FOOD INTAKE, BODY WEIGHT GAIN, AND BODY COMPOSITION OF THE YOUNG OBESE (ob/ob) MOUSE

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ABSTRACT

FOOD INTAKE, BODY WEIGHT GAIN, AND BODY COMPOSITION
OF
THE YOUNG OBESE (ob/ob) MOUSE

BY
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Estimates of food intake and body weight gain were obtained in obese (ob/ob) mice from 7 to 56 days of age. Milk intake was daily estimated from 7 to 21 days of age by removing the pups from their mothers' cage for 6 hours, then returning them and measuring their increase in body weight during the next hour for nursing. There was no difference between obese and lean mice in milk intake. From 14 to 21 days of age, obese mice gained more body weight than lean mice. At 21 days of age, obese mice contained about twice as much body fat as lean mice did, whereas the body protein content was not different. Mice were weaned at 21 days of age, and individually fed a commercial diet or a high-fat diet. During the first several days after weaning, obese males failed to consume as much food as did the lean mice. Obese mice consumed equal quantities of food, but gained more body weight than lean mice from 35-42 days of age for males, or from 28-35 days of age for females when they were fed the commercial diet. After that, obese mice consumed more food and gained more weight. Obese mice fed the high-fat diet started to eat more food and gain more weight from 28 days of age.

Pi-Yao Lin

At 56 days of age, the carcass of obese mice contained 4-5 times as much fat as did lean mice, but contained significantly less protein than lean mice. For the 5 week post-weaning period, obese mice converted about 3-4 times as much dietary energy to body energy as did lean mice, whereas obese mice consumed only 20-40% more energy. At the same time, obese mice converted only about 70% as much dietary protein to body protein as did lean mice. The high-fat diet enhanced the energy efficiency in mice. The present studies suggest that alterations in energy metabolism, as well as protein metabolism may play a primary role in the development of obesity in these mice. Hyperphagia may be of secondary importance.

Key indexing workd: food intake, body weight gain, body composition, obesity, ob/ob mice.

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INTRODUCTION

I. OBESITY

Obesity, which can be defined as an excessive accumulation of adipose tissue in the body(1,2), is a common and serious nutritional disease especially in affluent countries. The prevalence of obesity in children and adolescents depends upon various socioeconomic conditions. It is estimated that about 2.7% of all school children are obese in England, about 2% in Sweden, about 8% in Canada, and about 11% in the United States(reviewed in Ref. 3). The importance of childhood obesity as a forerunner of obesity in adult life has been documented. Approximately 80% of all obese children remain so as in adults(2,4). If adults who exceed 20 to 25% body fat are classified as obese, then, about 15 to 40% of the adult population in the United States would be classified as obese(5). The data from various other countries are based on somewhat different criteria of obesity and thus are not strictly comparable with the U.S. data. Roughly, 20-40% of the adult population in industrial countries suffer this nutrition related problem.

Obesity is, or at least is suspected to be a risk factor in many disorders including diabetes mellitus(6) and cardiovascular diseases such as vascular lesions of the central nervous system, hypertension and coronary heart disease(6,7). The prominence of stria blood vessels are common in the grossly obese. A few obese develop carbon dioxide insensitivity, hypoventilation, uneven ventilation and somnolence(8). In obesity, there is an

increase in the severity of complications of established diseases as well as an enhanced risk during surgery and recovery(9). Psychological disorders may be initiated of enhanced by obesity, and these disorders may alter metabolic patterns.

Obesity has been classified according to the following mechanisms, although some overlapping exists, a) regulatory or metabolic(10-12), b) hyperplastic or hypertrophic adipocyte growth(10,13), c) period of onset as juvenile, mature, or pregnancy(13,14), and d) actual stage of obesity as static or dynamic(13). Regulatory obesity is a result of a primary impairment in the regulation of food intake; and metabolic obesity is caused by an inborn or acquired error in the regulation of metabolism in tissues other than those directly regulating food intake. With respect to the development of the adipose tissue, hypertrophic obesity is described as a marked cell enlargement; and hyperplastic obesity, as a marked increase in the cell number. The obese-hyperglycemic mouse is classified as an example of hypertrophic-hyperplastic obesity, which is the result of both hyperplasia and hypertrophy of varying degrees in adipocytes. Johnson and Hirsch(1972) indicated that animal models having obesity of the hypertrophic-hyperplastic type might present the most suitable model for studies directed at understanding the human condition because of the similarities of this model to childhood onset or the hyperplastic type of human obesity (15). Therefore, the obese-hyperglycemic mouse has been utilized extensively during the past 20 years as an animal model in obesity studies.

II. THE GENETICALLY OBESE MOUSE

The obese mouse(ob/ob) was first identified in the V stock at the Jackson Laboratories, Bar Harbor, Maine in the summer of 1949(16). These obese mice were first distinguishable at about 4 to 6 weeks of age. Obese mice increase body weight rapidly until they are up to four times the weight of normal animals. The recessive gene(ob) of the mutant causes sterility in the homozygote. Infertility is characteristic of the ob/ob females(17,18). The ovaries and uterus in adult mice were atrophic, a finding not usually present in gold thioglucose(GTG) or hypothalamic obese(HTO) mice(19), and thus may reflect the genetic derangement in this species. The general characteristics of the obese syndrome are described by the following features: obesity, hyperglycemia, insulin resistance, and hyperinsulinemia(20). The activity of glycolytic enzymes in the livers of the obese mice is significantly higher than that in lean controls(21). In contrast to expectations, the increased insulin levels do not suppress hepatic gluconeogenic enzymes, except phosphoenolpyruvate carboxykinase (PEPCK) in obese mice(22). Activity of the lipogenic enzymes is also increased in the livers of the obese mice(23-25).

Hellman(26), and Westman(27) studied the development of the obese syndrome in these mice and found the obesity could not unequivocally identified before 26 days of age. Recently, increased body fat deposition in the obese mice by 21 days of age has been suggested by Chlouverakis, et al.(28) based on the analysis of body composition in a group of 21 female

offspring from known heterozygotes. Joosten, et al.(29) measured the fat cell diameters of 12-17 day old obese pups and indicated the obese mouse had enlarged adipocytes. This finding has been further studied and confirmed by Kaplan, et al.(1976)[1]. Total fat content in the hindlimbs of obese mice was significantly higher at 3-4 weeks of age than their lean counterparts(30).

Besides the differences in body fat, obese mice also show other different features before weaning. The body temperature of obese mice was about 0.3° lower from 8 to 16 days of age, and about 1.0° lower onwards than in their lean siblings(31). A lower locomotor activity has been detected in obese mice at only 7 days of age(32). The fact that young adult obese mice consumed only one-half as much oxygen as did lean mice(33,34) has been applied to predict obesity in young obese mice at 3 and 4 weeks of age(35). Based on studies of thyroid, the obese mouse may be hypothyroid as early as 5 weeks of age(31). The plasma-insulin concentration of these mice has also been measured. Hyperinsulinemia is not manifested before the beginning of the fourth week. Elevated levels of circulating insulin are present by one month of age, and reach a peak level by 6 months(36). Hyperglycemia, a prominent feature in fully developed obese mice(37), does not appear until after the onset of obesity and hyperinsulinemia, and reaches its peak by 12 weeks of age(26,38). "Obese-hyperglycemic" has been commonly used to specify this genetically obese mice. However, recent evidence suggests that metabolic alterations may already be present in these animals at a very early age.

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[1]Kaplan, M.L., Trout,J.R., and Leveille, G.A. unpublished observations.

III. EFFECTS OF FOOD INTAKE AND DIETARY NUTRIENTS ON THE DEVELOPMENT OF OBESITY

With respect to food intake, obese mice have been reported to consume significantly more food than lean mice. For example, Mayer, et al.(39) in 1951 found that obese mice, placed on a "free-choice diet", selected more fat, and ate about 25% more diet than non-obese mice. Even though animals were exercised, obese adult mice still consumed more food and gained more weight than their lean sibs(40). When the diet was diluted with cellulose, or made bitter, obese mice at 7-15 weeks of age still consumed more food than lean mice, suggesting that a defect exists in the regulation of food intake in obese mice(41). The obese syndrome in these mice was thought at one time to be similar psychophysiologically to hypothalamic obesity in rats. However, genetically obese mice do not display the behavior patterns associated with the rodents made obese by hypothalamic damage(41-43). Thus, the normal physiological and behavior correlates involved in the regulation of food intake have been suggested to remain intact in the genetic obesity.

Obese mice can become obese even in the absence of excessive food intake(41). When food is restricted, the body weight of the obese mouse returns to normal or becomes lighter, whereas the body fat concentration of obese mice still remains higher than that in lean mice(42,43). Similar results have also been found in some other studies in which food restriction was utilized to study energy metabolism(44-46). Therefore, hyperphagia is not necessary factor to cause obesity in these obese mice. The studies of Bray and his coworkers(47) also indicate that some differences in

hypothalamic endocrine function may not result from hyperphagia, but may be secondary to an alteration of the homoeostatic level of "adipose tissue mass"(41). In all these studies, adult animals have been used; in which obesity was already present. There is a paucity of data on food intake of the young obese mouse, in which the obese syndrome has not been complicated. Studies on the young obese are thus needed to clarify to the role of energy intake in the development of obesity in these animal models.

Several environmental factors interplay in the development of obesity. Diet is one of those factors, which has been well demonstrated to induce obesity in rodents(reviewed in Ref. 48). Before weaning, milk is normally the main diet for pups. Nutrients in milk vary with the food components which dams consumed(49). When dams were fed a 15% fat diet, the fat concentration(wet weight) in the milk produced by those dams is about 25%. When dams were fed a fat-free diet, their milk containes about 16% fat. Although it has been suggested that obese pups may have more milk intake due to their high growth rate(32), the effect of milk intake on the development of obesity in mice has not been elucidated, possibly because simplified techniques for measurement of 24-hour milk intake of pups are not available. Nowadays, a proper technique for the estimate of milk intake of pups is to seperate pups from dams for a short period, then to return them and measure the body weight difference due to nursing(50). The milking procedure did not affect the growth rate of these pups.

After weaning, the diet effect on the development of obesity depends

on the nutrients contained in the diet. When a normal diet containing 3-4 kcal/g, such as a commercial diet, is diluted with indigestible material, such as cellulose or kaolin, the mass of food ingested is adjusted to provide maintenance of total caloric intake(51). When diets with an increased caloric density are made by increasing the percentage of fat, some species of animals will show precise adjustment and will maintain body weight in spite of the increased caloric density of the diet(52). However, most strains of rats are unable to make a complete adjustment and will store excessive quantities of energy and become obese. The obesity induced by high-fat diets in normal animals is called dietary obesity. In genetically obese mice, obesity developed when only carbohydrate was provided as caloric source. When only 5% fat was added the fat-free diet, obese mice contained about the same body fat as other obese mice fed a 30% fat diet(53). In another words, obese mice are more efficient in converting dietary energy to body energy than lean mice, apparently regardless of the diet components(44,48). Most of this increased body energy contributes to fat accumulation. On the contrary, lean body mass of the obese mice is lower than that of their lean counterparts(42, 54,55). Bergen, et al.(30) studied muscle growth in obese mice and indicated that a possible defect in muscle may play a role in the etiology of genetic obesity. However, the possible role of nutrition in the early development of obesity has not been determined.

IV. OBJECTIVES

The present study was designed to follow the food intake and growth pattern of obese and lean mice from 1 to 8 weeks of age. Before weaning at 3 weeks of age, milk intake of the pups was estimated daily. From 3 to 8 weeks of age, obese and lean mice were fed a high-carbohydrate commercial diet or a high-fat semipurified diet. Food intake and body weight changes were monitored. In order to observe the differences in energy retention, fat retention, and protein retention between obese and lean mice, body composition was determined in 3 and 8 week old mice.

MATERIALS AND METHODS

Heterozygote breeding mice(C57BL/6J, ob/+)[2] were housed in solid bottom cages with wood shavings for bedding, and fed ad libitum a commercial diet[3]. Pregnant dams were removed from the breeding cages and placed in separate similar cages. The average litter contained 6-7 pups. Litters containing less than 4 pups or more than 9 pups were not utilized in the experiment. Pups were individually identified at 5 days of age. Room lights in the temperature-controlled(22-24°) were turned on at 0700 hours and off at 1900 hours each day. All animals had free access to water.

From 7 to 21 days of age, estimates of milk intake and growth rate were recorded daily. To obtain estimates of milk intake, pups were separated from their mother at 0900 hours daily and returned to the mother's cage 6 hours later. Pups were allowed access to water, but not to food during this time. Pups were weighed at 0900 hours, 6 hours later, and again after one hour nursing. The body weight gain of each pup during one hour of nursing was utilized as an estimate of the pups' milk intake(50). The sum of all the pups' milk intake in one litter was utilized as an estimate of milk production of the dam. Body weights measured daily at 0900 hours were utilized to determine the growth rate of the pups. Body weight differences between

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[2]Jackson Laboratory, Bar Harbor, Maine.

[3]Wayne Lab-Blox, Allied Mills, Inc., Chicago, Illinois.

0900 hours and 6 hours later were recorded as body weight loss during fasting. The obese(ob/ob) and lean(ob/+ or +/+) mice were identified retrospectively after it became obvious by visual inspection which mice were obese.

At 21 days of age, obese were identified by their lower oxygen consumption(35) and by their relatively fat appearances. From 21 to 56 days of age, male and female obese mice and their lean lettermates were individually housed in metal cages with wire-screen bottoms. They were fed a pelleted commercial diet or a high-fat diet(Table 1). Identification of the obese mice utilized in this feeding experiment was also confirmed retrospectively by visual inspection.

Male and female obese mice and their lean littermates were killed at 21 and 56 days of age. Food residue in the stomach was removed from the carcass. The carcass was then softened in an autoclave at 100° for 15 minutes prior to homogenization. The homogenate was heat in a water bath at 50°, mixed, and sampled. A cholroform/methanol (2:1) mixture was used to extract fat from the carcass homogenate. Carcass fat was determined gravimetrically. The nitrogen content of the carcass was determined by a semimicro-Kjeldahl method(58). The protein content was computed by multiplying the nitrogen content by 6.25. A bomb calorimeter[4] was utilized to determine the energy content of mouse carcasses. The carcass homogenates were dried(59) in a vacuum oven at 50° for 24 hours prior to combustion in

[4]Parr Adiabatic Calorimeter with Oxygen Bomb (Parr 1241). Parr Instrument Co., Moline, Illinois.

the calorimeter.

Statistical analysis of the results was conducted with a 2 or 3-factor model of crossed classification[5](60).

Table 1

Approximate diet composition

	Gross energy[c] (kcal/g)	% of Energy[d]		
		Fat	Protein	Carbohydrate
Commercial diet[a]	4.19	25	23	52
High-fat diet[b]	7.16	82	18	0

[a]Wayne Lab-Blox. Allied Mills, Inc., Chicago, Illinois.

[b]Weight percentage: casein 31.2; tallow 47.0; corn oil 7.8; methionine 0.5; mineral mix 6.3 (see ref.56); vitamin mix 0.6 (see ref. 57); choline 0.3; cellulose 6.3.

[c]Determined in a bomb calorimeter.

[d]Computation based on 4 kcal per g protein and carbohydrate and 9 kcal per g fat. Carbohydrate content of the commercial diet computed by difference. It was assumed that the cellulose in both diets was not utilized by the mouse.

[5]Gill, J.L. (1977) Crossed Classification: Factorial Experiments. In: Design and Analysis of Experiments in the Animal and Medical Sciences. Iowa State University Press, Ames. (In Press).

RESULTS

The milk production of 15 dams is shown in Figure 1. The rate of milk produced for the one hour period increased from 7 to 15 days of lactation, and then declined rather rapidly, especially from 19 to 21 days of lactation. The maximum milk yield per hour appeared at about 14 days of lactation.

From 7 to 20 days of age, young obese and lean mice consumed an equal amount of milk during one hour nursing periods. Similar results were obtained when pups nursed for 90 minutes before weighing. However, obese mice gained more body weight than their lean sibs(Table 2). As shown in the normal growth pattern(61), mice grew faster from 7 to 14 days of age than from 14 to 20 days of age. The growth curves of the mice during this period are shown in Figure 2. At 21 days of age, the body weights of obese mice were heavier ($p<0.05$) than their lean counterparts. The young mice lost body weight when they were isolated from their mother's cage for 6 hours. Obese mice lost less weight than lean mice from 7 to 14 days of age but not from 14 to 20 days of age. All mice lost less body weight from 7 to 14 days of age than from 14 to 20 days of age.

At 21 days of age, both male and female obese mice contained about twice the carcass fat and total body energy observed in their lean littermates; the carcass protein content was not influenced by the phenotype(Table 3). On a concentration basis, obese mice contained about 27% fat, and 16% protein; lean mice contained about 14% fat, and 18% protein.

Figure 1.

Lactation curve of 15 dams. Each dam nursed 5 to 8 pups. Each point is mean $\pm$ SEM of 15 dams.

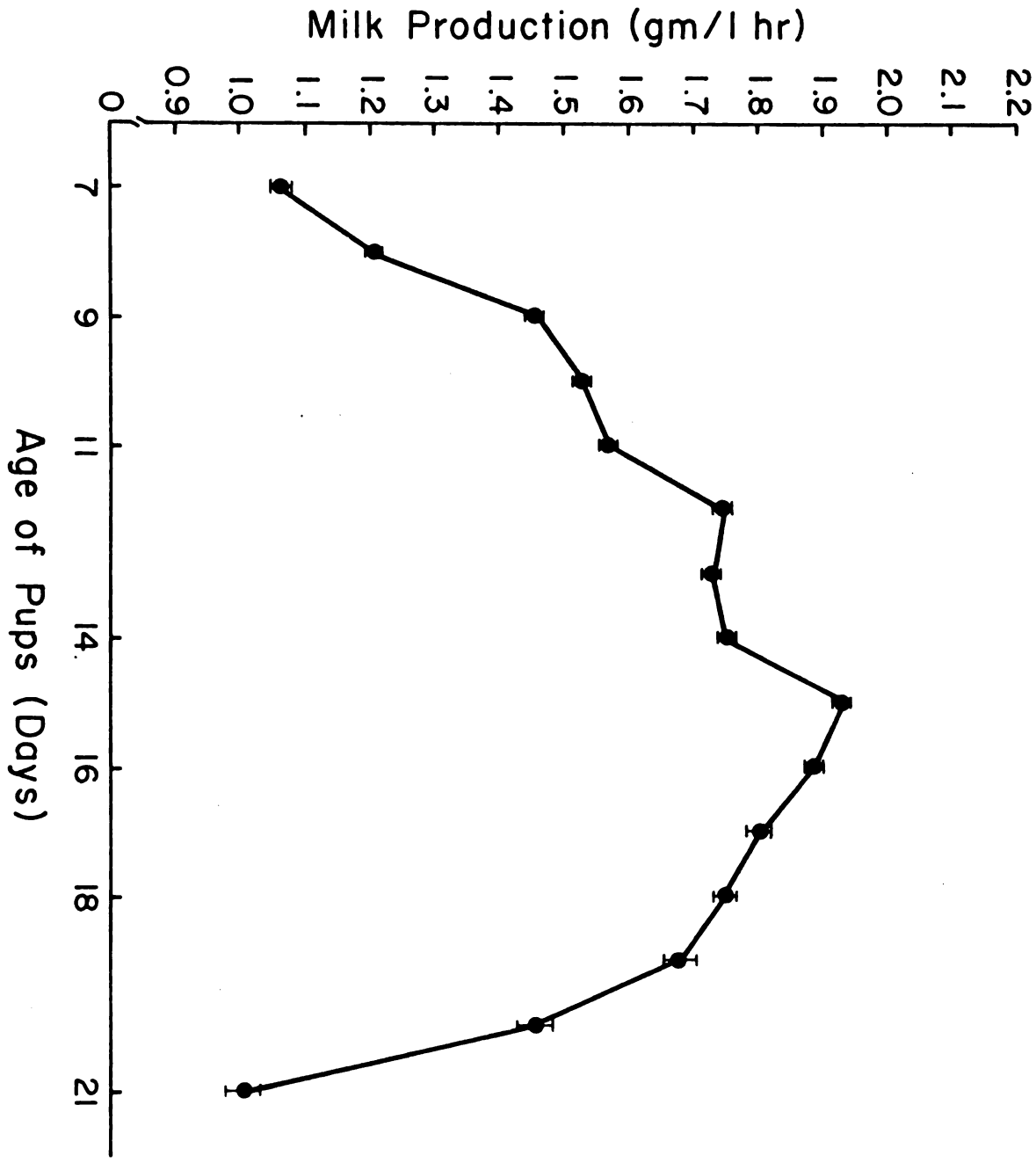
Milk Production (gm/l hr)
0
0.9
1.0
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9
2.0
2.1
2.2
Age of Pups (Days)
7
9
11
14
16
18
21

Table 2

Milk intake, body weight gain and body weight loss during fasting in mice

	Male		Female			
Age-days	Obese (15)[a]	Lean(30)	Obese (10)	Lean (30)	MS_E[c]	ANOVA[d]
			Milk Intake (mg/ 1 hr)			
7-14	250[b]	240	220	220	8,869	NS
14-20	280	260	240	300	13,886	NS
			Daily Body Weight Gain (mg)			
7-14	360	320	370	310	320	P
14-20	190	110	190	140	447	P
			Body Weight Loss (mg per 6 hr fasting)			
7-14	50	70	50	70	767	P
14-20	100	130	110	150	9,838	NS

[a]Number of mice. [b]Mean; estimated to the nearest 10 mg. [c]MS_E = Mean Square Error

[d]Analysis of Variance. P designates a significant ($p < 0.05$)phenotype effect. NS indicates not significant.

Figure 2.

Growth curves of mice before weaning. Each point is mean $\pm$ SEM. There are 15 obese males, 30 lean males, 10 obese females, and 30 lean females.

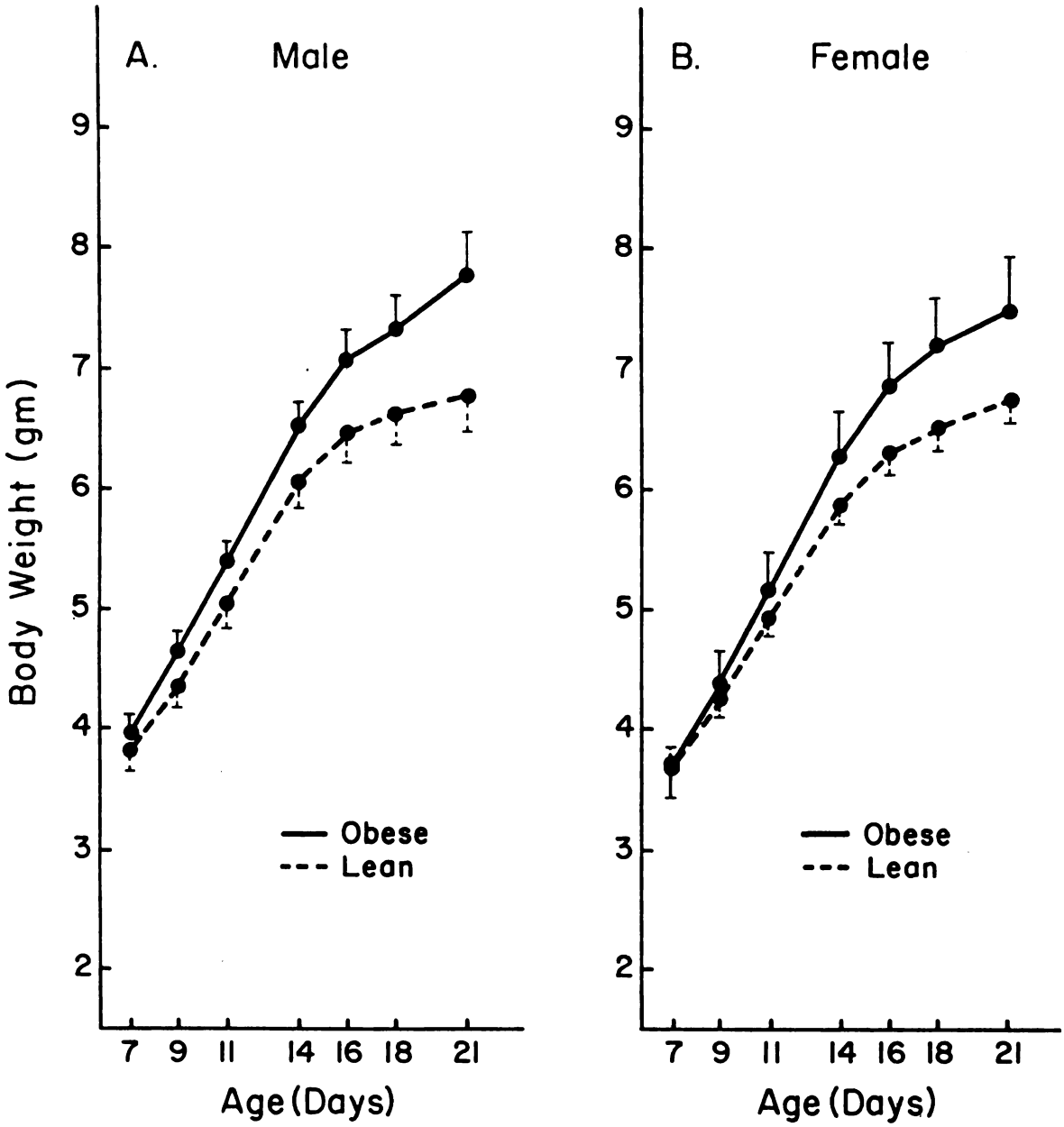
A.
Male
B.
Female
Body Weight (gm)
9
8
7
6
5
4
3
2
7 9 11 14 16 18 21
Age (Days)
Obese
Lean

Table 3

Body weight, fat, protein and energy content of mice at 21 days of age.

Body	Male		Female		MS_E[c]	ANOVA[d]
	Obese	Lean	Obese	Lean		
Weight, g	9.20[a]	8.18	9.17	7.85	1.59	P
Fat, g	2.58	1.19	2.25	0.94	0.17	P
Protein[b], g	1.43	1.48	1.47	1.42	0.04	NS
Energy, kcal	26.0	13.4	25.6	12.8	18.6	P

[a]Mean for eight mice per group.

[b]Protein = nitrogen content x 6.25

[c]MS_E = Mean Square Error.

[d]Analysis of Variance. P designates significant($p < 0.05$) phenotype effect. NS indicates not significant.

During the first several days immediately after weaning, the mice showed some differences in adaptation to the diets(Table 4). From 21 to 23 days of age, obese males consumed less energy and gained less body weight than lean males, while obese females consumed less energy but gained as much weight as did their lean counterparts (results based on average of both diets). At the same time, the diet fed did not affect the body weight gain of mice. However, mice consumed more energy when fed the commercial diet than fed the high-fat diet. From 23 to 25 days of age, obese mice consumed less energy and gained less body weight than lean mice when the results from both diets were averaged. Male and female mice fed the commercial diet consumed equal energy and gained equal weight, while males fed the high-fat diet consumed less energy and gained less weight than females. From 25 to 28 days of age, obese mice fed the commercial diet still consumed less energy but started to gain more weight than lean mice. Obese mice fed the high-fat diet consumed more energy and gained more weight than their lean sibs. After 28 days of age, the effects of the diet and the phenotype on body weight gain and food intake in mice became more pronounced (Table 5). The obese mice gained more body weight. From 35-42 days of age, obese mice fed the commercial diet started to consume more energy than lean mice, whereas obese mice fed the high-fat diet started to consume more energy than lean mice at an earlier age (25-28 days of age).

Before 21 days of age, the sex of the mice did not influence the observations. From 21 to 56 days of age, the sex of mice had only a minimum effect on food intake, but body weight gain of the mice was influenced by

Table 4

Effect of diet on body weight gain and food intake of mice

Days of Age	Diet[a]	Male		Female		MS_E[c]	ANOVA[d]
		Obese	Lean	Obese	Lean		
		Body Weight at Day 21 (g)					
	CD	9.2[b]	8.0	8.4	8.0	1.25	P
	HFD	7.3	6.5	8.0	7.2	1.39	
		Body Weight Gain (g)					
21-23	CD	0.1	1.0	0.4	0.8	0.29	P,PxS
	HFD	-0.2	0.4	1.0	0.7		
23-25	CD	0.9	1.4	1.0	1.3	0.20	P,S,DxS
	HFD	0.4	0.9	1.7	1.7		
25-28	CD	1.8	1.8	1.9	1.5	0.20	P,D
	HFD	3.1	2.4	3.0	2.7		
		Food Intake (kcal)					
21-23	CD	10	18	12	19	40.4	P,D,S
	HFD	< 1	5	10	9		
23-25	CD	14	22	16	22	48.4	P,S,DxS
	HFD	9	14	25	21		
25-28	CD	28	33	32	36	67.9	D,S,PxD
	HFD	39	32	44	40		

[a] CD = Commercial Diet; HFD = High-Fat Diet.

[b] Mean for eight mice per group.

[c] MS_E = Mean Square Error.

[d] Analysis of Variance. P,D, and S designate significant ($p < 0.05$) phenotype, diet and sex effects, respectively.

Table 5

Effect of diet on weekly body weight gain and food intake of mice

Days of Age	Diet[a]	Male		Female		MS_E[c]	ANOVA[d]
		Obese	Lean	Obese	Lean		
		Weekly Body Weight Gain (g)					
28-35	CD	4.0[b]	5.2	4.9	3.6	2.4	P,D,S,PxD,PxS
	HFD	10.1	7.4	9.2	5.5		
35-42	CD	5.2	3.1	5.6	2.3	1.7	P,D,S,PxD,DxS
	HFD	9.9	4.6	8.4	1.7		
42-49	CD	5.0	1.7	4.6	0.4	1.5	P,D,PxD,PxS
	HFD	6.2	2.4	7.4	1.1		
49-56	CD	4.2	1.1	3.7	0.5	1.0	P,D,S,PxD
	HFD	6.8	1.3	5.2	0.9		
		Weekly Food Intake (kcal)					
28-35	CD	82	93	97	94	404	P,D,PxD
	HFD	136	104	138	106		
35-42	CD	118	110	131	102	376	P,D,PxD
	HFD	166	130	164	118		
42-49	CD	141	112	145	101	248	P,D,PxD,PxS
	HFD	167	123	178	112		
49-56	CD	150	108	145	100	227	P,D,PxD
	HFD	174	118	179	117		
		Total Food Intake (kcal)					
21-56	CD	541	496	577	474	4574	P,D,PxD
	HFD	694	528	737	522		
		Total Protein Intake (g)					
21-56	CD	31.9	29.2	34.2	27.0	10.45	P,D,PxS
	HFD	25.8	19.6	27.4	19.4		

[a] CD = Commercial Diet; HFD = High-Fat Diet.

[b] Mean for eight mice per group.

[c] MS_E = Mean Square Error

[d] Analysis of Variance. P, D, and S designate significant ($p < 0.05$) phenotype, diet, and sex effects, respectively.

their sex. Male and female obese mice gained body weight at similar rates, but lean males gained more weight than lean females.

Growth curves of the mice from 21 to 56 days of age are summarized in Figure 3. Consumption of the high-fat diet increased the body weight of the obese mice whereas lean mice fed the high-fat diet had body weights similar to those observed in lean mice fed the commercial diet. Obese mice fed the high-fat diet weighed more than lean mice by 35 days of age, whereas obese mice fed the commercial diet weighed more than lean mice after 42-49 days of age.

Energy, fat, and protein retention of the mice from 21 to 56 days of age were computed from the results of carcass analysis, and are shown in Table 6. At 56 days of age, obese males contained 52% fat, and 12% protein; lean males contained 16% fat, and 20% protein. For the females, obese mice contained 57% fat, and 10% protein; lean mice contained 21% fat, and 18% protein. These results are computed from the average data which both diets were utilized . The three retention values , in terms of energy, fat, and protein, are resulted by substracting the mean value at day 21 from the individual value at day 56. Clearly, the obese mice retained more energy and exhibited a higher energy efficiency than lean mice. Obese mice retained 4-5 times as much energy and were about 3 times as efficient in converting dietary energy to body energy as their lean counterparts. Consumption of the high-fat diet enhanced the energy retention and energy efficiency to a greater extent in obese mice than in lean mice. As expected, fat retention was closely related to energy retention. Both obese and lean females

Figrue 3.

Growth curves of mice after weaning. Each point is mean $\pm$ SEM of eight mice per group.

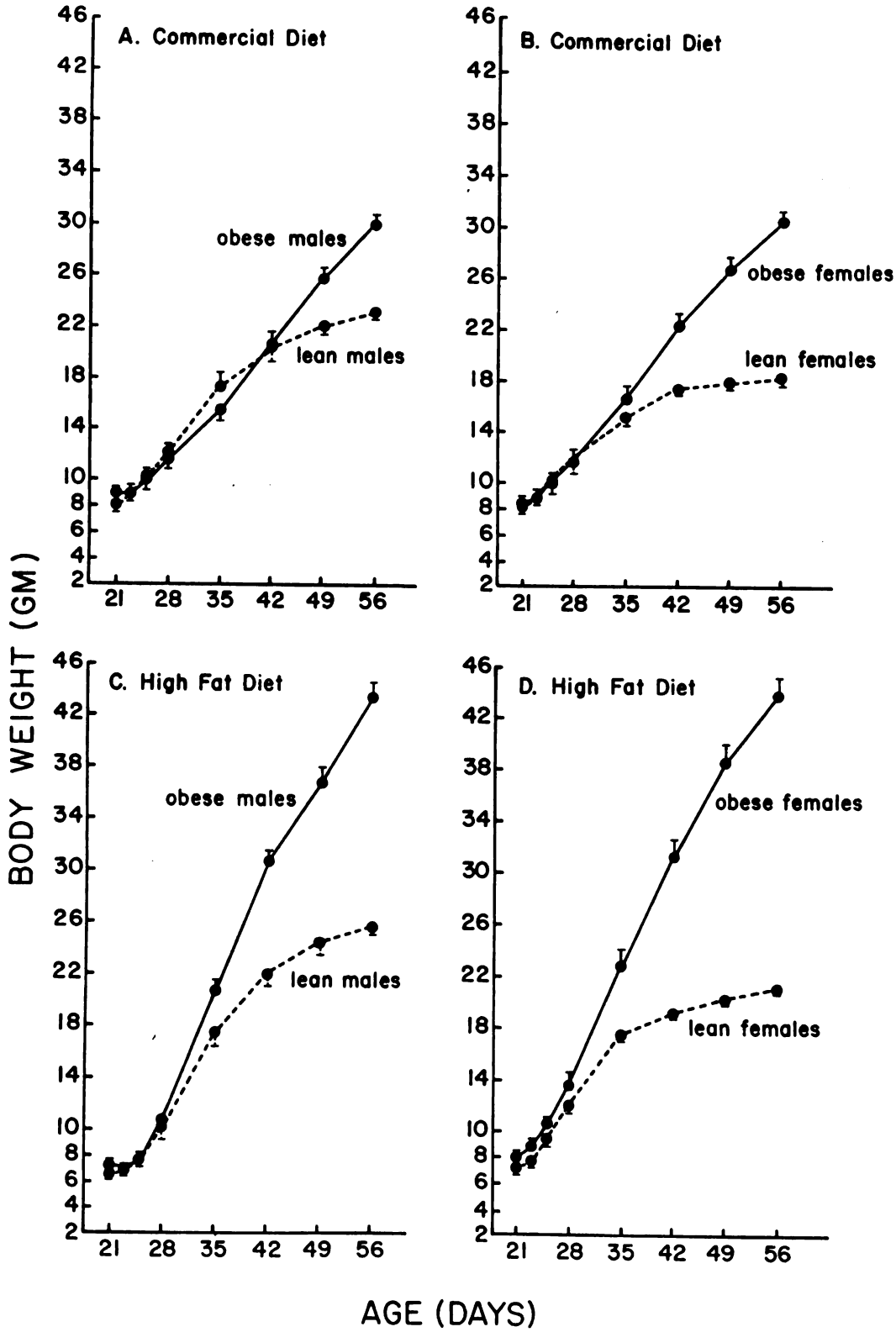
A. Commercial Diet
obese males
lean males
B. Commercial Diet
obese females
lean females
C. High Fat Diet
obese males
lean males
D. High Fat Diet
obese females
lean females
BODY WEIGHT (GM)
AGE (DAYS)
2
4
6
8
10
14
18
22
26
30
34
38
42
46
21
28
35
42
49
56

Table 6

Effect of diet on energy, fat and protein retention in mice from 21 to 56 days of age

	Male		Female			
Diet[a]	Obese	Lean	Obese	Lean	MS_E[c]	ANOVA[d]
	Energy Retention (kcal)					
CD	108[b]	35	118	28	188.4	P,D,PxD,PxS
HFD	210	45	233	42		
	Energy Efficiency (%)[e]					
CD	19.9	7.3	20.4	6.3	2.1	P,D,PxD,PxS
HFD	30.4	8.4	31.7	8.0		
	Fat Retention (g)					
CD	11.9	2.5	13.5	2.6	3.2	P,D,S,PxD,PxS
HFD	20.0	2.9	23.6	3.8		
	Protein Retention (g)					
CD	2.4	3.2	2.0	2.0	0.1	P,D,S,PxS
HFD	3.0	3.4	2.5	2.2		
	Protein Efficiency (%)[f]					
CD	7.6	11.0	6.0	7.3	1.4	P,D,S,PxD,PxS,DxS
HFD	11.5	17.5	9.2	11.6		

[a] CD = Commercial Diet; HFD = High-Fat Diet.

[b] Mean for eight mice per group.

[c] MS_E = Mean Square Error.

[d] Analysis of Variance. P, D, and S designate significant ($p < 0.05$) phenotype, diet and sex effects, respectively.

[e] Energy retention (kcal) / Energy intake (kcal).

[f] Protein retention (g) / Protein intake (g).

retained more fat than did their male counterparts.

Protein intake of mice was calculated and is shown in Table 5. Obese mice had a higher protein intake than lean mice. Mice consumed more protein when fed the commercial diet than when fed the high-fat diet; the protein to energy ratio was also higher in the commercial diet. Obese males retained less protein than did lean males; however, differences were not observed when obese and lean females were compared. Regardless of diet fed, obese mice were less efficient in converting dietary protein to body protein than lean mice. However, the high-fat diet caused a higher protein retention and protein efficiency than the commercial diet in mice.

DISCUSSION

As early as 7-14 days of age, small differences in body weight gain were evident in the obese mice. It has been suggested that increased milk intake may be a congenital factor in the development of obesity (32). However, no differences in milk intake were observed, suggesting that the obese mice might have a greater feed efficiency than lean mice. It is also possible that the one hour estimate of milk intake did not reflect the total daily milk intake. The lactation curve obtained was similar to previous reports (50, 62), although the daily milk yield was somewhat lower than reported previously (50,63). Genetic background(64) as well as the number of pups suckled per litter (65) would influence the results. Losses in body weight of the pups due to urination were not considered in the estimation of milk intake. Milk intake data for the 21st day were not presented because solid food was found in the gut of pups at this age. Pups have been reported to eat solid food when they were 15-16 days old (66). However, intake of solid food in pups less than 21 days of age was not quantitated in the present study.

Obese pups from 7 to 14 days of age lost less weight than lean mice during 6 hours of fasting; an observation similar to that found in adult obese and lean mice (67). Adult obese mice excrete much less creatinine than lean mice, during the first 36 hours of fasting, suggesting that the obese mice may utilize less muscle mass than lean mice during the fast(68).

Possibly obese mice rely to a greater extent on fat metabolism during a fast than do lean mice. However, the composition of the weight loss in the young pups during the fast is unknown. When the ratio of the daily weight gain to weight loss during the fast was calculated from 14 to 20 days of age, the values were 1.65 $\pm$ 0.12 and 0.84 $\pm$ 0.07 for the obese and lean mice, respectively. When values within the 99% confidence intervals were then used to predict the phenotype of an additional 7 litters, 6 of the 7 obese pups were correctly identified whereas 37 of the 39 lean mice were properly identified. Two lean mice were mistakenly identified as obese and one obese nouse was incorrectly identified as lean. When the values were calculated for the two week period (7 fo 20 days of age), similar results were also obtained. It may be possible to refine this technique into a useful tool for screening and identifying young obese and lean mice. It would be especially useful when large numbers of animals are needed; individual oxygen consumption measurements (35) would be impractical in this situation.

At 21 days of age, obese mice contained about 130% more body fat, but were only 14% heavier than lean mice; an observation in agreement with a report by Chlouverakis, et al. (28). Adipocyte cell size is increased in obese mice as early as 12-17 days of age (29). Similarily, the genetically obese rat (fa/fa) may have an increased body fat content as early as 14 days of age[6]. These observations suggest that significant differences in energy

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[6]Bell, G.E., and Stern, J.S. (1976) Development of obesity and hyperinsulinemia in the Zucker obese rat (fa/fa). Federation Proc. 35, 657.

metabolism probably occur very early in the life of the obese mouse.

The time required for mice to adapt to a new diet immediately after weaning varied with the diet consumed and the sex of the mice. Obese males just maintained their body weight immediately after weaning whereas both obese and lean females started to gain weight immediately after weaning, especially when the high-fat diet was fed. Mice were placed in wire bottomed cages in a room maintained at 22-24° when weaned. This may have stressed the obese males, which are known to adapt poorly to cold (69,70), to a greater extent than the other mice. The critical temperature, which is defined as the temperature below which there is an increase in the metabolic rate, for young mice is 30-32° (71,72).

In the present study, obese mice did consume more food than the lean mice during the 5 weeks feeding period. However, even when obese and lean mice were pair-fed from 24 days of age (41) or when the body weight of the adult obese mice was reduced to the level of the lean mice (42,44,45) obese mice still contained more fat than lean mice. These studies as well as those of others(43,46) indicate that excess food intake can enhance the degree of the obesity, but may not be necessary for its development. Hyperphagia is suggested to be secondary to a primary metabolic defect (41,73).

The obese mice, in agreement with earlier reports (reviewed in ref. 48), were much more efficient in retaining dietary energy than the lean mice when either one of the experimental diets was used. Comparisons between the commercial diet and the high-fat diet have to be made with reservation because one was formulated with natural ingredients and the other with semi-purified ingredients. Nevertheless, obese mice fed the high-fat diet were more efficient than were obese mice fed the high-carbohydrate commercial

diet. The high-carbohydrate commercial diet was selected because it was available in pelleted form, which greatly facilitated collection of food intake data in these young mice. In other studies a high-fat diet has also been shown to potentiate the development of obesity in rodents (53, 74-76). The obese mouse has a lower oxygen consumption (33,34), a lower body temperature (31), and lower locomotor activity (32,77,78) than lean mice. The involvement of these factors in the dietary-induced potentiation of obesity is not clear.

Obese animals also showed differences in their ability to retain dietary protein. While obese mice consumed more protein than lean mice, they retained less protein. Lean mice retained 35-45% their body energy as protein, whileas obese mice retained only 7-11%. These values are similar to those reported by others (44,46). Direct estimates of muscle mass also indicate a difference in protein accumulation between obese and lean mice (30). The role of protein metabolism in the development of obesity in these mice remains to be elucidated.

SUMMARY

From 7 to 20 days of age, obese mice consumed an equal quantity of milk but gained more body weight than lean mice. When mice were fasted for 6 hours every day, obese mice lost less weight than lean mice from 7 to 14 days of age. It is possible that obese utilize less muscle mass, and rely to a greater extent on fat metabolism during a fast than do lean mice. The ratio of the daily body weight gain to the weight loss during a fast also showed a significant difference between obese and lean mice. This may be a useful tool for screening and identifying the phenotype of mice, especially when large numbers of animals are needed. At 21 days of age, the fat content of obese mice was double that of lean mice, whereas the protein content was not changed. These observations suggest that significant differences in energy metabolism probably occur very early in the life of the obese mouse.

During the first several days after weaning, obese males failed to consume as much food as did the lean males. Obese mice consumed equal quantities of food, but gained more body weight than lean mice from 35-42 days of age for males, or from 28-35 days of age for females when they were fed the commercial diet. After that, obese mice consumed more food and gained more weight. Obese mice fed the high-fat diet started to eat more food and gained more weight from 28 days of age. The present observations imply that excess food intake can enhance the degree of the obesity, but may not be necessary for its development. Hyperphagia is suggested to

be secondary to a primary metabolic defect.

Obese mice, in agreement with early reports, were much more efficient in retaining energy than lean mice when either one of the experimental diets was used. The high-fat diet has been shown to potentiate the development of obesity in mice. When the results of carcass analysis were averaged, obese males contained 52% fat, and 12% protein; lean males contained 16% fat, and 20% protein at 56 days of age. At the same age and for the females, obese mice contained 57% fat, and 10% protein; lean mice contained 21% fat, and 18% protein.

Obese mice also showed differences in their ability to retain dietary protein. Obese males retained less protein than did lean males; however, differences were not observed when obese and lean females were compared. Regardless of diet fed, obese mice were less efficient in converting dietary protein to body protein than lean mice. These findings support the suggestion that a possible defect in protein metabolism may play a role in the etiology of the genetic obesity.

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APPENDIX

Appendix I. Analysis of Variance.

A. For milk intake of mice

Source	df	Mean Square	F ratio	p
1). From 7 to 14 days of age				
Phenotype	1	0.0039	0.0218	0.88
Sex	1	0.3952	2.1834	0.14
Phenotype x Sex	1	0.0250	0.1380	0.71
Error	81	0.1810		
2). From 14 to 20 days of age				
Phenotype	1	0.2789	0.7230	0.99
Sex	1	0.4470	1.1590	0.28
Phenotype x Sex	1	1.2776	3.3123	0.07
Error	81	0.3857		

B. For body weight gain

Source	df	Mean Square	F ratio	p
1). From 7 to 14 days of age				
Phenotype	1	0.0439	6.7177	0.01
Sex	1	0.00005	0.0074	0.93
Phenotype x Sex	1	0.0025	0.3700	0.54
Error	81	0.0065		
2). From 14 to 20 days of age				
Phenotype	1	0.0702	5.6575	0.02
Sex	1	0.0023	0.1831	0.67
Phenotype x Sex	1	0.00079	0.0635	0.80
Error	81	0.01242		

Appendix I. Analysis of Variance. (continued).

C. For body weight loss during a fast

Source	df	Mean Square	F ratio	p
1). From 7 to 14 days of age				
Phenotype	1	0.2441	15.6028	< 0.0005
Sex	1	0.0014	0.0887	0.77
Phenotype x Sex	1	0.0032	0.2046	0.65
Error	81	0.0156		
2). From 14 to 20 days of age				
Phenotype	1	0.9751	3.5682	0.06
Sex	1	0.1720	0.6294	0.43
Phenotype x Sex	1	0.0220	0.0805	0.78
Error	81	0.2733		

Appendix II. Analysis of Variance for body weight, fat, protein and energy content of mice at 21 days of age.

Source	df	Mean Square	F ratio	p
1). Body weight.				
Phenotype	1	10.8461	6.8240	0.02
Sex	1	0.2538	0.1596	>0.50
Phenotype x Sex	1	0.1846	0.1161	>0.75
Error	28	1.5894		
2). Body fat.				
Phenotype	1	14.5672	84.6909	<0.0005
Sex	1	0.6694	3.8915	0.06
Phenotype x Sex	1	0.0133	0.0774	0.78
Error	28	0.1720		
3). Body protein.				
Phenotype	1	0.00003	0.0008	0.98
Sex	1	0.00195	0.0563	0.81
Phenotype	1	0.02023	0.5841	0.45
Error	28	0.03464		
4). Body energy content.				
Phenotype	1	1,297.0381	69.5544	<0.001
Sex	1	2.1636	0.1160	0.75
Phenotype x Sex	1	0.0223	0.0011	>0.75
Error	28	18.6478		

Appendix III. Analysis of Variance.

A. For body weight at 21 days of age, mice for feeding experiments.

Source	df	Mean Square	F ratio	p
Phenotype	1	10.1442	6.2256	0.53
Sex	1	0.6480	0.3977	0.02
Phenotype x Sex	1	0.2525	0.1550	0.70
Error	60	1.6294		

B. For body weight gain of mice,

1). From 21 to 23 days of age

Source	df	Mean Square	F ratio	p
Diet	1	o.0492	0.2463	0.62
Sex	1	0.5898	2.9540	0.10
Diet x Sex	1	0.4550	2.2785	0.14
$Error_1$	28	0.1997		
Phenotype	1	0.5902	6.8717	0.01
Diet x Phenotype	1	0.2817	3.2797	0.08
Sex x Phenotype	1	0.3546	4.1288	0.05
Diet x Sex x Phenotype	1	0.0515	0.5999	0.44
$Error_2$	28	0.0859		

Appendix III. Analysis of Variance. (continued).

B. For body weight gain of mice,(continued),

Source	df	Mean Square	F ratio	p
2). From 23 to 25 days of age				
Diet	1	0.0019	0.0158	0.90
Sex	1	1.0842	8.9485	0.006
Diet x Sex	1	1.1718	9.6716	0.004
$Error_1$	28	0.1212		
Phenotype	1	0.3813	4.6656	0.039
Diet x Phenotype	1	0.0276	0.3382	0.57
Sex x Phenotype	1	0.1234	1.5096	0.23
Diet x Sex x Phenotype	1	0.0315	0.3855	0.54
$Error_2$	28	0.0817		
3). From 25 to 28 days of age				
Diet	1	2.0682	19.4992	<0.0005
Sex	1	0.0005	0.0049	0.94
Diet x Sex	1	0.0235	0.2218	0.64
$Error_1$	28	0.1061		
Phenotype	1	0.2437	8.9802	0.006
Diet x Phenotype	1	0.0437	1.6118	0.22
Sex x Phenotype	1	0.0009	0.0340	0.86
Diet x Sex x Phenotype	1	0.0558	2.0548	0.16
$Error_2$	28	0.0271		

Appendix III. Analysis of Variance. (continued).

C. For food intake of mice.

Source	df	Mean Square	F ratio	p
1). form 21 to 23 days of age				
Phenotype	1	364.5732	9.0285	0.003
Diet	1	1151.7868	28.5234	<0.0001
Sex	1	295.3878	7.3152	0.008
Phenotype x Diet	1	134.5935	3.3331	0.08
Phenotype x Sex	1	113.8932	1.1782	0.26
Phenotype x Diet x Sex	1	24.0397	0.5953	>0.25
Error	56	40.3804		
2). from 23 to 25 days of age				
Phenotype	1	202.9400	4.1967	<0.05
Diet	1	36.1092	0.7467	>0.25
Sex	1	567.7390	11.7405	0.001
Phenotype x Diet	1	162.3743	3.3578	>0.05
Phenotype x Sex	1	110.1548	2.2779	>0.10
Diet x Sex	1	416.5340	8.6137	<0.005
Phenotype x Diet x Sex	1	41.8452	0.8653	>0.25
Error	56	48.3573		
3). from 25 to 28 days of age				
Phenotype	1	3.0055	0.0443	>0.75
Diet	1	604.0487	8.9009	0.004
Sex	1	364.4429	5.3702	0.025
Phenotype x Diet	1	468.9448	6.9101	0.01
Phenotype x Sex	1	5.0784	0.0748	>0.75
Diet x Sex	1	34.0746	0.5021	>0.25
Phenotype x Diet x Sex	1	12.1805	0.1795	0.75
Error	56	67.8638		

Appendix IV. Analysis of Variance.

A. For weekly body weight gain.

Source	df	Mean Square	F ratio	p
1). from 28 to 35 days of age				
Diet	1	209.9239	90.0296	<0.0005
Sex	1	11.8078	5.0640	0.03
Diet x Sex	1	4.6494	1.9940	0.17
$Error_1$	28	2.3317		
Phenotype	1	40.4337	19.1735	<0.0005
Diet x Phenotype	1	41.8447	19.8426	<0.0005
Sex x Phenotype	1	12.2763	5.8214	0.02
Diet x Sex x Phenotype	1	2.4846	1.1782	0.29
$Error_2$	28	2.1088		
2). from 35 to 42 days of age				
Diet	1	69.3056	42.9081	<0.0005
Sex	1	22.6100	13.9982	0.001
Diet x Sex	1	14.7264	9.1173	0.005
$Error_1$	28	1.6152		
Phenotype	1	302.4121	169.5491	<0.0005
Diet x Phenotype	1	42.6736	23.9252	<0.0005
Sex x Phenotype	1	6.6693	3.7392	0.06
Diet x Sex x Phenotype	1	0.0210	0.0118	0.91
$Error_2$	28	1.7836		

Appendix IV. Analysis of Variance. (continued).

A. For weekly body weight gain.(continued).

Source	df	Mean Square	F ratio	p
3). from 42 to 49 days of age				
Diet	1	27.4576	17.0462	<0.0005
Sex	1	2.7390	1.7004	0.20
Diet x Sex	1	2.5921	1.6092	0.22
$Error_1$	28	1.6108		
Phenotype	1	314.8850	201.6399	<0.0005
Diet x Phenotype	1	7.0225	4.4969	0.04
Sex x Phenotype	1	11.5600	7.4026	0.01
Diet x Sex x Phenotype	1	3.0276	1.9388	0.18
$Error_2$	28	1.5616		
4). from 49 to 56 days of age				
Diet	1	21.5528	19.5784	<0.0005
Sex	1	9.4403	8.5754	0.007
Diet x Sex	1	0.6520	0.5923	0.45
$Error_1$	28	1.1008		
Phenotype	1	260.7418	255.3022	<0.0005
Diet x Phenotype	1	11.7478	11.5027	0.002
Sex x Phenotype	1	1.1289	1.1054	0.30
Diet x Sex x Phenotype	1	1.1790	1.7125	0.20
$Error_2$	28	1.0213		

Appendix IV. Analysis of Variance. (continued).

B. For weekly food intake of mice.

Source	df	Mean Square	F ratio	p
1). from 28 to 35 days of age				
Phenotype	1	3,054.7768	7.5654	0.01
Diet	1	14,218.5377	35.2133	<0.001
Sex	1	393.8216	0.9753	>0.25
Phenotype x Diet	1	5,171.0093	12.8064	<0.001
Phenotype x Sex	1	168.7930	0.4180	>0.50
Diet x Sex	1	160.5047	0.3975	>0.50
Phenotype x Diet x Sex	1	225.3448	0.5581	>0.25
Error	56	403.7836		
2). from 35 to 42 days of age				
Phenotype	1	14,220.5589	37,7742	<0.001
Diet	1	13,843.7132	36.7732	<0.001
Sex	1	81.8611	0.2174	>0.50
Phenotype x Diet	1	2,016.0863	5.3554	0.025
Phenotype x Sex	1	945.9063	2.5126	>0.10
Diet x Sex	1	402.7055	1.0697	>0.25
Phenotype x Diet x Sex	1	145.7417	0.3871	>0.50
Error	56	376.4619		
3). from 42 to 49 days of age				
Phenotype	1	32,733.6349	131.8903	<0.001
Diet	1	7,190.6092	28.9724	<0.001
Sex	1	105.1365	0.4236	0.50
Phenotype x Diet	1	1,217.2640	4.9046	0.03
Phenotype x Sex	1	1,507.7565	6.0750	0.02
Diet x Sex	1	10.0311	0.0404	>0.75
Phenotype x Sex x Diet	1	64.1228	0.2584	>0.50
Error	56	248.1884		

Appendix IV. Analysis of Variance. (continued)

B. For weekly food intake of mice. (continued).

Source	df	Mean Square	F ratio	p
4). from 49 to 56 days of age				
Phenotype	1	41,122.4498	180.9054	<0.0005
Diet	1	7,221.2759	31.7678	<0.001
Sex	1	88.7014	0.3902	>0.50
Phenotype x Diet	1	1,052.2350	4.6290	0.03
Phenotype x Sex	1	97.7378	0.4300	0.50
Diet x Sex	1	267.0496	1.1748	0.25
Phenotype x Diet x Sex	1	9.7340	0.0428	>0.75
Error	56	227.3146		

C. For total food intake of mice from 21 to 56 days of age.

Source	df	Mean Square	F ratio	p
Phenotype	1	265,812.3274	58.1135	<0.001
Diet	1	181,643.2575	39.7120	<0.001
Sex	1	6,793.1161	1.4851	0.25
Phenotype x Diet	1	40,241.1151	8.7977	0.004
Phenotype x Sex	1	5,519.2147	1.2066	>0.25
Diet x Sex	1	1,319.7581	0.2885	>0.50
Phenotype x Diet x Sex	1	461.8543	0.1009	0.75
Error	56	4,574.0139		

Appendix IV. Analysis of Variance. (continued).

D. For total protein intake of mice from 21 to 56 days of age.

Source	df	Mean Square	F ratio	p
Phenotype	1	581.5392	55.6525	<0.001
Diet	1	903.4217	86.4562	<0.001
Sex	1	2.0838	0.1994	>0.50
Phenotype x Diet	1	17.7010	1.6940	>0.25
Phenotype x Sex	1	41.7457	3.9550	0.05
Diet x Sex	1	1.5920	0.1524	>0.50
Phenotype x Diet x Sex	1	7.0578	0.6754	>0.50
Error	56	10.4495		

Appendix V. Analysis of Variance.

A. For energy retention of mice from 21 to 56 days of age

Source	df	Mean Square	F ratio	p
Phenotype	1	269,740.6864	1,431.8289	<0.0001
Diet	1	58,052.5994	308.1529	<0.001
Sex	1	561.8344	2.9823	0.08
Phenotype x Diet	1	38,164.8620	202.5855	<0.001
Phenotype x Sex	1	1,894.9905	10.0589	<0.01
Diet x Sex	1	267.9560	1.4223	>0.10
Phenotype x Diet x Sex	1	88.7201	0.4709	>0.25
Error	56	188.3889		

B. For energy efficiency of mice.

Source	df	Mean Square	F ratio	p
Phenotype	1	5,254.6588	2,588.7894	<0.0001
Diet	1	607.7261	289.2281	<0.001
Sex	1	0.1920	0.0913	>0.75
Phenotype x Diet	1	353.4738	168.2247	<0.001
Phenotype x Sex	1	10.7676	5.7676	0.02
Diet x Sex	1	1.7324	0.8244	>0.25
Phenotype x Diet x Sex	1	0.0521	0.0247	>0.75
Error	56	2.1012		

Appendix V. Analysis of Variance. (continued).

C. For fat retention of mice from 21 to 56 days of age.

Source	df	Mean Square	F ratio	p
Phenotype	1	3,318.5135	1,026.0376	<0.0001
Diet	1	390.1550	120.6304	<0.001
Sex	1	35.7279	11.0465	0.002
Phenotype x Diet	1	276.5699	85.5115	<0.001
Phenotype x Sex	1	15.0477	4.7638	0.04
Diet x Sex	1	8.5025	2.6288	0.10
Phenotype x Diet x Sex	1	1.0714	0.3312	>0.50
Error	56	3.2343		

D. For protein retention of mice from 21 to 56 days of age

Source	df	Mean Square	F ratio	p
Phenotype	1	0.8101	7.2654	0.008
Diet	1	2.5049	22.4654	<0.001
Sex	1	10.3472	92.8000	<0.0005
Phenotype x Diet	1	0.3466	3.1085	0.07
Phenotype x Sex	1	2.5422	22.8000	<0.001
Diet x Sex	1	0.0002	0.0017	>0.75
Phenotype x Diet x Sex	1	0.0346	0.3103	>0.50
Error	56	0.1115		

Appendix V. Analysis of Variance. (continued).

E. For protein efficiency of mice from 21 to 56 days of age.

Source	df	Mean Square	F ratio	p
Phenotype	1	175.8939	123.4949	<0.001
Diet	1	323.0854	226.8380	<0.001
Sex	1	180.4636	126.7033	<0.001
Phenotype x Diet	1	14.0093	9.8359	<0.005
Phenotype x Sex	1	33.9394	23.8288	<0.001
Diet x Sex	1	8.4511	5.9335	0.02
Phenotype x Diet x Sex	1	1.9092	1.3404	0.25
Error	56	1.4243		