MONOSODIUM GLUTAMATE AFFECTS SOMATIC DEVELOPMENT, CALORIC INTAKE AND SOME GLUCOREGULATORY MECHANISMS

by

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Most importantly, I thank my family members for all their love and patience during what has surely been a very long and confusing time for everyone involved. I expressly dedicate this document to the memory of my Dad. He bestowed upon me the ability to develop the fortitude and the determination to carry on, no matter the obstacles in my path. His favorite saying, "You gotta be tough," will be a lifelong commitment for myself.

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CHAPTER I

BRIEF INTRODUCTION AND METHODOLOGY

Introduction

Initial experiments using monosodium glutamate (MSG) to induce neuropathology reported damage only to cells within the inner retina (Lucas and Newhouse, 1957). Observations of obesity following systemic administration of MSG led Olney (1969a) to hypothesize that lesions would be found within the nuclei of the hypothalamus. Previous documentation of obesity resulting from damage to the ventromedial nucleus of the hypothalamus (VMH) had been widely accepted and used extensively as an experimentally-induced model of obesity (Hetherington and Ranson, 1940). For a more thorough review of hypothalamic models and obesity, see Appendix A, Part I.

Histological verification provided evidence of lesions within the arcuate nucleus of the hypothalamus (ARH) following neonatal treatment with MSG (Olney, 1969a, 1969b; Olney, Ho, and Rhee, deGubareff, 1971; Olney, Sharpe and Feigen, 1972). The lesions are characterized by intracellular edema, followed by neuronal necrosis (Scallet and Olney, 1986). Monosodium glutamate represents a unique lesioning technique because there is a loss of specific neuronal cell bodies from the ARH without damage to axons

terminating in or coursing through the region (Scallet and Olney, 1986; Tanaka, Shimado, Nakao, and Kusunoki, 1978).

Glutamate ions have been shown to preferentially accumulate in the ARH following oral ingestion or systemic injection of MSG (Perez and Olney, 1972). Either method of administration will result in lesioning, with the severity of the concomitant neuronal damage and/or alterations in physiological and behavioral manifestations being affected in a dose-dependent fashion (Olney, 1971).

Obesity, the most obvious consequence of MSG administration, led Olney (1969a) to suspect that the ARH must somehow be involved in the regulation of energy balance. The obesity resulting from MSG administration is unique in a variety of ways, the most notable being that obesity is characterized by a slow steady weight gain with increased lipid deposition developing in the absence of hyperphagia (Araujo and Mayer, 1973; Pizzi and Barnhart, 1976).

This finding led to a possible proposal of reduced activity levels being associated with the developing obesity, thereby allowing for an increase in energy storage (Olney, 1969a). Olney (1969a), and Pizzi and Barnhart (1976) did note that MSG-treated mice were less active than controls. It was concluded from these studies that the increase in lipid deposition was a direct result of the reduction in energy expenditure, since no increase in food

intake was present. Araujo and Mayer (1973) and Nikoletseas (1977), however, reported hyperactivity in MSG-treated mice using tilt-type activity wheels and wheel activity cages, respectively. These studies revealed that the increased weight gain could not be accounted for by reductions in energy expenditure. On the other hand, Prabhu and Oester (1971) reported no significant differences using tilt-type activity cages between animals that had received MSG and controls. Unpublished data from our laboratory, using an open-field apparatus, supported a conclusion of increased activity. Differences in the measures of activity used, the age(s) at which the measurement took place, age(s) of MSG administration, the dose of MSG, and/or species differences have all been used as potential explanations for the contradictory findings presented above (Redding, Schally, Arimura, and Wakabayashi, 1971).

Without consistent evidence for a decrease in activity level, an alternative explanation was imperative to account for the increase in fat deposition occurring in the absence of increased food intake. An explanation offered by Himms-Hagen (1976, 1984a, 1984b), and Hogan and Himms-Hagen (1983) indicated that a potential intrinsic tissue defect could result from hypothalamic damage allowing for an increased metabolic efficiency. That is, fuel utilization is enhanced such that all ingested foods are converted to increased storage substances (e.g., glycogen or fat). An increased

metabolic efficiency, therefore, could account for an increased fat deposition in the absence of increased consumption. An increase in lipid deposition could occur even under conditions of increased energy expenditure (e.g., exercise) (Nikoletseas, 1977). An increased metabolic efficiency has been reported in MSG-treated animals (Djazayery, Miller, and Stock, 1979). The exact mechanisms and contributions of this increased metabolic efficiency, however, have yet to be determined with respect to contributions to weight gain or effects on patterns of food or caloric intake.

Subsequent studies have confirmed that MSG administration in the neonatal period can generate a variety of pituitary function disturbances in addition to obesity. These disturbances include arrested skeletal development and endocrine abnormalities (Redding et al., 1971; Nemeroff, Konkol, Bissette, Youngblood, Martine, Brazeau, Rone, Prange, Breese and Kizer, 1977).

Hyperinsulinemia and enlarged pancreatic islets are well-documented characteristics of most hypothalamic obesities (Hales and Kennedy, 1964; Kennedy and Parker, 1963). The extent to which they occur in the MSG animal is highly dependent upon the dose administered and the diet on which the animal is maintained (Cameron, Cutbush, and Opat, 1978; Chisholm and O'Dea, 1987). Insulin plays a major role in the development of the obese syndrome by governing the

levels of circulating blood glucose (Hers and Hue, 1983; LeMagnen, 1984). Carbohydrate metabolism, in turn, is regulated by the action of insulin on levels of circulating blood glucose via its actions on key enzymes within glycolytic and gluconeogenic pathways (Elliot, Hems, and Beloff-Chain, 1971; Gorbman, Dickhoff, Vigna, Clark, and Ralph, 1983).

Badillo-Martinez, Nicotera, Butler, Kirchgessner, and Bodnar (1984) have indicated that insulin secretion and its regulation are altered by the destruction of the arcuate nucleus following MSG administration. Insulin secretion responds in an exaggerated manner to both exogenous and endogenous insulin and glucose (Nemeroff et al., 1977). The changes in insulin secretion following MSG administration are related to a syndrome of "transient" hyperglycemia, whereby levels of circulating glucose are more variable (Cameron, Cutbush and Opat, 1978). Changes in insulin regulation suggest that differences would exist in the regulation of carbohydrate metabolism, which could be reflected in enzyme activity patterns.

In previous studies in our laboratory, (refer to Appendix A, Part II, for a more complete description of the pilot data obtained) the growth rates of MSG animals were examined from 20 to 120 days of age. While initial body weight recordings were lower for MSG animals (e.g., Y-intercept), the rates of growth (e.g., beta-weights or

slopes) were greater for MSG-treated animals. In fact, the MSG animals did not exceed the control animals in weight until approximately 80-85 days of age. It was noted that even when the body weights were lower, the MSG animals exhibited a "fatty" appearance and feel. Measurements of body fat content, however, were not obtained during this study.

The patterns of growth were also shown to differ (e.g., trends analysis). While the control animals exhibited a characteristic plateau in weight development, the MSG animals exhibited an increasing weight gain, with no such plateau effect evident.

From the ages of 90 to 100 days of age, a two-choice taste preference paradigm was employed, using plain ground chow versus a high caloric diet. The MSG animals exhibited a greater preference (see percentages of the diets consumed) for the high caloric diet, even though the overall consumption did not increase. The MSG animals exhibited a greater weight gain during this same period. From this study, however, it could not be determined if this was merely a reflection of the increased growth rates or if the increased caloric diet affected the MSG animals differently.

This study was designed with two purposes in mind: 1) to examine the effects of consumption of an increased caloric diet on growth rates of younger MSG and saline-treated animals; and 2) to examine patterns of activity

levels of some rate-limiting enzymes regulating glycolysis and lipogenesis, which would lead to an increased fat deposition in animals predisposed to obesity.

The study was designed such that daily measures of consumption, gram amount and kilocalorie amount per day, could be obtained, along with measures of growth, including body weights (grams), nasoanal body lengths (cm) and a measure of body fat content called the obesity index or Lee Index.[1] The physiological recordings include: plasma glucose; and from the liver, the rate-limiting enzymes chosen were glucose 6-phosphate dehydrogenase (G6PDH), glucokinase (GK), pyruvate kinase (PK), and malate dehydrogenase (MDH). See Appendix B, for specific hypotheses.

Methods

Animals

Twenty (20) nulliparous CD-1 mice (Charles Rivers, MA.) served as breeders in our laboratory. The offspring of these mothers were the subjects in the present studies. A total of 112 pups from these breeders served as subjects in the following study. All animals were housed in translucent plastic cages filled with wood shavings and covered with wire tops. They were maintained in an animal colony room

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[1] $(\sqrt[3]{\text{Body Weight (grams)}}) /$ Nasoanal length (cm)

with controlled temperature (20-22°C) and a 12-12-hour (hr) light-dark cycle (lights on: 0700 hr).

Procedures

Preweaning period. At birth, Day 1, all litters born within 12 hours of one another were pooled and culled such that eight pups (4 females, 4 males) could be randomly assigned to each mother. Before returning pups to their home cages, they were randomly assigned to either MSG-injected groups or saline-injected groups. Pups in the MSG-injected groups received subcutaneous injections of 3 mg/gr body weight of the monosodium salt of L-glutamic acid (MSG) (Sigma Chemical Co., St. Louis, MO). Control animals were injected with an equivolume of physiological (0.09%, pH 7.5) saline solution. Injections of both MSG and saline were given to each respective group on Days 1, 2, 3 and 6, 7, and 8 following the modified injection schedule used by Cohen (1967) and previously used in our laboratory.

Postweaning. Animals were weaned at 20 days of age. All mice were separated from their mothers and housed individually in standard laboratory translucent plastic cages with wood shavings under the same previously described conditions.

The animals were left undisturbed until 40 days of age. At that time, they were randomly assigned to one of two diet condition groups. No more than two pups of the same sex from each litter were assigned to the same condition. The

animals were given pre-weighed portions (gr ± .01) of either ground chow (CHOW groups) or a high-fat, high-sugar (HFS groups) diet. The CHOW diet had a caloric value of 3.6 Kcal/gr, while the HFS diet had a caloric value of 5.1 Kcal/gr. The diet was placed in jars designed to avoid spillage or soiling of food by urine and feces. The HFS diet consisted of 3/5 (by weight) ground chow, 1/5 Crisco, and 1/5 sugar. This diet has been shown to be a highly preferred, palatable diet in our laboratory (See Appendix A, Part II and Sclafani, 1972).

Consumption was estimated from the difference between food administered and food remaining on a daily basis (gram ± .01) and then averaged over a two-day period to correspond with the same schedule of weight and length recordings for each animal. If excessive spillage occurred on the day of measurement, no recording was made. Freshly weighed portions of the diet were returned to each cage daily.

Body weights (gram ± .01) and body lengths, measured nasoanally (cm ± 0.1) for the determination of Lee Indices, were recorded every second day and averaged over the two-day period.

All measurements of amount consumed, body weight and body length were recorded at the same time of day (i.e., between 10:00 and 11:30 A.M.).

At 60 days of age, the animals were decapitated for the collection of blood and tissue samples. Animals were not subjected to any type of deprivation schedule prior to decapitation. Samples were collected between 1:00 P.M. and 2:30 P.M. for all groups. Trunk blood was collected in heparinized hematocrit tubes, and liver sections were removed and immediately frozen in dry ice. Blood was centrifuged for five minutes in a Clay-Adams hematocrit centrifuge and the plasma recovered and stored in polypropylene tubes at -20°C for later analysis. Liver was placed in the freezer (-100°C) until homogenization and analysis.

Assays

All chemicals used in the plasma and tissue analysis were obtained from Sigma Chemical Co., St. Louis, Missouri.

The following abbreviations are of the compounds used in the assay section: ATP = Adenosine triphosphate; ADP = Adenosine diphosphate; NAD^+ = Nicotinamide adenine dinucleotide (oxidized); NADH = Nicotinamide adenine dinucleotide (reduced); $NADP^+$ = Nicotinamide adenine dinucleotide phosphate (oxidized); NADPH = Nicotinamide adenine dinucleotide phosphate (reduced); G-6-P = Glucose 6-phosphate; 6-PG = 6-Phosphogluconolactone; PEP = Phosphoenolpyruvate; Pyr = Pyruvate; Lac = Lactate; $MgCl_2$ = Magnesium chloride; KCl = Potassium chloride.

All fluorometric readings were made in a Farrand fluorometer using the following filters: Primary, 5860; Secondary, 5562, 4308, 3387.

Plasma. The plasma was used for the fluorometric determination of glucose following the enzymatic procedure of Lowry and Passoneau (1972). The reaction was carried out in a 3-ml tube with 1 ml of 50 mM Tris buffer (pH 8.1) containing 1 mM $MgCl_2$, 0.5 mM dithiothreitol, 300 µM ATP, 30 uM $NADP^+$, 0.14 U/ml hexokinase and 0.2 U/ml glucose 6-phosphate dehydrogenase. The generation of NADPH was measured fluormetrically against glucose standards ranging from 1-10 nanomoles.

Liver. Frozen liver samples were weighed on the Sartorius (to the nearest .00001 gr) and immediately homogenized in 2 ml of ice-cold 0.1 M potassium phosphate buffer (pH 7.5) in a 2-ml Pyrex tissue grinder. Equal aliquots of the homogenized samples were then stored in small polypropylene tubes at -30°C until the following analyses of each sample could be completed. All enzymatic assays followed the procedures of Lowry and Passoneau (1972).

Protein determination was obtained for each aliquot according to the Lowry procedure (1972). The samples were read in glass cuvettes in a Beckman spectrophotometer at 750 nm against 1 mg/ml bovine serum albumin (BSA) standards. Enzyme activity is reported as nanomoles/min per mg protein.

The activity of a glucose 6-phosphate dehydrogenase (G6PDH), was fluorometrically determined by examining the generation of NADPH from the following reaction:

$$G\text{-}6\text{-}P + NADP^{+} \Longrightarrow 6\text{-}PG + NADPH.$$

This reaction was carried out for five minutes in 1 ml of 0.05M AMP_2 pH 9.2 buffer, containing 0.1 mM $NADP^{+}$ and 0.01% BSA at 37°C.

Hepatic glucokinase (GK), also known as hexokinase IV, activity was fluorometrically determined by coupling the production of glucose 6-phosphate to the reduction of $NADP^{+}$ in the presence of excess glucose 6-phosphate dehydrogenase. The same buffer solution as that used for plasma glucose is used here, with hexokinase omitted and an excess of the substrate, glucose, added. The generation of NADPH was assessed following an incubation period of five minutes at 37°C as can be seen in the following reaction sequence:

$$Glucose + ATP \Longrightarrow ADP + G6P + NADP^{+} \Longrightarrow 6\text{-}PG + NADPH$$

The activity of pyruvate kinase (PK) was measured by examining the depletion of NADP, or, in other words, the formation of NAD^{+} as seen in the following reaction:

$$ATP + PEP \longleftrightarrow ATP + Pyr + NADP \Longrightarrow Lac + NAD^{+}.$$

The buffer for this reaction was a 0.05 M Tris, pH 7.8, containing 0.2 mM ADP, 5.0 mM $MgCl_2$, 50 mM KCl, 0.2 mM PEP, 0.01 mM NADH. The reaction was carried out for five minutes at 37°C.

Malate dehydrogenase (MDH) activity was determined by following this reaction:

Malate + NAD^+ <===>> oxaloacetate + NADP

in a 0.1 M Tris buffer containing 0.02% BSA, 1 mM NAD^+ and a large excess of substrate to drive the reaction (20 mM malate). Once again, this reaction was carried out at 37°C for a period of five minutes and read in the fluorometer.

CHAPTER II

RESULTS

Design

Analyses of these data follow a completely randomized factorial (CRF) design with three independent variables, each with two levels:

Treatment: MSG-treated (MSG)
Saline-treated (CONT)

Sex: Female (FEM)
Male (MALE)

Diet: High-fat, high sugar (HFS)
Regular ground chow (CHOW),

yielding a CRF-222 (Kirk, 1982). Each cell contained an equal number of subjects (n = 14) for a total subject pool of 112 (See Appendix B; Figure 15). This subject pool was used for the dependent measures of amount consumed, Kcal consumed, body weight, body length, and Lee Indices. The remaining dependent measures of blood glucose and enzyme activity were analyzed using cells which contained a random selection of 10 samples from the pooled plasma and liver samples.

Statistical Analysis

Consumption, grams and calories consumed, did not vary from day to day and, therefore, were collapsed across all nine time periods of measurement to form one mean which was

then analyzed by the 2x2x2 ANOVA. Body weights, body lengths, and Lee Indices were submitted to regression analyses across all nine periods of measurements. A priori comparisons between the individual beta-weights (slopes) obtained for each of the regression curves were used for the determination of differences between rates of growth.

An analysis of change scores between Day 40 measurements and Day 60 measurements was used to analyze the relative changes of body weights, body lengths and Lee Indices from the beginning to the end of the study.

Estimations of the magnitude of significant effects were calculated using omega squared (w^2) for all significant main effects and interactions (Kirk, 1982).

Correlations were obtained between all the enzymes and glucose recordings for the individual groups to provide further descriptions of the mechanisms of the obesity.

A priori comparisons were based on Tukey's planned contrasts for all possible pairwise comparisons ($p < .05$) for samples with an equal number of subjects per cell (Kirk, 1982).

Consumption and Morphometric Data

Table 1 displays the means (+SEM) for the amount consumed averaged across all days of measurement, from 40 to 60 days of age, for all eight groups. The brackets indicate significant (*$p < .05$) meaningful comparisons showing that all MSG animals consumed less than the saline controls.

Table 1.

Mean (+SEM) dietary consumption (gr/day)
averaged across all time periods of measurement.

GROUP	Means (+SEM)			
MSG-FEM-HFS	4.60	(.09)	.05	
MSG-FEM-CHOW	5.61	(.13)		.05
CONT-FEM-HFS	5.83	(.12)	.05	
CONT-FEM-CHOW	6.97	(.13)		.05
MSG-MALE-HFS	5.06	(.10)	.05	
MSG-MALE-CHOW	6.13	(.14)		.05
CONT-MALE-HFS	5.39	(.13)	.05	
CONT-MALE-CHOW	6.64	(.08)		.05

Note: Significant ($p < .05$) and meaningful comparisons (Tukey's) of appropriate controls have been indicated (All possible comparisons are given in Appendix C, Table 20).

Treatment and Diet main effects for gram amount consumed accounted for 27.08% and 42.44%, respectively, of the total variance. For complete analysis performed and all comparisons among the means, see Appendix C, Tables 18 and 19.

Means (+SEM) of caloric consumption (Kcal/day) are presented in Table 2, with the meaningful comparisons of the appropriate controls indicated ($p < .05$). See Appendix C, Table 20 for complete source table. Again, MSG-treated animals consumed significantly less ($p < .0001$) than their respective controls. The same pattern of differences were noted for caloric consumption, as that seen for total gram consumed. Furthermore, Treatment and Diet main effects accounted for the largest portion of variance, at 40.05% and 16.98%, respectively. A main effect for Sex was noted, however, it accounted for less than 1% of the variance.

At birth, no differences were noted for body weights, nor were there differences in body weights at the beginning of the study (Day 40), with the exception of the CONT-MALE groups which differed significantly ($p < .05$) from one another at that time. At Day 40 the CONT-HFS males were already heavier than all other male groups. All MSG-groups started out with lower body weights than their saline-counterparts as shown with the Y-intercepts of the regression curves, however they show increased rates of growth (See Figures 1-4).

Table 2.

Mean (+SEM) dietary consumption (Kcal/day) averaged across all time periods of measurement.

GROUP	Means (+SEM)	
MSG-FEM-HFS	23.43 (.31)	.05
MSG-FEM-CHOW	20.18 (.32)	.05 .05
CONT-FEM-HFS	29.56 (.34)	.05
CONT-FEM-CHOW	24.56 (.38)	
MSG-MALE-HFS	25.76 (.36)	.05
MSG-MALE-CHOW	22.03 (.36)	.05 .05
CONT-MALE-HFS	27.45 (.38)	.05
CONT-MALE-CHOW	23.60 (.27)	

Note: Significant ($p < .05$) and meaningful comparisons (Tukey's) of appropriate controls have been indicated.

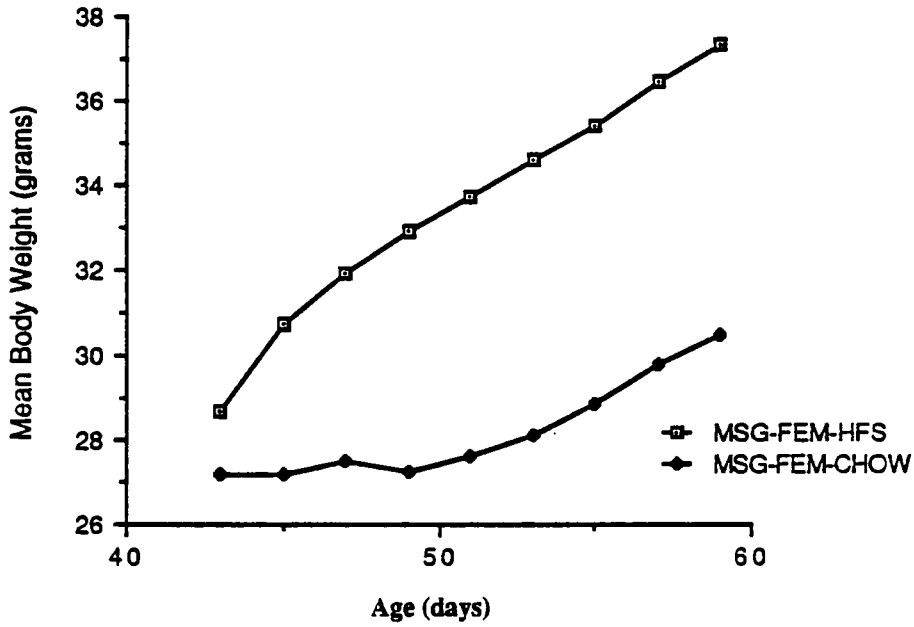

Figure 1. Mean body weight (grams) showing growth rates for MSG-female groups. The regression formulae (*p < .05):

MSG-FEM-HFS: 28.49 + 1.01(x)*

MSG-FEM-CHOW: 26.16 + 0.40(x)

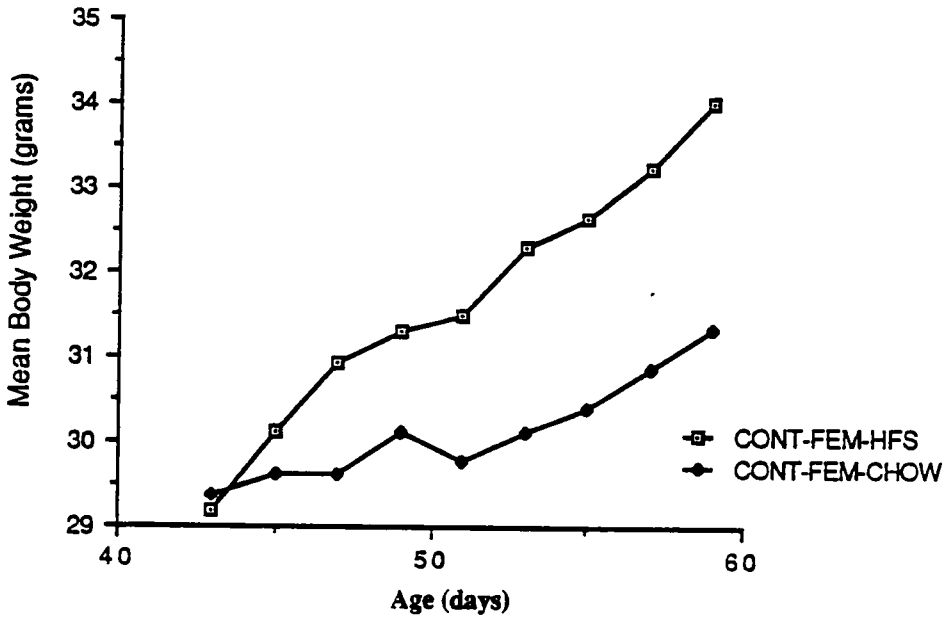

Figure 2. Mean body weight (grams) showing growth rates for CONT-female groups. The regression formulae:

CONT-FEM-HFS: 28.94 + 0.45(x)
CONT-FEM-CHOW: 29.04 + 0.40(x)

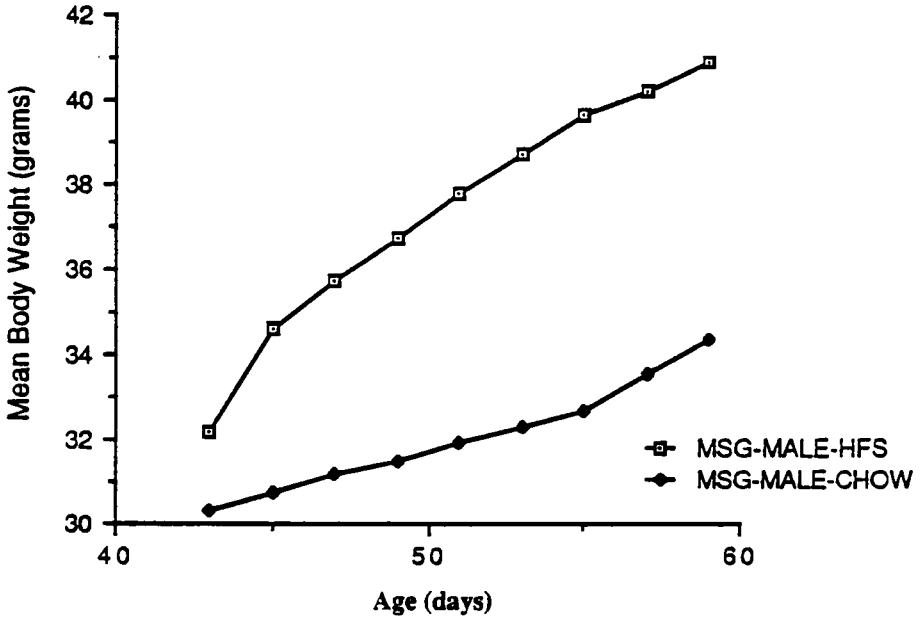

Figure 3. Mean body weight (grams) showing growth rates for MSG-male groups. The regression formulae (*p < .05):

MSG-MALE-HFS: 32.28 + 1.03(x)*

MSG-MALE-CHOW: 29.67 + 0.45(x)

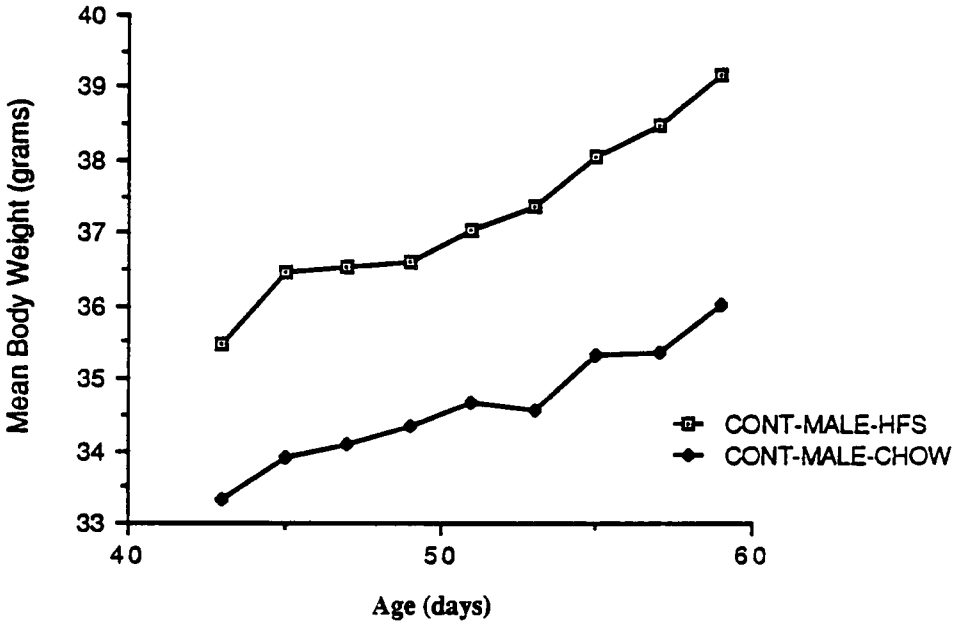

Figure 4. Mean body weight (grams) showing growth rates for CONT-male groups. The regression formulae (*p < .05):

CONT-MALE-HFS: 35.18 + 0.64(x)*

CONT-MALE-CHOW: 33.14 + 0.37(x)

All groups maintained on HFS diets had greater increases in weight than those on CHOW diets, with the MSG-HFS groups exceeding the rates of growth for all CONT animals, as revealed by the differences in beta-weights or slopes of each function. Table 3 and Table 4 show comparisons between the individual beta-weights for females and males, respectively.

The relative changes in body weights that ensue from Day 40 to Day 60 are presented in Figure 5, for both females and males. There is a highly significant effect ($p < .0001$) revealed for Treatment levels, which account for 15.6% of the variance, and for Diet levels, accounting for another 23.9% of the variance. This effect is most evident for the MSG-HFS groups which showed the greatest significant weight gain, 8.71 grams for both females and males in the 20-day period of measurement. The CONT-HFS groups gained only 3.31 and 4.07 grams for females and males, respectively, while the CHOW groups gained the least. While a significant ($p < .0001$) interaction between these two variables exists, when combined they add only another 2.08% of variance which could be accounted for (Appendix C, Table 21). For complete set of absolute differences for the changes in body weight for both females and males, see Appendix C, Tables 22 and 23.

Table 3.

Differences between beta-weights (e.g., slopes) for females on the regression curves for body weight.

$t(24) = 1.71$ (*$p < .05$)

Group	(1)	(2)	(3)	(4)
(1) MSG-FEM-HFS (1.01)	----	----	----	----
(2) MSG-FEM-CHOW (0.41)	4.00*	----	----	----
(3) CONT-FEM-HFS (0.45)	4.38*	0.29	----	----
(4) CONT-FEM-CHOW (0.40)	4.99*	0.08	0.47	----

Table 4.

Differences between beta-weights (e.g., slopes) for males on the regression curves for body weight.

$t(24) = 1.71$ (*$p < .05$)

Group	(5)	(6)	(7)	(8)
(5) MSG-MALE-HFS (1.03)	----	----	----	----
(6) MSG-MALE-CHOW (0.45)	4.02*	----	----	----
(7) CONT-MALE-HFS (0.64)	2.40*	1.40	----	----
(8) CONT-MALE-CHOW (0.37)	4.75*	0.61	2.07*	----

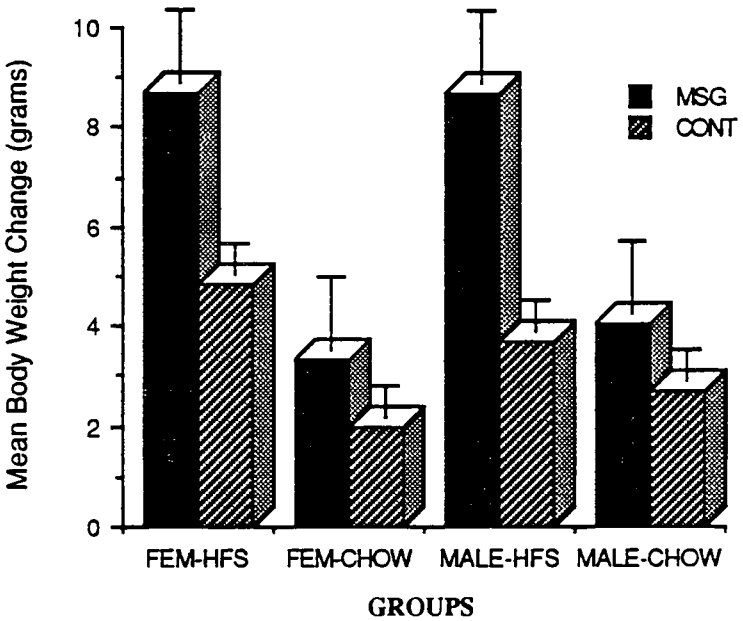

Figure 5. Changes in mean (+SEM) body weight (grams) from Day 40 to Day 60 for females and males.

Figure 6 and Figure 7, respectively, show the mean body lengths for females and males of MSG-treated and CONT-animals plotted across the nine periods of measurement. The characteristic stunting is evident in MSG-treated animals, with the body lengths being significantly shorter than saline-treated controls across all times. This significant ($p < .0001$) treatment effect accounted for 82.67% of the total variance. The differences among beta-weight for females and males, respectively, are revealed in Tables 5 and 6. The regression formulae are given below each figure for the respective function.

The absolute differences between mean changes in body length from Day 40 to Day 60 are shown in Table 7. Significant comparisons are indicated between appropriate groups. See Appendix C, Table 24, for ANOVA of change scores for body length.

For all time periods, Lee Indices of obesity showed that MSG-treated animals had substantially ($p < .0001$) more body fat than the saline-treated controls (Figure 8, females; and Figure 9, males). The difference in Lee Indices observed among the groups cannot be solely accounted for by differences in body length, since the MSG-groups maintained on CHOW were not only shorter, but they weighed less than controls. Tables 8 and 9 reveal the differences between beta-weights for the functions associated with Lee indices for females and males.

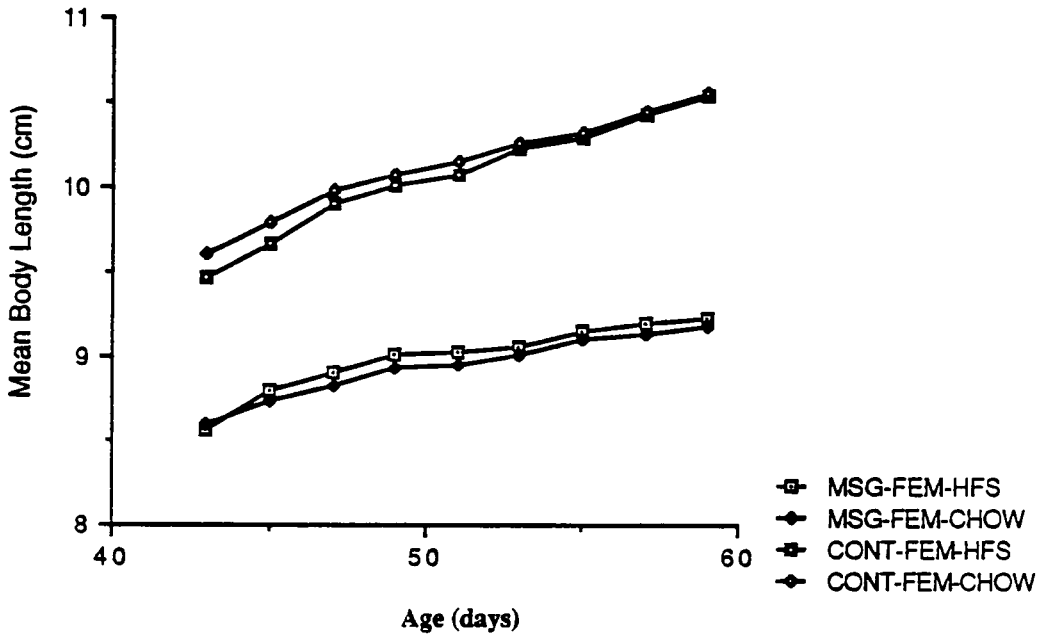

Figure 6. Mean body length (cm) development from Day 40 to Day 60 for all female groups. Different letter indicates difference ($p < .05$):

(A) MSG-FEM-HFS: 8.62 + .08(x)
(A) MSG-FEM-CHOW: 8.59 + .07(x)
(B) CONT-FEM-HFS: 9.44 + .13(x)
(B) CONT-FEM-CHOW: 9.61 + .10(x)

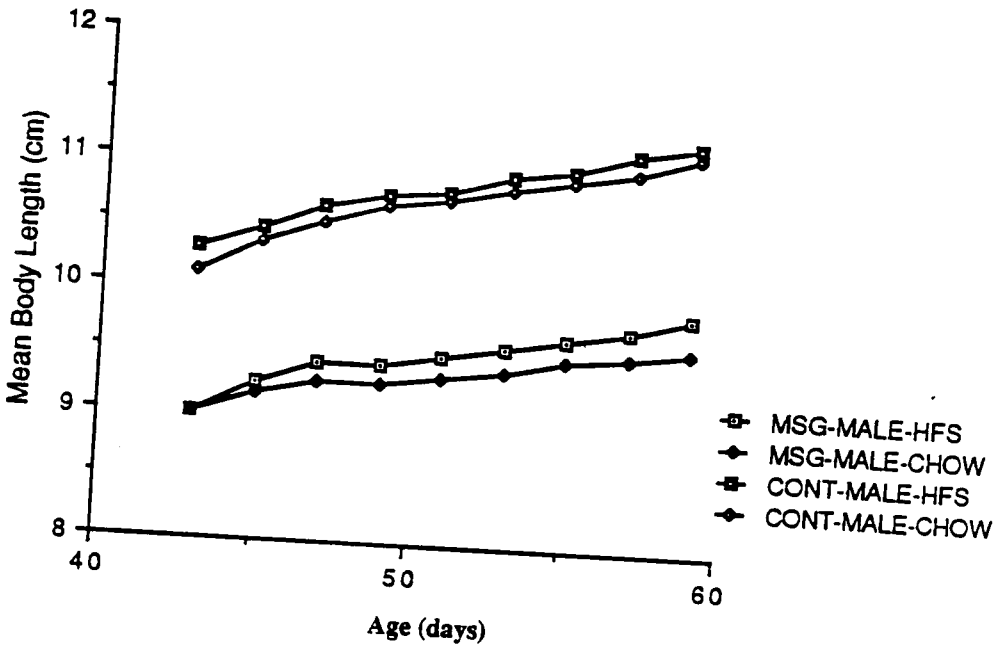

Figure 7. Mean body length (cm) development from Day 40 to Day 60 for all male groups. Different letter indicates difference ($p < .05$):

(A) MSG-MALE-HFS: 9.02 + .04 (x)
(A) MSG-MALE-CHOW: 9.00 + .05 (x)
(B) CONT-MALE-HFS: 10.24 + .11 (x)
(B) CONT-MALE-CHOW: 10.12 + .12 (x)

Table 5.

Differences between the individual beta-weights for females of the regression curves for body length.

$t(24) = 1.71$ (*$p < .05$)

Group	(1)	(2)	(3)	(4)
(1) MSG-FEM-HFS (.007)	----	----	----	----
(2) MSG-FEM-CHOW (.006)	1.09	----	----	----
(3) CONT-FEM-HFS (.006)	5.42*	7.07*	----	----
(4) CONT-FEM-CHOW (.110)	3.03*	4.34*	2.17*	----

Table 6.

Differences between the individual beta-weights for males of the regression curves for body length.

$t(24) = 1.71$ (*$p < .05$)

Group	(5)	(6)	(7)	(8)
(5) MSG-MALE-HFS (.008)	----	----	----	----
(6) MSG-MALE-CHOW (.009)	1.57	----	----	----
(7) CONT-MALE-HFS (.008)	1.77*	3.32	----	----
(8) CONT-MALE-CHOW (.007)	2.82*	4.39*	0.94	----

Table 7.

Absolute differences for mean changes (+SEM) of body lengths (cm) from Day 40 to Day 60.

GROUP	Mean changes (+SEM)
MSG-FEM-HFS	0.68 (0.15)
MSG-FEM-CHOW	0.59 (0.14)
CONT-FEM-HFS	1.08 (0.13)
CONT-FEM-CHOW	0.95 (0.13)
MSG-MALE-HFS	0.86 (0.12)
MSG-MALE-CHOW	0.61 (0.11)
CONT-MALE-HFS	0.95 (0.12)
CONT-MALE-CHOW	1.03 (0.11)

Note: Significant comparisons (Tukey's) of appropriate controls have been indicated ($p < .05$).

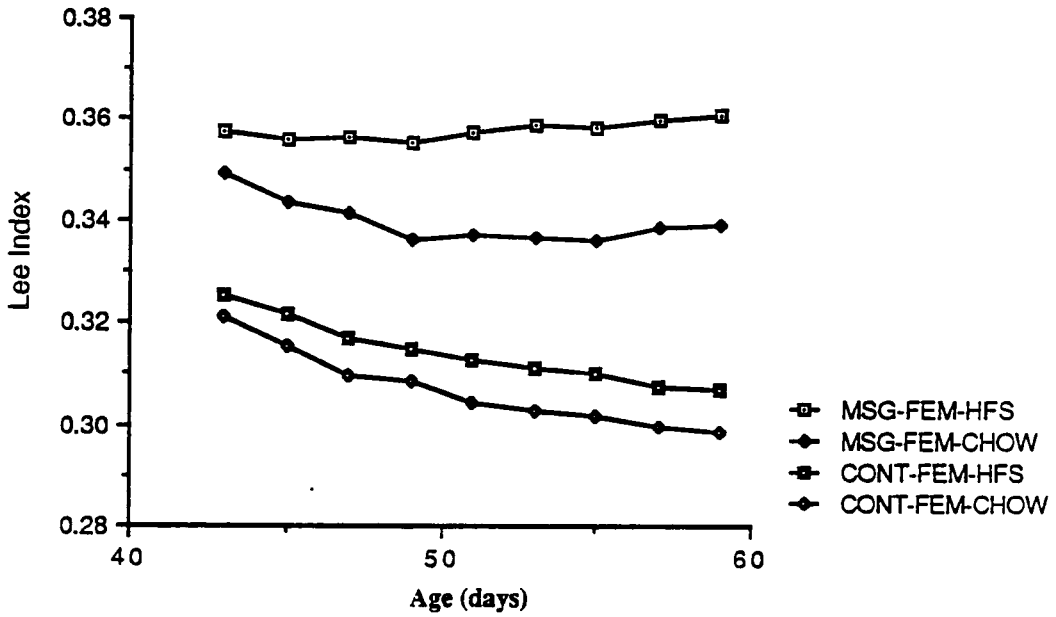

Figure 8. Lee Indices for all female groups across all time periods of measurement. Different letter indicates difference ($p < .05$):

(A) MSG-FEM-HFS .3572 + .0006(x)
(B) MSG-FEM-CHOW .3454 + (-.0011)(x)
(C) CONT-FEM-HFS .3251 + (-.0022)(x)
(C,D) CONT-FEM-CHOW .3195 + (-.0024)(x)

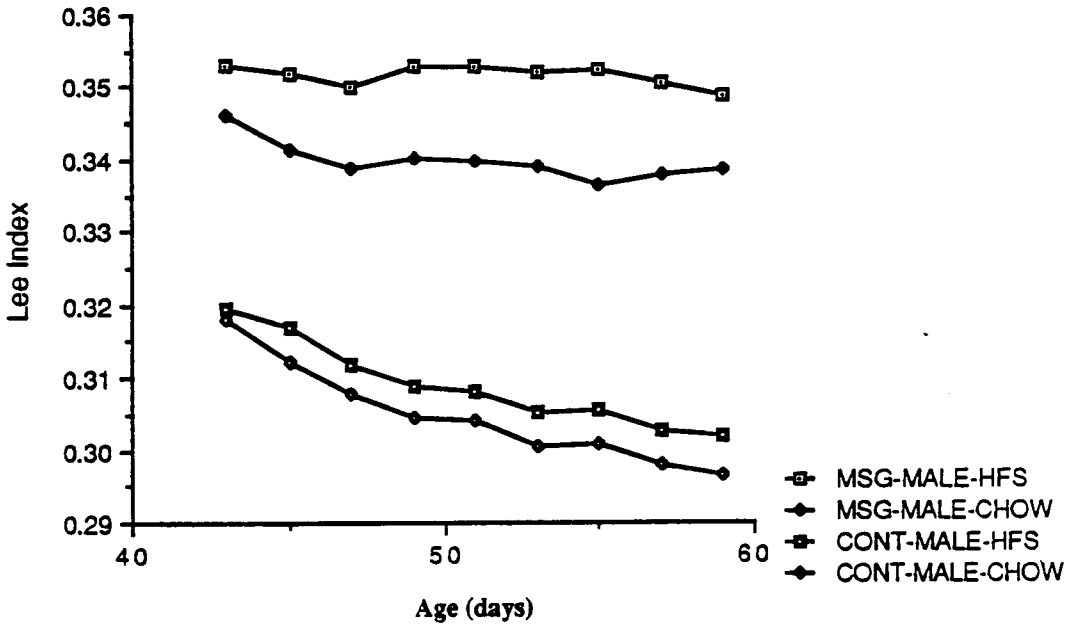

Figure 9. Lee Indices for all male groups across all time periods of measurement. Different letter indicates difference ($p < .05$):

(A)	MSG-MALE-HFS	.3530 + (-.0003)
(A)	MSG-MALE-CHOW	.3437 + (-.0008)
(B)	CONT-MALE-HFS	.3198 + (-.0021)
(B,C)	CONT-MALE-CHOW	.3170 + (-.0025)

Table 8.

Absolute differences between individual beta-weights of the regression curves for Lee Index for females.

t(24) = 1.71; (*p < .05)

Group Mean (+SEM)	(1)	(2)	(3)	(4)
(1) MSG-FEM-HFS .0006 (.0004)	----	----	----	----
(2) MSG-FEM-CHOW -.0011 (.0004)	3.01*	----	----	----
(3) CONT-FEM-HFS -.0022 (.0003)	5.60*	2.20*	----	----
(4) CONT-FEM-CHOW -.0024 (.0002)	6.71*	2.91*	0.56	----

Table 9.

Absolute difference between individual beta-weights of regression curves for Lee Index for males.

t(24) = 1.71; (*p < .05)

Group Mean (+SEM)	(5)	(6)	(7)	(8)
(5) MSG-MALE-HFS -.0003 (.0004)	----	----	----	----
(6) MSG-MALE-CHOW -.0008 (.0003)	1.00	----	----	----
(7) CONT-MALE-HFS -.0021 (.0002)	2.91*	3.61*	----	----
(8) CONT-MALE-CHOW -.0025 (.0002)	4.92*	4.71*	1.41	----

HFS-feeding significantly increased the body fat of both MSG- and saline-treated animals, when compared to CHOW-fed animals. While all MSG animals, including those on CHOW diets, maintained a higher level of adiposity across all time periods. The differences for overall changes in Lee Indices (See Appendix C, Table 25) demonstrate that Treatment effect accounted for 29.5% and the Diet effect accounted for 2.5% of the total variance. Changes from Day 40 to Day 60 for Lee Indices are also evident, as shown in Tables 26 and 27, Appendix C.

Glucose and Enzyme Measurements

Glucose levels were higher in CONT animals ($F(1, 104) = 48.93$; $p < .0001$); in MALE's ($F(1, 104) = 77.16$; $p < .0001$; and in those animals maintained on the HFS diet ($F(1, 104) = 118.90$). The means (+SEM) of plasma glucose are can be seen in Figure 10. The MSG-FEM-CHOW group differed from all others ($p < .05$), except the MSG-MALE-CHOW group. No other differences in means were evident. The MSG animals all showed slightly decreased amounts of plasma glucose. Complete ANOVA source table and the set of absolute differences are presented in Appendix C, Tables 28 and 29.

Figure 11 displays the mean liver activity levels of G6PDH for females and males, respectively. Table 30 (Appendix C) gives source table and Tables 31 and 32 (Appendix C) indicate the appropriate significant comparisons for females and males, respectively.

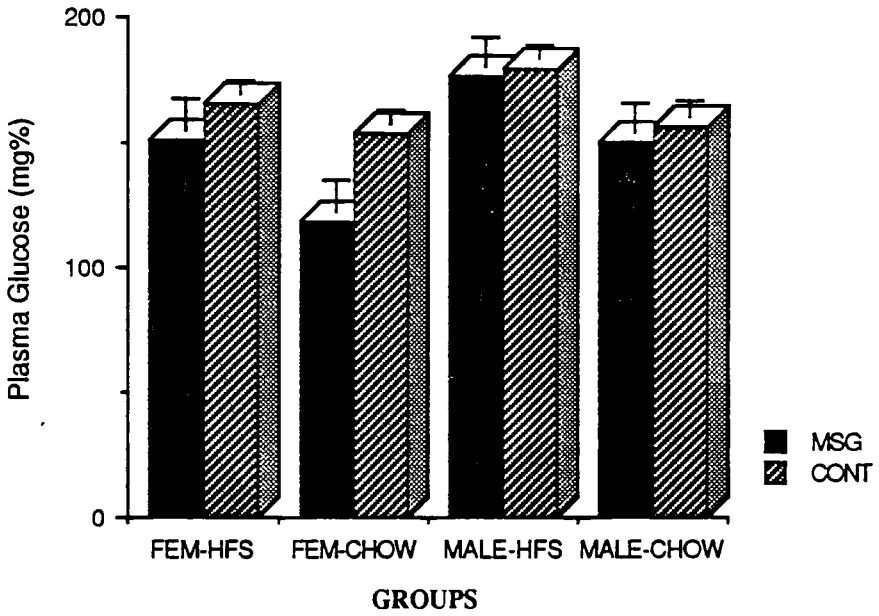

Figure 10. Mean (+SEM) plasma glucose (mg%) for unfasted animals from all groups.

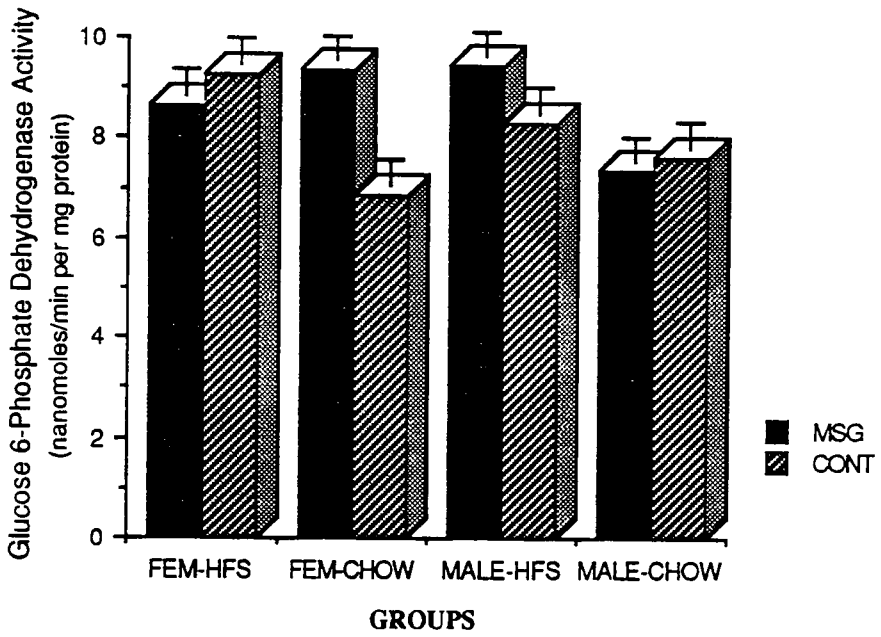

Figure 11. Mean (+SEM) glucose 6-phosphate dehydrogenase activity (nanomoles/min per mg protein) for both females and males.

A highly significant difference ($p < .0001$) at levels of Diet ($w^2 = 9.82\%$) and at the interaction of Treatment by Sex by Diet ($w^2 = 9.92\%$). The absolute differences indicate that the comparisons of the MSG-FEM-CHOW group with the CONT-FEM-HFS group, are the only two means that do not differ from one another for female groups ($p < .05$). All male groups differ from one another.

The same pattern of GK activity is evident for both females and males (Figure 12). The MSG-CHOW animals have the highest GK activity at 8.94 and 7.86 nanomoles/min per mg protein, while the MSG-HFS animals have the lowest activity at 4.98 and 5.37, respectively. This Treatment by Diet interaction accounts for approximately 61.38% of the variance, while the main effects for Treatment and Diet, alone account for less than 10% of the total variance. (See Appendix C, Tables 33 and 34, for extra tables.)

Mean PK (Figure 13) activity levels indicate that MSG-treated animals have significantly ($p < .0001$) greater activity levels than saline-treated controls. (See Appendix C, Tables 35, for source table and Tables 36 and 37, respectively, for absolute differences between means for females and males.)

Figure 14 demonstrates the mean MDH activity levels for all groups. There are no differences between any of the means. (See Appendix C, Table 38, for analysis source table and Table 39, for all comparisons of means.)

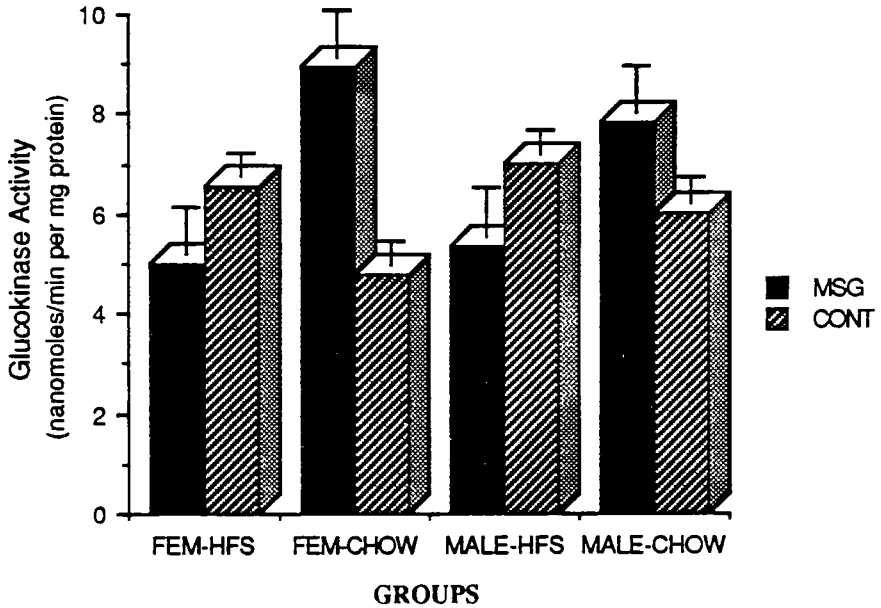

Figure 12. Mean (+SEM) glucokinase activity (nanomoles/min per mg protein) for females and males.

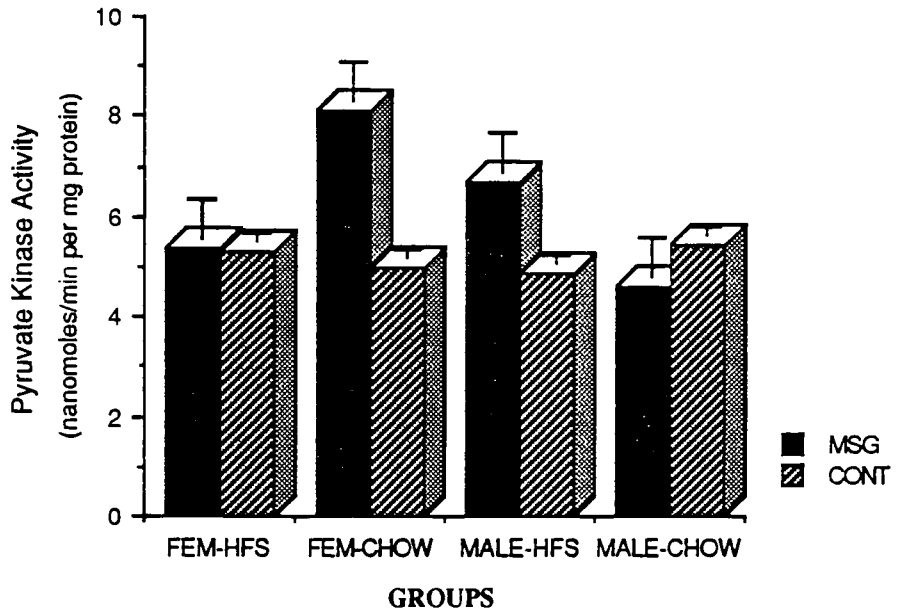

Figure 13. Mean (+SEM) pyruvate kinase activity (nanomoles/min per mg protein) for both females and males.

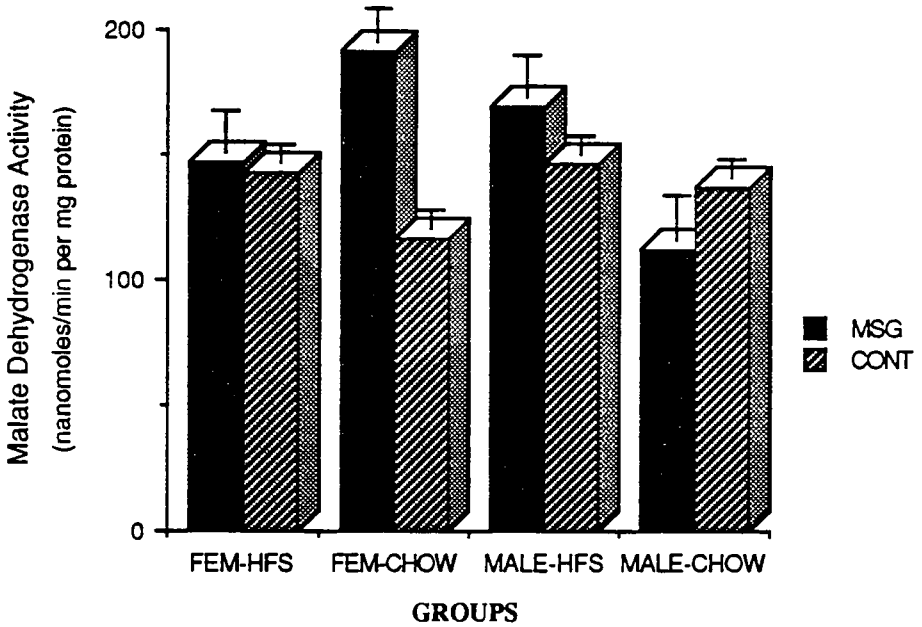

Figure 14. Mean (+SEM) malate dehydrogenase activity (nanomoles/min per mg protein) for both females and males.

Correlations were obtained among the physiological measures of enzyme activity and plasma glucose within each treatment group. Table 10 indicates the significant correlations (Pearson) among the physiological measures for eah individual group. No correlations were found for any of the items measured in either of the MSG-MALE groups, or for CONT-MALE-CHOW group. (See Appendix C, Tables 40-47, for the correlations found within each of the groups.)

Table 10.

Significant Pearson correlations found within each group

GROUP (n = 10)	Items	Correlations
MSG-FEM-HFS	GK & G6PDH	(-.75; $p < .01$)
	PK & Glucose	(0.64; $p < .05$)
MSG-FEM-CHOW	PK & G6PDH	(0.85; $p < .01$)
	GK & Glucose	(-.82; $p < .01$)
CONT-FEM-HFS	PK & G6PDH	(0.85; $p < .05$)
	PK & MDH	(0.70; $p < .05$)
CONT-FEM-CHOW	PK & GK	(0.78; $p < .01$)
MSG-MALE-HFS	-----	-----
MSG-MALE-CHOW	-----	-----
CONT-MALE-HFS	PK & MDH	(0.70; $p < .05$)
	PK & G6PDH	(0.78; $p < .01$)
	MDH & G6PDH	(0.68; $p < .05$)
CONT-MALE-CHOW	-----	-----

CHAPTER III

DISCUSSION AND CONCLUSIONS

Feeding and Obesity

This study confirms and extends some previously recorded characteristics of the obesity related to the hypothalamic damage following MSG toxicity. All major hypotheses of consumption were confirmed: (1) that the MSG animals would consume less food across all time periods, (2) that more Kcal/day would be consumed in the groups that were maintained on HFS diets.

All groups that were maintained on the HFS diet demonstrated approximately the same percentage decrease in the total amount consumed (17-18%), however, this decrease in amount was not enough to make up for the increase in caloric intake. All animals in HFS groups, consequently, consumed more calories (16-18% increase), even though food intake was reduced. The third hypothesis of consumption stated that females in the HFS groups would consume more than the males in the same groups. This was found to only hold true for the control females. The MSG females that were maintained on the HFS diet exhibited a lower caloric intake than did males of the same group. As pointed out below, it was the MSG females that gained the most weight.

The characteristic stunting effect was also confirmed. All MSG animals weighed less at the beginning of the study. Only those MSG animals consuming the HFS diet, ever surpassed the weight of the controls. This occurred relatively early in development for the MSG females (43-46 days of age), whereas, the MSG males took a few more days (50-55) to surpass the weight of the controls. At this time, though, the weights of the females are lower than that for the males.

The rates of growth for the MSG animals, as seen in the regression functions, are more rapid in the MSG animals maintained on HFS. The CHOW-fed MSG animals, on the other hand, exhibit the same rate of growth as the CHOW-fed controls during the time periods used in this study. The MSG females and males maintained on HFS diet demonstrated an increased weight gain (or change in weight) over all other groups. In fact, during the 20 days of testing, their weight gain was double that of the control groups maintained on the same HFS diet.

The real evidence of the increased adiposity is seen with the obesity index or Lee index. Across all times, the MSG animals weighed less than their saline controls, but the corresponding Lee index was much higher. What was unexpected was that the animals appeared to be at the maximum level of obesity at the very beginning of the study, since there was little change in the index over time. The

control animals did show a progressive decline in the index, which would be indicative of the increasing amount of lean body mass (e.g., muscle) that would be occurring during this developmental time period. The only group that showed an increase in their fat deposition, was the MSG females on HFS. Al other regression functions indicated a decline would be occurring, although it would be less so in the other MSG groups.

All of the data presented above leads one to the conclusion that this obesity would most definitely have to classified as being metabolic in nature. The MSG- animals consumed less food overall and also showed a decrease in the amount consumed when presented with a highly palatable, high caloric diet. This suggests that these animals attempted to correct for caloric content and that the developing obesity is not due to some internal "calorie-counter" that has gone awry as a result of MSG injections. These data also demonstrated that animals given neonatal injections of MSG resulted in increased rates of growth which could be affected so dramatically by altering the dietary content would suggest an increased efficiency in energy utilization. This rapid increase in weight has been demonstrated in the VMH-lesioned animal, but only very early in the actual development of the obesity (LeMagnen, 1984). Some interesting future work in this area might include a study to see if caloric restriction would cause an increase in

consumption such that a decline in body weight could be avoided. It would be interesting to examine animals after they have been taken off the diets, to see if there is a levelling off or slowing down of the rate of weight gain.

Carbohydrate Metabolism

From the data presented above and from other literature, it has generally been concluded that obesities resulting from hypothalamic damage are metabolic in nature. In this section we will see that there also appears to be a "metabolic push" in the direction of increased fat deposition.

The central feature common to all of the hypothalamic obesities is a concomitant rise in plasma insulin. Insulin is known to be a primary regulator of intermediary metabolism. As will be seen below, insulin is an inducer of two of the major rate-limiting enzymes involved in carbohydrate metabolism. Corticosteroids, catecholamines and glucagon also play a major role in carbohydrate regulation, although each of these act in an antagonistic manner towards the action of insulin.

Activities of enzymes are controlled *in vivo* by a variety of mechanisms: (1) concentration of physiologic compounds, other than immediate substrates and/or products, (2) catalytic efficiency or V_{max}; (3) binding of substrate at the active site (K_m); (4) oxygen supply; (5) availability of hormones; (6) cAMP-dependent processes of

phosphorylation/dephosphorylation; and (7) the general nutritional condition (Denton and Pogson, 1976).

Properties of enzymes noted for activities in vivo may not be the same as those measured in vitro such that maximum activities observed in the laboratory should only be used as rough guides, as they may actually be far removed from the actual physiological conditions.

The study of intermediary metabolism includes an examination of the behavior or fluxes within an integrated complex system of glycolysis and lipogenesis (Krebs and Eggleston, 1965). Glycolysis is the process which accounts for the conversion of glucose to pyruvate or lactate and lipogenesis is the process which converts either one of these products back to fatty acids or triglycerides. The fluxes through these pathways are regulated by the activities of very specific rate-limiting enzymes and by factors that could affect blood glucose levels (Hers and Hue, 1983).

The enzymes selected in this study represent either key, rate-limiting enzymes or enzymes found at the "junction" of two pathways and could be involved in determining which pathway would be activated based on the steady-state condition of the organism.

Under conditions of carbohydrate feeding or in the "fed state," there will typically be an increase in activity of glucose 6-phosphate dehydrogenase (Denton and Pogson, 1976).

This enzyme is responsible for the conversion of glucose 6-phosphate to 6-phosphogluconolactone, which is the entry point to the hexose monophosphate shunt (HMP). The presence of active lipogenesis or increased synthesis of fatty acids will stimulate an active degradation of glucose via this shunt. Estimates of activity of this shunt pathway could give an indication of what is happening in the overall scheme of carbohydrate metabolism (Elliot, Hems and Beloff-Chain, 1971; Bernardis, Rosen, Goldman, and Martin, 1977).

In MSG females and males maintained on HFS, there was large increase in the activity of G6PDH. These two groups differed from all others. There was also an increase seen particularly with the CONT females on the HFS diet. This would suggest that there would be an increased tendency for lipogenesis and fatty acid synthesis.

Glucokinase (GK) is primarily responsible for removing glucose from the blood following a meal and allowing for glucose to enter the glycolytic pathway by a process of phosphorylation (Seidman, Horland, and Teebor, 1967). The activity of GK is insulin-inducible and strongly affected by changes in nutritional state (Chisholm and O'Dea, 1987). Under physiological conditions this level in glycolysis is regarded as an irreversible, rate-limiting reaction. In fat-fed animals, there is a reduction in some insulin regulated pathways of glucose metabolism (Krebs and Eggleston, 1965).

All of the measures of GK activity differed from one another. While all the means differed from one another, the two groups which seem to be most revealing are the MSG female and male groups that were maintained on the HFS diet. Both of these groups showed a decline in GK activity, perhaps pointing to the reduction of insulin regulated pathways, particularly in light of the fact that these animals are typically hyperinsulinemic (Bernardis, Rosen, Goldman, and Martin, 1977).

Pyruvate kinase (PK) is also insulin-inducible and is the enzyme responsible for the conversion of phosphoenolpyruvate to pyruvate (Krebs and Eggleston, 1965). Variations in PK activity play a key role in the regulation of gluconeogenesis and are thought to be the site of regulation for the switch from glycogen storage pathways to fat storage pathways (Weber, Stamm, and Fisher, 1965). It is also a rate-limiting enzyme in the glycolytic process. Increases in PK activity have been related to conditions of high-carbohydrate feeding (Weber, Stamm, and Fisher, 1965).

The measures of activity obtained for PK also lends itself to the suggestion that the MSG animals are geared to the production of fatty acids. There were increases seen in both the female and male MSG animals above that for the other groups.

Finally, activity levels of malate dehydrogenase (MDH) have also been used as an indicator of lipogenic activity, as well as, an increase in activity under "well-fed" conditions, closely paralleling fatty acid synthesis (Vrana and Kazdova, 1986).

Although some differences seem to have existed upon the initial statistical analysis, the interpretation was hampered when no differences between the means were found. Generally speaking, it would appear that there is a trend toward increased activity with the all MSG groups, except the males maintained on CHOW. The activity measured here also suggests that an increase in lipogenesis is ongoing within the MSG animals.

An attempt was made to individually examine each one of these enzymes, but more meaningful information for some of the groups was found in examining the correlations obtained among variables within the same groups. The groups having correlations among the enzymes which control rates of lipogenesis are found either within the groups that were maintained on HFS or they are the MSG treated animals consuming either one of the diets (particularly females). Those groups maintained on CHOW seem to show the highest correlations with the enzymes responsible for gluconeogenesis and glycolysis, combined. The fact that no correlation existed for any of these enzymes within all the

MSG male groups and the CONT-CHOW male group is unexplainable.

Extreme caution should be taken when examining the enzyme data, however, in this particular study, no attention was given to the V_{max} properties, the K_m properties, or other possible mechanisms that have effects on the enzymes selected. Accordingly, any conclusions based on this data are tentative at best.

General Discussion

From the data presented here there would seem to be an overwhelming consensus that this hypothalamic model is of a metabolic nature. Many attempts have been made to explain the possible mechanisms involved in the development of obesity. Much of the recent work on the origins of the obesity resulting from hypothalamic damage has suggested an overactive parasympathetic nervous system (PNS). This notion has been incorporated into the proposal of an "autonomic hypothesis" (Bray and York, 1979), which considers that the obesity is the result of a shift in the balance of the two components of the autonomic nervous system. It is suggested that an overactivity of some components of the sympathetic nervous system (SNS) would lead to hyperinsulinemia and heightened taste responsiveness, which in turn produces an increase in consumption and enhanced lipogenesis leading to an overall increase in fat deposition.

The MSG-induced obese animals in our study failed to exhibit all the characteristics of the typical hypothalamic model. There are human obesities that have the same characteristics of the MSG animal, with a decreased food intake and enhanced metabolic efficiency. In light of this, more work needs to be done to further elucidate the actual mechanisms that underlie the development of such an obesity.

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APPENDIX A

LITERATURE REVIEW

PART I

GENERAL REVIEW

Introduction

Obesity in humans is a social and biomedical problem of increasing severity (Cohen, 1985). While no death certificate has ever indicated obesity as the cause of death, an increased mortality rate has been associated with complications resulting from obesity (Cleave and Campbell, 1966; Wilson and Wilson, 1969; Donald, 1973; Mann, 1974).

One of the major difficulties confronting investigators and clinicians alike is the lack of criteria for identifying different types of human obesity. All too often, obesity is considered as a homogeneous entity, when in reality, it is a collection of disturbances in which obesity is an objective feature.

Several mechanisms have been suggested which would lead to obesity: imbalances between energy storage resulting from excessive intake (Bray, 1975), lack of activity causing decreased energy expenditure, or more efficient use of ingested food energy (Inoue and Bray, 1979). Each of these may, in turn, be influenced by a host of psychosocial and physiological factors (McMinn, 1984). The presence of obesity, alone, tells us nothing about the mechanism(s) which contributed to this imbalance. A broad interest in

experimental research has been generated examining the physiological mechanisms involved in the regulation of body weight (LeMagnen, 1983). Despite the tremendous amount of work, a clear understanding of the etiology of obesity and treatment of this multifaceted phenomenon has yet to emerge (LeMagnen, 1984).

In an effort to understand these mechanisms a host of animal models of experimental-induced obesities have been used (LeMagnen, 1983). Extensive reviews of this research have confirmed that the most common methods of inducing obesity have used distinct methods to create disruptions of assorted regions of the hypothalamus (Bray and York, 1979; LeMagnen, 1983).

The hypothalamic areas affected and the severity of the obesity resulting from these diverse methods within each of the models may differ. Nevertheless, there are some common mechanisms apparent in the diverse models. The three central features of the obesity syndrome seen in almost all the models involve a triad of hyperinsulinemia, reduced physical activity and exaggerated taste responsiveness (Bray and York, 1979). Whether each of these is necessary or to what extent they play a role in the development of obesity has yet to be determined. The following review examines more closely the obesity syndrome related to hypothalamic damage concentrating specifically on a unique method

ofinducing hypothalamic damage using monosodium glutamate as a chemical agent to produce lesions.

Historical Foundations

Mohr (1840) published the first description of obesity associated with hypothalamic injury. This isolated report stood unsupported until the nearly simultaneous publication of two landmark papers by Frohlich (1900) and Babinski (1901), which documented the presence of tumors in the region of the hypothalamus that were associated with obesity, atrophy of the gonads, decreased vision, signs of increased intracranial pressure, and short stature.

In 1940, the landmark observations by Hetherington and Ranson recorded that bilateral electrolytic lesions restricted to the ventromedial region of the hypothalamus (VMH) could be associated with the development of obesity. According to their formulation, the obesity was the result of the destruction of a region of the brain which inhibited feeding, the so-called "satiety center." As a result of "knocking out" this center, there was an increased food intake, termed hyperphagia, and consequently the animals became quite obese.

Several years after finding that VMH lesions produced obesity, Anand and Brobeck (1951) demonstrated that food intake was decreased (hypophagia) or totally abolished (aphagia) after injury to the lateral region of the hypothalamus (LH). This area became known as the "feeding

center." With hyperphagia and obesity associated with VMH destruction and LH injury resulting in reduced food intake, a dual-center hypothesis was formulated (Stellar, 1954).

The functional value of this hypothesis has been great as evidenced by the large volume of research generated. Initial support for the dual-center hypothesis illustrated the reciprocity of these two regions of the hypothalamus. Inhibition of feeding occurred with electrical stimulation of the VMH (Sclafani and Maul, 1974) whereas, stimulation of LH regions lead to increased feeding (Steffens, 1975). Neurophysiological recordings during periods of hunger confirmed that the VMH had decreased levels of activity when compared to the LH (Anand, Dua, and Singh, 1941). Under conditions of glucose infusions there is an augmented frequency of spikes from the VMH and a concomitant reduction in spike frequency from the LH (Oomura, 1975). This pattern of electrical activity also follows the normal circadian pattern of food intake with the lowest activity in the VMH and the highest in the LH seen in the night cycle during periods of increased eating. The opposite pattern is apparent in the day-light cycle (Koizoma and Nishino, 1976). Anatomic studies intimated direct connections between the VMH and the LH (Arees and Mayer, 1967). Studies with chemical agents also supported the dual-center hypothesis and hinted that, in addition, the VMH might function as a "glucoreceptive" tissue (Oomura, 1975). The obesity induced

by injection of gold-thioglucose (GTG) proved to be a pivotal link in the dual-center hypothesis and in the role of the VMH as a glucose-sensitive region of the brain (Marshall, Barnett, and Mayer, 1955).

Although the dual-center hypothesis is a satisfying construct for relating the effects of hypothalamic injury to changes in food intake followed by the development of obesity, there are several lines of evidence indicating that it is inadequate. After extensive review, Rabin (1972), has shown that destruction of the VMH, the "satiety" center, was not essential for the development of hyperphagia. Hyperphagia and obesity were not produced when the lesion was restricted entirely to this nucleus, but could also be produced by injuries to other areas not involving the ventromedial region (Gold, 1973). Hypothalamic damage created by knife cuts produced such a wide variety of behavioral responses dependent upon the specific nature and location of the cuts that it was suggested that there must be other areas involved in the regulation of eating behavior (Gold, 1970). The demonstration that the hypothalamic obesity could occur without hyperphagia offered another major challenge to the dual-center hypothesis (Bernardis and Frohman, 1970; Han, 1968).

The dual-center hypothesis was followed by several theories of energy metabolism conceived to define the influence of the hypothalamus as it regulates energy balance

by controlling energy input (Brobeck, 1946; Kennedy, 1953; Mayer, 1953). Briefly, Brobeck (1960) proposed a theory of food intake based on patterns of thermogenesis. That is, animals will eat in order to keep warm and will stop eating after body temperature has risen. The original theory lacked empirical support (Lytle, 1977). For example, eating and fasting are not associated with consistent changes in overall core body temperature. In fact, the minimal fluctuations in core body temperature are not correlated with measurable changes in metabolic rate and food intake (Brobeck, 1960).

While Brobeck's initial formulation proved to be inadequate, several studies have renewed interest in thermogenesis and have provided an explanation for variations in energy requirements of a specialized lipid tissue known as the brown adipose tissue or BAT. Brown adipose tissue, as opposed to white adipose tissue (WAT), appears to serve as a buffering mechanism in the regulation of food intake (Himms-Hagen, 1976; Rothwell and Stock, 1979; Trayhurn, 1979). When stimulation of BAT occurs, energy derived from ingested food is dissipated as heat. Two types of stimuli are known to trigger thermogenesis: exposure to cold ("non-shivering" thermogenesis) and ingestion of food ("diet-induced" thermogenesis) (Himms-Hagen, 1984a). Although different terms are used to describe this, the underlying metabolic mechanisms are similar. Under certain

conditions, thermogenesis induced by cold or diet, can be a major component of energy expenditure and variations in its magnitude can determine whether energy balance is achieved (Miller, Mumford, and Stock, 1967; Mitchell, 1976; Nicholls, 1979).

Mayer's (1953) first formulation of the glucostatic theory proposed that the overall levels of blood glucose would be the signal for food intake. When glucose falls below normal, consumption increases until such time as glucose returns to normal. Mayer (1953) offered the suggestion that the ventromedial nucleus of the hypothalamus (VMH) must be sensitive to circulating levels of glucose and that these were in large part responsible for satiety. This concept was based on several lines of reasoning: 1) the central nervous system (CNS) is dependent on the supply of glucose, 2) carbohydrates are preferentially utilized rather than stored, and 3) there is greater change in carbohydrates than of fat and protein reserves between meals (Mayer, 1953).

There is also considerable evidence suggesting the existence of glucoreceptors in the hypothalamus which control feeding, as previously indicated with recordings of increased electrical activity of the VMH under hyperglycemic conditions or feeding, and a decrease during hypoglycemic or fasting conditions (Anand, Dua, and Singh, 1961). While these findings of reciprocal changes in

glucose utilization and feeding are interesting, they do not rule out the possibility that other regions of central nervous system (CNS) might detect changes in glucose utilization and relay this information to the VMH or LH (Leibowitz, 1976).

There were other discrepancies in this theory that were unexplainable, as with the extreme hunger evident in an untreated diabetic even though hyperglycemia is present. Mayer (1960) later revised the theory to account for this incongruity by indicating that it was necessary to understand how the glucose was being utilized in order to understand the mechanisms of energy balance. Accordingly, this theory has now taken into account the principal hormones (insulin, glucagon, and corticosterone) which are known to be primary regulators of glucose utilization.

In contrast to carbohydrates which seem to play a predominant role in short-term energy regulation, it has been suggested that long-term energy regulation is the result of fat stores being maintained as a constant proportion of total body weight (Keesey and Powley, 1975; Kennedy, 1950). Consequently when fat stores are low, feeding increases to regulate body weight until the appropriate level has been reached.

To this day, debate continues as to which of these hypotheses provides the most satisfactory explanation for the increased fat storage and what the mechanisms for this

might be. An alternative hypothesis for interpreting mechanisms relating to energy balance and ultimately to understanding the alterations observed in the syndrome of hypothalamic obesity has been suggested by Bray and York (1979) and is labelled the autonomic hypothesis. This hypothesis argues that many of the altered behavioral and metabolic features of the hypothalamic-obese syndrome could be explained in large part by an exaggerated positive-feedback system, initiated by taste receptors and amplified over the parasympathetic nervous system. Imbalances of the parasympathetic and sympathetic nervous are incurred following hypothalamic injury allowing for the parasympathetic component to be hyperactive, and the sympathetic side to reveal reduced responsiveness (Powley, 1977).

PART II

MONOSODIUM GLUTAMATE AND HYPOTHALAMIC OBESITY

Monosodium Glutamate

Monosodium glutamate (MSG) has become associated with an aggregation of clinical manifestations known as the Chinese restaurant syndrome (Ho Man Kwok, 1968; Schaumberg and Byck, 1968). Several incidents occurred in which people became violently ill after having eaten in a Chinese restaurant where MSG is used liberally in the preparation of food. The symptoms included burning sensations, chest pains, headaches and facial pain, which have all been related to the toxicity of MSG (Schaumberg, Byck, Girstle, and Mashman, 1969). The notoriety of the incidences generated a series of investigations into the toxic effects of MSG on a variety of physiological effects.

Glutamic acid is a naturally occurring excitatory neurotransmitter found predominantly in the arcuate nucleus of the hypothalamus (ARH). At approximately 10 mM/Kg, it is the highest concentration of any amino acid within the central nervous system (Olney, 1969b).

Exogenous monosodium glutamate stimulates firing of neurons in many if not all regions of the CNS. The excitatory activity of MSG is shared by aspartate (ASP), cysteic acid (CYS) and certain other structural analogues

(Bakke, Lawrence, Bennet, Robinson, and Bowers, 1978; Curtis and Watkins, 1960; Olney, Sharpe, and Feigen, 1972). These structurally related compounds are thought to be the transmitters released at the majority of excitatory synapses in the mammalian brain (Watkins, 1975).

Lucas and Newhouse (1957) were the first to report that subcutaneous administration of MSG to infant mice resulted in acute degeneration of neurons in the inner layer of retina. Olney (1969a; 1969b) further demonstrated that MSG caused acute necrosis of neurons in the ARH, with some scattered damage visible in the median eminence (ME). The site and the extent of the damage has been confirmed in our laboratory, as well. Hypothalamic lesions could be induced by either oral or subcutaneous administration of MSG (Burde, Schainker, and Kayes, 1971; Cohen, 1967; Perez and Olney, 1972; Price, Olney, Lowry, and Buschbaum, 1981; Olney and Ho, 1970).

It has been observed that the toxicity of MSG results from the depolarizing action impinging selectively upon the dendrosomal portions of the neurons (Olney, 1971; Olney, Ho, and Rhee, 1971; and Olney, Sharpe, and Feigin, 1972). These initial observations of excessive depolarization led to the "excitotoxic" concept (Olney, 1984) which describes the action of MSG on synaptic receptors specialized for glutamatergic (or aspartergic) transmission resulting in death of the neuron.

The susceptibility of the developing nervous system to damage from MSG has been observed in every species of experimental animal tested thus far -- mice, rats, rabbits, dogs, and monkeys (Gordon, 1971; Olney and Sharpe, 1969). Monosodium glutamate lesions are characterized by intracellular edema and neuronal necrosis which develop within hours after treatment even with very low doses (Olney, 1969b). Monosodium glutamate is a unique lesioning technique; it removes specific neuronal cell bodies from ARH without damaging axons terminating in or coursing through the region (Scallet and Olney, 1986; Tanaka, Shimada, Nakao, and Kusunoki, 1978). The degeneration of the ARH is dose-dependent, irreversible and remarkably acute (Holzwarth-McBride, Hurst, and Knigge, 1976; Olney, 1971). There has been no report of damage to any other areas of the hypothalamus identified with the development of obesity (i.e., VMH, LH or paraventricular nucleus), nor is pituitary damage involved, although there are altered functions of the pituitary as a result of the lesion (Miyabo, Ooya, Yamamura, Hayashi, 1982).

Olney (1969) was the first to suspect that hypothalamic lesions might be associated with MSG treatment, when several months after neonatal mice were treated with MSG, for the purpose of inducing retinal pathology (Cohen, 1967), they were found to be quite obese. With evidence of the previously documented cases of hypothalamic damage, Olney

(1969b) hypothesized that the ARH must somehow be involved in the regulation of energy balance.

Studies involving the anatomical characteristics and the accompanying endocrine disturbances of the lesion have been considerably more extensive than the study of the behavioral aspects of the syndrome and the specific mechanisms accountable for the resulting obesity (Bakke et al., 1978; Olney, 1969a, 1969b; Nemeroff, Konkol, Bissette, Youngblood, Martine, Brazeau, Rone, Prange, Brees, and Kizer, 1977; Redding, Schalley, Arimura, and Wakabayashi, 1971).

Comparisons of Hypothalamic Obesity Syndromes

Weight Gain

The MSG-treated animal is characterized by a slow, steady weight gain with the increase in fat deposition noticeable far before the actual weights exceed those of controls (Araujo and Mayer, 1973; Pizzi and Barnhart, 1976), as measured by the "Lee Index" (Bernardis and Patterson, 1968). This suggests that weight alone is not an adequate measurement of fat deposition. This characteristic weight gain has been confirmed in our laboratory. The developmental weight patterns for MSG-treated animals and saline-treated animals are presented in Tables 11 and 12 for females and males, respectively. It is apparent that the MSG-treated animals exhibit the characteristic stunting as

Table 11.

Mean weights (± 95% Confidence Intervals) for all female groups from pilot data (*p < .05).

Age (Days)	MSG-female (N=16)		CONT-female (N=9)
20	8.53 (0.35)	*	10.86 (0.52)
30	14.14 (0.83)	*	20.33 (0.93)
40	19.83 (0.81)	*	24.87 (1.36)
50	23.15 (0.86)	*	27.08 (1.01)
60	26.88 (1.20)		29.01 (1.92)
70	28.51 (1.33)		29.59 (1.42)
80	30.04 (1.48)		30.79 (1.84)
90	34.35 (1.85)		32.65 (1.62)
100	37.12 (1.90)		33.37 (2.29)
110	37.14 (1.76)	*	32.96 (1.52)
120	37.51 (1.65)	*	33.08 (1.39)

NOTE: Between the ages of 90 through 100 days, the animals were given a two-choice taste preference (HFS-diet vs Chow).

Table 12.

Mean weights (± 95% Confidence interval)
for all male groups from pilot data (*p < .05).

Age(Days)	MSG-males (N=7)		CONT-males (N=8)
20	8.59 (0.70)	*	11.17 (0.47)
30	15.43 (2.91)	*	23.86 (0.54)
40	21.77 (2.91)	*	29.04 (0.93)
50	26.12 (3.60)	*	32.29 (1.24)
60	30.70 (3.48)		35.14 (1.02)
70	32.35 (4.52)		35.70 (1.25)
80	34.36 (4.70)		36.92 (1.55)
90	39.53 (4.97)		38.46 (0.92)
100	43.31 (3.92)		39.54 (1.57)
110	43.67 (3.94)	*	39.93 (1.55)
120	44.44 (3.74)	*	39.65 (1.27)

NOTE: Between the ages of 90 through 100 days, the animals were given a two-choice taste preference (HFS-diet vs Chow).

evidenced by the reduced body weights until approximately 90 days of age. When comparisons are made of the overall rates of development by examining the regression formulae, it is obvious that the MSG animals demonstrate the faster rate of growth:

MSG females..... Y = 7.05 + .280(X)
CONT females..... Y = 14.94 + .138(X)
MSG males..... Y = 6.78 + .341(X)
CONT males..... Y = 16.72 + .231(X)

Comparisons of the beta weights (Marascuilo and Levin, 1983) revealed significant differences between MSG-female and CONT-females ($\underline{t}$ (.01, 21)=6.95; and between MSG-male and CONT-male ($\underline{t}$ (.01, 11)=5.12, illustrating that MSG animals have distinctly different growth rates than the controls, and that the differences in weight can not be totally accounted for by the stunting alone. These curves also demonstrate that the MSG animals show no signs of ceasing their rate of development, whereas the normals show the characteristic plateau in their weight gains. Table 13 shows comparisons of trends analysis on the data illustrating further support for the hypothesis that MSG-treated animals exhibit distinctly different growth rates.

Table 13.

Trends analysis* on body weight regression functions with percent variance accounted for in pilot data.

MSG - animals		
Female:	Linear: $F(1, 175)=2323.97$;	88%
	Quad.: $F(1, 175)=128.37$;	5%
	Total variance accounted for =	93%
Male:	Linear: $F(1, 76)=601.64$;	86%
	Quad.: $F(1, 76)=28.38$;	4%
	Total variance accounted for =	90%
CONT - animals		
Female:	Linear: $F(1, 87)=2984.50$;	73%
	Quad: $F(1, 87)= 706.12$;	17%
	Cubic: $F(1, 87)= 58.01$;	1%
	Total variance accounted for =	91%
Male:	Linear: $F(1, 98)=2314.59$;	75%
	Quad: $F(1, 98)= 549.14$;	18%
	Cubic: $F(1, 98)= 68.59$;	2%
	Total variance accounted for =	95%

*All were significant ($p < .0001$).

Food Intake

Quantitative differences. An increase in consumption of 100% or more has been associated with the obesity resulting from VMH, GTG and genetic obesities. This was originally suggested as being a primary factor in the increased fat deposition (Brobeck, 1946; 1960; Kennedy, 1950; Mayer, 1953). An increased food intake has also been associated with hypothalamic obesity in humans (Bray and York, 1971). The augmentation of food intake is not a requirement for the development of obesity and is not seen in lesioned weanling animals or in MSG-lesioned animals (Rabin, 1974). Both of these models have been characterized as hypophagic, yet they maintain high levels of adiposity (Olney, 1969a; Poon and Cameron, 1978). Since the obesity associated with MSG occurs in the absence of hyperphagia, it is assumed that the obesity can be explained in terms of a reduction in energy expenditure (Bunyan, Elspeth, Murrell, and Shah, 1976). The hypophagic tendencies were also confirmed in our laboratory as presented in Table 14. There is very little variation in daily consumption with MSG-treated animals consistently eating less than controls across all time periods.

Regression analyses on consumption revealed significant ($p < .0001$) differences on the intercepts between CONT and MSG, but not the slopes:

Table 14.

Mean consumption (± 95% Confidence interval) for all groups from pilot data (*p < .05).

Age (days)	MSG-female n=16		CONT-female n=9		MSG-male n=7		CONT-male n=8	
20	3.23	(.15) *	4.61	(.16)	3.38	(.54) *	5.17	(.18)
30	3.75	(.12) *	4.37	(.39)	4.04	(.43) *	5.04	(.17)
40	3.68	(.11) *	4.31	(.22)	4.17	(.37) *	4.86	(.28)
50	3.66	(.17) *	4.36	(.23)	4.09	(.47) *	4.84	(.15)
60	3.56	(.22) *	4.45	(.23)	3.96	(.31) *	4.99	(.28)
70	3.39	(.17) *	4.34	(.28)	3.84	(.60) *	4.73	(.18)
80	3.48	(.13) *	4.53	(.28)	3.82	(.62) *	5.00	(.26)
90	3.49	(.17) *	4.74	(.35)	4.05	(.43) *	5.04	(.27)
100	3.99	(.33) *	4.65	(.40)	4.18	(.34) *	5.02	(.20)
110	3.80	(.23) *	4.65	(.28)	3.92	(.20) *	5.00	(.14)
120	3.62	(.14) *	4.65	(.17)	3.99	(.23) *	5.03	(.10)

MSG females..... Y = 3.43 + .003(X)
CONT females..... Y = 4.31 + .003(X)
MSG males..... Y = 3.80 + .002(X)
CONT males..... Y = 4.97 + .000(X)

Trends analysis revealed only a slight linear trend for the MSG females, $F(1,175)=10.10$; $p < .01$. This trend accounted for less than 5% of the variance.

Regardless, an elevation of food intake is not a necessary requirement for the development of obesity as seen in our MSG animals and this has also been reported in weanling VMH-lesioned animals (Rabin,1974). Animals within each of these models have been characterized as having hypophagic tendencies, while maintaining a high level of adiposity (Olney, 1969a; Poon and Cameron, 1978). Since the obesity associated with MSG occurs in the absence of hyperphagia, it is assumed that the obesity must then be accounted for by a reduction in energy expenditure (Bunyan, Murrell, and Shah, 1976). The reduced energy expenditure is also a central part of the VMH-syndrome, GTG-syndrome and genetically-based syndromes (Tanaka et al., 1978), although, it could also be due to a more highly efficient energy utilization (Himms-Hagen, 1984).

Qualitative differences. Qualitative differences also exist following VMH lesions. Normally, food intake follows a biphasic pattern with the largest amount consumed during the night cycle (Brooks, 1946; Inoue and Bray, 1977). After lesioning, there is a loss of diurnality seen with the primary increase in consumption occurring in the day-light cycle (LeMagnen, 1983). This loss of cyclicity has been demonstrated in the weanling-lesioned animal, as well (Bernardis, 1973). This is not essential for the development of obesity. Hales and Kennedy (1964) demonstrated that when food was made available only during the day or night, hyperphagic tendencies persisted and the animals still became obese. This loss of diurnality has not been reported with the MSG-induced obesity.

Most hypothalamic obese syndromes have been associated with the unwillingness to consume quinine-adulterated foods (Ferguson and Keesey, 1976). When a two-choice taste preference paradigm is used to compare the intake of chow and any other highly palatable foodstuff, the lesioned animals show an even stronger preference for the more palatable foods than normal rodents (Carlisle and Stellar, 1969), suggesting the inability to control caloric intake and/or heightened taste responsiveness. This effect was confirmed in our laboratory using a high-fat, high sugar diet (HFS-diet), which has been shown to be a highly preferred food (Sclafani, 1972). The preference (Table 15)

Table 15.

Mean consumption (grams) of HFS, CHOW, Total and %HFS from pilot data (*p < .01).

Diet	MSG-female	CONT-female	MSG-male	CONT-male
HFS	2.94	2.96	3.25	2.81
CHOW	1.05	1.97	0.93	2.22
Total	3.99	4.93	4.18	5.03
%HFS	74.82 *	55.76	77.58 *	55.83

is shown by looking at the percentage of total intake that consisted of the special diet. Our mice consumed approximately 75% of their total daily intake in HFS-diet, compared to the normals which consumed just over 55%. It should be noted that there was a slight (but statistically insignificant) increase in the total amount consumed by the MSG's, whereas, a slight decrease in total amount consumed was evident in the controls. This could have implications for how energy balance is regulated. When the weight gains for the same time periods are examined, it is evident that there is significantly greater increase in weight during this period of time.(Table 16). It may be possible that the excess in calories consumed was enough to account for the increase in weight, or it could be that the weight gain is just part of their overall growth curves, since it is also evident in Tables 11 and 12 that just prior to the introduction of the diet the animals seemed to experience an increase in growth, without the addition of a highly palatable diet. An alternative hypothesis is also in need of examining, and that has to do with the occurrence of increased energy efficiency which may be indicative of a defect in thermogenesis or some defect within intermediary metabolism.

When cellulose (a nonabsorbable agent) is added, the VMH-lesioned animal responds just as the normals, by correcting for the caloric deficit (Kennedy, 1953; 1957).

Table 16.

Change in weight (grams) from pre-diet (Day 90) level and post-diet (Day 100) level from pilot data (*p < .01).

	MSG-female	CONT-female	MSG-male	CONT-male
Day 90	34.35	32.65	39.53	38.46
Day 100	37.52	33.37	43.31	39.54
change	2.77*	.72	3.78*	1.08

NOTE:
MSG-female.....t(15)=8.95*
MSG-male.....t(6) =5.25*
CONT-female.....t(8) =1.68
CONT-male.....t(7) =2.94

There is also a suppression of food intake in response to intragastric feeding (Panskepp, 1971). When gastric loads of any of the macronutrients (fat, protein, or carbohydrate) were administered, feeding was suppressed, but this did not reduce the rate of fat deposition in VMH-lesioned animals (Panskepp, 1972).

Motivational deficits are evident in VMH-lesioned animals as seen in reports of the unwillingness to work for food as a reinforcer (Grossman, 1966; 1967), derangements in avoidance behavior and in the acquisition of fear responses (Colpaert, 1975). When animals are maintained at or below the prelesion weight, there is less finickiness (Beatty, 1973; Sclafani, 1972), increased bar-pressing for food reward (Marks and Remley, 1972), and increased acquisition of learned responses (Sclafani, 1973). These data suggest that the behaviors are the consequence of the obesity and not of the lesion (Beatty, Vilberg, and Shisk, 1975) and therefore these same results would be expected for MSG-treated animals. This holds true for only some of the physiological and behavioral measurements. A number of these are examined more closely below.

Physical Activity and Metabolic Rate

Reduced physical activity occurring in both the adult and weanling rat following VMH lesions was initially interpreted as the major mechanism for the development of obesity (Hetherington and Ranson, 1942). The increased

level of activity usually associated with food deprivation is absent in VMH-lesioned obese rats but an increase still ensues when animals are placed in an environment with lowered ambient temperature (Teitelbaum, 1957).

Beyond the observation that MSG-treated animals are hypophagic, little is known about their feeding behavior. The MSG mouse is assumed to be an example of a metabolic obesity. That is, an obesity that results from a defect in tissue function rather than from failure to control food intake (Araujo and Mayer, 1973). Monosodium glutamate-treated mice exhibit a greater metabolic efficiency than controls (Djazayery, Miller, and Stock, 1973), but a metabolic defect does not preclude the possibility that behavioral defects are present and contribute to the obesity syndrome.

Decreases in activity levels and in thermogenesis could also be factors in the development of obesity. At present, data on activity levels are contradictory. Araujo and Mayer (1973) were the first to demonstrate an unexpected increase in activity using tilt-type activity cages. This finding was supported by Nikoletseas (1977) with the use of wheel-activity cages. Decreases in activity have been reported in wheel rotations (Pradhan and Lynch, 1972), and in open-field behavior (Pizzi and Barnhart, 1976; Pizzi, Tabor, and Barnhart, 1978), while Prabhu and Oester (1971) found no differences in activity levels using tilt-type activity

cages. These contradictions have been attributed to a variety of factors, including: dose of MSG; length of administration; age of administration; and age of testing (Goldman and Stowe, 1985).

Thermogenesis of adipose tissue. Facultative thermogenesis is composed of two components: cold-induced, non-shivering thermogenesis and diet-induced thermogenesis. Brown adipose tissue (BAT) has been postulated to be the major organ site responsible for these (Himms-Hagen, 1976; Rothwell and Stock, 1979). Defects within this tissue have been noted within other hypothalamic, as well as, genetic models of obesity (Perkins, Rothwell, Stock, and Stone, 1981; Takahashi and Shimazu, 1982) as measured by oxygen consumption and heat dissipation.

The cells and blood vessels in the BAT are richly innervated by the sympathetic nervous system. The metabolic activity is regulated primarily by norepinephrine secreted by the nerve supply and by circulating levels of the hormones, insulin and epinephrine (Nicholls, 1984). Oxidation of triglycerides stored in BAT occurs following sympathetic stimulation. Brown adipose tissue mitochondria possess a specific proton conductance pathway by which oxidative phosphorylation can be partially uncoupled from the electron transport system, such that heat rather than adenosine triphosphate (ATP) is produced as a result of fatty acid oxidation.

An increase in thermogenic activity has been postulated as a mechanism that minimizes increased weight gain following high caloric intake. Brown adipose tissue has been implicated for its role in adaptive metabolic variance in obesity. Glick, Teague, and Bray (1981) demonstrated that metabolic and BAT mass can be influenced even after a single meal. Rothwell and Stock (1979) demonstrated that there was an increase in BAT thermogenic activity in chronically over-fed rats, reducing the rate of weight gain. Feeding a highly palatable, energy dense "cafeteria diet" has also been shown to increase BAT thermogenic activity (Sclafani and Springer, 1976).

The mechanism for the thermogenic activity of BAT is related to the process of oxidative phosphorylation, which occurs in the mitochondria of all cells. This process is controlled primarily through the glycolytic and gluconeogenic pathways. Glycolysis is a multiple chain reaction that converts glucose to pyruvate which is a precursor to a molecule that can enter the energy-yielding tricarboxylic acid (TCA) cycle, also known as Kreb's cycle (Gorbman, Dickhoff, Vigna, Clark and Ralph, 1983). Through glycolysis, glucose is converted to cellular energy. Changes in activity of rate-limiting or key enzymes along the pathway would be indicative of the oxidative-phosphorylation process, which ultimately, is an indicator of metabolism. In addition to glycolysis, there can be a

conversion of cellular energy back to its original form of glucose, by the process of gluconeogenesis, which is the conversion of non-carbohydrate sources, fatty acids and amino acids, into glucose (Gorbman et al., 1983).

Endocrine System

The hypothalamus secretes specific releasing hormones which are carried to the anterior pituitary gland via the hypophyseal portal vessels where they exert certain influences on the synthesis and release of trophic hormones. Chemical or physical damage to the hypothalamus may result directly in changes in the content and release of hormones. Consequently, there could be alterations in the respective target endocrine organs. Decreased weights of pituitaries, along with decreased weights of thyroid, adrenals, and gonads have all been reported in MSG-induced obesity (Redding, et al., 1971).

Pancreatic hormones. Animals made obese by electrolytic destruction of the ventromedial region of the hypothalamus or by injection of gold-thioglucose (GTG) share some of the endocrine disturbances of the MSG mouse (Frohman and Bernardis, 1968). Hyperinsulinemia and enlarged pancreatic islets are well documented for hypothalamic obese syndromes (Hales and Kennedy, 1964; Kennedy and Parker, 1963). The hyperinsulinemia is the direct result of the hypothalamic damage and is thought to be responsible for the

hyperphagic tendencies (Bernardis and Frohman 1970; 1971; Frohman, Goldman, Schnatz, and Bernardis, 1971).

The characteristic rise in insulin following meal consumption consists of two phases: an anticipatory phase (Epstein, 1959; Powley, 1977), dependent upon an intact vagus nerve (Louis-Sylvestre, 1975) and a later rapid rise with food absorption (Strubbe and Steffens, 1975). The insulin released in both of these phases is enhanced following VMH lesioning (Inoue, Campfield, and Bray, 1977; Steffens, 1969; 1970). Steffens (1970) suggested that the rise in insulin may play the most important role in the development of the syndrome of obesity as it relates to the control of the glycolytic and gluconeogenic pathways. Marks and Davison (1976) demonstrated that animals treated with insulin and VMH-lesioned animals were comparable on measures of activity, taste preference, weight gain, and fat deposition. Steffens (1969) further demonstrated an increase in food intake during the static phase of weight gain following intravenous infusions of insulin. While there are several lines of evidence implicating insulin in a pathogenic role (Friedman, 1972; Inoue and Bray, 1978; Vilberg, and Beatty, 1975; York and Bray, 1972), it is important to point out that if destruction of pancreatic islets occurs after the development of obesity, there is no reversal of the existing obesity (Inoue, Bray and Mullen, 1978).

Reports on glucagon concentrations have been inconsistent following hypothalamic damage. Inoue et al. (1977) found reduced levels, implicating the involvement of the sympathetic nervous system in hypothalamic obesity. Bernardis, Rosen, Goldman and Martin (1977) found no changes whereas, Karakash, Hustvedt, Lovo, Lemarchand and Jeanrenaud (1977) found higher levels. These differences may only reflect assay variations.

Scallet and Olney (1986) have noted that the ARH contains the primary binding sites for insulin and that with the MSG syndrome there are also the increased levels of insulin, which is not consistent with the lowered intake if the above hypothesis of insulin involvement is correct. Badillo-Martinez, Nicotera, Buttler, Kirchgessner, and Bodnar (1984) have suggested that the negative feedback loops associated with insulin regulation may by disrupted by damage to the ARH.

The MSG-animal shows an increase in the glucagon to insulin ratio, indicative of ketogenesis. This problem becomes even more noticeable under fasting conditions, whereby, high insulin levels are not suppressed during fasting (Cameron, Cutbush and Opat, 1978), and both syndromes have been noted as having "transient" hyperglycemia. The VMH syndromes have shown some signs of insulin resistance and glucose intolerance (LeMagnen, 1984). On the contrary, MSG animals seem to show a hyper-

responsiveness to both exogenous and endogenous insulin (Nemeroff, et al., 1977).

Growth and growth hormone. A consistent observation of animals with hypothalamic damage in the ventromedial region is stunted growth (Bernardis and Skelton, 1965/1966; Han and Liu, 1966; Han, Liu, and Lepkovsky, 1963; Hetherington and Ranson, 1940) and is directly related to the size of the lesion (Bernardis and Frohman, 1970). Diminished growth has also been observed in humans with hypothalamic obesity (Bray and Gallagher, 1975) and after MSG administration (Araujo and Mayer, 1973; Pizzi and Barnhart, 1976; Redding, et al., 1971). Nagasawa, Yanai, and Kikuyama (1974) have suggested that the metabolic imbalance associated with the reduction of pituitary secretion of growth hormone (GH), a lipolytic hormone, is responsible for the development of obesity. When there is an alleviation of circulating GH and pituitary content of GH, there will be an elevation in fat deposition (Cameron, Cutbush, and Opat, 1978; DePaolo and Steger, 1985). It is necessary to point out that stunting and diminished GH secretion does not occur following hypothalamic damage using knife cuts (Palka, Liebelt, and Critchlow, 1971), implying that altered growth hormone secretion can not be the sole mechanism responsible for the development of obesity.

Pituitary-adrenal axis. The effect on adrenocorticotropin hormone (ACTH) following damage to the

VMH has not been examined closely. A few have examined the involvement of the pituitary-adrenal axis by performing adrenalectomies (Mook, Fisher, Durr, 1975) and by using replacement doses of corticosteroids (Wilson, 1977). There was little, if any effect of either one. Wilson (1977) did note that a pharmacological dose of methylprednisone, a synthetic steroid, completely reversed the obesity in weanling VMH-lesioned animals.

On the other hand, with MSG-induced damage, there has been a more extensive investigation into the hypothalamic regulation of pituitary functioning (Nemeroff et al., 1977; Nemeroff, Lamartiniere, Mason, Squibb, Hong, and Bondy 1981). Monosodium glutamate selectively destroys the hypothalamic area (ARH) containing the only neuronal bodies which contain ACTH, beta-lipotropin, and beta-endorphin (Bodnar, Abrams, Zimmerman, Kreiger, Nicholson, and Kizer, 1980). Destruction of the ARH results in reduced ACTH and beta-lipotropin within the hypothalamus, but not in the pituitary, with no alterations visible in plasma ACTH (Bodnar et al., 1980; Kreiger and Liotta, 1979).

Despite the gross optic atrophy, the animals all have shown circadian rhythms entrained to the light-dark cycle, indicating that the retinohypothalamic tract is not the primary tract essential for light entrainment (Miyabo, Ooya, Yamamura, and Hayashi, 1982). Nemeroff and colleagues (1977) suggested that the rhythms remain intact because the

accessory optic tracts which project to the suprachiasmatic nuclei (SCN) are preserved.

Destruction of ARH neurons causes a loss of endorphinergic pathways, thereby inducing an up-regulation of post-synaptic opiate receptors in the midbrain (Simantov and Amir, 1983). This results in a hyper-responsiveness to both morphine and naltrexone. Destruction of endorphinergic system and adrenal axis would lend itself to altered behavioral outcomes after painful or stressful situations. There is a reduction in the analgesic response typically following cold-water swims and other stressors (Badillo-Martinez et al., 1984; Badillo-Martinez, Nicotera, and Bodnar, 1984).

Pituitary-gonadal axis. A frequently noted abnormality exhibited in the syndrome of hypothalamic obesity is diminished reproductive capability (Nemeroff et al., 1981). Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secretion from the pituitary are controlled through the release of luteinizing hormone-releasing hormone (LHRH), which is found predominantly in the arcuate nucleus. Premature sexual development in females and loss of estrous cycles are often the consequence of hypothalamic damage (Kennedy and Mitra, 1963; Trentini, Botticelli, and Botticelli, 1973). The degree of gonadal atrophy in the male is dependent on the amount of hypothalamic damage

(Gladfelter, 1971; Olney, Cicero, Meyer, and deGubareff, 1976).

The LHRH terminals appear to be hyper-responsive to potassium and prostaglandin stimulation, indicative of defective mechanisms for triggering LHRH release (DePaolo and Negro-Vilar, 1982). Several studies have intimated that the hyperresponsiveness of the LHRH terminal is due to the denervation of dopamine (DA) neurons in the ARH (Dada and Blake, 1985; Inkster and Whitehead, 1983; Inkster, Clayton, and Whitehead, 1985). Dopamine is the most potent inhibitory agent for prolactin secretion. Accordingly, the loss of DA neurons results in an increase in prolactin (Heiman and Ben-Jonathan, 1983).

Pituitary-thyroid axis. A number of abnormalities of thyroid function have been revealed in VMH-lesioned animals: a reduced thyroxine secretion (Himms-Hagen, 1976); reduced thyroid weight and thyroid cell size, suggestive of decreased thyroid-stimulating hormone (TSH) (Seidman, Horland and Teebor, 1967); hypometabolism, (York, Hershman, Utiger, and Bray, 1972) and reduced uptake and release of radioactively labelled iodine (Bray and York, 1971).

MSG-induced obese animals have normal levels of TSH and no evidence of hypothyroidism (Nemeroff et al., 1981), which would be indicative of normal metabolic patterns.

Using three doses of MSG administration, a marked reduction in endochondrial bone, incomplete ossification,

and reduced cartilage development were demonstrated, with the severity increasing as the dose increased (Dhindsa, Omran, Riff, and Bhup, 1978). Dhindsa et al. (1978) attributed the decreased bone deposition as the result of disruptions in the balance of parahormone and calcitonin which is necessary for the proper histogenesis of bone to occur.

Autonomic nervous system. Many physiological processes are partly or entirely under the control of sympathetic and/or parasympathetic systems. The process of energy balance and regulation falls into this category (Dulloo and Miller, 1986). The sympathetic nervous system activity in innervated organs can be estimated by the measurement of norepinephrine (NE) turnover. Following hypothalamic damage, the turnover rate of NE is reduced in the heart and interscapular brown adipose tissue (IBAT), but not in the pancreas (Yoshida, Nishioka, Nakamura, and Kondo, 1984; Yoshida, Nishioka, Nakamura, Kanatsuma, and Kondo, 1985). A decreased rate of NE turnover is also correlated with hypophagia, whereas an increased NE turnover is identified with hyperphagia and sucrose feeding (York and Bray, 1972). This represents a contradiction for the autonomic hypothesis since the eating behavior of VMH-lesioned animals is characterized as hyperphagic. The hypothesis remains tenable for the MSG-induced obese syndrome.

Electrical activity and release of catecholamines are affected by the nutritional status and acute changes in circulating levels of insulin and glucose (Young and Landsberg, 1980). Insulin levels are directly under the control of the parasympathetic nervous system via the vagus nerve. Repeatedly, the importance of the integrity of the vagus has been demonstrated; if vagotomy is performed following VMH-lesioning, there is a reversal of the obesity (LeMagnen, 1984).

A brief summary of the most salient characteristics relating to hypothalamic damage are presented in Table 17.

Table 17.

Summary of characteristics of three hypothalamic models of obesity.

Feature	MSG	VMH-A	VMH-W
Obesity	+++	+++	+++
Hyper-phagia	-	+++	-
Finicki-ness	+	+++	++
Hyper-glycemia	±	+	±
Hyper-insulinemia	++	+++	++
Insulin Resistance	-	++	++
Thermogenesis*			
DIT	++(?)	-	-
TRT	- (?)	++	++

*DIT = Diet-induced; TRT = Non-shivering;

(+++) Severe; (++) Moderate; (+) Mild; (±) Variable; (-) Absent;

Note: VMH-A = Adult-lesioned animal;
VMH-W = Weanling-lesioned animal;

APPENDIX B

DESIGN AND HYPOTHESES

Experimental Design
CRF-222

	A_1=MSG		A_2=CONT	
	C_1=HFS	C_2=CHOW	C_1=HFS	C_2=CHOW
B_1= FEMALE	n=14	n=14	n=14	n=14
B_2= MALE	n=14	n=14	n=14	n=14

Figure 15. Completely randomized 2 (A: neonatal treatment) x 2 (B: sex) x 2 (C: diet) design.

Hypotheses

Consumption

All MSG-treated animals will consume less food across all time periods when compared to appropriate saline-treated controls.

While more Kcal/day will be consumed from the HFS diets, the overall consumption will be less.

Females will consume more Kcal/day on the HFS-diet, than males.

Body Weight

MSG-CHOW animals, both males and females, will weigh less than all other groups across all time periods measured. The MSG-HFS groups, while their beginning weights will be lower, will surpass their appropriate saline-treated control groups, such that, at the end of time period of measurement they will weigh more than CONT-HFS groups.

All males will be heavier than females.

The regression functions for MSG-CHOW will be less than CONT-CHOW's, while the MSH-HFS's will show an increase in the rate of weight gain, above all groups.

The absolute changes in body weight will be far greater for the MSG-HFS groups than all other groups, followed by the CONT-HFS male and females groups.

Body Length

MSG animals will be significantly shorter across all time periods of measurement as will be shown with the regression functions.

The absolute changes of body length growth will remain less for all MSG animals. A difference in growth will also be seen in the interaction with diet. That is, the CHOW animals will show lower growth rates, than the HFS-groups.

Lee Indices

The MSG-treated animals will show higher Lee indices across all time periods of measurement.

All HFS-animals have higher indices of obesity. The highest Lee index recording will be found with the MSG-FEM-HFS group.

Physiological Measures

From previous recordings of plasma glucose being higher in the MSG animals, it is thought that these recordings will likewise be higher than controls, especially for those animals within the high-fat, high-sugar diet groups. At this time, actual levels of activity for the enzymes can not be made, however, some calculated guesses will be made, based on general knowledge of the response of these particular enzymes to nutritional conditions.

Glucokinase (GK) activity is expected to be lower in the HFS-fed groups, since there tends to be a reduction in insulin-regulated pathways for glucose metabolism under "fat-fed" conditions.

Pyruvate kinase (PK) activity is hypothesized as being lower in the high caloric conditions. This enzyme plays a major role in the regulation of gluconeogenesis. Therefore, under conditions of high carbohydrate-feeding, activity of this enzyme should decrease.

The activity level of G6PDH is suggested as being very high for those animals in the HFS diet conditions. This will give a strong indication for an increase in lipogenesis.

Malate dehydrogenase activity is also going to show an increase in those groups fed HFS and in the MSG animals, which will also serve as an indicator of increased lipogenic activity.

APPENDIX C

EXTRA STATISTICAL TABLES

Table 18.

Source table for analysis of the dependent variable of consumption (gr/day).

Source	df	MS	F
Treatment	1	33.24	239.54**
Sex	1	1.08	7.80*
Diet	1	51.54	371.40**
Treatment * Sex	1	12.98	93.52**
Treatment * Diet	1	2.82	20.35**
Sex * Diet	1	1.27	9.14*
Treat * Sex * Diet	1	1.63	11.75*
Error	104	.14	

* $p < .01$ ** $p < .0001$

Table 19.

Absolute value difference between means for diet consumption (gr/day).

MS_{error} = 0.14; p = 8; n = 14; C.V. = 0.44; (*p < .05)

Group Mean (+SEM)	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
(1) MSG-FEM-HFS 4.60 (.09)	----	----	----	----	----	----	----	----
(2) MSG-FEM-CHOW 5.61 (.13)	10.1*	----	----	----	----	----	----	----
(3) CONT-FEM-HFS 5.83 (.12)	12.3*	2.20*	----	----	----	----	----	----
(4) CONT-FEM-CHOW 6.97 (.13)	23.7*	13.6*	11.4*	----	----	----	----	----
(5) MSG-MALE-HFS 5.06 (.10)	4.60*	5.50*	7.70*	19.1*	----	----	----	----
(6) MSG-MALE-CHOW 6.13 (.14)	15.3*	5.20*	3.00*	8.40*	10.7*	----	----	----
(7) CONT-MALE-HFS 5.39 (.13)	7.90*	2.20*	4.40*	15.8*	3.30*	7.40*	----	----
(8) CONT-MALE-CHOW 6.64 (.08)	20.4*	10.3*	8.10*	3.30*	15.8*	5.10*	12.5*	----

Table 20.

Source table for analysis of the dependent variable of consumption (Kcal/gr).

Source	df	MS	F
Treatment	1	553.49	217.99**
Sex	1	14.72	5.80@
Diet	1	244.91	96.46**
Treatment * Sex	1	221.15	87.10**
Treatment * Diet	1	7.97	3.14
Sex * Diet	1	19.51	7.68*
Treat * Sex * Diet	1	9.89	3.89
Error	104	2.54	

@ $p < .05$ * $p < .01$ ** $p < .0001$

Table 21.

Source table for analysis of the changes in mean body weight from Day 40 to Day 60.

Source	df	MS	F
Treatment	1	235.39	41.45**
Sex	1	.21	0.04
Diet	1	339.54	59.79**
Treatment * Sex	1	2.41	0.42
Treatment * Diet	1	65.84	11.59*
Sex * Diet	1	12.26	2.16
Treat * Sex * Diet	1	2.20	0.39
Error	104	5.68	

* $p < .01$ ** $p < .0001$

Table 22.

Absolute differences between means for body weight changes for females from Day 40 to Day 60.

MS_{error} = 5.68; p = 4; n = 14; C.V. = 2.63 (*p < .05)

Group	(1)	(2)	(3)	(4)
(1) MSG-FEM-HFS (8.71)	----	----	----	----
(2) MSG-FEM-CHOW (3.31)	8.43*	----	----	----
(3) CONT-FEM-HFS (4.85)	6.03*	2.41	----	----
(4) CONT-FEM-CHOW (1.96)	10.6*	2.11	4.52*	----

Table 23.

Absolute differences between means for body weight changes for males from Day 40 to Day 60.

MS_{error} = 5.68; p = 4; n = 14; C.V. = 2.63 (*p < .05)

Group	(5)	(6)	(7)	(8)
(5) MSG-MALE-HFS (8.71)	----	----	----	----
(6) MSG-MALE-CHOW (4.07)	7.25*	----	----	----
(7) CONT-MALE-HFS (3.70)	7.83*	0.58	----	----
(8) CONT-MALE-CHOW (2.70)	9.39*	2.14	1.56	----

Table 24.

Source table for analysis of the changes in mean body length from Day 40 to Day 60.

Source	df	MS	F
Treatment	1	2.80	53.32**
Sex	1	.04	.75
Diet	1	.27	5.15@
Treatment * Sex	1	.11	2.08
Treatment * Diet	1	.15	2.86
Sex * Diet	1	.00	.04
Treat * Sex * Diet	1	.25	4.78@
Error	104	.05	

@ $p < .05$ ** $p < .0001$

Table 25.

Source table for analysis of the mean changes in Lee indices from Day 40 to Day 60.

Source	df	MS	F
Treatment	1	.0067	60.64**
Sex	1	.0000	.27
Diet	1	.0012	10.86*
Treatment * Sex	1	.0001	.85
Treatment * Diet	1	.0001	1.17
Sex * Diet	1	.0002	1.81
Treat * Sex * Diet	1	.0002	1.76
Error	104	.00011	

@ $p < .05$ ** $p < .0001$

Table 26.

Absolute differences between mean changes in Lee indices for females from Day 40 to Day 60.

MS_{error} = 0.0001; p = 4; n = 14; C.V. = .0098 (*p < .05)

Group	(1)	(2)	(3)	(4)
(1) MSG-FEM-HFS (.0039)	----	----	----	----
(2) MSG-FEM-CHOW (-.0101)	5.19*	----	----	----
(3) CONT-FEM-HFS (-.0182)	8.19*	3.00*	----	----
(4) CONT-FEM-CHOW (-.0226)	9.82*	4.63*	1.63*	----

Table 27.

Absolute differences between mean changes in Lee indices for males from Day 40 to Day 60.

MS_{error} = 0.0001; p = 4; n = 14; C.V. = 0.0098 (*p < .05)

Group	(5)	(6)	(7)	(8)
(5) MSG-MALE-HFS (-.0043)	----	----	----	----
(6) MSG-MALE-CHOW (-.0077)	1.26*	----	----	----
(7) CONT-MALE-HFS (-.0174)	4.85*	3.59*	----	----
(8) CONT-MALE-CHOW (-.0217)	6.44*	5.19*	1.60*	----

Table 28.

Source table for the analysis plasma glucose.

Source	df	MS	F
Treatment	1	4448.85	48.93**
Sex	1	7016.26	77.16**
Diet	1	10811.25	118.90**
Treat * Sex	1	1858.21	20.44**
Treat * Diet	1	761.87	8.38*
Sex * Diet	1	26.06	.29
Treat * Sex * Diet	1	343.04	3.77
Within (error)	72	90.93	
Total	79		

* $p < .01$ ** $p < .0001$

Table 29.

Absolute value difference between means for plasma glucose.

MS_{error} = 90.93; p = 8; n = 10; C.V.= 16.89; (*p < .05)

Group Mean (+SEM)	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
(1) MSG-FEM-HFS 151.32 (.88)	----	----	----	----	----	----	----	----
(2) MSG-FEM-CHOW 118.90 (.98)	10.7	----	----	----	----	----	----	----
(3) CONT-FEM-HFS 165.56 (.94)	4.72	15.5	----	----	----	----	----	----
(4) CONT-FEM-CHOW 153.77 (.97)	0.81	11.6	3.90	----	----	----	----	----
(5) MSG-MALE-HFS 176.69 (1.01)	8.40	19.1*	3.69	7.59	----	----	----	----
(6) MSG-MALE-CHOW 150.27 (.91)	0.35	10.4	5.06	0.93	8.75	----	----	----
(7) CONT-MALE-HFS 179.93 (1.32)	9.47	20.2*	4.76	8.66	1.07	9.82	----	----
(8) CONT-MALE-CHOW 157.57 (1.05)	2.07	12.8	2.65	1.26	6.33	2.42	7.40	----

Table 30.

Source table for the analysis of G6PDH activity.

Source	df	MS	F
Treatment	1	9.30	7.17*
Sex	1	2.37	1.83
Diet	1	25.09	19.35**
Treat * Sex	1	1.20	.92
Treat * Diet	1	3.57	2.76
Sex * Diet	1	1.37	1.06
Treat * Sex * Diet	1	25.25	19.47**
Within (error)	72	1.29	
Total	79		

* $p < .01$ ** $p < .0001$

Table 31.

Absolute differences between means for G6PDH activity for females.

MS_{error} = 1.29; p = 4; n = 10; C.V. = 1.56 (*p < .05)

Group Mean (+SEM)	(1)	(2)	(3)	(4)
(1) MSG-FEM-HFS 8.67 (0.36)	----	----	----	----
(2) MSG-FEM-CHOW 9.36 (0.34)	1.92*	----	----	----
(3) CONT-FEM-HFS 9.29 (0.33)	1.72*	0.19	----	----
(4) CONT-FEM-CHOW 6.88 (0.32)	4.97*	6.89*	6.69*	----

Table 32.

Absolute differences between means for G6PDH activity for males.

MS_{error} = 1.29; p = 4; n = 10; C.V. = 1.56 (*p < .05)

Group Mean (+SEM)	(5)	(6)	(7)	(8)
(5) MSG-MALE-HFS 9.46 (0.33)	----	----	----	----
(6) MSG-MALE-CHOW 7.38 (0.35)	5.78*	----	----	----
(7) CONT-MALE-HFS 8.33 (0.33)	3.13*	2.64*	----	----
(8) CONT-MALE-CHOW 7.64 (0.34)	5.06*	0.72*	1.92*	----

Table 33.

Source table for the analysis of glucokinase activity.

Source	df	MS	F
Treatment	1	9.05	30.89**
Sex	1	1.38	4.69@
Diet	1	17.07	58.24**
Treat * Sex	1	7.38	25.17**
Treat * Diet	1	106.01	361.73**
Sex * Diet	1	.58	1.93
Treat * Sex * Diet	1	6.56	22.39**
Within (error)	72	.29	
Total	79		

@p < .05 * p < .01 ** p < .0001

Table 34.

Absolute value difference between means for glucokinase activity.

MS_{error} = 0.29; p = 8; n = 14; C.V. = 0.90; (*p < .05)

Group Mean (+SEM)	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
(1) MSG-FEM-HFS 4.98 (.23)	----	----	----	----	----	----	----	----
(2) MSG-FEM-CHOW 8.94 (.23)	23.5*	----	----	----	----	----	----	----
(3) CONT-FEM-HFS 6.57 (.26)	9.35*	13.9*	----	----	----	----	----	----
(4) CONT-FEM-CHOW 4.80 (.23)	1.06*	24.4*	10.4*	----	----	----	----	----
(5) MSG-MALE-HFS 5.37 (.23)	2.29*	21.0*	7.06*	3.35*	----	----	----	----
(6) MSG-MALE-CHOW 7.86 (.24)	16.9*	6.35*	7.59*	18.0*	14.7*	----	----	----
(7) CONT-MALE-HFS 7.04 (.22)	12.1*	11.2*	2.76*	13.2*	9.80*	4.82*	----	----
(8) CONT-MALE-CHOW 6.06 (.23)	6.35*	16.9*	3.00*	7.41*	4.06*	10.6*	5.77*	----

Table 35.

Source table for the analysis pyruvate kinase activity.

Source	df	MS	F
Treatment	1	21.25	8.89**
Sex	1	5.43	2.27
Diet	1	1.10	.46
Treat * Sex	1	6.06	2.53
Treat * Diet	1	.29	.12
Sex * Diet	1	19.43	8.13*
Treat * Sex * Diet	1	40.94	17.13**
Within (error)	72	2.39	
Total	79		

* $p < .01$ ** $p < .0001$

Table 36.

Absolute difference between means
for pyruvate kinase activity for females.

MS_{error} = 2.39; p = 4; n = 10; C.V. = 2.12 (*p < .05)

Group Mean (+SEM)	(1)	(2)	(3)	(4)
(1) MSG-FEM-HFS 5.36 (0.42)	----	----	----	----
(2) MSG-FEM-CHOW 8.13 (0.44)	6.65*	----	----	----
(3) CONT-FEM-HFS 5.33 (0.41)	0.06	5.71*	----	----
(4) CONT-FEM-CHOW 5.00 (0.41)	0.74	6.39*	0.67	----

Table 37.

Absolute differences between means
for pyruvate kinase activity for males.

MS_{error} = 2.39; p = 4; n = 10; C.V. = 2.12 (*p < .05)

Group Mean (+SEM)	(5)	(6)	(7)	(8)
(5) MSG-MALE-HFS 6.71 (0.36)	----	----	----	----
(6) MSG-MALE-CHOW 4.64 (0.35)	4.22*	----	----	----
(7) CONT-MALE-HFS 4.91 (0.40)	3.67*	0.55	----	----
(8) CONT-MALE-CHOW 5.47 (0.35)	2.53*	1.69	1.14	----

Table 38.

Source table for the analysis of malate dehydrogenase activity.

Source	df	MS	F
Treatment	1	7249.10	38.18**
Sex	1	1133.04	5.97*
Diet	1	2863.58	15.08*
Treat * Sex	1	8222.53	43.31*
Treat * Diet	1	666.72	3.51
Sex * Diet	1	8912.01	46.94**
Treat * Sex * Diet	1	17278.68	91.01**
Within (error)	72	189.85	
Total	79		

* $p < .01$ ** $p < .0001$

Table 39.

Absolute value difference between means for malate dehydrogenase activity.

MS_{error} = 189.85; p = 8; n = 10; C.V. = 23.22; (*p < .05)

Group Mean (+SEM)	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
(1) MSG-FEM-HFS 147.01 (1.11)	----	----	----	----	----	----	----	----
(2) MSG-FEM-CHOW 191.32 (1.27)	10.6	----	----	----	----	----	----	----
(3) CONT-FEM-HFS 142.86 (1.14)	0.95	11.1	----	----	----	----	----	----
(4) CONT-FEM-CHOW 116.86 (1.23)	6.92	18.1	5.96	----	----	----	----	----
(5) MSG-MALE-HFS 169.71 (1.06)	5.21	4.96	6.16	12.2	----	----	----	----
(6) MSG-MALE-CHOW 113.01 (1.11)	7.80	18.0	6.85	0.88	13.0	----	----	----
(7) CONT-MALE-HFS 147.33 (1.22)	0.07	10.1	1.03	6.99	5.13	7.87	----	----
(8) CONT-MALE-CHOW 137.87 (1.20)	2.10	12.3	1.15	4.82	7.30	5.70	2.17	----

Table 40.

Correlations (Pearson) among physiological variables for MSG-FEM-HFS Group.

Variables	Glucose	G6PDH	GK	PK	MDH
Glucose	1.00	---	---	---	---
G6PDH	0.04	1.00	---	---	---
GK	0.15	-0.75*	1.00	---	---
PK	0.66@	0.56	-0.35	1.00	---
MDH	0.12	0.21	-0.03	0.38	1.00

@ $p < .05$ * $p < .01$

Table 41.

Correlations (Pearson) among physiological variables for MSG-FEM-CHOW Group.

Variables	Glucose	G6PDH	GK	PK	MDH
Glucose	1.00	---	---	---	---
G6PDH	0.24	1.00	---	---	---
GK	-0.82*	-0.33	1.00	---	---
PK	-0.07	0.85*	-0.11	1.00	---
MDH	-0.51	0.25	0.29	0.46	1.00

* $p < .01$

Table 42.

Correlations (Pearson) among physiological variables for MSG-MALE-HFS Group.

Variables	Glucose	G6PDH	GK	PK	MDH
Glucose	1.00	---	---	---	---
G6PDH	-0.06	1.00	---	---	---
GK	0.27	-0.17	1.00	---	---
PK	0.11	0.49	-0.15	1.00	---
MDH	0.18	0.37	0.00	0.57	1.00

Table 43.

Correlations (Pearson) among physiological variables for MSG-MALE-CHOW Group.

Variables	Glucose	G6PDH	GK	PK	MDH
Glucose	1.00	---	---	---	---
G6PDH	0.19	1.00	---	---	---
GK	0.25	0.05	1.00	---	---
PK	0.11	-0.24	0.16	1.00	---
MDH	0.13	0.24	-0.36	0.01	1.00

Table 44.

Correlations (Pearson) among physiological variables for CONT-FEM-HFS Group.

Variables	Glucose	G6PDH	GK	PK	MDH
Glucose	1.00	---	---	---	---
G6PDH	-0.05	1.00	---	---	---
GK	-0.18	0.38	1.00	---	---
PK	-0.60	0.63@	0.40	1.00	---
MDH	-0.14	0.49	0.27	0.72@	1.00

@ $p < .05$

Table 45.

Correlations (Pearson) among physiological variables for CONT-FEM-CHOW Group.

Variables	Glucose	G6PDH	GK	PK	MDH
Glucose	1.00	---	---	---	---
G6PDH	-0.31	1.00	---	---	---
GK	0.16	0.47	1.00	---	---
PK	0.35	0.34	0.78*	1.00	---
MDH	0.21	0.46	0.59	0.55	1.00

* $p < .01$

Table 46.

Correlations (Pearson) among physiological variables for CONT-MALE-HFS Group.

Variables	Glucose	G6PDH	GK	PK	MDH
Glucose	1.00	---	---	---	---
G6PDH	-0.17	1.00	---	---	---
GK	-0.30	0.31	1.00	---	---
PK	-0.05	0.78*	0.55	1.00	---
MDH	0.29	0.68@	0.07	0.70@	1.00

@ $p < .05$ * $p < .01$

Table 47.

Correlations (Pearson) among physiological variables for CONT-MALE-CHOW Group.

Variables	Glucose	G6PDH	GK	PK	MDH
Glucose	1.00	---	---	---	---
G6PDH	-0.45	1.00	---	---	---
GK	0.46	-0.07	1.00	---	---
PK	-0.61	0.26	0.11	1.00	---
MDH	0.06	0.38	0.42	0.19	1.00