ob/obs and +/+s on the high fat diet. The ob/+s, on the other hand, displayed a steady decrease in body weight on the high fat diet. These trends are illustrated in Figures 2, 3, and 4. In addition, the Diet X Week interaction had significant linear, $F(2, 74) = 77.18$, $p<.001$, and quadratic, $F(2, 74) = 9.59$, $p<.001$, trends.

Body Temperature

Analysis of the percentage change in Tc across testing revealed that the ob/obs' Tcs increased most, followed by the +/+s and ob/+s, Genotype: $F(2, 74) = 3.49$, $p<.05$. Although the same Genotype effect was found for the seven change scores, no significant diet effect was obtained for the change scores or the percentage change scores. Analysis of the raw data (for purposes of trend analysis) revealed significant Genotype X Week, $F(6, 216) = 4.43$, $p<.001$, and Diet X Week, $F(6, 216) = 2.45$, $p<.05$, interactions. The Genotype X Week interaction had a significant quadratic trend, $F(2, 72) = 10.74$, $p<.001$. This trend, displayed in Figure 5, is manifested in the ob/obs' increase in Tc in Week 2, followed by decreases in Weeks 3 and 4. A significant linear trend, $F(2, 72) = 3.66$, $p<.05$, to the Diet X Week interaction emphasized that Tc generally increased across diets and weeks.

Cold Challenge

Analysis of the raw cold challenge data revealed genotype, $F(2,71) = 70.84$, $p<.001$, diet, $F(2, 71) = 4.35$, $p<.05$, and sampling time, $F(4, 284) = 217.10$, $p<.001$, main effects. As well, mice on the

Figure 2. Mean (±SEM) change in body weight (g) during testing for the ob/ob mouse.

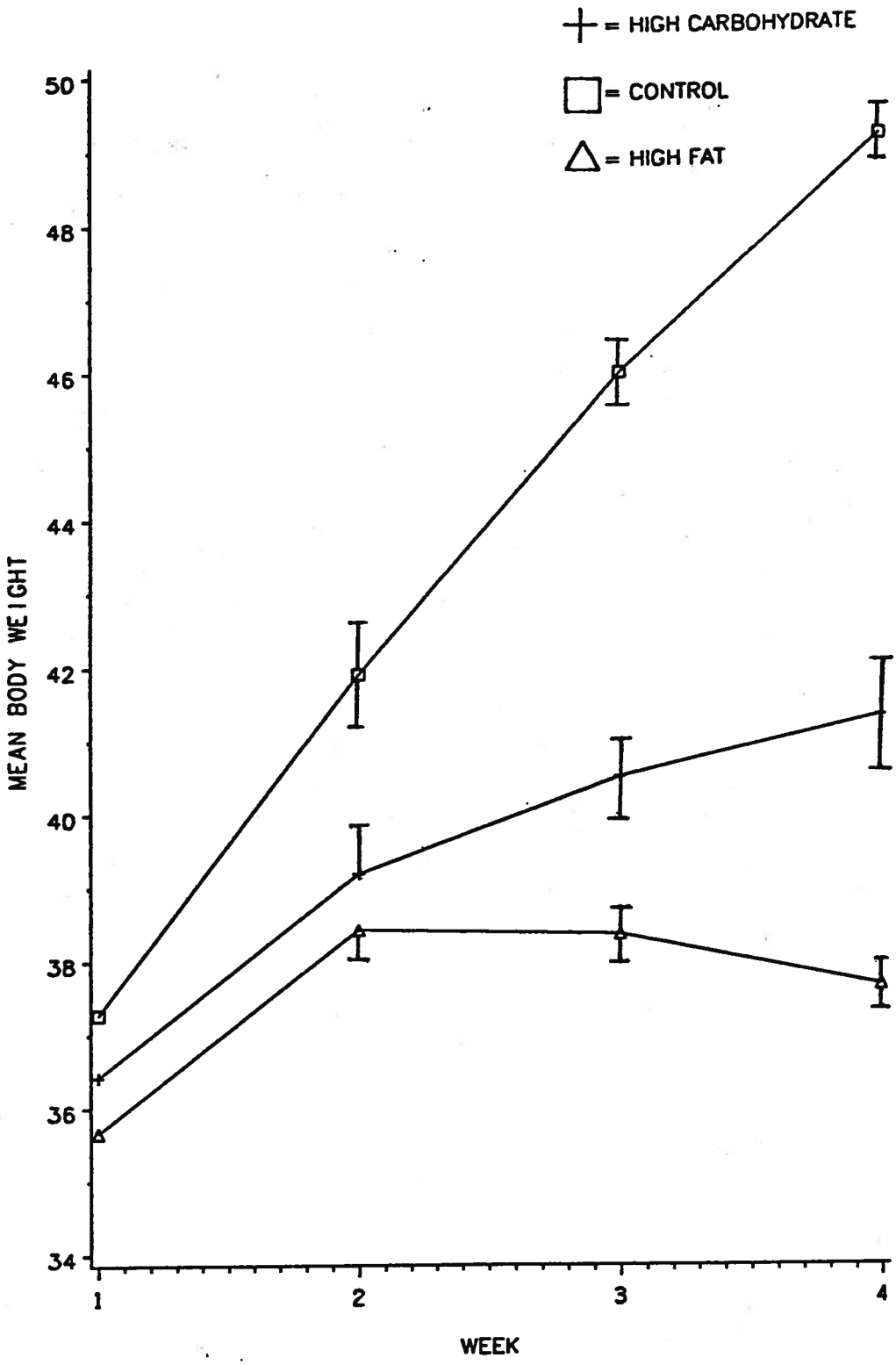

+ = HIGH CARBOHYDRATE
□ = CONTROL
△ = HIGH FAT
MEAN BODY WEIGHT
50
48
46
44
42
40
38
36
34
1
2
3
4
WEEK

Figure 3. Mean (±SEM) change in body weight (g) during testing for the *ob*/+ mouse.

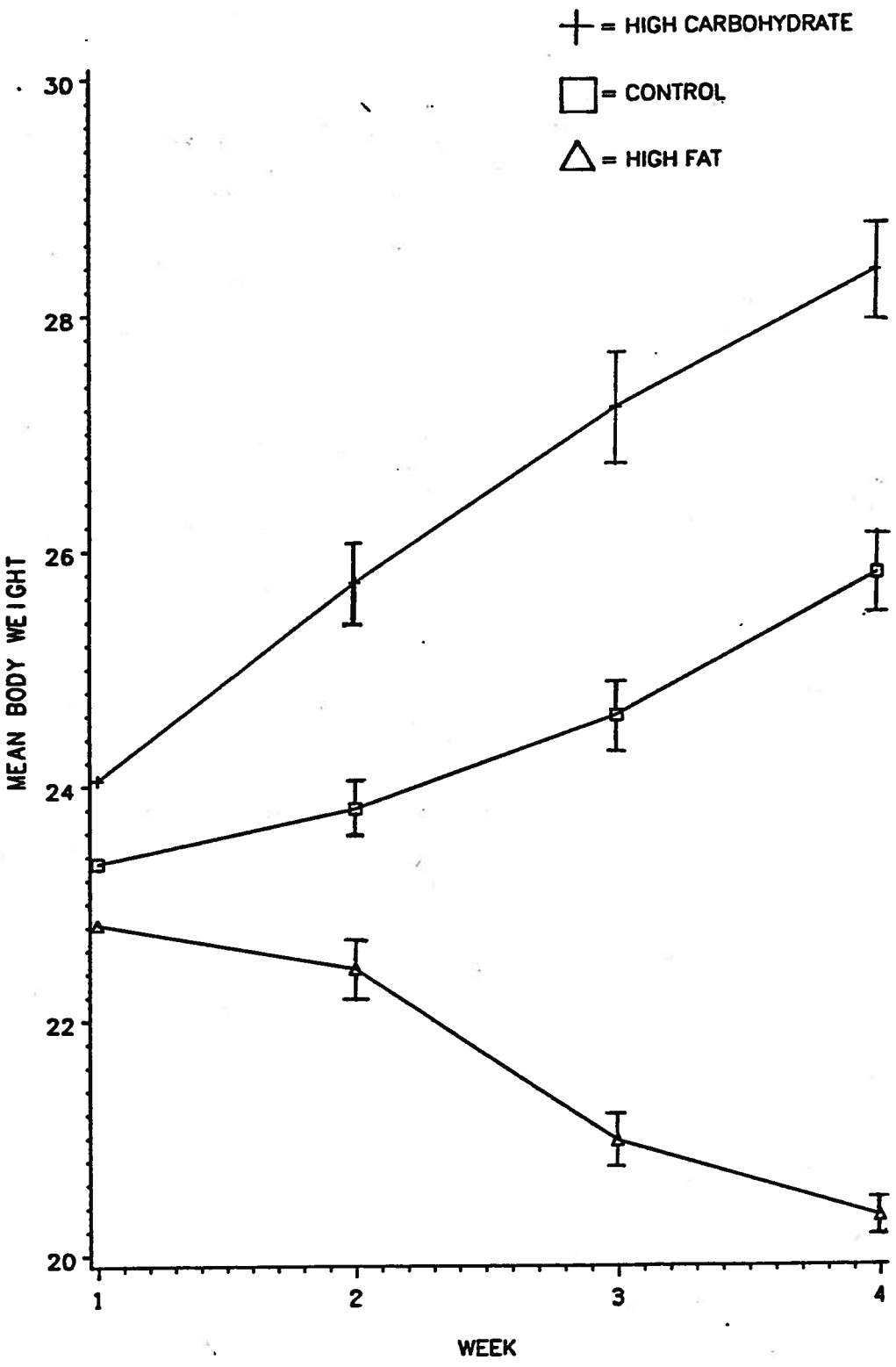

+ = HIGH CARBOHYDRATE
□ = CONTROL
△ = HIGH FAT
MEAN BODY WEIGHT
30
28
26
24
22
20
1
2
3
4
WEEK

Figure 4. Mean (±SEM) change in body weight (g) during testing for the +/+ mouse.

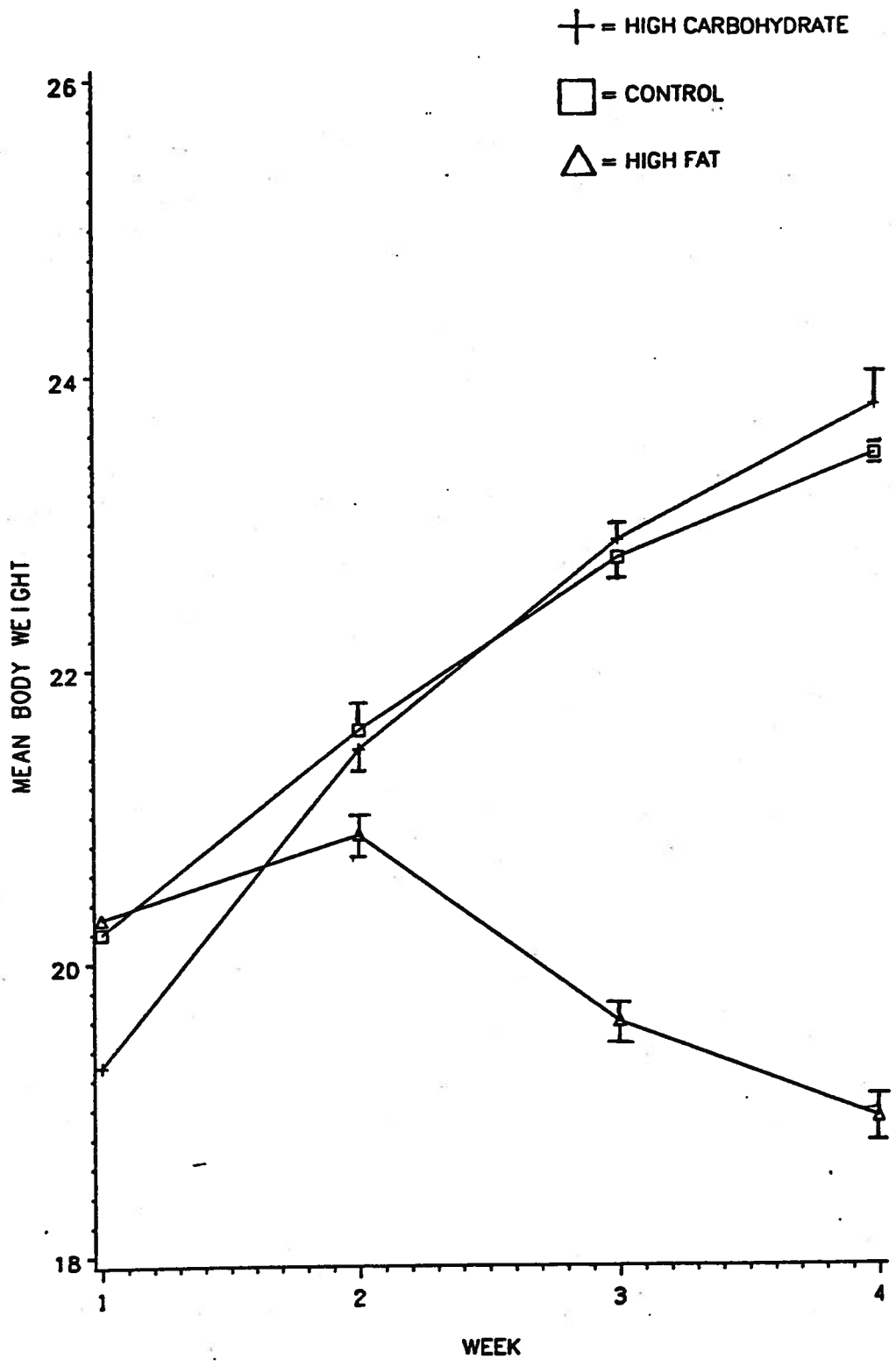
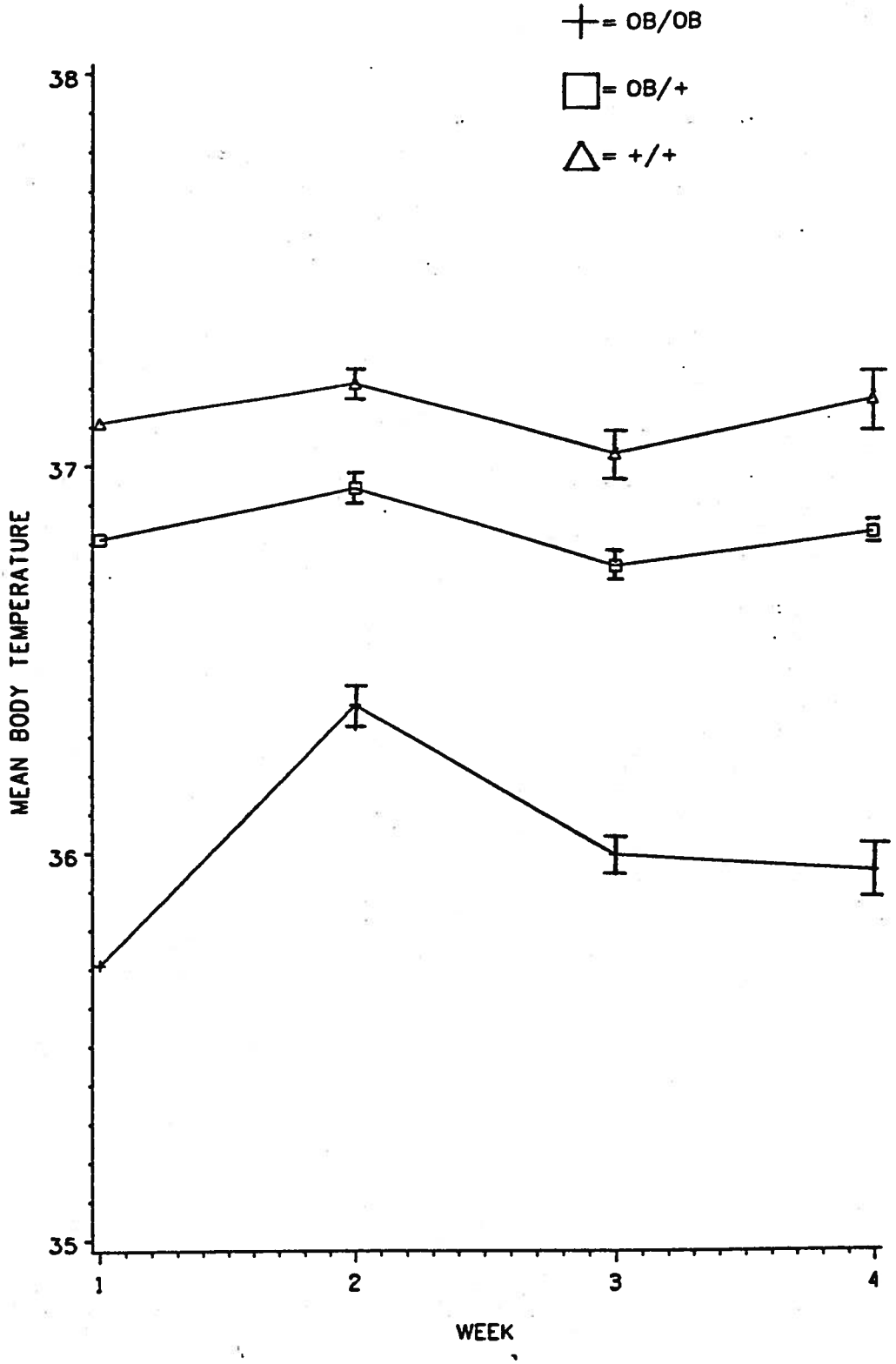

+ = HIGH CARBOHYDRATE
□ = CONTROL
△ = HIGH FAT
MEAN BODY WEIGHT
26
24
22
20
18
1
2
3
4
WEEK

Figure 5. Mean (±SEM) change in body temperature (^{o}C) across diet conditions during testing for ob/ob, ob/+, and +/+ mice.


+ = OB/OB
□ = OB/+
△ = +/+
38
37
36
35
MEAN BODY TEMPERATURE
1
2
3
4
WEEK

HC diet displayed the greatest mean Tc, while mice on the control and HF diets displayed lower Tcs. These data are displayed in Table 1. Orthogonal trend analysis of these data revealed a significant linear trend to the genotype effect, $F(2, 71) = 45.95$, $p<.001$. This trend reflects the steady linear decrease in Tc, over time at 5°C, shown by all mice. In addition, mice with different genotypes showed different Tc declines over the five sampling times in the cold, Genotype X Time in Cold interaction: $F(8, 284) = 32.80$, $p<.001$. The interaction contained a strong linear trend, $F(2, 71) = 47.70$, $p<.001$, indicating a genotype-specific sensitivity to the cold stress, with ob/obs' Tcs declining more steeply than those of ob/+ or +/+ mice.

Analysis of the change scores (ColdTc5 - ColdTc1) revealed a significant effect for genotype, $F(2, 71) = 45.85$, $p<.001$ (see Table 1). Tukey's post hoc analysis of this effect confirmed my hypothesis in that ob/obs displayed the greatest drop in Tc during the cold challenge, followed by +/+s and ob/+s. Diet, however, did not interact with genotype to differentially affect mice's responses to the cold challenge.

Fat Pad Weight

Analysis of the raw fat pad weights showed that across all diet conditions, the ob/obs had heavier fat pads than ob/+s, who had significantly heavier pads than +/+s, Genotype: $F(2, 74) = 804.86$, $p<.001$. When data were collapsed across genotype, mice on the HC diet had the heaviest pads, followed by mice on the control diet, and mice

Table 1

Mean (±SEM) Body Temperature (°C) and Change in Body Temperature During Cold Stress as a Function of Genotype or Diet

Genotype Comparisons

Genotype	Temperature*	Change in Temperature*
ob/ob	35.12 (0.22)	-4.79 (0.46)
ob/+	37.65 (0.06)	-1.44 (0.11)
+/+	37.42 (0.12)	-1.82 (0.30)

* $p < .05$ for ob/ob-ob/+ and ob/ob-+/+ comparisons

Diet Comparisons

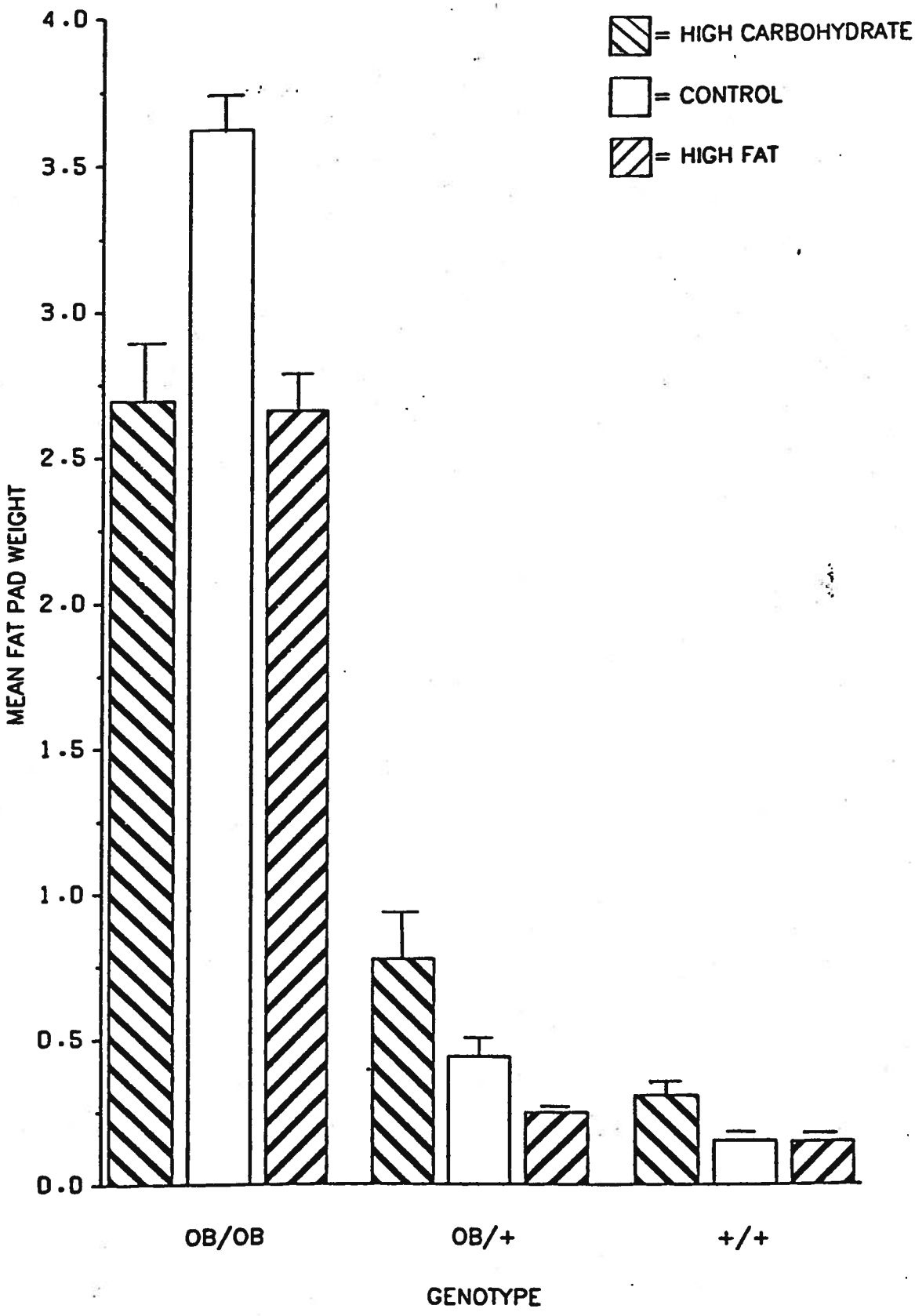
Diet	Temperature*	Change in Temperature
High Carbohydrate	37.35 (0.13)	-2.01 (0.30)
Control	36.88 (0.16)	-2.63 (0.44)
High Fat	36.64 (0.15)	-2.47 (0.37)

* $p < .05$ for High carbohydrate-High fat comparison

on the high fat diet, Diet: $F(2, 74) = 14.13$, $p<.001$. A Genotype X Diet interaction, $F(4, 74) = 16.13$, $p<.001$, indicated several intra-genotype differences, which are displayed in Figure 6. The ob/obs' pad weights were heaviest on a control diet and lightest on the HF diet, with no difference between HC and HF diets. The weights of the fat pads of those ob/+s on the HC diet were greater than those of the ob/+s on the control diet, which were equal to those of the ob/+s on the HF diet. There were no differences in pad weights among the +/+s on the different diets. Thus, one could speculate that, in contradiction to previous work, the ob/obs shunted more fat to the fat pads on the control diet than on the other diets. In order to assess this speculation, an analysis was run on the derived score of fat pad weight divided by body weight at time of sacrifice. This measure provided an index of pad size relative to total body mass.

Analysis of pad/gram BW indicated a genotype effect, $F(2, 74) = 816.15$, $p<.001$, a diet effect, $F(2, 74) = 3.91$, $p<.001$, and a Genotype X Diet interaction, $F(4, 74) = 9.18$, $p<.001$. However, examination of these data (see Figure 7) illustrated that the ob/obs on the high fat and control diets had pad/BW scores which were not different from the pad/BW scores on the HF diet. Thus ob/obs did not differentially shunt diets into storage. However, across all diets, on both pad weight and pad/BW measures, ob/obs did shunt significantly more energy into fat than either the ob/+s or +/+s. Furthermore, ob/+s were more proficient than +/+s at depositing the HC and control diets into storage.

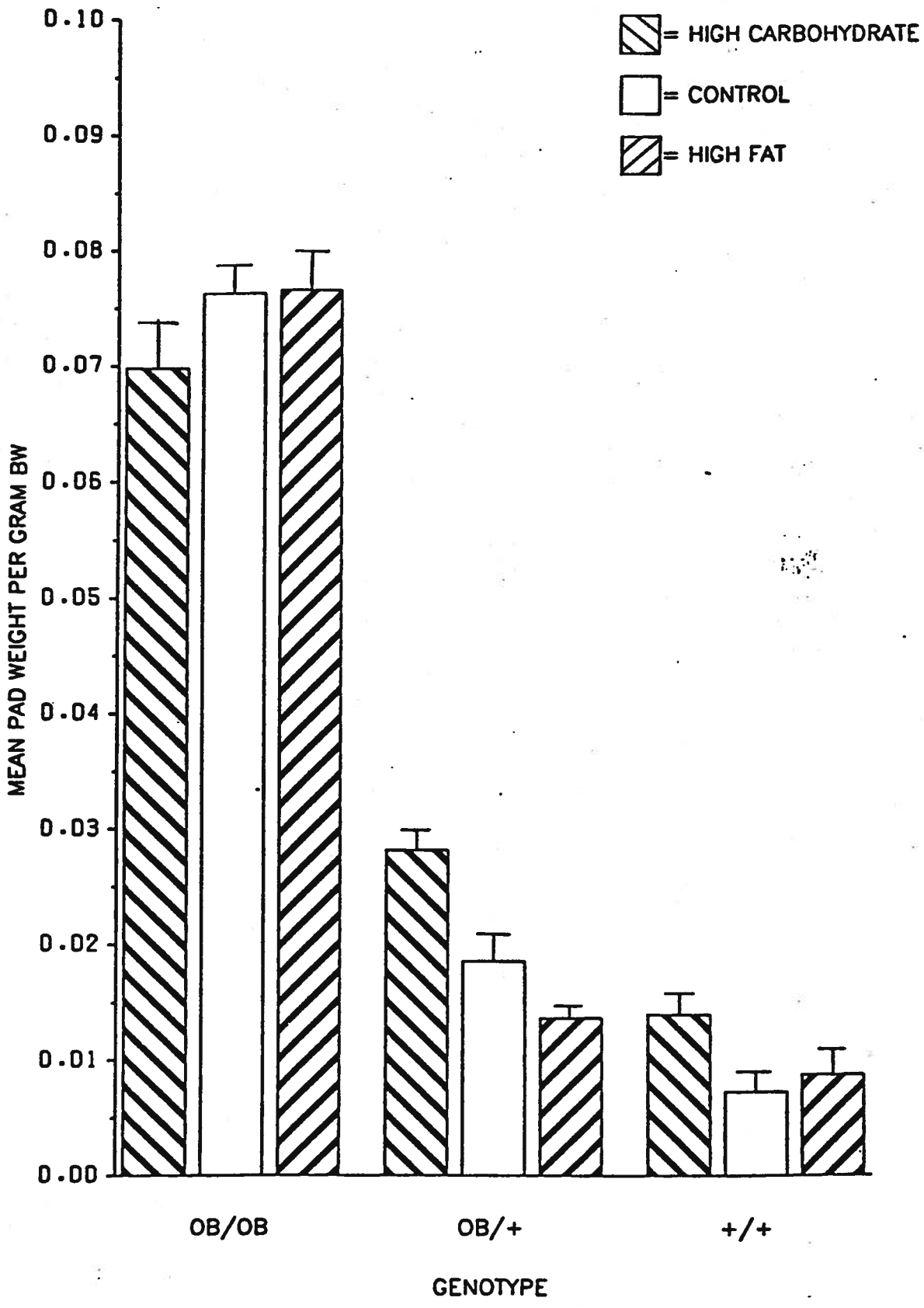
FIGURE 6. Mean (±SEM) epididymal fat pad weights (g) across diets, for ob/ob, ob/+, and +/+ mice.


= HIGH CARBOHYDRATE
= CONTROL
= HIGH FAT
MEAN FAT PAD WEIGHT
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
OB/OB
OB/+
+/+
GENOTYPE

High Fat

38

Figure 7. Mean (±SEM) epididymal fat pad weights per gram body weight across diets, for ob/ob, ob/+, and +/+ mice.


= HIGH CARBOHYDRATE
= CONTROL
= HIGH FAT
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
MEAN PAD WEIGHT PER GRAM BW
OB/OB
OB/+
+/+
GENOTYPE

Thus, it is evident from Figures 6 and 7 that on both measures of fat deposition ob/obs and ob/+s behaved similarly.

Plasma Glucose

An ANOVA on the raw glucose data showed that, consistent with Mayer, Russell, Bates, and Dickie's (1952) comparison of ob/obs and +/?s, ob/obs were hyperglycemic, ob/+s less so, and +/+s normal, Genotype: $F(2, 69) = 13.95$, $p<.001$. As well, a diet effect was detected, $F(2, 69) = 56.63$, $p<.001$, with glucose levels highest in the mice on the HC diet, lower in mice on the control diet, and lowest for mice on the HF diet. These data are presented in Table 2. All intergenotype and interdiet comparisons were significant with a Tukey's post hoc analysis.

A Genotype X Diet interaction almost achieved significance ($p=.075$). It was hypothesized that missing data resulted in this lack of significance. The missing data were a result of failing to obtain enough blood plasma from some animals (1 ob/+ on the control diet, 3 ob/+s on the HF diet, and 1 +/+ on the control diet) to do an assay. To test this speculation, group means were inserted in place of the missing data, for these 5 mice, with error degrees of freedom adjusted accordingly (viz., decreased by 5), and the 3 X 3 ANOVA was re-run. Both Genotype and Diet effects are also present in this analysis. However, the Genotype X Diet interaction was not significant. In order to assess a priori hypotheses regarding diets' effect on glucose level (across genotypes, see p. 18) linear

Table 2

Mean (±SEM) Blood Plasma Glucose Levels as a Function of Genotype or Diet

Genotype Comparisons

Genotype	Glucose*(mg/dl)
ob/ob	238.48 (20.97)
ob/+	203.55 (13.95)
+/+	154.31 (12.55)

* $p < .05$ for all obese-lean and lean-lean comparisons

Diet Comparisons

Diet	Glucose*(mg/dl)
High Carbohydrate	262.14 (14.11)
Control	227.90 (10.99)
High Fat	106.41 (7.27)

* $p < .05$ for all diet comparisons

contrasts were run. Within the HC condition, only the ob/ob -+/+ comparison was significant, while in the control diet condition, ob/ob-+/+ and ob/+-+/+ comparisons were significantly different. However, in the high fat condition, there were no intergenotype differences, suggesting that the high fat diet was less effective than the HC and control diets in differentially affecting glucose levels across genotypes. These data are presented in Figure 8.

Plasma Insulin

As was the case with the glucose data, some insulin values were missing (one ob/ob on the HF diet and one +/+ on the HF diet) due to a lack of blood plasma. The results of two analyses (one on data with the missing values and one on data with the group means substitiuted in place of the missing values) were so similiar that only the former analysis will be described.

The ob/obs had the highest plasma insulin levels, with no significant difference between the levels of the ob/+s and the +/+s, Genotype: $F(2, 72) = 74.62$, $p<.001$, (Ms (in ng/ml) = 3.96, 0.84, and 0.95, respectively). Diet affected insulin levels, $F(2, 72) = 17.85$, $p<.001$, with insulin levels on the HC and control diets being greater than those on the HF diet, although levels on the HC and control diets did not differ (Ms (in ng/ml) = 2.00, 1.98, and 0.84, respectively).

Examining for a Genotype X Diet effect revealed significance, $F(4, 72) = 10.31$, $p<.001$ (see Figure 9). However, the only

Figure 8. Mean (±SEM) plasma glucose level (mg/dl) across diets, for ob/ob, ob/+, and +/+ mice.

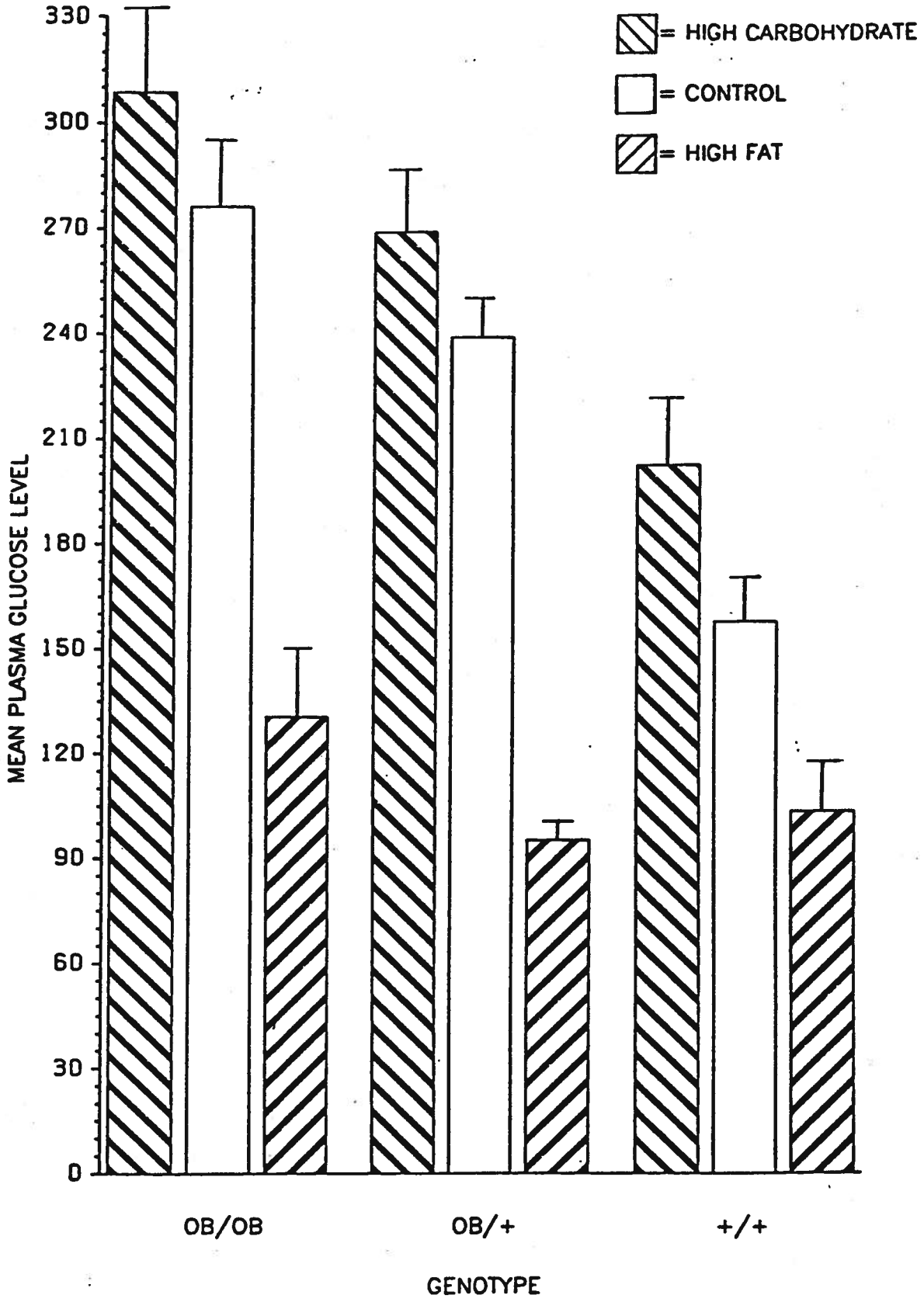
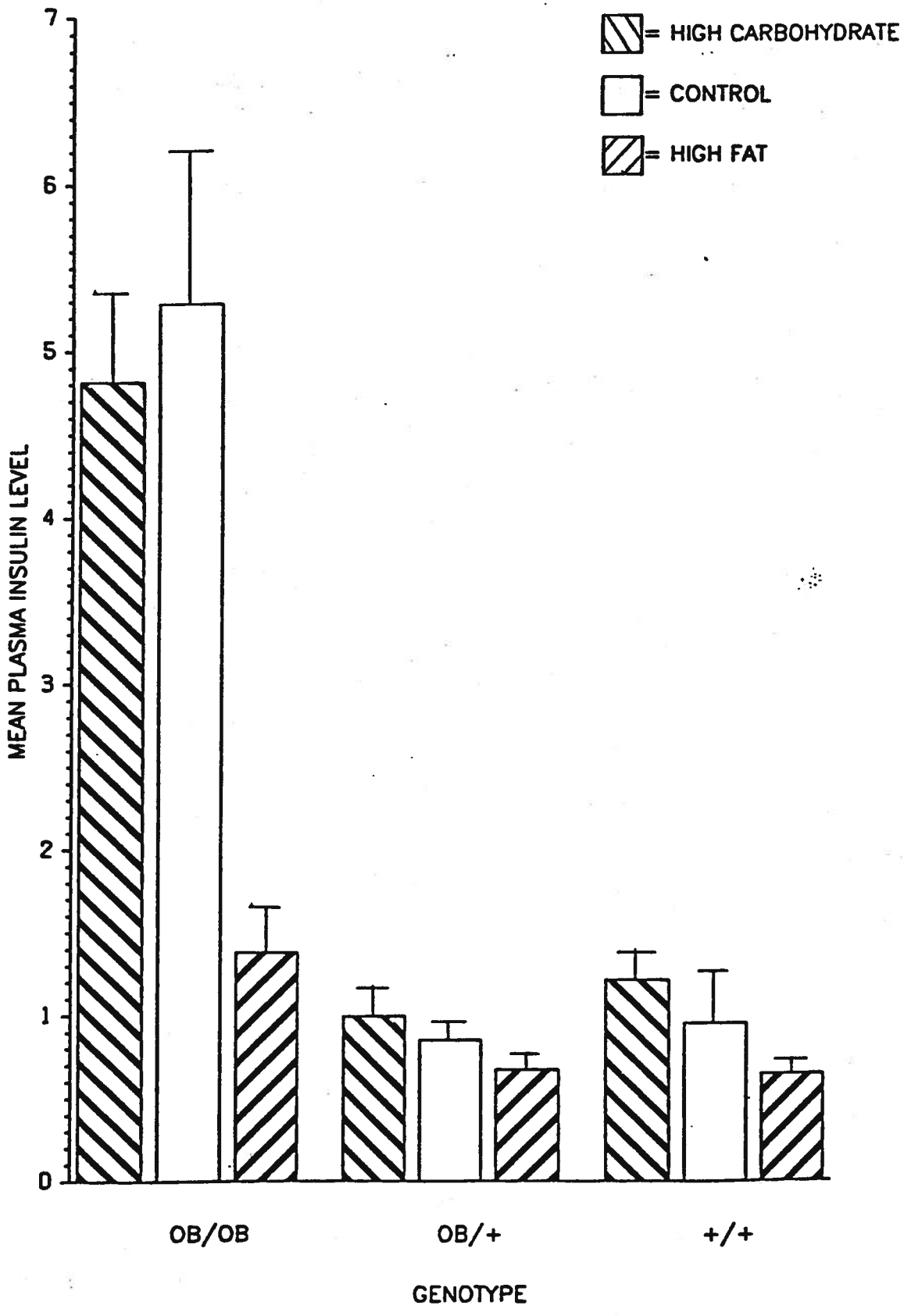

330
300
270
240
210
180
150
120
90
60
30
0
MEAN PLASMA GLUCOSE LEVEL
= HIGH CARBOHYDRATE
= CONTROL
= HIGH FAT
OB/OB
OB/+
+/+
GENOTYPE

Figure 9. Mean (±SEM) plasma insulin level (ng/ml) across diets, for ob/ob, ob/+, and +/+ mice.


= HIGH CARBOHYDRATE
= CONTROL
= HIGH FAT
MEAN PLASMA INSULIN LEVEL
0
1
2
3
4
5
6
7
OB/OB
OB/+
+/+
GENOTYPE

intra-genotype differences were within the ob/obs, in which levels on the HC and control diets were each greater than the level on the HF diet.

Discussion

Due to the difficulties in measuring food intake (see p. 25), hypotheses regarding this dependent variable cannot be discussed directly. In accordance with my hypotheses and previous research (Fanelli & Kaplan, 1978; Vandertuig, Romsos, & Leveille, 1980), obese mice gained more weight than their lean counterparts. This increase in body weight was uniform across the HC and control diet conditions. However, on the high fat diet, all mice lost weight from their first day on that diet to the conclusion of testing, with the obese mice displaying a smaller drop in BW than the ob/+s. The first question to be answered is why did all mice lose weight on the one diet, for which they were supposed to have the greatest affinity (Castonguay, Rowland, & Stern, 1985; Fanelli & Kaplan, 1978; Hamilton, 1964) and which was the highest energy source (22.91 kj/g). Hamilton (1964) showed that as the fat (lard) content of diets increased, so did the rats'preference for that diet. Similarly, Castonguay, Rowland, and Stern (1985) demonstrated that, relative to lean controls, young Zucker fatty rats, when allowed to compose their own diet from three macronutrient sources (i.e., casein, starch, and corn oil), selected diets higher in fat. Fanelli and Kaplan (1978) fed obese and lean mice either a high fat or high carbohydrate diet and found that ob/obs displayed an increased food intake on the high fat diet,

relative to the high carbohydrate diet. No difference in food intake was found between the carbohydrate- and fat-fed lean mice. Thus studies using various strains of mice and rats, including those with genetic predispositions to obesity, have demonstrated that diets with a higher fat content are preferred. Although this study failed to report intake data (as a measure of food consumption), the direct correspondence between intake and body weight found in other research (Fanelli & Kaplan, 1978) tends to support the notion that increased food intake contributed to increased body weight. Why this study failed to find similiar body weight results may include the following possibilities: (a) The nutrient composition of the high fat diet made it less nutritionally appealing than the other diets; (b) the texture and palatability of the high fat diet was not adequate when compared to the HC and control diets.

All diets were prepared in accordance with American Institute of Nutrition standards to ensure adequate mineral and vitamin content. Thus, it is unlikely that the HF diet was less favored because of a vitamin or mineral deficiency. Another possibility involves the relative abundance of the various macronutrients (i.e., carbohydrate, fat, and protein). The diets used were similar to those described by Romsos, Hornshuh, and Leveille (1979). While this study modelled those authors' diets, there was one departure. Whereas Romsos et al. used 19.4g of tallow in their high fat diet, this study added 27.1g to the HF diet. However, because the HF diet used by Romsos et al. (1979) summed to 71.4g, the percentage of fat in this study is

identical to that of Romsos et al. and therefore should have contributed to a weight gain across genotypes. Romsos et al. prepared their diets on an equal energy basis, whereas the energy levels in this study decreased proportionally from HF to control to HC diets. Because the HC diet is lowest in energy content and the leans had the highest body weights on the HC diet, it is likely that (contradictory to hypotheses) their food intake was greatest on the HC diet. This would be likely if they preferred a HC diet, because they would have to eat more of a lower energy source (HC diet) to increase weight, than they would on a higher (control or HF) energy source--all else being equal. Moreover, the carbohydrate and protein content of the diets was also altered to keep the fat/carbohydrate and fat/protein ratios the same as those of Romsos et al. (1979), so that although absolute quantities of macronutrients were different between this diet and that of Romsos et al., the proportional contribution of the major macronutrients to the completed diet remained unchanged. Furthermore, the difference in fat content alone probably wouldn't have caused such a drop in food intake, when one considers studies (e.g., Salmon & Flatt, 1985), which show that as the percentage of fat per diet increases, so does consumption of the diet. Salmon and Flatt (1985), using albino mice, demonstrated that as the fat-to-carbohydrate ratio of their diets increased (yielding diets whose percentages of calories as fat ranged from 1% to 64%) so did the mice's consumption of the diets. With increasing dietary fat content, body fat stores increased, indicating that the excess

ingested energy was not being expended. Because Salmon and Flatt got these results using a diet with as much as 64% dietary fat, the present study's 34.1% fat was considered a reasonable amount with which to induce body weight and fat mass increases. Moreover, the control diet is a "high fat" diet, relative to the HC diet, and ob/obs displayed the greatest weight gain on the control diet in the present study. Therefore, it is possible that the HF diet did contain too much fat. However, as can be seen in Appendix E, the three protein/calorie ratios are very close and therefore the amount of fat cannot account for the apparent weight loss.

Another possibility why the mice lost weight on the high fat diet involves the sensory characteristics of the diets. When originally prepared, the HC and control diets were powdered. Water was added to these diets in an attempt to make them firmer and thereby easier for the mice to handle. Unfortunately, evaporation left the HC and control diets in a granular form. The high fat diet, on the other hand, remained firm and moist throughout testing. Thus, the three diets differed in terms of consistency and texture. Both the taste and texture of a diet can influence the rate of its consumption, with granular diets being preferred over powdered and gel-type diets (Blundell, 1983; McArthur & Blundell, 1986). Thus, mice could have been reluctant to consume the high fat diet simply because of its moist texture. However, most, if not all, of the studies that have reported feeding differences based on the sensory characteristics of the diets, use a selection paradigm in which each mouse has a choice

of two or more diets. The mice in this study had no choice, and therefore to resist the high fat diet meant eating little food. It seems unlikely that the texture or taste alone could have been aversive enough to prompt the mice to choose weight loss over eating a nonpreferred diet. Nevertheless, these characteristics could have reduced intake.

A final related idea why the mice lost weight on the HF diet concerns tallow's relative indigestibility. Whereas lard, as a source of fat, is approximately 99% digestible, tallow is far less so (Dr. D. Fitzpatrick, Personal communication, January, 1987). However, Romsos et al. (1979) and Knehans and Romsos (1984) also used tallow as their source of fat, and they found increased consumption of the high fat diet by both lean and obese mice. Moreover, Romsos is currently involved in a study using the same diet and he has reported that all mice are ingesting great quantities of the diet (Dr. D. R. Romsos, Personal Communication, February, 1987). Therefore, the indigestibility of tallow alone seems to be an insufficient explanation for the weight loss.

The data confirmed my hypotheses regarding body temperature, in that ob/obs displayed the lowest Tc, +/+s the highest, and ob/+s an intermediate body temperature. There were no indications that diet differentially influenced Tc. It was expected that ob/+s on the high fat diet would display the lowest Tc among the leans. However, because of the weight loss seen in all mice on the high fat diet, it is difficult to say if the lack of a thermic effect was due to fat

not influencing Tc or not enough fat being ingested. As predicted, Tcs of ob/+s on the HC and the control diets did not differ. Apparently 15% more glucose (HC vs control) was not enough to significantly affect Tc. Although there were no diet effects or main effect interactions, two significant interactions for week appeared. A Genotype X Week interaction manifested itself primarily through the large difference between the ob/obs and leans (ob/+ and +/+ together; see Figure 5). It appears that changing from lab chow to specially constituted diets did have an initial effect of elevating Tcs in the mice. However, as time went on, this effect diminished. While this initial rise in Tc could be attributed to stress, because nothing in the experimental protocol changed, except the diets themselves, it's unlikely that anything but the diets caused this initial elevation. The Diet X Week interaction revealed a tendency for mice on the high fat diet to conclude testing with lower Tcs. However, the lack of a Genotype X Diet interaction makes it impossible to specify which genotypes were most affected.

When mice were cold-stressed, the ob/obs displayed the lowest mean Tc during the challenge. However, the ob/+s' average Tc across the cold stress was equal to that of the +/+s. Thus, the presence of a single ob gene did not appear to significantly affect the ob/+s' ability to thermoregulate and thereby maintain its Tc in the face of a cold challenge. Similarly, while the ob/obs displayed the greatest overall drop in Tc across the challenge, ob/+s and +/+s did not differ on overall Tc drop. The only diet effect revealed that those

mice on the HC diet had a higher poststress Tc. This result was unexpected in light of Fanelli and Kaplan's (1978) research, which pointed to HC diets decreasing O_2 consumption and HF diets increasing O_2 consumption. Therefore it was expected that mice on the HC diet would have had more difficulty in defending their Tcs under a cold stress, while mice on the high fat diet, aided by the diet's positive effect on O_2 consumption, would maintain a higher Tc. However, research by Landsberg and Young (1981) and Schwartz, Young, and Landsberg (1983) demonstrated that both carbohydrate- and fat-enriched diets led to enhanced NE turnover in rat heart, BAT, and pancreas. Because NE turnover is often used as a direct indicator of sympathetic nervous system activity, and because BAT's activity in NST and DIT is sympathetically mediated, both diets should aid the mice in maintaining their Tcs in the cold challenge. However, because all mice gained more weight on the HC and control diets than on the HF diet, it is possible that not enough of the high fat diet was consumed to exert a significant thermogenic influence. It is also possible that the high energy cost of assimilating a high fat diet diminished the thermogenic capacity of the mice. Diet did not affect overall decline in Tc across testing, although mice on the HC and control diets had higher average Tcs than mice on the HF diets. Himms-Hagen, Hogan, and Zoror-Behrens (1986) have shown that a carbohydrate- and fat-supplemented cafeteria diet normalized the chronically hypothermic Tcs of obese mice, yet left lean mice's Tcs unaffected. This reflects enhanced sensitivity of ob/ob's BAT to NE

activation. In addition, cafeteria feeding improved both lean and obese mice's ability to withstand a cold stressor, compared to chow fed mice. The finding of a diet effect on average Tc in the cold in the present experiment is consistent with this recent research, although diet did not have a differential impact on the cold tolerance of the three mouse genotypes.

As anticipated, obese mice had significantly heavier fat pads than lean mice. Furthermore, ob/+s' fat pads were significantly heavier than those of the +/+s. These results confirm previous research (Fanelli, & Kaplan, 1978) and reiterate the ability of the ob/ob to deposit excess energy as fat. Moreover, because past research has failed to differentiate between ob/+s and +/+s, this study extends the current literature by highlighting the ob gene's positive influence on fat deposition. Diet also affected fat pad weight, although not entirely as expected. Contrary to my hypotheses, fat pads were heaviest on the control diet, lighter on the HC diet, and lightest on the HF diet. However, this was not the case within each genotype. The ob/obs had the heaviest fat pads on the control diet, the ob/+s' pads were heaviest in the HC diet condition, and there were no significant differences in pad weights for the +/+s in the three diet conditions. As Figure 6 shows, overall, pads were lightest for the ob/+s on the diets higher in fat. As well, Figure 3 illustrates that the ob/+s were the only mice to lose weight from Day 1 on the HF diet. Therefore, it would seem appropriate that they should be the ones whose pad weights on diets higher in fat differ

from pad weights in the HC diet condition. Ob/obs, on the other hand, had equal pad weights on the HC and HF diets. This result makes sense, when one considers that of all the mice, the ob/obs lost the least weight on the HF diet and therefore should have the heaviest pads on this diet (see Fig. 2). When pad weight (PW) was considered as a function of body weight (PW/g BW), ob/obs in the high fat and control diet conditions were not significantly different. Ob/+s on the HF diet did not have greater PW/BW scores than +/+s on the same diet. However, ob/+s pad/BW scores in the control diet condition were greater than those of the +/+s on the control diet and therefore, despite the problems with the high fat diet, the ob gene clearly impacted on the ob/+s on the control diet. Finally, the +/+s showed no significant difference among the three diet conditions. It is possible that their ability to expend energy more efficiently than the ob/+s could explain why their pad weights on the HC diet are not greater than those in the other diet conditions.

Analysis of the glucose data confirmed several hypotheses. Ob/obs were hyperglycemic, ob/+s less so, and +/+s normoglycemic. Diet influenced glucose level, with levels being greatest on the HC diet, intermediate on the control diet, and lowest on the high fat diet. The genotype results agree with previous work (Mayer, Bates, & Dickie, 1951) in pointing to the ob/obs' hyperglycemia. As well, the intermediate value for the ob/+s illustrates the effect of the ob gene on blood glucose regulation. This finding corroborates the fact that ob/+s maintain an intermediate rate of glucose oxidation

(Yen, Lowry, & Steinmetz, 1968). It also confirms the results of Flatt and Bailey (1981), who found similar baseline differences in plasma glucose between ob/+s and +/+s. Probing within genotypes across diet conditions, revealed differences between this study and past research. In contrast to York (1975) this study found a HC diet increased the glucose levels of ob/obs. Genuth (1976) and Herberg and Kley (1975) demonstrated that a high fat diet lowered the glucose levels of the ob/ob, relative to lean controls. Similarly, a HC diet increased the glucose levels of both ob/+s and +/+s, and the HF diet had no effect on these levels. Although York (1975) did not differentiate between ob/+s and +/+s, his results are in disagreement with those of this study, in that a HC diet raised glucose levels in lean and obese mice. The present results extend these earlier findings, by showing that ob/ob and ob/+ mice share similar elevated plasma glucose levels on the HC and control diets, with plasma glucose levels for +/+ mice being significantly lower than those of ob/+ mice on the control diet. On this dimension the diet given seems to interact with the ob gene to push heterozygous lean mice closer to obese mice in their glucose profiles. On the HF diet, however, all mice had depressed glucose levels, regardless of genotype.

Overall, obese mice displayed greater insulin levels than the leans, thus confirming an initial hypothesis. This result corroborates other researchers' work (Genuth, 1969; York, 1975). However, contrary to hypothesis, the insulin level of the ob/+s was not significantly different from that of the +/+s. This is noteworthy

because ob/+s had significantly greater glucose levels than +/+s. One could interpret the hyperglycemia without hyperinsulinemia of the ob/+ to indicate an insulin resistance, similar to that seen in the ob/ob, except instead of an oversecretion of insulin, the ob/+ doesn't secrete enough to accommodate its blood glucose level, resulting in the hyperglycemia. Diet also affected insulin. Levels were significantly higher on the HC and control diets than on the HF diet, but levels of mice on the HC and control diets did not differ. The suppression of insulin in mice by the HF diet mirrors similiar reports by York (1975). Examination of the Genotype X Diet interaction for insulin (see Fig. 9) suggests that the lack of difference between HC and control diets may be due to the lack of significant differences between these diets for the ob/+s and +/+s. Only the ob/obs showed an intra-genotype difference for diet.

Conclusions

The responses of the ob/+s to the three diets in this study were, in several cases, intermediate to those of the ob/obs and +/+s. However, the diets alone were not successful in "pushing" the ob/+s to respond like the ob/obs, with the exception of diet effects on fat pad weights, pad/BW scores, and plasma glucose levels. Problems with consumption of the high fat diet may have altered the results in such a way as to suppress the ob gene's potential to predispose the ob/+ towards obesity. It's likely that had the animals gained weight on the high fat diet more consistent with past research, the ob/+s may have more closely resembled the ob/obs. This is not to say that

other environmental factors are unimportant. External stimuli, such as room temperature and humidity, noise levels, diet taste and texture, and amount of handling all likely interact to influence the feeding behavior of the mice, although treatment with respect to these variables was identical for all mice. It is clear that future research is needed to assess the relative contributions of these (and other) factors to the eating patterns of the obese mouse and its lean counterparts.

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Appendix A

Glucose Analysis

The glucose hexokinase reagent was prepared in accordance with the instructions supplied with the kit. Then 1.5 ml of the reagent was placed in a properly labeled cuvette and its absorbance recorded at 340nm versus water as a reference. This absorbance was termed INITIAL A. To this reagent, 0.01 ml of the plasma sample was added and mixed by gentle inversion. The cuvet (containing the reagent and the sample) was then incubated at room temperature (21-24^{o}C) for 5 min. The absorbance was again recorded at 340nm vs water as a reference. This reading was termed FINAL A. This entire procedure was repeated, if possible, for a duplicate sample of plasma. A hemolysis control was determined for each sample, by adding 0.01 ml of plasma to 1.5 ml sodium chloride and reading the absorbance. Subtracting INITIAL A from (FINAL A + Hemolysis Control) yielded the change in absorbance. The glucose concentration of the sample (mg/dl) was determined via the following:

$$\text{Glucose Concentration} = (\text{change in A}) \times \frac{TV \times MW \times 100}{6.22 \times LP \times SV \times 1000}$$

This is equivalent to Change in A X 437,

where

change in absorbance = FINAL A - INITIAL A

TV = Total volume (ml)

MW = Molecular weight of Glucose (180.16)

100 = Converts millilitres to decilitres

6.22 = Millimolar absorptivity of NADH at 340nm

LP = Lightpath in centimetres

SV = Sample volume

1000 = Converts micrograms to milligrams

To convert the glucose concentration (mg/dl) into S.I. units, the value could have been multiplied by 0.0556 to yield a glucose concentration in mM.

Appendix B

Double-Antibody Radioimmunoassay Procedure

In accordance with Kennedy (Personal communication, May, 1986), the following protocol was followed in performing the radioimmunoassay.

The materials used in this assay system were (a) 0.08M sodium barbital buffer, pH 8.6 (5g sodium barbital, 2.57g sodium acetate.$3H_2O$, 34.2 ml 0.1 NHCL and 0.1g thimerasol as preservative per litre); (b) 0.5M phosphate buffer, pH 7.5; (c) purified rat insulin standard (Sigma, St. Louis, MO, 24.5 $IU.Mg^{-1}$; at 0, 0.3125, 0.625, 1.25, 2.5, 5, 10, and 20 ng/ml) originally prepared in phosphate buffer and subsequently diluted in barbital buffer containing 5% bovine serum albumin (BSA); (d) porcine [^{125}I]-insulin (New England Nuclear, 100 uCi/g) dissolved in barbital buffer containing 0.5% BSA (0.5% diluent) and used at a concentration of approximately 20,000 cpm/0.2 ml; (e) guinea pig antibovine insulin (1st antibody, Miles Laboratories, Elkhart, IN, 65-101-1) dissolved in 0.5% diluent and used at an initial titre of 1:30,000 with a 46.7% total binding; (f) goat antiguinea pig IgG (2nd antibody; Antibodies Inc. G4-1) diluted with 0.5% diluent to a titre of 1:60; and (g) normal guinea pig serum (carrier protein; Miles 83-111, Lot 25-R) diluted with 0.5% diluent to a titre of 1:300.

Aliquots (0.1 ml) of the standard solutions (in triplicate) and serum samples (in duplicate, if possible) were incubated overnight

at 4°C with 0.2 ml of the 1st antibody. The next morning 0.2 ml of [^{125}I]-insulin was added to all tubes. Three "total count tubes" containing only 0.5% diluent and trace were included in the assay run. Following another 48 hours of incubation at 4°C, 0.1ml each of 2nd antibody and carrier protein were added to all tubes except the "total count tubes" and tubes were then incubated until the next morning when sample and standard tubes were centrifuged at 3500 RPM at 4°C for 30 min. The supernatant was aspirated and the precipitate counted for 1 min with Wallac-LKB 1282 CompuGamma (Kennedy, personal communication, May, 1986).

Standard curve and extrapolation of insulin concentration values were performed automatically using the CompuGamma Spline program. All samples were run in the same assay to prevent inter-assay variation.

Appendix C-1

Raw Data in a 3 X 3 (Genotype X Diet) ANOVA

Percentage Change in Body Weight

Main Effects

Source	Statistic	F	df	p
Genotype	SS = 665.40 MS = 332.70	5.78	2, 74	0.0046
Diet	SS = 10857.88 MS = 5428.94	94.36	2, 74	0.0001
Genotype X Diet	SS = 1882.79 MS = 470.70	8.18	4, 74	0.0001
Error	SS = 4257.56 MS = 57.53			

Appendix C-2

Raw Data in a 3 X 3 X 4 (Genotype X Diet X Week) ANOVA

Absolute Change in Body Weight

Main Effects and Orthogonal Trends

Source	Statistic	F	df	p
Week	SS = 3135.76 MS = 1045.25	168.63	3, 222	0.0000
Week X Genotype	SS = 1430.70 MS = 238.45	38.47	6, 222	0.0000
Week X Diet	SS = 2505.41 MS = 417.57	67.37	6, 222	0.0000
Week X Genotype X Diet	SS = 819.59 MS = 68.30	11.02	12, 222	0.0000
Error	SS = 1376.08 MS = 6.20			
Genotype X Week: Linear	SS = 1303.50 MS = 651.75	41.13	2, 74	0.0000
Error	SS = 1172.58 MS = 15.85			
Genotype X Week: Quadratic	SS = 126.71 MS = 63.35	37.32	2, 74	0.0000
Error	SS = 125.61 MS = 1.70			

Source	Statistic	F	df	p
Diet X Week: Linear	SS = 2446.04 MS = 1223.02	77.18	2, 74	0.0000
Error	SS = 1172.58 MS = 15.85			
Diet X Week: Quadratic	SS = 32.55 MS = 16.27	9.59	2, 74	0.0000
Error	SS = 125.61 MS = 1.70			

Appendix C-3

Raw Data in a 3 X 3 (Genotype X Diet) ANOVA

Percentage Change in Body Temperature

Main Effects

Source	Statistic	F	df	p
Genotype	SS = 32.59 MS = 16.30	3.49	2, 74	0.0358
Diet	SS = 3.24 MS = 1.62	0.35	2, 74	0.7086
Genotype X Diet	SS = 14.83 MS = 3.71	0.79	4, 74	0.5333
Error	SS = 345.89 MS = 4.67			

Appendix C-4

Raw Data in a 3 X 3 X 4 (Genotype X Diet X Week) ANOVA

Absolute Change in Body Temperature

Main Effects and Orthogonal Trends

Source	Statistic		F	df	p
Week X Genotype	SS =	16.81	4.43	6, 216	0.0003
	MS =	2.80			
Week X Diet	SS =	9.28	2.45	6, 216	0.0261
	MS =	1.55			
Week X Genotype X Diet	SS =	4.76	0.63	12, 216	0.8174
	MS =	0.40			
Error	SS =	136.47			
	MS =	0.63			
Genotype X Week: Quadratic	SS =	12.73	10.74	2, 72	0.0001
	MS =	6.37			
Error	SS =	42.69			
	MS =	0.59			
Diet X Week: Linear	SS =	6.71	3.66	2, 72	0.0307
	MS =	3.36			
Error	SS =	66.11			
	MS =	0.92			

Appendix C-5

Raw Data in a 3 X 3 X 5 (Genotype X Diet X Sampling Time) ANOVA

Absolute Change in Body Temperature

During Cold Stress

Main Effects and Orthogonal Trends

Source	Statistic		F	df	p
Genotype	SS =	445.19	70.84	2, 71	0.0000
	MS =	222.60			
Diet	SS =	27.33	4.35	2, 71	0.0165
	MS =	13.67			
Genotype X Diet	SS =	5.79	0.46	4, 71	0.7645
	MS =	1.45			
Error	SS =	223.11			
	MS =	3.14			
Sampling Time	SS =	317.46	217.10	4, 284	0.0000
	MS =	79.36			
Sampling Time X Genotype	SS =	95.91	32.80	8, 284	0.0000
	MS =	11.99			
Sampling Time X Diet	SS =	3.83	1.31	8, 284	0.2379
	MS =	0.48			
Sampling Time X Genotype X Diet	SS =	6.19	1.06	16, 284	0.3962
	MS =	0.39			
Error	SS =	103.82			
	MS =	0.37			

Source	Statistic	F	df	p
Genotype X Sampling Time: Linear	SS = 95.40 MS = 47.70	45.95	2, 71	0.0000
Error	SS = 73.70 MS = 1.04			

Appendix C-6

Raw Data in a 3 X 3 (Genotype X Diet) ANOVA

Total Change in Body Temperature During Cold Stress

Main Effects

Source	Statistic	F	df	p
Genotype	SS = 154.23 MS = 77.11	45.85	2, 71	0.0001
Diet	SS = 5.61 MS = 2.81	1.67	2, 71	0.1958
Genotype X Diet	SS = 9.49 MS = 2.37	1.41	4, 71	0.2392
Error	SS = 119.42 MS = 1.68			

Appendix C-7

Raw Data in a 3 X 3 (Genotype X Diet) ANOVA

Absolute Change in Fat Pad Weight

Main Effects

Source	Statistic		F	df	p
Genotype	SS =	107.25	804.86	2, 74	0.0001
	MS =	53.62			
Diet	SS =	1.88	14.13	2, 74	0.0001
	MS =	0.94			
Genotype X Diet	SS =	4.30	16.13	4, 74	0.0001
	MS =	1.07			
Error	SS =	4.93			
	MS =	0.07			

Appendix C-8

Raw Data in a 3 X 3 (Genotype X Diet) ANOVA

Absolute Change in Fat Pad Weight per Gram Body Weight

Main Effects

Source	Statistic		F	df	p
Genotype	SS =	0.05	816.15	2, 74	0.0001
	MS =	0.03			
Diet	SS =	0.0003	3.91	2, 74	0.0244
	MS =	0.0001			
Genotype X Diet	SS =	0.001	9.18	4, 74	0.0001
	MS =	0.0003			
Error	SS =	0.002			
	MS =	0.00003			

Appendix C-9

Raw Data in a 3 X 3 (Genotype X Diet) ANOVA

Absolute Change in Plasma Glucose Level

Main Effects

Source	Statistic	F	df	p
Genotype	SS = 72597.51 MS = 36298.76	13.95	2, 69	0.0001
Diet	SS = 294605.05 MS = 147302.53	56.63	2, 69	0.0001
Error	SS = 179490.29 MS = 2601.31			

Appendix C-10

Raw Data in a 3 X 3 (Genotype X Diet) ANOVA

Absolute Change in Plasma Insulin Level

Main Effects

Source	Statistic	F	df	p
Genotype	SS = 132.23 MS = 66.11	74.62	2, 72	0.0001
Diet	SS = 31.64 MS = 15.82	17.85	2, 72	0.0001
Genotype X Diet	SS = 36.55 MS = 9.13	10.31	4, 72	0.0001
Error	SS = 63.79 MS = 0.89			

Appendix D

Number of Non-Food Spilling Mice

Per Group

DIET	GENOTYPE ob/ob	ob/+	+/+
High Carbohydrate	4	1	0
Control	7	0	0
High Fat	7	12	4

Appendix E

Energy and Nutrient Composition of the Test Diets

(constituents expressed as a percentage of the total diet composition)

Constituent:	High Carb	High Fat	Control
Casein	20.0	28.0	24.0
D-L Methionine	0.3	0.42	0.35
Mineral Mix	3.5	5.0	4.0
Vitamin Mix	1.0	1.4	1.2
Choline Bitartrate	0.2	0.28	0.24
Cellulose	4.0	5.5	5.0
Corn Oil	5.0	7.0	6.0
Glucose	66.0	25.3	45.21
Tallow	-----	27.1	14.0
Total	100.0	100.0	100.0
Calculated Energy (calories)	4.09 cal/g	5.48 cal/g	4.80 cal/g
Energy in Kilojoules (Kj)	17.10 kj/g	22.91 kj/g	20.06 kj/g
Protein: Calorie Ratio	4.89	5.11	5.00
Fat: Carbohydrate Ratio	.17	3.03	1.00
Fat: protein Ratio	.56	2.74	1.88
Carbohydrate: Protein Ratio	3.30	.90	1.88