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INFLUENCE OF PATTERN OF FEEDING ON WEIGHT GAIN, NITROGEN BALANCE, AND BODY COMPOSITION IN RATS

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ABSTRACT

INFLUENCE OF PATTERN OF FEEDING ON WEIGHT GAIN, NITROGEN BALANCE, AND BODY COMPOSITION IN RATS

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Sixteen experiments were designed to study the influence of meal pattern on body weight gain, nitrogen balance, and body composition in rats.

First, a series of experiments was conducted to evaluate the influence of meal frequency on weight gain, nitrogen balance, and body composition of rats. Rats were either fed two hours per 24 or 48 hours (meal-eaters), or pair-fed to meal-eaters with an automated feeding machine (nibblers). Rats weighing approximately 250 g initially were fed 10%, 20%, or 30% casein, high-carbohydrate diets or a 20% casein, high-fat diet for seven to eight weeks. Meal-eaters gained essentially the same amount of body weight as the nibblers. Meal-feeding once per 24 or 48 hours did not adversely influence nitrogen balance or body composition of the rats. In one experiment, smaller rats, weighing approximately 150 g initially, were utilized. Meal-eaters, again,

retained as much nitrogen as nibblers, and contained less body fat than the nibblers.

Next, rats weighing 110 to 150 or 250 g initially were utilized to determine the effect of the form of the diet (dry *versus* liquid) and the pattern of feeding (meal-feeding, force-feeding, nibbling, or *ad libitum*) on body weight gain and body fat. A high-carbohydrate, 20% casein, or 20% lactalbumin, diet was fed for four to eight weeks. Consumption of a diet mixed with an equal weight of water increased weight gain in one of three experiments. Body fat content of the rats was not influenced by addition of water to the diet. Neither force-feeding nor meal-feeding influenced body fat gain provided the respective control rats were pair-fed during the initial adaptation period. Likewise, when rats were pair-force-fed to *ad libitum* fed rats without an initial adaptation, meal frequency did not influence body fat gain. When meal-fed rats were switched to *ad libitum* intake, their food intake increased to equal that of rats which had been continuously fed *ad libitum*; however, rats which had been switched gained more body fat than did rats continuously fed *ad libitum*.

To determine the effect of initial food restriction on subsequent body weight gain and body fat accumulation, rats were restricted to 75%, 50%, or 25% of the intake

of control rats for one week and were subsequently pair-fed on a food intake basis to the control rats. As expected, restricted rats gained weight at a slower rate and had less body fat at the end of the restricted period than control rats. Upon re-feeding the same amount of food as consumed by the control rats, these re-fed rats gained more body fat than control rats. This compensatory fat gain occurred regardless of whether the rats were force-fed twice daily, meal-fed once daily, or allowed to consume the food throughout the day. Both a high-carbohydrate and a high-fat diet produced compensatory fat gain. Compensation was apparent as early as the first week of re-feeding and was greater in rats which had been restricted to 50% or 25% of *ad libitum* intake than in rats less severely restricted. Restricted rats were also re-fed on a body weight basis. These gained as much weight and fat as the *ad libitum* fed controls, indicating that the restricted rats were more efficient in converting dietary energy to body fat when re-fed than rats fed *ad libitum* continuously.

The results of these experiments suggest that meal-eating does not cause a depression in nitrogen retention or an increase in body fat deposition in rats, provided the experimental animals are pair-fed to the control rats throughout the entire experiment. A shift to a higher

level of food intake may cause an increased food efficiency and greater rate of fat deposition than in rats continuously fed the higher level of intake. The initial food restriction inherent in many studies involving meal frequency may cause the subsequent increased food efficiency and greater accumulation of body fat often attributed to an alteration in meal pattern.

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INTRODUCTION

In the evolutionary process, higher organisms develop adaptive mechanisms that allow them a degree of independence from their environment.

Rats with the hypothalamic-hyperphagic syndrome contain more fat, less water, and less nitrogen when fed *ad libitum* than pair-fed control animals. When the animals are offered their entire daily ration at one time, the hypothalamic-hyperphagic animals quickly consume their food while the controls take all day to eat their diet. Under these conditions, the differences in body fat between experimental and control animals are exaggerated (Van Putten *et al.*, 1955). These studies were among the first to suggest that in addition to the effect of dietary constituents offered to an animal, the timing of food ingestion may play a role also in the economy of calorie disposition (Cohn and Joseph, 1959).

The significance of studying the influence of periodicity of eating on body metabolism may be two-fold. First, at birth man is normally a nibbler, but custom, convenience, working habits, and sociability often make him a meal eater. Changes of the living pattern in highly

industrialized countries lead to the concentration of food intake in the evening hours, the calorie intake during the remainder of the day often being low (Fabry and Tepperman, 1970). It is quite possible that a number of diseases associated with abnormalities in fat, protein, and carbohydrate metabolism may be caused or aggravated by eating habits (Cohn and Joseph, 1960). Epidemiologic studies revealed that excessive weight and, in elderly groups, also hypercholesterolemia, impaired glucose tolerance, and ischemic heart disease were more common among persons with an infrequent meal pattern than among those who customarily ate five or more meals per day (Fabry and Tepperman, 1970).

Secondly, if the timing of protein intake influences overall nitrogen utilization in the human, alterations in the distribution of protein among meals would be expected to have more distinct effects on infants and growing children than on adults (Taylor *et al.*, 1973). This will have important impact in developing countries. Large-scale efforts have been made to improve the nutritional status of school children by providing one or, occasionally, two meals a day in the school. On a smaller but growing scale, similar efforts are being made to improve the nutritional status of preschool children considered to be much more at risk. If adequate nitrogen retention and growth can be

maintained with most of the protein being given at a single meal and *ad libitum* energy with lower amounts and/or quality of protein being consumed at other meals during the day, the possibility of increasing the value of food supplementation programs would exist (Maclean *et al.*, 1975).

CHAPTER I

LITERATURE REVIEW

Nutritional Adaptations

Nutritional adaptation is an example of the ability of the body to adapt to changes in the external environment. Animals and men are able to adapt within a relatively wide range of dietary changes, provided that the supply of essential nutrients is ensured (Fabry, 1969). A change in the amount or composition of the diet leads to a change in the activity of systems involved in the metabolism of the nutrient. This change may be induced either by the direct effect of the substrate on the enzyme or indirectly by influencing other physiological mechanisms controlling the activity of some key enzymes. Nutrition has a marked influence on the activity of different endocrine glands and on the adaptation of target tissues and their enzyme systems (Fabry, 1969).

The pancreas responds to an excess of some nutrients by the excretion of several enzymes; however, continued administration of the diet leads to an adaptively increased synthesis of the appropriate eynzyme. Digestive functions seem to adapt not only to the quantity of a

main nutrient but also to a certain type of the nutrient. A diet rich in glucose, fructose, or galactose leads to an increased absorption of the appropriate sugar. If the change in the dietary regimen persists for a prolonged period, morphological adaptation of the small intestine may finally occur. Changes in the composition of the diet, for instance, a high lactose or a high fat diet, can lead, in fully grown animals, to an enlargement of the small intestine (Fabry, 1969).

A special situation is the adaptation of the digestive system to changes in the nutritional pattern in mammals in the early stages of postnatal life when the animal changes from suckling to the diet of a fully grown animal. The absorption rate of nutrients and the activity of some enzymes in the intestine during lactation corresponds to that of a diet rich in fat and lactose and it changes markedly when the young animal changes to the diet of adult animals (Fabry, 1969).

A changed supply of nutrients is reflected also in the tissue metabolism. An altered supply of a nutrient leads to the alteration of the systems associated with its metabolism and its preferential use as a source of energy. An adaptive increase or decrease in the utilization of a nutrient may take place in the processing of the appropriate substrates from endogenous as well as exogenous sources, depending on the availability of the nutrient.

An animal adapted to a high carbohydrate diet has increased activity of the enzymes catalyzing the stages in the glucose metabolism, an increased capacity to synthesize glycogen from glucose, a higher glucose tolerance, and is more sensitive to the administration of insulin or hypoglycemic substances (Mitchell, 1965; Bassler, 1969).

Animals adapted to a high fat diet have an increased ability to metabolize fat which is manifested by an increased oxidation of fatty acids, increased formation of ketone bodies in the liver or their increased oxidation in peripheral tissues, and the preferential use of fat reserves as a source of energy in calorie undernutrition (Mitchell, 1965). One of the metabolic effects of feeding fat is reduced lipogenesis from acetate and glucose in the liver and adipose tissue.

In the metabolic adaptation to a high protein diet, there is a striking increase in the activity of a number of enzymes associated with catabolism of proteins and amino acids, which are broken down to an increased extent as a source of energy and needed glucose. The increased amino acid catabolism in animals adapted to a high protein diet is manifested not only by an increased formation of urea but also by an enhanced breakdown of proteins during fasting (Fabry, 1969). On a low protein diet, the activity of a number of amino acid catabolizing enzymes declines, which

is an example of a metabolic adaptation to a reduced supply of substrate.

A reduction in energy supply results in a decrease in total energy output. Depending on the length and severity of the energy deficit in man and experimental animals, the basal metabolism is reduced, the urinary nitrogen excretion declines, and the physical activity is lowered. In experimental animals subjected to complete or partial starvation, tissue respiration and the activity of some tissue enzymes are reduced. Similarly, an increased energy intake also leads to metabolic adaptation manifested by a higher energy output (Fabry, 1969).

Frequency of Feeding and Body Metabolism

Experimental Methods of Feeding Frequency. The laboratory rat ingests food almost continuously in small amounts during the day, with a maximum intake during the night. When this nocturnal feeding pattern is changed by periodic feedings and fastings, rats "learn" to ingest all the daily food ration within a few hours. Many of Fabry's experiments (Fabry, 1969; Fabry and Kujalova, 1960; Fabry *et al.*, 1961 and 1962) were carried out on intermittently fasting rats adapted to gradually extended periods of fasting between which there were days when free access to food was permitted. These rats gradually compensated for the periods of fasting by a larger food intake on the days of feeding;

however, this periodically increased intake did not cause a complete compensation of food intake or weight gain. Intermittently fed rats were about 30% lighter than *ad libitum* fed controls (Fabry, 1969).

When the controlled feeding is necessary, feeding the animals with a stomach tube has been frequently applied. Cohn (Cohn and Joseph, 1959, 1963, 1968) fed rats twice a day by tube and indicated that this feeding pattern leads to marked metabolic changes brought about by the periodic loads of nutrients and not by forced feeding.

In addition to intermittent and force-feeding, rats have also been "trained" to consume "meals," that is, only two or three hours daily. During the first few days of meal feeding, these animals consumed very little food but they gradually increased the food intake. Trained rats consumed 15-20% less food than the *ad libitum* fed rats. The weight gain of these animals is also less than the *ad libitum* fed rats (Leveille, 1970, 1975; Philippens *et al.*, 1977).

Morphological and Functional Changes in Gastrointestinal Tract (GIT) Associated with Feeding Frequency. The laboratory rat is basically a "nibbler" and, if food is readily available, eats small quantities of food more or less continuously, reaching a maximum in the nighttime. If, however, the access to food is restricted to 1 to 2 hours

per day, or if rats are fed intermittently only 3 days a week, alternating with 1 to 2 day periods of fasting, the animal soon learns to ingest large amounts of food at a time (Fabry and Tepperman, 1970). The GIT displays marked functional and morphological changes including increased activity of digestive enzymes of the pancreas, increased enzyme activity in the intestinal mucosa, and an increased rate of glucose absorption from the intestine (Fabry and Tepperman, 1970).

A striking consequence of intermittent hyperphagia in rats and mice is enlargement of the GIT, especially that of the stomach (Fabry and Tepperman, 1970; Philippens *et al.*, 1977). The enlargement is apparent in the absolute weight of the stomach and its portions (although the total body weight of the animal is reduced), as well as in histological reactions with a hypertropy of the mucosa and musculature (Fabry, 1969). Intermittent hyperphagia produces morphological and functional changes in the digestive system which allows the animal to consume and to digest a large quantity of food within a short time (Pose *et al.*, 1969). The functional consequence of the enlarged stomach is its increased capacity. The organ also serves as a food reservoir during the following period of fasting. Even after a 24 hour fast, a considerable amount of food may be found in the stomach of adapted rats (Fabry, 1969).

The small intestine also becomes significantly enlarged (Leveille and Chakrabarty, 1968; Romsos and Leveille, 1974a). In Fabry's experiments (Fabry and Kujalova, 1960), the absolute weight of the small intestine was about 35% heavier than the weight of the small intestine of *ad libitum* fed controls, even though the animals weighed about 30% less than controls. Histological appearance of the intestine from meal-eaters does not differ significantly from the appearance of intestines of controls (Fabry and Kujalova, 1960).

After six weeks of infrequent feeding, there is a markedly increased rate of glucose absorption from the intestines (Fabry, 1969). Leveille (1970) reported that the rate of glucose absorption increased by about 40% in the meal-fed rats. The stimulus leading to enhanced intestinal absorption in intermittently fed rats is not the reduction of the energy intake alone, but periodic hyperphagia on days of free access to food. Contrary to findings of an enhanced glucose and fat absorption, amino acid absorption does not change (Fabry, 1969). The values of glucose absorption *in vitro* in intermittently fed animals are more than 60% higher than in *ad libitum* fed controls (Fabry, 1969).

The weights of the liver, kidneys, femur, small intestine, and stomach were significantly greater in meal-fed rats than in continuously fed control animals (Pocknee

and Heaton, 1976).

Circadian Rhythms and Feeding Frequency. The response of an animal to meal-feeding may depend on the timing of the meal in relation to the animal's circadian rhythms or to other periodic factors that influence these rhythms. When food is continuously available, the timing of circadian rhythms in rats and mice is determined primarily by the daily alteration between light and darkness (Nelson *et al.*, 1975).

Mice restricted to feeding in early darkness consumed less food and had a lower body weight than those feeding only in early light (Nelson *et al.*, 1975). Nelson *et al.* (1975) also found a large increase in the overall mean for liver glycogen and serum corticosterone concentration in mice restricted to 4 hours daily food accessibility as compared with animals feeding *ad libitum*. The relation among the several variables examined can be considerably different at any given elapsed time after food presentation depending on whether food is presented near the onset of light or near the onset of darkness.

Philippens *et al.* (1977) studied circadian rhythms in rats in relation to meal timing. Liver weight varied during the 24-hour period in the rats fed *ad libitum* and subjected to a light-dark cycle. The maximum liver weight occurred during the early part of the light period. Restricting the availability of food intake to certain times

had an effect on the waveform of the rhythm; the amplitude was increased in restricted feeding groups. Maximum liver weight usually occurred 9 hours after the food was removed. When the overall 24-hour means of each group were compared, relative liver weight (liver weight per body weight) was significantly increased in meal-fed rats compared to *ad libitum* fed rats. However, relative weight was smaller when food was presented during the first part of the light period. Stomach weight directly reflected the restricted time of food intake, with maximum stomach weight soon after the food was made available (Philippens *et al.*, 1977). The overall 24-hour mean weights of stomach of restricted feeding groups were approximately doubled when compared with the *ad libitum* fed groups. The rats fed during mid-light had a significantly lower stomach weight than other restricted feeding groups. The restricted feeding groups gained less weight than the control groups. Those rats with feeding confined to the light phase gained less weight than the rats with feeding restricted to the dark phase.

Generally, lower values of serum protein were noted when the 24-hour means for the restricted groups were compared with those of the control groups (Philippens *et al.*, 1977). The glucose rhythm was synchronized to the restricted feeding schedules. The phase of the

oscillation always occurred just prior to the time when food was made available; peak values occurred shortly thereafter. Those rats fed during the dark exhibited higher overall 24-hour blood sugar levels when compared with controls; also, their levels were higher when compared to those rats fed during the lights.

Fuller and Diller (1970) also showed that there was a high amplitude circadian rhythm in liver glycogen in *ad libitum* fed rats. Restricted mean timing altered the waveform of the rhythm as well as increasing its amplitude. Restricted groups demonstrated a significant increase when compared with the *ad libitum* fed rats. Rats fed during the dark had higher overall 24-hour glycogen levels than rats fed during the light. In those rats fed during early light or dark periods, the phasing of liver protein closely followed the *ad libitum* pattern, with the major peaks occurring the first hour of feeding. The pattern of total protein in the liver was the inverse of that for glycogen. The total protein content of restricted groups was lower than that of the *ad libitum* fed rats, especially in those groups fed during the dark period (Fuller and Diller, 1970).

Therefore, optimal nutrition may depend not only on what is eaten but when it is eaten in relation to other schedules and demands.

Carbohydrate Metabolism. The tissues of the meal-fed animal utilize glucose more rapidly than do those of nibbling rats (Leveille, 1970). Peripheral tissues of the meal-fed rat might convert a considerable portion of this glucose to storage forms, glycogen and lipid. For up to 8 hours after the initiation of the daily meal, the respiratory quotient (RQ) is in excess of unity, indicating that glucose is serving as the major oxidative fuel and that lipogenesis is proceeding at a rapid rate. From 8 to 14 hours following meal initiation, the RQ decreases to a value which suggests that carbohydrate, probably glycogen, and lipid are serving as the oxidative fuel. Finally, from 14 hours after the start of the meal until the initiation of the next meal, the RQ indicates lipid is the major source of energy (Leveille, 1970).

After oral or parenteral glucose administration to intermittently fed rats or in rats adapted to consuming food for 2 hours per day, the rise in blood glucose is less and the rate of its decline more rapid than in *ad libitum* fed rats (Hoffmann *et al.*, 1972). The tolerance for glucose is high because of the increased capacity of liver and peripheral tissues of the adapted animal to handle the absorbed glucose, mainly by converting it to glycogen and fat (Fabry and Tepperman, 1970). This improved clearance capability of glucose in the meal-fed

rat is the product of higher circulating insulin levels both in fed and fasted states and of greater tissue sensitivity to insulin (Wiley and Leveille, 1970).

Romsos and Leveille (1974b) conducted intraperitoneal glucose tolerance tests in meal-fed and nibbling rats fed high carbohydrate or high fat diets with either glucose or sucrose as the source of carbohydrate. Regardless of diet fed, meal eaters exhibited a greater ability to clear glucose from circulation than did *ad libitum* fed controls. The increase in blood glucose after the glucose load was similar in both groups of rats; however, blood glucose values in meal-fed rats returned towards the basal level sooner than did the values for nibblers. Source of dietary carbohydrate was without effect in nibbling rats or in meal-fed rats fed the high fat diets. Replacement of dietary glucose with sucrose impaired glucose tolerance in meal-fed rats fed the high carbohydrate diet (Romsos and Leveille, 1974b).

Studies in humans (Wadhwa *et al.*, 1973) showed that the mean blood glucose level of the subjects was higher when they gorged than when they nibbled. Post glucose levels were significantly affected by the frequency of feeding, being higher in gorgers than in nibblers (Wadhwa *et al.*, 1973). A delayed response of serum immune reactive insulin to glucose load in

gorging subjects suggests that decreased sensitivity of peripheral tissues to insulin may be a contributory factor to greater than normal circulating blood glucose levels.

This difference in the effect of meal-feeding on glucose tolerance in humans and in rats cannot be readily explained. One explanation might be the difference in diet composition (Leveille and Romsos, 1974). In studies with rats, a high carbohydrate diet has generally been used whereas, in human studies, a diet much higher in fat was used. Also, the human diets contained significant quantities of sucrose but the rat diets did not. The study of Romsos and Leveille (1974b) is in agreement with the suggestion that both fat and sucrose impaired glucose tolerance; however, this impairment was noted both in meal-fed and nibbling rats.

Animals adapted to ingesting food for 1 to 2 hours per day or to intermittent feeding have a higher liver glycogen content than *ad libitum* fed rats (Fabry, 1969; Tepperman and Tepperman, 1958). In the heart muscle of intermittently fed animals, the glycogen concentration is increased to about double the value found in controls (Fabry, 1969). Glycogen stores in adipose tissue greatly increase above those found in nibbling animals after only five days of adaptation to meal eating and reach

a point in fully adapted rats several fold higher after a meal than in nibbling rats. However, prior to the meal, glycogen concentrations in adipose tissue are similar to those found in fed or fasted nibbling rats (Leveille, 1967).

The activity of liver hexokinase in rats adapted to intermittent feeding is significantly increased not only in the state of satiety but also after 24 or 48 hours fasting as compared with controls fed *ad libitum* or fasted for an equal period (Fabry, 1969). An increased hexokinase activity in the liver and adipose tissue is found also in rats fed for 1 hour per day (Fabry, 1969) compared with *ad libitum* fed rats.

Intestinal hexokinase activity is elevated above control nibbling rats when rats were forced to consume food within a 2-hour period each day. Meal-fed animals respond to a large meal with a gradual increase in hexokinase activity (Romsos and Leveille, 1974a). Intestinal pyruvate kinase activity is similar in meal-fed and nibbling rats (Romsos and Leveille, 1974a), whereas pyruvate kinase activity of adipose tissue from meal-fed rats is significantly elevated above control nibbler values (Leveille, 1970).

Adaptation in carbohydrate metabolism in meal-fed rats, specifically an increased capacity for glucose

absorption and utilization and an increased glycogenesis in muscle, adipose tissue, and liver, are thought to be mediated by insulin, in at least two ways: circulating plasma insulin levels are elevated for at least a portion of the day; and, in adipose tissue, sensitivity to the hormone is increased. It has been suggested that some of the actions of insulin may be mediated by changes in tissue concentrations of cyclic-AMP and/or -GMP (Ip *et al.*, 1977).

Hoffman *et al.* (1972) observed that meal-feeding did not enhance the insulinogenic response to intravenous glucose. Meal-feeding elevated the concentration of insulin in the pancreas. In intermittently fed rats, the insulin activity is higher in the serum of fasted animals as compared with controls. It may be that insulin is released in increased amounts from the pancreas of intermittently fed rats; however, its reserve in the pancreas is relatively small and when the requirements and the utilization of the hormone in tissue are raised, its concentration in the bloodstream diminishes (Fabry, 1969). In the meal-fed rats, 2 hours after administration of food, a short-term hyperinsulinemia develops and insulin concentrations exceed the maximum levels in *ad libitum* fed controls (Fabry, 1969).

Studies with humans showed that gorging elevated the insulin response of the subjects exhibiting relatively normal insulin levels following oral glucose. Upon nibbling, the insulin responses of both "normal" and "abnormal" groups (subjects exhibiting delayed insulin response to oral glucose) were lowered (Pringle *et al.*, 1976).

The meal-eating rats exhibited a sharp increase in circulating glucagon levels that coincided with hyperinsulinemia. The *ad libitum* fed rats showed a longer sustained increase which was spread over much of the dark period. The amplitude of the diurnally changing glucagon concentrations tended to be larger in the meal-fed rats (Ip *et al.*, 1977).

Protein Metabolism. Frequent meals resulted in a considerably greater retention of absorbed nitrogen than did two meals per day in rats (Cohn and Joseph,1963) although the digestibility and fecal nitrogen were not affected by the frequency of meals (Han, 1973). The mechanisms underlying the effect of frequency of meals on the excretion of nitrogen are not clearly known. Since protein synthesis *in vivo* is a rate-limiting enzymatic reaction, it seems possible that there is a limit to the quantity of amino acids absorbed which can be channelled into protein biosynthesis reaction per unit of time. Also, it seems probable that when overloading with substrates occurs, as it might under

the conditions of infrequent feeding of large meals, some of the amino acids would be deaminated and excreted in the urine. Thus, rats would have to adapt to overloading by altering the pathways of enzymatic breakdown (Cohn and Joseph, 1963; Han, 1973).

Most studies with experimental animals suggest that nitrogen utilization is reduced when isocaloric and isonitrogenous meals are provided infrequently, but this has not been the general finding for human subjects. There was no difference in nitrogen balance of young women consuming 30 g protein and given 3 or 6 meals during the day (Shortridge and Linkswiler, 1963). No change was observed in nitrogen utilization in young women with alterations in meal frequency (Swindells *et al.*, 1968). Bortz *et al.* (1966) also found similar nitrogen utilization and weight loss in obese subjects given a low energy intake and either 72 or 13 g protein consumed in 1, 3, or 9 meals daily.

It was observed in a number of experiments that, in force-fed (Cohn and Joseph, 1959, 1963) and in intermittently or meal fed (Fabry, 1969) rats, the proportion of total body protein declines in connection with an increase in body fat.

In mammals, the absorption of food protein is not continuous yet, after meals, there is little accumulation of free amino acids in the tissues (Garlick *et al.*, 1973).

The liver is usually considered to modulate the inflow of amino acids from the intestines and the concentrations of amino acids in the systemic blood fluctuate much less after a meal than the concentrations in the portal vein. Muscle tissues are exposed to more constant concentrations of plasma amino acids than the liver. The synthesis of muscle proteins may not respond to sudden dietary changes to the same extent. However, experiments *in vitro* have shown that muscle protein synthesis is increased by insulin. Changes in the metabolism of muscle protein could therefore occur after a feed, despite the small alterations in plasma amino acid concentrations, as a result of changes in plasma insulin concentrations (Garlick *et al.*, 1973). During cyclic feeding or after feeding, dramatic changes in liver weight and protein content occur. The major effect of food absorption in liver appears to be a decrease in the rate of protein breakdown, with a subsequent increase in the protein content of the tissue in rats on scheduled meals (Garlick *et al.*, 1973; Oblet *et al.*, 1975).

The rhythm in tyrosine aminotransferase activity is generated primarily by the periodicity of food intake (Cohn *et al.*, 1970). The amplitude of the 24-hour rhythm in hepatic tyrosine aminotransferase activities of rats fed hourly was markedly reduced when compared with the activities of the enzyme in rats eating *ad libitum* (Cohn *et al.*, 1970).

At any time of the day, the feeding of a protein meal resulted in a rise in tyrosine aminotransferase and a second protein meal induced a second rise. This induction was not mediated by a release of either insulin or corticosteroids. The lowest enzyme activity always occurred approximately 14 hours after induction by a protein meal. There was a total absence of tyrosine aminotransferase rhythm in animals fed a protein-free diet (Girard Globa and Bouldal, 1973).

The rhythm in tryptophan pyrrolase is also markedly altered by changing the pattern of food intake. The peak of the rhythm in meal-fed rats was approximately at the nadir in *ad libitum* fed rats, and *vice versa*. In addition, tryptophan pyrrolase activity was generally much higher in meal-fed rats (Cohn *et al.*, 1970).

Lipid Metabolism. Rats trained to eat their food in a single 2-hour daily meal have a tremendously increased capacity to synthesize fat from carbohydrates (Leveille, 1975; Palmquist *et al.*, 1977; De Bont *et al.*, 1975; Hollifield and Parson, 1962; Cohn and Joseph, 1967). These changes occurring in liver and in adipose tissue have been termed "adaptive hyperlipogenesis" by Tepperman and Tepperman (1965). The increased lipogenic activity occurs hand in hand with an increased activity of different enzymes involved in the fat formation as well as increased synthesis of proteins and nucleic acids in the fat cells

Fabry and Tepperman, 1970). This phenomenal augmentation of fatty acid synthesis occurs after only four days of training during which time the gorging animals eat less than *ad libitum* fed controls and always lose weight (Palmquist *et al.*, 1977).

Following the consumption of a meal, the rates of liver fatty acid synthesis rapidly rise such that, within two hours, the rates are five times pre-meal values (Palmquist *et al.*, 1977; Lowenstein, 1971; Sullivan *et al.*, 1974). Although fatty acid synthesis rates immediately after a meal are several fold higher in livers of meal-trained rats than nibbling animals fasted 22 hours and re-fed two hours, the rate of lipogenesis in meal-fed rat liver does not reach that of the fed nibbler until 5 hours after meal initiation. The rate of hepatic fatty acid synthesis in meal-eating rats gradually returns to pre-meal values over the subsequent 22 hour period of food deprivation (Lowenstein, 1971; Sullivan *et al.*, 1974).

Rats which were fed a high carbohydrate diet during a single 3-hour feeding period each day, developed increased rates of lipogenesis that persisted for 12 to 16 hours after the meal was terminated. To determine the cause of this prolonged lipogenesis, trained rats were fed meals ranging in size from 0.5 to 10.5 g (Wittman *et al.*, 1973). Lipogenesis, gluconeogenesis, and absorption-related parameters

which might affect these pathways were measured at scheduled times after feeding. All meal sizes were equally effective in causing high rates of lipogenesis within 30 to 40 minutes; however, the duration of these rates varied directly with the size of the meal. Stomach contents, luminal glucose in the intestine, and portal blood glucose and insulin concentrations varied with the rates of lipogenesis. Gluconeogenesis did not increase until 4 to 8 hours after lipogenesis returned to pre-feeding rates (Wittman *et al.*, 1973; Miller *et al.*, 1973). Adaptation to the meal-feeding regimen results in an increased rate of food ingestion and a prolonged period of intestinal absorption. The latter is associated with increased blood sugar and insulin levels which, in turn, may cause metabolic changes producing the increased lipogenesis (Wittman *et al.*, 1973).

The stimulation of adipose tissue lipogenesis which occurs after consumption of the daily meal may be related to the rapid absorption of glucose and subsequent production of α-glycerophosphate in adipose tissue. This, in turn, provides for fatty acid esterification and reversal of potential inhibition by elevated levels of tissue-free fatty acids or CoA derivatives (Leveille, 1967). Romsos and Leveille (1972a) suggested that the enhanced lipogenic capacity observed in the adipose tissue from meal-fed rats

resulted from a true metabolic adaptation and not a change in cell size. The authors found that the differences in *in vitro* lipogenic capacity of the epididymal fat pads from meal-fed and *ad libitum* rats were of similar magnitude whether the results were expressed on a tissue weight basis or on a fat cell basis.

A number of enzymes are involved in the conversion of ingested carbohydrates to storage forms of energy; fatty acids and glycogen (Leveille and Romsos, 1974). These enzymes are: (a) involved in the phosphorylation of glucose and its conversion to α-glycerophosphate and pyruvate; (b) involved in fatty acid synthesis; and (c) involved in the generation of NADPH for the support of fatty acid synthesis.

The activities of α-glycerophosphate dehydrogenase and pyruvate kinase were increased as a consequence of meal-feeding, whereas there was no change in phosphofructokinase activity (Leveille, 1970, 1975; Leveille and Romsos, 1974).

Citrate cleavage enzyme, acetyl coenzyme A carboxylase and fatty acid synthetase were all over 100% higher in adipose tissue of meal-fed as compared with nibbling rats (Fabry, 1969; Leveille, 1970; Leveille and Romsos, 1974; Armstrong *et al.*, 1976). The activity of the fatty acid synthetase complex was not in excess of that of acetyl CoA carboxylase. Although acetyl CoA carboxylase may be the

rate-limiting enzyme for fatty acid biosynthesis, this regulatory role would result from the allosteric nature and not the total activity of this enzyme. High activity of citrate cleavage enzyme in adipose tissue of meal-fed rats implies that the generation of acetyl CoA *via* this enzymatic step is not likely to limit fatty acid synthesis (Leveille, 1970).

During enhanced lipogenesis, the pentose pathway supplies more than half of the reduced NADP needed for the synthesis of fatty acids in rodent adipose tissue (Fabry, 1969). The activities of two pentose pathway enzymes, glucose-6-phosphate and 6-phospho-gluconate dehydrogenase, were increased as a result of meal-feeding (Leveille, 1970; Tepperman and Tepperman, 1958, 1961, 1964; Leveille, 1967; Hollifield and Parson, 1962; Muiruri and Leveille, 1970). Of the enzymes involved in the transhydrogenation cycle, the activities of pyruvate carboxylase and malic enzyme in rats (Leveille, 1970; Muiruri and Leveille, 1970; Tepperman and Tepperman, 1964; Leveille and Hanson, 1966) and mice (Romsos and Leveille, 1972b) were markedly increased by meal-feeding. The activity of malic dehydrogenase was not significantly increased but was high in contrast to that of the other two enzymes of the transhydrogenation cycle and not likely to be limiting (Leveille, 1970). The activity of isocitrate dehydrogenase was not increased

in rat epididymal adipose tissue as a result of meal feeding.

There is a marked difference between rats and mice with respect to adaptations to a gorging pattern of food intake. Thus far, there is no evidence from *in vivo* tracer experiments that rates of fatty acid synthesis from carbohydrate are any greater in gorging mice than in nibblers (Palmquist *et al.*, 1977). Despite the apparent species differences in lipogenic activation of rats and mice, both species respond similarly with respect to accumulation of depot fat. In both species, total food consumption is drastically reduced and in neither species does body fat accumulate during the early stages of food restriction to a single large meal per day.

Mice that are on an intermittent fasting (24 hours) and refeeding (48 hours) schedule show no change in body composition relative to *ad libitum* fed mice. However, relative rates of fatty acid synthesis in adipose tissue and liver vary considerably (Romsos and Leveille, 1972b).

In the guinea pig, there was a reduction in average daily weight gain and gross feed efficiency after meal feeding. *In vivo* lipogenesis indicated a definite diurnal pattern on both meal feeding and *ad libitum* feeding, with lower glucose incorporation into lipid by the meal-fed animals (Kuhl and Reid, 1973).

DiGirolamo and Rudman (1966) compared the rate of *in vitro* conversion of glucose to fatty acid by slices of epididymal adipose tissue from rat, guinea pig, rabbit, and hamster. The relative rates were 157 : 83 : 12 : 1, respectively. Adding insulin to the culture medium resulted in 188-290% increase in the rate for rat tissue, but it had no effect on hamster tissue. Hamster adipose tissue may also convert glucose to fatty acids at a low and rather inflexible rate *in vivo* (Silverman and Zucker, 1976). Noncompensation may result from intake of low fat diet being held to current metabolic needs plus the small amount which can be converted to fat. Presumably, intake in excess of this amount inhibits further consumption through negative feedback (Silverman and Zucker, 1976). In contrast to results obtained in adipose tissue of intermittent fasting hamster, synthesis of liver lipids and glycogen increase after the meal, as has been established in the rat (Simek, 1975).

In the livers of chickens adapted to a single daily meal, the rate of fatty acid synthesis is increased (Leveille, 1967; Leveille and Hanson, 1965a). Ingestion of the daily 2-hour meal increases hepatic lipogenesis to a level which is more than twice that observed for livers of *ad libitum* fed chicks. Hepatic lipogenic enzyme activity in the chick does not appear to be increased by meal feeding (Leveille, 1967; Leveille and Hanson, 1965a). The 22 hour

period of fast between daily meals depresses hepatic lipogenesis much less in adapted chicks than in a similar period of food restriction in *ad libitum* fed chicks (Leveille *et al.*, 1975).

The capacity for fatty acid synthesis was significantly reduced in both meal-fed and nibbling rats as a consequence of increasing the percentage of energy derived from fat. The response to meal feeding decreased as the level of dietary fat increased, although the rate of lipogenesis in adipose tissue of meal-fed animals was still higher than that of tissue from nibbling rats. The activities of malic enzyme (ME) and glucose-6-phosphate dehydrogenase were higher in tissues of meal-fed animals and decreased as the level of dietary fat was increased (Leveille, 1970, 1975).

As the level of protein in the diet increased and, hence, the level of carbohydrate decreased, the rate of fatty acid synthesis was diminished. The difference between meal-fed and nibbling rats was apparent at all levels of dietary protein. As in the studies with dietary fat, the activity of ME paralleled the observed rates of fatty acid synthesis. The observed effects could be due to a reduction in dietary carbohydrate or to the increased amount of protein or fat ingested (Leveille, 1975).

Hepatic lipogenesis and the activities of ME and

citrate cleavage enzyme are reduced by feeding chicks high fat or high protein diets (Yeh and Leveille, 1969). Increased dietary fat, at the expense of carbohydrate but with a constant calorie/protein ratio, resulted in a significant impairment of lipogenesis as did increasing the level of dietary protein (Leveille *et al.*, 1975). This suggests that dietary protein and fat exert a direct effect on the capacity for fatty acid synthesis which cannot be totally accounted for by reduction in dietary carbohydrate.

Intermittent feeding leads, in addition to enhanced ability of the liver to synthesize fatty acids, to an increased ability to oxidize fatty acids (Fabry, 1969). A new equilibrium between anabolic and catabolic processes means that the enhanced lipid formation during the period of free access to food is balanced by more intense catabolic processes during the subsequent period of fasting. In the course of subsequent fasting, anabolic changes decline to an even lower level, while catabolic changes become markedly enhanced (Petrasek *et al.*, 1969).

Cohn *et al.* (1959, 1960, 1963, 1968) reported, in a series of experiments, that rats force-fed twice daily gained the same amount of weight but significantly more fat than *ad libitum* fed controls on an equal food intake. The increase in fat was at the expense of nitrogenous

constituents.

In the experiments of Fabry *et al.* (1969), the ratio of body fat in relation to body weight or total body protein was significantly higher in intermittently fed rats or in rats fed for 2 hours a day, than in control groups. On the other hand, Stevenson *et al.* (1964) confirmed the enhanced lipogenesis in adipose tissue but found a slightly reduced ratio of fat as compared with heavier controls fed *ad libitum*.

Leveille and Romsos (1974) found that approximately 70% of ingested energy is stored during the absorptive period following the initiation of a meal in the rat: 20% in the form of glycogen, and 50% in the form of fat. The remaining 30% of ingested energy serves to support metabolic functions during the absorptive period.

Muscle is the major site of glycogen storage in both meal-fed and *ad libitum* fed animals, accounting for over 90%. Most of the remainder is stored in the liver in the case of nibbling animals, and in adipose tissue in the case of meal-fed rats. Fifty percent of ingested energy stored in the form of lipid is synthesized and stored in adipose tissue (Leveille and Romsos, 1974). Protein ingested during the meal period in excess of that needed for immediate protein synthesis would be converted to lipid for storage.

Cohn and Joseph (1968) suggested that, during a weight reduction regimen for obese rats, the magnitude of the load of ingested nutrients and the frequency of its consumption play a role in the body weight loss and in the alterations in body composition. After rats had been made obese by force-feeding excess calories, they were allowed to ingest their food *ad libitum* or were pair force-fed against a control. The rats with free access to food ate sparingly until a body weight consistent with their age and sex was achieved; at this time, the food intake increased to approximately normal with consequent slow gain in body weight. The force-fed animals not only tended to lose less and regain more weight than their partners eating *ad libitum* but, in addition, these rats fed by a tube ended the experiment containing more body fat and less protein and water. When the amount of food eaten was restricted, there was no influence of feeding frequency on the rate of weight loss and on body composition.

Han (1973) demonstrated that rats force-fed two meals a day contained more fat and energy but less water than did *ad libitum* fed rats. The proportion of tissue gained as fat was much higher in rats fed two meals per day than in those fed *ad libitum*. Han (1973) suggested that higher fat deposition in the rats fed two meals per day indicated that overloaded carbohydrate may stimulate the lipogenesis

of the adipose tissues.

In mice, witholding food two or three separate 24-hour periods per week produced increased percentages of body fat but had no effect upon food intake or weight gains during experimental periods ranging from 14 to 44 days (Welch, 1968).

Han (1967) reported that sheep ingesting 8 meals per day gained body protein, fat, and energy at a more rapid and efficient rate than sheep fed one meal per day.

Lepkovski *et al.* (1960) showed that chickens trained to eat their daily feed in two hours were no different in body fat content than chickens fed the same amount of feed *ad libitum*. Han *et al.* (1967) also concluded that frequency of meals caused no effect on the body composition of chickens. However, Feigenbaum *et al.* (1962) obtained results with chickens which were contrary to those obtained by Cohn with rats. The fat content of chicks fed two meals per day was considerably lower than that of the control group fed *ad libitum* meals. These observations suggest that body fat accumulation varies from species to species, and even within the same species different results may be obtained under different experimental conditions.

When restricted diets with a predominance of carbohydrates are used, the ratio of body fat in relation to the body weight or total body protein was significantly

higher in intermittently fasting rats or in rats fed for two hours per day, than in control *ad libitum* groups (Fabry, 1969). The differences in the fat content were, in most instances, smaller than in Cohn's experiments. With the exception of Cohn's experiments on tube-fed rats where an equal caloric intake is ensured, the increment of body fat is quantitatively not so marked and readily reproducible in intermittent fasting animals. One might have expected a large increment of body fat in intermittently fasting animals in view of the high lipogenic activity in these animals (Fabry, 1969).

Rats fed a high fat diet had a higher concentration of body fat and deposited more fat daily than those fed a low fat diet (Wood and Reid, 1975). Fatty acid synthesis was also greatly increased as a result of meal feeding with the low fat diet but not with the high fat diet. The formation of body fat from dietary fat resulted in a greater deposition of fat in epididymal and perirenal fat pads, as well as in the carcasses of the rats given the high fat diet. Rats meal-fed the low fat diet, as a result of their greatly increased capacity for lipogenesis, had larger fat pads than nibbling rats fed the low fat diet. Conversely, rats meal-fed the high fat diet had smaller fat pads than nibbling rats fed the high fat diet. These results suggest that meal-fed and nibbling rats respond differently to changes in diet composition.

Wardlaw *et al* (1969) observed that decreased feeding frequency did not affect fat deposition in mature rats, whereas, in the younger animals, infrequent feeding increased body fat content. Heggeness (1965) used a feeding procedure for rats which involved alternation between three days of *ad libitum* food intake and three days of food restriction. He found an increase in body fat as a result of the alternation in feeding pattern in animals which were placed on the experiment at 25 days but not in those placed on experiment at 55 days of age. Friend (1967) was unable to show a significant difference in fat deposition of rats between single feeders and multiple feeders except when young rats with an initial weight of 117 g were used. With these young rats, there was an increased deposition of fat when only one feeding was given. In the studies of Van Putten *et al* (1955) and Cohn (1959, 1960, 1963, 1968) in which increased deposition of fat with decreasing feeding frequency was demonstrated, rats which were relatively immature at the beginning of the experiment were utilized.

The fat that is deposited in adipose tissue and other organs such as the liver may be derived from either dietary fat or *de novo* synthesis. Both synthesis and mobilization are processes that are self-regulating to some extent but many hormones influence the rates of lipid metabolism and, thus, the balance between lipogenesis and lipolysis (Meier

and Burns, 1976).

Much of the lipogenic influence of insulin is attributed to its role in carbohydrate metabolism. It promotes the utilization of glucose with the formation of α-glycerol phosphate and accelerates the conversion of pyruvate to acetyl CoA. Insulin also promotes lipogenesis in adipose tissue by making glucose available through facilitated transport. Insulin promotes the production of NADPH by directing carbohydrate metabolism through the hexose monophosphate shunt. In addition to its rapid lipogenic influence, insulin also has a delayed effect by increasing the synthesis of enzymes involved in lipogenesis. Insulin is a potent inhibitor of fat mobilization. It facilitates the movement of glucose into the adipocytes with subsequent production of α-glycerol phosphate. Thus, when glucose is available, most of the fatty acids mobilized from the triglycerides are re-esterified and little fatty acid is released to the blood. Insulin also has an inhibitory influence on the activity of cyclic-AMP, an effect that could reduce mobilization of fat (Ip *et al.*, 1977; Meier and Burns, 1976).

The lipogenic effect of insulin on fat cells from fasted/re-fed rats was found to be enhanced (Braun and Fabry, 1969) as indicated by an increased incorporation of ^{14}C-1-glucose into total lipids of fat cells isolated from

adipose tissue of fasted/re-fed rats as compared to fat cells from *ad libitum* fed rats. Similarly, an increased lipogenic effect of exogenous insulin on adipose tissue from periodically fed rats has been demonstrated *in vitro* and *in vivo* (Braun *et al.*, 1967). Meal-fed rats show a greater insulin sensitivity than do *ad libitum* fed rats (Reiser and Hallfrisch, 1977; Wiley and Leveille, 1970).

Glucagon has an inhibitory effect on lipogenesis. The opposing activities of insulin and glucagon may be related to their inhibitory and stimulatory effects, respectively, on cyclic-AMP which inhibits hepatic lipogenesis (Meier and Burns, 1976).

Although growth hormone has an immediate inhibitory effect on fatty acid synthesis *in vitro*, administration of the hormone for several days can repair lipogenesis in hypophysectomized rats and pigeons. Obesity resulting from chronic growth hormone administration is also accompanied by the development of progressive pancreatic islet hypertropy and hyperplasia indicative of increased insulin production (Meier and Burns, 1976).

Prolactin has been shown to have marked stimulatory influence on body fat stores. *In vitro* studies of fish indicate that prolactin stimulates the rapid formation of hepatic lipids. Prolactin given *in vivo* also stimulates the activities of several enzymes involved in lipogenesis

in the pigeon (Meier and Burns, 1976). Studies of rat adipose tissue indicate that prolactin has several lipogenic activities that are similar to those of insulin. Provided that glucose is present, it stimulates the synthesis of fatty acids *in vitro* from acetate and pyruvate, and directs carbohydrate metabolism through the hexose monophosphate shunt (Meier and Burns, 1976). On the other hand, prolactin does not repair fatty acid synthesis in alloxan-diabetic rats whereas insulin does. Prolactin delays the degradation of insulin thereby increasing its activity (Meier and Burns, 1976). Prolactin stimulates increases or decreases in fat storage depending on whether it is present in larger quantities during daily intervals of lipogenic or lipolytic sensitivities (Meier and Burns, 1976).

Hormonal regulation of fat mobilization centers on the hormone-sensitive lipase which catalyzes the conversion of triglycerides to fatty acids. Epinephrine, norephinephrine, and glucagon have strong stimulatory influences on this reaction. ACTH has similar effects *in vitro* but it is not clear whether it has a physiological role. Cyclic-AMP also promotes the conversion of triglycerides to fatty acids and it is generally accepted that many of the lipolytic activities of hormones are consequences of their effects on adenyl cyclase which catalyzes the conversion of ATP to c-AMP.

Thyroxin has a supportive effect on lipolysis, perhaps by way of augmenting catecholamine activity. Glucocorticoids also have supportive influences on the mobilization of fat (Meier and Burns, 1976).

Results of several experiments in different species suggest that infrequent feeding might cause a rise of serum cholesterol levels and worsening of experimental atherosclerosis. Okey *et al.* (1960) investigated the serum cholesterol levels of *ad libitum* fed rats and rats fed for one hour per day. On the high cholesterol diet, female rats fed one hour had higher blood but lower liver cholesterol content than rats fed *ad libitum*.

Cohn *et al.* (1961) fed chicks an atherogenic diet either *ad libitum* or for only two hours per day. After five weeks of the experiment, the chicks fed for two hours had doubled their serum cholesterol levels and had an increased incidence of atherosclerotic vascular lesions relative to birds fed *ad libitum*, despite the fact that the *ad libitum* fed chicks consumed more food and ingested more cholesterol. When the chicks were switched to a cholesterol-free diet, the return of the cholesterol levels to normal values and the regression of vascular plaques were more rapid in *ad libitum* fed animals. Leveille and Hanson (1965a, 1965b) also reported an increased cholesterol synthesis in liver in rats and chicks fed for two hours per day.

On a high-fat diet, monkeys fed twice for 30 minutes daily had significantly greater serum cholesterol levels than monkeys with free access to food (Gopalan *et al.*, 1962). In humans, a dietary pattern of large infrequent feedings is associated with higher serum cholesterol levels than a pattern of frequent smaller meals (Fabry and Tepperman, 1970). In some hyperlipidemic patients, the level of serum lipids declined if the diet, otherwise unchanged, was divided into a greater number of small portions (Cohn, 1961).

Enhanced biosynthesis of lipids and triglycerides can play a significant part in the development of obesity if the energy balance is favorable for energy storage (Fabry and Tepperman, 1970). A study of several years' duration evaluated the feeding patterns in a group of subjects with "resistant" obesity and a group of lean but otherwise healthy individuals (Pawan, 1972). In the obese group, 60% of the women and 54% of the men were found to have a feeding pattern of two meals daily, the larger meal being consumed in the evening, whereas only 16% of the women and 32% of the men consumed more than three meals daily.

The relationship between frequency of eating and adiposity was also studied in a cross-sectional population of men and women ages 35 to 69 (Metzner, 1977). Frequency of eating was related inversely to the adiposity index. The

proportion of overweight people tended to decrease as frequency of meals increased from three or fewer to five or more per day. The proportion of men with normal weight increased with meal frequency. Studies by Young *et al.* (1971a, 1971b) showed that there was a slightly greater weight loss among subjects when taking more frequent meals.

The increased cholesterol synthesis in liver can participate in the hypercholesterolemic effects of infrequent feeding observed in many species (Fabry and Tepperman, 1970).

Serum phospholipid concentrations were higher in the subjects having one meal per day than in those on more frequent regimens. A similar effect was observed in the values for serum triglycerides (Young *et al.*, 1971a).

The percentage of subjects in which the ischemic heart disease was diagnosed decreased significantly with the increased meal frequency (Fabry, 1969).

In conclusion, meal frequency might play a significant role in regulation of metabolism and may even cause pathological changes under certain conditions.

Force-fed rats were utilized in previous studies to demonstrate that feeding frequency increased nitrogen excretion and body fat accumulation. It is possible that force feeding *per se* caused the metabolic alterations. Consequently, these experiments were undertaken to determine the influence of meal frequency on body weight gain, body composition, and nitrogen balance in rats.

CHAPTER II

INFLUENCE OF DIET COMPOSITION ON NITROGEN BALANCE AND BODY COMPOSITION IN MEAL-EATING AND NIBBLING RATS

Introduction

The time distribution of food intake has been shown to influence metabolism in several species. When the laboratory rat, which is by nature a nibbler, is forced to become a meal-eater, the alterations in the ingestion and absorption of food may produce changes in various enzymatic activities and in body composition (Leveille, 1970, 1975). Pair-fed rats given food by a stomach tube twice daily gained essentially the same amount of body weight as did rats with free access to food; however, the force-fed rats had a marked increase in total fat content in comparison to rats eating *ad libitum* (Cohn and Joseph, 1963; Han, 1973). Cohn *et al.* (1963) also showed in rats that decreasing the number of meals was accompanied by increased urinary nitrogen excretion, nitrogen intake being constant. They concluded that the capacity of rate-limiting enzymatic reactions concerned with protein anabolism may have been exceeded by the load of nutrients presented to the animal. Thus, an increased quantity of absorbed amino acids was catabolized; the nitrogen was

excreted as urea, and the residual carbon chains utilized for fat synthesis.

In addition to alteration of meal pattern by force-feeding, rats have also been trained to eat meals. Rats trained to consume their food in a short period of time each day usually ingest only 60% to 80% of the amount of nutrients eaten by the control rats with free access to food. However, the trained rats gain as much body weight as the *ad libitum* fed rats (Leveille, 1970; Cohn and Joseph, 1970). Thus, these trained rats are more efficient in converting food to body weight than *ad libitum* fed rats. These results suggest that the pattern of food intake may alter protein and energy metabolism. The objectives of this study were to investigate the influence of diet composition on nitrogen balance and body composition in rats fed one 2-hour meal per 24 or 48 hours compared with pair-fed rats continuously fed with a feeding apparatus.

Materials & Methods

Male Sprague-Dawley rats[1] weighing approximately 150 g or 250 g initially, were individually housed in metal cages having raised wire floors. The room was lighted from 0700 hours to 1900 hours. Water was available *ad libitum*. Five experiments were conducted. The composition of the four semipurified diets used in the

various experiments is presented in Table 1. The high-fat diet was prepared by substituting tallow on an equal energy basis for all the glucose in the 20% casein diet. Food intake was recorded daily and body weights were recorded weekly.

Meal-fed rats were given access to food for only 2 hours per 24 or 48 hours (0900-1100 hours). Nibblers were pair-fed to their meal-eating pair-mates by means of an automated feeding apparatus (Romsos and Leveille, 1974b). The feeding apparatus continuously delivered food to the nibblers. The rats consumed the diet as it was delivered to the food cup, without allowing the diet to accumulate in the food cup.

During the fourth or fifth week of the experiment, the rats were placed in metabolic cages to collect urine and feces separately. About 1 ml of 4 N HCl was added to each urine collection flask. The cages were rinsed daily to quantitatively recover urine. Daily specimens of urine and feces were pooled at the end of 7 days.

At the termination of the experiments, rats were decapitated at 1200 hours and the carcasses were frozen until analyzed. Carcass weight represented the weight of the rat minus blood loss and minus weight of the stomach contents. Carcasses were dried to constant weight, and ground for the body composition analyses.

TABLE 1

COMPOSITION OF THE DIETS

	Diet			
	1	2	3	4
Casein, g	10.0	20.0	30.0	20.0
Basal,[1] g	13.9	13.9	13.9	13.9
Glucose,[2] g	76.1	66.1	56.1	-
Tallow,[2] g	-	-	-	28.0
Total	100.0	100.0	100.0	61.9

[1]The basal mix contained (in g/13.9 g): methionine, 0.3; vitamin mix, 0.4 (see Yeh and Leveille, 1969); mineral oil mix, 4.0 (see Leveille and O'Hea, 1967); choline chloride, 0.2; cellulose, 4.0; and corn oil, 5.0.

[2]Energy values used were: glucose = 3.64 kcal/g, tallow = 8.59 kcal/g (Brambila and Hill, 1966).

Urinary, fecal, feed, and carcass nitrogen were determined by the semi-micro or micro Kjeldhal procedure (Horwitz, 1960) and body protein was calculated as nitrogen × 6.25. Body fat was determined gravimetrically after chloroform: methanol (3:2 v/v) extraction. In experiments 3 and 4, the body fat and protein were calculated from the following equations which were derived from direct carcass analysis of 48 rats:

$$\% \text{ fat} = 60.62 - 0.77 \times \% H_2O$$

$$(r = 0.76;\ p < 0.01)$$

$$\% \text{ protein} = -19.48 + 0.559 \times \% H_2O$$

$$(r = 0.80;\ p < 0.01)$$

The data were analyzed statistically by means of the Student's t-test or analysis of variance. When the F value was significant, the means were compared by Scheffé test (Gill, 1977).

Results

Experiment 1. The food intake and body weight of the rats fed the 30% casein, high-carbohydrate diet are shown in Table 2. There was no difference in weight gain between the meal-eaters and the pair-fed nibblers. Average increases in body weights were parallel during the 8 week study. Urine and feces were collected for 7 days during the sixth week of the experiment. Meal-eaters excreted slightly more urinary, but less fecal nitrogen. There was

TABLE 2

FOOD INTAKE, WEIGHT GAIN, NITROGEN BALANCE, AND CARCASS COMPOSITION OF RATS FED A 30% CASEIN, HIGH CARBOHYDRATE DIET (EXP. 1)[1]

Parameter	Meal-Eater	Nibbler
Food intake (g/day)	17.4 ± 0.3	17.4 ± 0.3
Weight gain (g)	145.0 ± 3.0	155.0 ± 4.0
Urinary nitrogen (mg/day)[2]	446.0 ± 24.0	383.0 ± 26.0
Fecal nitrogen (mg/day)[2]	44.0 ± 2.0	54.0 ± 4.0[3]
Nitrogen balance (mg/day)	334.0 ± 18.0	347.0 ± 24.0
Carcass weight (g)	371.0 ± 8.0	395.0 ± 7.0
Carcass protein (%)	19.2 ± 0.8	18.9 ± 0.6
Carcass fat (%)	12.8 ± 1.2	14.6 ± 1.3

[1]Values represent mean ± SEM for 10 rats weighing 251 ± 3 g initially and fed for 8 weeks. Meal-eaters = one meal per 24 hours.

[2]Urine and feces were collected during the sixth week.

[3]Significant change ($p < 0.05$), meal-eaters *vs* nibblers.

no difference in overall nitrogen balance between the two groups (Table 2). Carcass weight, and carcass protein and fat are presented in Table 2. Carcass protein values agree with our nitrogen balance data in that there were no differences in either carcass protein or nitrogen balance between the two groups. Carcass weights and fat contents of meal-fed and nibbling groups also were similar.

Experiment 2. Diets containing 10%, 20%, or 30% casein were fed to investigate whether the level of protein would affect the nitrogen balance and the body composition of meal-fed rats. Meal-eaters and nibblers fed the same amount of protein gained practically the same amount of body weight. Weight gain increased as the level of casein in the diet increased.

Urinary and fecal nitrogen excretions increased as the level of casein in the diet increased ($p < 0.01$) (Table 3). There was no significant difference in urinary nitrogen excretion between the meal-eaters and nibblers. Fecal nitrogen excretion tended to be less in meal-eaters than in nibblers and was significantly lower ($p < 0.05$) in meal-eaters than in nibblers fed 30% casein. Again, meal frequency did not influence nitrogen balance when the three levels of casein were fed (Table 3).

The quantity of protein (nitrogen × 6.25) in the carcasses, in contrast to force-feeding studies (Cohn and

TABLE 3

FOOD INTAKE, WEIGHT GAIN, NITROGEN BALANCE, WATER INTAKE, AND CARCASS COMPOSITION OF RATS FED DIFFERENT LEVELS OF DIETARY PROTEIN (EXP. 2)[1]

Parameter	10% Casein		20% Casein		30% Casein			
	Meal-eater	Nibbler	Meal-eater	Nibbler	Meal-eater	Nibbler	MS_E[2]	AOV[3]
Food intake (g/day)	13.2	13.2	16.5	16.5	17.3	17.3	10.4	NS
Weight gain (g)	31.0	19.0	107.0	93.0	116.0	105.0	502.0	D
Urinary nitrogen[4] (mg/day)	144.0	152.0	350.0	343.0	521.0	551.0	1627.0	D
Fecal nitrogen[4] (mg/day)	19.0	24.0	35.0	44.0	40.0	62.0	415.0	D,M
Nitrogen balance (mg/day)	51.0	38.0	333.0	332.0	349.0	280.0	3776.0	D
24-hr water intake[5] (ml)	35.0	17.0	49.0	24.0	49.0	28.0	3.0	M,D,MxD
Water/food (ml/g)	2.3	1.2	2.3	1.3	2.8	1.4	0.6	M
Urine volume (ml/day)	18.0	8.0	31.0	11.0	36.0	13.0	60.0	M,D,MxD
Carcass weight (g)	317.0	308.0	393.0	381.0	401.0	388.0	905.0	D
Carcass protein (%)	19.8	17.8	18.4	16.4	16.4	15.7	3.1	D,M
Carcass fat (%)	7.2	9.0	9.1	11.1	10.8	11.5	26.4	NS
Carcass moisture (%)	68.2	65.2	67.6	64.2	65.9	63.3	6.7	M

[1]Values represent mean for 8 rats weighing 290 ± 2 g initially fed for 8 weeks; meal-eaters = one meal per 24 hours.

[2]MS_E = mean square of error.

[3]AOV = analysis of variance: NS = not significant; D = diet; M = meal pattern, significant ($p < 0.05$) change .

[4]Urine and feces were collected during the fourth week.

[5]Water intake was measured for 5 days during the fifth week.

Joseph, 1959; Han, 1973), was higher ($p < 0.05$) in meal-fed rats consuming the 10% and 20% casein diets than in nibblers fed the same diets (Table 3). At each level of protein intake, nibblers had slightly more body fat than the meal-eaters, but the differences were not statistically significant.

During the experiment, a greater water consumption by the meal-eaters was observed; therefore, water intakes of these rats were measured during the fifth week. Total water intake of meal-eaters was higher ($p < 0.01$) than for the nibblers at all three levels of dietary casein. When the water intake was expressed as ml water consumed per g food eaten, meal-eaters had a higher water to food ratio; the ratio was not influenced by the level of protein in the diet. Urine volumes (24 hour) of the meal-eaters were also greater ($p < 0.01$) than observed for the nibblers (Table 3).

Experiment 3. In this experiment, source of dietary energy was varied. The high-fat diet (Table 1, Diet 4) contained 45% tallow by weight (71% of the energy) and was carbohydrate-free. The protein to energy ratio of this diet was similar to that of the high-carbohydrate, 20% casein diet. Each of the four groups of rats received the same amount of dietary energy. Body weight gains were similar in meal-eaters and nibblers within

both high-fat and high-carbohydrate groups (Table 4). However, rats fed the high-carbohydrate diet gained significantly more ($p < 0.05$) weight than rats fed the high-fat diet (Table 4). The increased weight gain of rats fed the high-carbohydrate diet was not expected.

Nitrogen balance was not altered by meal-feeding either of the two diets. Less nitrogen was retained in rats fed the high-fat diet ($p < 0.01$). There were no significant differences between meal-eaters and nibblers in either protein or fat content of the carcasses.

The influence of the high-fat diet on water consumption of meal-fed and nibbling rats was compared with the results obtained when a high-carbohydrate diet was fed (Table 4). Over a 24-hour period, meal-eaters fed the high-fat diet consumed only slightly more water than did the nibblers. Urine volumes were not significantly influenced by the meal frequency when the high-fat diet was fed. In agreement with experiment 2, rats meal-fed the high-carbohydrate diet consumed more water ($p < 0.01$), approximately double the amount consumed by the nibblers during a 24-hour period. Thus, meal feeding increased water intake to a greater extent when a high-carbohydrate diet was fed than when a high-fat diet was fed. The source of dietary energy (glucose or tallow) had little influence on water intake of nibbling rats. These differences might

TABLE 4

FOOD INTAKE, WEIGHT GAIN, NITROGEN BALANCE, WATER INTAKE, AND CARCASS COMPOSITION OF RATS FED HIGH-FAT *versus* HIGH-CARBOHYDRATE DIETS (EXP. 3)[1]

Parameter	High-Fat Diet		High-CHO Diet			
	Meal-eater	Nibbler	Meal-eater	Nibbler	MS_E[2]	AOV[3]
Food intake (kcal/day)	60.0	60.0	60.0	60.0		NS
Weight gain (g)	110.0	95.0	132.0	134.0	398.0	D
Urinary nitrogen[4] (mg/day)	498.0	521.0	426.0	356.0	819.0	D,M,DxM
Fecal nitrogen[4] (mg/day)	53.0	45.0	39.0	48.0	118.0	DxM
Nitrogen balance (mg/day)	155.0	149.0	210.0	267.0	3334.0	D
24-hr water intake[5] (ml)	26.0	21.0	52.0	24.0	40.0	D,M,DxM
Urine volume (ml/day)	23.0	20.0	48.0	20.0	68.0	D,M,DxM
Carcass weight (g)	369.0	350.0	392.0	396.0	1078.0	D
Carcass protein (%)	15.2	14.5	15.4	15.3	1.9	NS
Carcass fat (%)	12.8	13.8	12.6	12.7	3.7	NS
Carcass moisture (%)	67.1	60.8	62.4	62.3	6.6	NS

[1]Values represent mean for 8 rats, weighing 246 ± 2 g initially, fed for 7 weeks; meal-eaters, one meal per 24 hours.

[2]MS_E = mean square of error.

[3]AOV = analysis of variance: NS = not significant; D = diet; M = meal pattern, significant ($p < 0.05$) change.

[4]Urine and feces were collected during the fifth week.

[5]Water intake was measured for 4 days during the fourth week.

be related to the greater osmotic effect of carbohydrate than of fat (Harper and Spivey, 1958).

Experiment 4. The results of the first three experiments indicated that neither the level of dietary protein nor the energy sources tested caused adverse effects on nitrogen balance or body fat content in rats meal-fed once per day. Thus, rats were fed a 20% casein, high-carbohydrate diet only once every other day to see if these rats would retain less nitrogen than the nibbling rats and whether this meal pattern would result in a change in body composition. After a week of adaptation to a 2-hour feeding once per day, the meal-fed rats were changed to the regimen of one 2-hour feeding every other day and were fed for 6 more weeks. Meal-eaters lost 48 g and nibblers lost 34 g weight (Table 5) during these 6 weeks. After the first week, because of a decrease in food intake, both groups lost considerable weight but, starting from the fifth week, they began to gain weight. Although they did not reach their initial weights, they were in positive nitrogen balance during the 5th and 6th weeks. The rats gained 5 to 6 g body weight during the nitrogen balance trial. The nibblers excreted more ($p < 0.05$) urinary and fecal nitrogen than meal-eaters, but the overall nitrogen balance was not influenced by the meal pattern (Table 5). There were no differences in carcass weight or in protein, fat, or moisture content of the carcasses between the

TABLE 5

FOOD INTAKE, WEIGHT GAIN, NITROGEN BALANCE, AND CARCASS COMPOSITION OF RATS MEAL-FED A 20% CASEIN, HIGH-CARBOHYDRATE DIET ONCE EVERY 48 HOURS (EXP. 4)[1]

Parameter	Meal-Eaters	Nibblers
Food intake (g/day)	8.2 ± 0.4	8.2 ± 0.4
Body weight at end of 1st week (g)	254.0 ± 5.0	261.0 ± 5.0
Final weight (g)	206.0 ± 9.0	227.0 ± 8.0
Urinary nitrogen (mg/day)[2]	206.0 ± 7.0	241.0 ± 11.0[3]
Fecal nitrogen (mg/day)[2]	15.0 ± 2.0	22.0 ± 2.0[3]
Nitrogen balance (mg/day)	107.0 ± 21.0	71.0 ± 11.0
Carcass protein (%)	18.3 ± 0.2	17.9 ± 0.2
Carcass fat (%)	8.6 ± 0.2	9.2 ± 0.3
Carcass moisture (%)	67.6 ± 0.3	66.8 ± 0.3

[1]Values represent mean ± SEM for 8 rats weighing 247 ± 3 g initially, fed for 7 weeks; meal-eaters were fed for 2 hours once every 24 hours for 1 week, then once every 48 hours for 6 weeks.

[2]Urine and feces were collected during the fifth week.

[3]Significant change ($p < 0.05$): meal-eaters *vs* nibblers

two groups (Table 5).

Experiment 5. Our experiments did not show any adverse effects of meal-feeding on nitrogen balance or body composition when 200 to 250 g rats were utilized. Since Cohn *et al.* (1959, 1968) utilized smaller rats in most of their studies, rats initially weighting 140 g were fed a 20% casein, high-carbohydrate diet. There was no difference in body weight gain between the meal-eating and nibbling rats (Table 6). Meal-eaters excreted slightly more urinary and less fecal nitrogen. Nitrogen balance tended to be less positive in meal-eaters than in nibblers but the differences were not statistically significant. There was no difference in carcass weight, protein, or moisture between the two groups. However, in this experiment, nibblers deposited more ($p < 0.05$) body fat than meal-eaters.

Discussion

In the present study, meal-eaters were fed for 2 hours per 24 or 48 hours, and the nibblers were pair-fed the same amount of food eaten by the meal-eaters. Body weight gains were not different for the two groups. This observation is consistent with the recent findings of Pocknee and Heaton (1976) who also used an automated feeding machine. Meal-eaters retained as much nitrogen as the

TABLE 6

FOOD INTAKE, WEIGHT GAIN, NITROGEN BALANCE, AND CARCASS COMPOSITION OF YOUNG MEAL-FED RATS (EXP. 5)[1]

Parameter	Meal-Eaters	Nibblers
Food intake (g/day)	11.6 ± 0.5	11.6 ± 0.5
Body weight gain (g)	111.0 ± 11.0	133.0 ± 12.0
Urinary nitrogen (mg/day)[2]	398.0 ± 78.0	284.0 ± 32.0
Fecal nitrogen (mg/day)[2]	22.0 ± 3.0	43.0 ± 3.0[3]
Nitrogen balance (mg/day)	192.0 ± 91.0	285.0 ± 46.0
Carcass weight (g)	294.0 ± 26.0	268.0 ± 29.0
Carcass protein (%)	21.84 ± 0.46	20.02 ± 0.83
Carcass fat (%)	11.01 ± 0.58	13.37 ± 0.58[3]
Carcass moisture (%)	68.38 ± 0.44	68.27 ± 0.70

[1]Values represent mean for 8 rats, weighing 140 ± 1 g initially, fed a 20% casein, high-carbohydrate diet for 8 weeks; meal-eaters, one meal per 24 hours.

[2]Urine and feces were collected during the fifth week.

[3]Significant change ($p < 0.05$), meal-eaters *vs* nibblers.

nibblers regardless of the composition of the diet fed or the size of the animals. Likewise, the meal-fed rats retained as much nitrogen as nibblers, even when the rats were fed only 2 hours per 48 hours. The percent body protein, like nitrogen balance, was not reduced in meal-fed rats. Actually, the tendency was towards greater body protein retention in meal-eaters, although the results were not significantly different except in one experiment. Several studies in humans also showed no significant differences in nitrogen balance or body weight resulting from an alteration in frequency of eating (Bortz *et al.*, 1966; Young *et al.*, 1971; Taylor *et al.*, 1973; MacLean and Graham, 1976). The mechanism(s) whereby the meal-fed rats, even when fed only once every other day, can retain as much nitrogen as pair-fed nibbling rats while force-fed rats retain less nitrogen (Cohn and Joseph, 1963; Han, 1973) remains to be established.

Under the conditions of controlled food intake, we did not observe an increase in the body fat content in the meal-fed rats. This observation is consistent with previous reports (Leveille, 1970; Muiruri and Leveille, 1970; DeBont *et al.*, 1975) which indicated that rats fed for 2 hours each day do not become fatter than *ad libitum* fed controls. Likewise, intermittently fed (offered food every other day or 2 to 3 days per week) rats do not gain

more body fat than *ad libitum* fed controls (Fabry, 1969; Morin-Jomain, 1966, 1969). The ratio of body fat to body weight is sometimes higher in intermittently-fed rats than in *ad libitum* fed controls, but the absolute gain in fat is less because the intermittently-fed rats gain considerably less body weight. Alterations in diet composition did not influence comparisons between meal-eaters and nibblers in the present study, in agreement with the results of Wood and Reed (1975). Both meal-fed and force-fed rats have an increased lipogenic capacity (Leveille, 1970, 1972, 1975; Muiruri and Leveille, 1970; DeBont *et al.*, 1975; Beaton *et al.*, 1964; Hollifield and Parson, 1962; Cohn and Joseph, 1967). However, the increase in lipogenesis exhibits a cyclic pattern, being high immediately after the meal and then low later (Leveille and O'Hea, 1967). Consequently, the increased lipogenic capacity does not necessarily result in an increase in body fat.

Several suggestions have been made in an attempt to explain the increase in body fat in force-fed rats. A decreased thyroid function in force-fed rats has been suggested (Cohn and Joseph, 1960) but thyroxin injections did not equalize body fat in the two groups. The age of the rats may be another factor affecting the results. In young force-fed rats, more fat and less protein accumulated than in *ad libitum* fed rats; these differences were not as

apparent in older rats (Cohn and Joseph, 1959; Wardlaw *et al.*, 1969). In our experiment with the younger rats, meal-eaters had as much body protein as nibblers, and they accumulated slightly less body fat than the nibbling rats. Physical activity may be altered by the meal pattern. Meal-fed rats have been reported to exhibit less spontaneous activity than *ad libitum* fed rats (Leveille and O'Hea, 1967). However, more recently it has been demonstrated that total activity of meal-fed rats, as estimated with electronic activity meters, was not reduced (Romsos and Leveille, 1977).

There are a number of differences between previous force-feeding experiments and our controlled meal-feeding study. In the controlled meal-feeding studies, the level of food intake was restricted. Different results might possibly have been obtained with higher levels of food intake; however, we have been unable to induce meal-fed rats to consume as much food as *ad libitum* fed rats eat. Another difference is the rate and form in which the diet was ingested. Food might be digested at a different rate when it is force-fed. DeCastro and Balagura (1975) indicated that immediately after the voluntary ingestion of food, a large proportion is found in the stomach; however, if the rats are force-fed over the same period of time, more of the nutrients are found in the intestine. It is

possible that increased fat deposition in force-fed rats results from increased rate of nutrient absorption. In force-feeding, the diet is mixed with water and given as a liquid suspension. Keane *et al.* (1962) reported that when water was added to their experimental diets, a significant growth-promoting effect was obtained over that found when water was not added. The form in which the diet is administered to force-fed rats may influence their body composition.

Both force-fed (Cohn and Joseph, 1959, 1963) and meal-fed (Leveille, 1970, 1972; DeBont *et al.*, 1975) rats require an initial adaptation to the feeding pattern; reduced quantities of food are consumed during this period. This adaptation might cause a metabolic adaptation and compensatory gain (DeBont *et al.*, 1975; Leveille, 1972; Szepesi and Vojnik, 1975; Szepesi *et al.*, 1976) during the rest of the experimental period. In our present experiments, nibblers were pair-fed the amount of food consumed by the meal-eaters; thus, both groups of rats underwent the same initial adaptation. Cohn and Joseph (1963) did report that when rats were force-fed a protein-free diet without initial adaptation, no significant difference was observed in body fat content between force-fed and *ad libitum* fed rats. They were able to pair force-feed this diet without initial adaptation because the *ad libitum* fed

rats consumed reduced quantities of the protein-free diet. It has also been shown that meal-fed rats appear more efficient in converting dietary energy to body weight for the first 3 to 6 weeks of meal-eating; however, in longer-term studies, this increased energetic efficiency was not apparent (DeBont *et al.*, 1975).

These observations suggest that factors other than the frequency of eating *per se* are involved in the regulation of the increased body fat accumulation in force-fed rats.

CHAPTER III

INFLUENCE OF A LIQUID DIET AND MEAL PATTERN ON BODY WEIGHT AND BODY FAT IN RATS

Introduction

Obesity is a serious public health problem in Western societies (Fabry, 1969) and the pattern of food intake, in addition to many other factors, has been suggested to influence obesity in humans (Fabry, 1969; Fabry and Tepperman, 1970; Fabry *et al.*, 1964; Gordon *et al.*, 1963). Studies with rats which were pair-fed with a stomach tube twice daily compared with *ad libitum* fed control rats supported the suggestion that meal pattern may affect body fatness. Cohn *et al.* (1955, 1959, 1963) observed that after force-fed rats had been gradually adapted to ingesting two meals daily for 1 week, they deposited more body fat than the *ad libitum* fed controls. On the other hand, rats trained to eat one meal per day (meal-eaters) consumed less food than the *ad libitum* fed controls but gained body weight at the same rate as the *ad libitum* fed rats (Leveille, 1970, 1975). These meal-fed rats gained as much fat as the *ad libitum* fed controls while consuming less food. Both the force-fed and the meal-fed rats consumed restricted amounts of food initially, but the food

intake of the control rats was not restricted. When the food intake of the control rats was restricted, with an automated feeding machine, to the level consumed by meal-fed rats, no differences in body fat gain were observed between the meal-fed and continuously pair-fed rats (Ozelci *et al.*, 1977). These results suggested that the increased body fat accumulation observed in force-fed rats (Cohn *et al.*, 1955, 1959, 1963; Han, 1973), which has been attributed to their altered meal pattern, may not occur when the feeding pattern of rats is altered by other mechanisms.

The present experiments were designed to determine the effects of two differences in experimental design between previous force-feeding and meal-feeding experiments on body weight gain and on body fat gain of rats. It is necessary to add water to the diet before force-feeding rats; however, *ad libitum* fed control rats have routinely consumed a dry diet. Addition of water to the diet has been reported by others (Keane *et al.*, 1962; Hopkins and Steinke, 1976) to increase growth of rats. Consequently, three experiments were conducted to evaluate the influence of feeding the diet in liquid form. Secondly, the level of food intake, *ad libitum* or restricted, may alter the response to the feeding pattern (Cohn and Joseph, 1968). Thus, in one experiment, rats were force-fed *ad libitum* levels of intake without previous adaptation to force-

feeding and, in another experiment, meal-fed rats were offered two meals per day rather than a single meal per day to induce them to increase their food intake.

Materials & Methods

Five experiments were conducted utilizing male Sprague-Dawley rats[1] weighing approximately 110 to 150 g or 250 g initially. Rats were individually housed in stainless steel cages in a temperature-regulated room (22 ± 2°C) with lights on from 0700 hours to 1900 hours. Water was available *ad libitum*. Table 7 presents the composition of the two purified diets utilized. Diet 2 was utilized in experiments where rats were force-fed. Lactalbumin was substituted for casein because of the greater water solubility of lactalbumin as compared to casein. This diet was cellulose-free and contained 35.05 parts glucose and 35.05 parts corn starch to duplicate the diet used in the force-feeding experiments of Cohn *et al.* (1959, 1963). Food intake (dry weight basis) was recorded daily; body weights were recorded twice a week.

Rats were killed by decapitation; epididymal fat pads, the liver (experiment 1 only), and stomach contents were removed. Carcass weight represented the weight of the rat minus blood loss and minus weight of the stomach contents. Carcasses were frozen until analyzed. Body fat

TABLE 7

COMPOSITION OF DIETS

	Diet (g)	
	1	2
Casein	20.0	-
Lactalbumin	-	20.0
Basal[1]	9.9	9.9
Cellulose	4.0	-
Glucose	66.1	35.05
Corn starch	-	35.05

[1]The basal mix contained (in g/9.9 g): methionine, 0.3; vitamin mix, 0.4 (Yeh and Leveille, 1969); mineral mix, 4.0 (Leveille and O'Hea, 1967); choline chloride, 0.2; and corn oil, 5.0.

was determined gravimetrically after chloroform:methanol (3:2 v/v) extraction of the carcasses which had been dried and ground or homogenized with an equal amount of water. Duplicate samples of the warm homogenate were taken immediately after blending. All the data were evaluated statistically by the Student's t-test or by analysis of variance. Treatment differences were compared by the least significant range procedure for *a posteriori* tests (Sokal and Rohlf, 1969).

Experiment 1. Rats weighing approximately 250 g initially were allotted to three treatment groups. Meal-eaters were fed for 2 hours per day (0900 hours to 1100 hours) and nibblers were continuously pair-fed with an automated feeding machine, as described in Chapter II (Romsos and Leveille, 1974b). The third group was fed the same amount of food mixed with an equal weight of water; these rats were fed at 0900 hours daily. The liquid-fed rats consumed about 50% of the diet by 1900 hours and the remaining 50% was consumed during the night.

Blood samples were taken on each of 3 days during the 6th week to determine blood glucose levels. At 0800 and 1200 hours, rats were bled by clipping their tails. Blood (0.1 ml) was collected into heparinized capillary tubes. Protein was precipitated with $Ba(OH)_2$ and $ZnSO_4$, and the samples were centrifuged. Blood glucose levels

were determined in the supernatant by the glucose oxidase procedure.[2]

At the termination of the experiment, *in vivo* rates of fatty acid synthesis were measured in liver and adipose tissue by the amount of tritiated water incorporated into fatty acids. Each rat was injected intraperitoneally at 1200 hours with 2 mCi tritiated water[3] in 0.8 ml physiological saline; rats were killed exactly 15 minutes later. Plasma samples were collected at the time of killing to determine the plasma specific activity. The liver and epididymal fat pads were removed and homogenized in equal volumes of water. Aliquots of the homogenates were saponified in 30% ethanol-KOH. The mixture was then acidified and extracted 3 times with petroleum ether. Radioactivity was determined in a scintillation counter after adding scintillation cocktail (4.0 g scintillant[4] dissolved in 230 ml absolute ethanol and toluene to 1 liter) to the extracted fatty acids. Body water specific activity was used as an index to calculate nmoles of ^{3}H incorporated into fatty acids per g tissue per minute (Jungas, 1968).

Carcasses were dried to constant weight and ground for body composition analyses. Carcass moisture was calculated as the difference between wet and dry weights. Carcass protein was calculated as nitrogen × 6.25 after nitrogen determination by the semi-micro Kjeldhal procedure

(Horwitz, 1960).

Experiment 2. This experiment was designed to investigate the effect of feeding the diet in liquid form as compared with force-feeding the diet. Rats (140 g) were fed Diet 2 for 4 weeks. One group was fed the diet in dry form, whereas another group received the diet mixed with an equal weight of water. Force-fed rats were given the liquid diet with a stomach tube[5] twice daily. Food intake was gradually increased during the first week to prevent food shock. During the first week, rats fed the liquid and dry diets were pair-fed to the force-fed group. Starting from the second week, rats consuming the diet in dry form were fed *ad libitum* and the other two groups of rats were pair-fed to these rats for three additional weeks. Food consumption patterns were similar for rats fed liquid and dry diets. Since the food intake was restricted during the first week, both groups consumed all the food in 1 to 2 hours. During the second week, both groups consumed about 40-50% of their food during the day. After the second week, the *ad libitum* fed and the pair liquid-fed rats consumed less than 40% of their food during the daylight hours.

The rats were killed at the end of 4 weeks. The carcasses were blended with an equal weight of water. Aliquots (about 1 g) were taken for body fat analyses.

Experiment 3. Both meal-fed and force-fed rats require an initial adaptation before food intake approaches *ad libitum* levels. Meal-eating rats consume small quantities of food initially. Average food consumption of meal-eating rats (one 2 hour meal per day) plateaus at about 70-80% of *ad libitum* intake after 7 to 10 days (Leveille, 1970). Force-fed rats have been given smaller amounts of food during the first days of tubing to prevent food shock (Cohn and Joseph, 1959, 1963). In this experiment, the control rats consumed food *ad libitum* and the liquid-fed rats were pair-fed to examine the influence of the liquid diet on body weight and body fat in rats consuming food at levels equal to *ad libitum* intake throughout the experiment. Rats weighing 250 g initially were either fed a 20% casein, high-carbohydrate diet *ad libitum* or pair-fed daily the diet mixed with an equal weight of water. The food consumption pattern of the liquid-fed group was very similar to the pattern observed in *ad libitum* fed rats. The experiment lasted 8 weeks. At the end of the experiment, rats were killed by decapitation and stomach contents were removed. Carcasses were blended with an equal weight of water and the body fat was determined.

Experiment 4. In previous force-feeding studies (Cohn and Joseph, 1959, 1963), food intake of rats has

been initially restricted to adapt the animals to stomach distention. It was suggested earlier that the initial food restriction might alter the metabolic efficiency of the force-fed rats (Ozelci *et al.*, 1977). Consequently, the experiment was designed to examine the influence of force-feeding, without initially restricting food intake, on body weight and on body fat. Rats weighing approximately 150 g initially were fed *ad libitum* or were pair-fed a 20% lactalbumin, high-carbohydrate diet with a stomach tube twice daily for 4 weeks. On the first day of the experiment, force-fed rats were fed three times to reduce the stress of stomach load. However, 20% of the force-fed rats still died during the first week. At the end of the first week, 10 *ad libitum* fed rats and 12 force-fed rats were killed. Remaining rats from both groups were killed at the end of 4 weeks. Carcasses were homogenized with an equal weight of water. Carcass fat was determined. Carcass energy was determined with an Adiabetic Bomb Calorimeter. Fat energy was calculated by multiplying the g of body fat by 8.5 kcal/g fat. This value was the average of several determinations of carcass fat extracts. Lean energy was calculated as the difference between the total carcass energy and the fat energy.

Experiment 5. In this experiment, rats were trained to eat two 1-hour meals per day, for several reasons:

(a) this two meal per day pattern was similar to the feeding pattern utilized in previous (Cohn and Joseph, 1958, 1963) force-feeding experiments; (b) rats trained to consume two 1-hour meals per day have been reported to consume more food than rats restricted to one 2-hour meal per day (Muiruri and Leveille, 1970); and (c) by switching rats trained to meal-eat from meal-eating to nibbling, it was possible to compare the two feeding patterns without a reduction in food intake. The feeding pattern of nibblers was controlled with an automated feeding machine (Romsos and Leveille, 1974b).

Five groups of rats were used. One group was fed *ad libitum* for 5 weeks and then killed. Four other groups of rats were fed two 1-hour meals per day (from 0900 hours to 1000 hours and, again, from 1600 hours to 1700 hours) for the first 2 weeks. One group was killed at this time; a second group continued to meal-eat for an additional 3 weeks; a third group was fed the same amount of food as the meal-eaters, with an automated feeding machine (nibblers) for the last 3 weeks; and the remaining group was fed *ad libitum* for the last 3 weeks of the 5 week study. Diet 1 was fed.

Body fat was determined at the end of 2 and 5 weeks. *Ad libitum* fed rats were killed at 5 weeks; their body fat content at 2 weeks was estimated. The estimate was

based on the body fat content observed in rats in our laboratory of similar age and weight fed the same diet *ad libitum*. Fat gain of the *ad libitum* fed rats was calculated as the difference between the final body fat and the calculated body fat at 2 weeks.

Results

Experiment 1. Meal-fed, nibbling, and liquid-fed rats consumed an average of 14.7 g food per day (Table 8). Meal-eaters gained about 20 g less weight than either nibblers or liquid-fed rats; however, the differences were not significant. Carcass weight and carcass composition, at the end of the 8 week experiment, were also similar in the three groups. Carcass protein did not differ among the meal-fed, nibbling, or liquid-fed rats. Carcass fat content was slightly, but not significantly, less in meal-eaters than in either nibblers or liquid-fed rats. All three groups had essentially the same carcass moisture content.

Blood glucose levels at 0800 hours were similar in the three groups of rats (Table 8). From 0800 hours to 1200 hours, blood glucose levels increased in meal-eaters, in agreement with previous studies (Gasquet and Pequignot, 1973). The circulating level of glucose also increased in liquid-fed rats but not in nibblers. Consequently, at 1200 hours, meal-fed rats had significantly higher

TABLE 8

FOOD INTAKE, WEIGHT GAIN, CARCASS COMPOSITION, BLOOD GLUCOSE LEVEL, AND FATTY ACID SYNTHESIS IN MEAL-EATING (ONE 2-HOUR MEAL DAILY), NIBBLING, AND LIQUID-FED RATS (EXP. 1)[1]

Parameter	Meal-Eaters	Nibblers	Liquid-Fed
Food intake (g/day)	14.7 ± 0.6$_a$	14.7 ± 0.6$_a$	14.7 ± 0.6$_a$
Weight gain (g)	77.0 ± 9.0$_a$	97.0 ± 10.0$_a$	93.0 ± 8.0$_a$
Carcass weight (g)	325.0 ± 11.0$_a$	343.0 ± 11.0$_a$	340.0 ± 9.0$_a$
Carcass protein (g)	53.1 ± 2.8$_a$	50.9 ± 2.5$_a$	51.9 ± 3.0$_a$
Carcass fat (g)	45.3 ± 2.1$_a$	53.7 ± 4.7$_a$	52.7 ± 1.9$_a$
Carcass moisture (g)	213.9 ± 6.8$_a$	212.9 ± 8.3$_a$	222.8 ± 7.3$_a$
Blood glucose (mg/100 ml): 0800 hrs	79.0 ± 3.0$_a$	80.0 ± 3.0$_a$	77.0 ± 2.0$_a$
1200 hrs	109.0 ± 6.0$_a$[2]	86.0 ± 2.0$_b$	99.0 ± 3.0$_{a,b}$[2]
Fatty acid synthesis:[3] Liver	709.0 ± 70.0$_a$	661.0 ± 127.0$_a$	625.0 ± 58.0$_a$
Epididymal fat pad	1453.0 ± 178.0$_a$	756.0 ± 209.0$_b$	1017.0 ± 199.0$_{a,b}$

[1]Values represent mean ± SEM for 8 rats, weighing 253 ± 2 g initially. The rats were fed for 8 weeks. Treatment means not sharing a common subscript letter differ significantly ($p < 0.05$).

[2]Significant difference ($p < 0.05$) between samples obtained at 0800 hours *vs* those obtained at 1200 hours.

[3]nmoles ^3H_2O incorporated into fatty acids/g tissue/minute. Liver weights averaged 11.8, 12.6, and 12.1 g and epididymal fat pad weights averaged 5.4, 5.4, and 6.9 g for meal-eaters, nibblers, and liquid-fed rats, respectively. Tissue weights were not influenced ($p > 0.05$) by dietary treatment.

blood glucose levels than the nibblers, and the blood glucose levels of the liquid-fed rats were intermediate.

The rates of fatty acid synthesis in liver, as measured by the incorporation of tritiated water into fatty acids, were similar among the three groups. In the adipose tissue (epididymal pad), however, meal-eaters incorporated tritiated water into fatty acids at twice the rate observed in the nibblers. Fatty acid synthesis in adipose tissue of the rats consuming the diet in liquid form occurred at an intermediate rate (Table 2). These results confirm previous observations (Leveille, 1970) that rates of fatty acid synthesis are elevated to a greater extent in epididymal adipose tissue than in liver of meal-fed rats, even when the control nibblers are pair-fed. This greater rate of fatty acid synthesis in the epididymal adipose tissue shortly after consumption of the high-carbohydrate meal did not result in increased body fat.

Experiment 2. Food intake, weight gain, carcass weight, and carcass fat of the rats are shown in Table 9. All three groups consumed essentially the same amount of food. Force-fed rats gained less body weight than either of the other two groups; likewise, the carcasses of the force-fed group weighed less than carcasses of the other two groups. However, there were no differences in the amount of carcass fat among the three groups of rats.

TABLE 9

FOOD INTAKE, WEIGHT GAIN, AND CARCASS FAT OF FORCE-FED, PAIR-FED, AND *AD LIBITUM* RATS (EXP. 2)[1]

Parameter	Force-Fed (0-4)[2]	Pair-Fed (0-4)	Pair-Fed (0-1) and *ad libitum* (2-4)
Form of diet	Liquid	Liquid	Dry
Food intake (g/day)	$20.8 \pm 0.7_a$	$18.2 \pm 1.9_a$	$20.9 \pm 0.6_a$
Weight gain (g)	$149.0 \pm 4.0_a$	$186.0 \pm 3.0_b$	$164.0 \pm 3.0_c$
Carcass weight (g)	$278.0 \pm 5.0_a$	$315.0 \pm 4.0_b$	$295.0 \pm 5.0_c$
Carcass fat (g)	$51.1 \pm 1.1_a$	$57.5 \pm 2.8_a$	$51.1 \pm 3.2_a$

[1]Values represent mean ± SEM for 10 rats weighing 140 ± 1 g initially and fed for 4 weeks. Treatment means on the same line not sharing a common subscript letter differ significantly ($p < 0.05$).

[2]Numbers in parentheses represent the weeks of the experiment.

Experiment 3. Table 10 presents the food intake, body weight gain, carcass weight, and fat of rats fed *ad libitum* or pair-fed the same diet in liquid form. Both groups consumed similar amounts of food during the 8 week experiment. Neither weight gain nor carcass weight differed between the *ad libitum* and liquid-fed rats. Carcass fat content was slightly greater in the liquid-fed rats but the difference was not significant. Based on the results of the first three experiments, we concluded that feeding the diet in liquid form did not contribute to the greater body fat content previously observed in force-fed rats (Cohn and Joseph, 1959, 1963; Han, 1973).

Experiment 4. Rats were killed after 1 and 4 weeks. During the first week, both *ad libitum* and force-fed rats consumed an average of about 16 g food per day (Table 11). *Ad libitum* fed rats had gained more body weight than the force-fed rats at the end of the first week. Carcass weight at the end of 1 week was also greater for the *ad libitum* fed rats than for the force-fed group. Total carcass energy did not differ between the two groups at the end of 1 week.

At the end of the experiment, both groups had consumed about 520 g food per rat (approximately 19 g per day). The body weight gained in the last 3 weeks was similar for

TABLE 10

FOOD INTAKE, WEIGHT GAIN, CARCASS WEIGHT, AND CARCASS FAT OF RATS FED *AD LIBITUM* OR PAIR-FED A LIQUID DIET (EXP. 3)[1]

Parameter	*Ad Libitum*	Liquid-Fed
Food intake (g/day)	22.6 ± 0.7	22.4 ± 0.5
Weight gain (g)	217.0 ± 12.0	206.0 ± 10.0
Carcass weight (g)	453.0 ± 11.0	442.0 ± 8.0
Carcass fat (g)	66.2 ± 4.3	71.4 ± 5.2

[1]Values represent mean ± SEM for 8 rats weighing 250 ± 3 g initially and fed for 8 weeks. Treatment effects were not significantly different ($p > 0.05$).

TABLE 11

FOOD INTAKE, WEIGHT GAIN, CARCASS WEIGHT, AND CARCASS ENERGY OF RATS FED *AD LIBITUM* OR PAIR FORCE-FED FOR 4 WEEKS (EXP. 4)[1]

Parameter		*Ad Libitum* Fed		Pair Force-Fed	
		End of Week 1	Weeks 2-4[2]	End of Week 1	Weeks 2-4[2]
No. rats per treatment		10	10	12	20
Total food intake (g)		95.0 ± 1.0	431.0 ± 8.0	93.0 ± 0	433.0 ± 0
Weight gain (g)		46.0 ± 4.0	133.0 ± 6.0	31.0 ± 1.0[3]	137.0 ± 2.0
Carcass weight (g)		180.0 ± 3.0	134.0 ± 5.0	168.0 ± 2.0[3]	137.0 ± 3.0
Energy (kcal/rat):	Total carcass	250.0 ± 8.0	380.0 ± 31.0	255.0 ± 5.0	436.0 ± 32.0
	Fat[4]	102.0 ± 10.0	259.0 ± 44.0	117.0 ± 7.0	341.0 ± 26.0
	Lean[5]	148.0 ± 10.0	121.0 ± 27.0	138.0 ± 4.0	95.0 ± 24.0

[1]Values represent mean ± SEM for rats weighing 146 ± 2 g initially and fed for 4 weeks. Rats were killed at the end of 1 and 4 weeks.

[2]Food intake and body weight gain expressed as totals for the 3-week period, whereas carcass weight and carcass energy expressed as increases during the 3-week period. Increase in carcass weight and carcass energy during the 3-week period calculated by subtracting the carcass weight and energy in rats killed after 1 week from values obtained at 4 weeks.

[3]Significantly different ($p < 0.05$) from the *ad libitum* fed group at the end of the first week.

[4]8.5 kcal/g fat × g body fat.

[5]Total carcass energy minus carcass fat energy.

ad libitum and force-fed rats. Changes in carcass weight were essentially the same for both groups. Force-fed rats gained slightly, but not significantly, more total carcass energy during the last 3 weeks than did the *ad libitum* fed rats. The energy gain contributed by fat was not greater in the force-fed rats than in the *ad libitum* fed rats. Thus, we conclude that force-feeding has only a minimal influence on body fat when the force-fed rats are pair-fed throughout the entire experiment.

Experiment 5. During the first 2-week period of meal-feeding (two 1-hour meals per day), rats consumed about 12 g of food, in contrast to consumption of about 17 g food by the *ad libitum* fed group (Table 12). The *ad libitum* fed group gained nearly twice as much weight compared to the meal-fed rats during this period.

During the last 3 weeks, *ad libitum* fed rats consumed about 25% more food than did the meal-fed rats or the pair-fed nibblers. Body weight gain was similar for meal-fed, nibbling, and *ad libitum* fed rats; however, rats which had been switched from meal-feeding to *ad libitum* feeding gained more weight than the other three groups during the last 3 weeks of the experiment. Carcasses of meal-fed and nibbling rats killed at the end of the 5 week experiment weighed significantly less than did carcasses of the other two groups.

TABLE 12

FOOD INTAKE, WEIGHT GAIN, CARCASS WEIGHT, AND CARCASS FAT OF RATS (EXP. 5)[1]

Parameter	Meal-Fed (0-2)[2]	*Ad Libitum* (0-2)	Meal-Fed (0-2) and Meal-Fed (3-5)[3]	Meal-Fed (0-2) and Nibbler (3-5)[3]	Meal-Fed (0-2) and *Ad lib* (3-5)[3]	*Ad lib* (0-2) and *Ad lib* (3-5)[3]
No. of rats	10	6	10	10	10	6
Food intake (g/day)	12.1 ± 1.3	17.3 ± 0.5	18.9 ± 0.7$_a$	18.8 ± 0.7$_a$	23.7 ± 0.3$_b$	23.4 ± 0.5$_b$
Weight gain (g)	50.0 ± 3.0	93.0 ± 4.0	118.0 ± 4.0$_a$	125.0 ± 2.0$_a$	164.0 ± 6.0$_b$	122.0 ± 8.0$_a$
Carcass weight (g)	156.0 ± 3.0	-	272.0 ± 4.0$_a$	278.0 ± 3.0$_a$	314.0 ± 5.0$_b$	314.0 ± 8.0$_b$
Carcass fat (g)	15.5 ± 1.5	25.0[4]	39.1 ± 2.2$_a$	42.6 ± 2.9$_a$	57.2 ± 3.8$_b$	52.9 ± 3.9$_{a,b}$
Fat gain (g)	-	-	23.6 ± 2.2$_a$	27.1 ± 2.9$_a$	41.7 ± 3.8$_b$	27.9 ± 3.9$_a$

[1]Values represent mean ± SEM for rats weighing 114 ± 0.4 g initially and fed for 5 weeks. Meal-eater = two 1-hr meals per day. Treatment means from weeks 3-5 not sharing a common subscript letter differ significantly ($p < 0.05$).

[2]Numbers in parentheses represent weeks of the experiment.

[3]Food intake and weight gain values in these columns represent data for weeks 3-5 of the experiment. Carcass weight and carcass fat was determined at the end of the 5 week experiment. Fat gain was determined by subtracting the estimated body fat at 2 weeks from the values obtained at 5 weeks.

[4]Estimated from values of fat content of *ad libitum* fed rats of similar weights in previous experiments.

At the end of the 5 week experiment, there was no difference in the carcass fat content between meal-eating and nibbling rats. Thus, provided rats are pair-fed throughout the experiment, meal frequency may have only a minimal influence on body fat. Both the *ad libitum* fed groups of rats had significantly greater body fat than observed in the meal-fed and nibbler groups. Fat gain (weeks 3 to 5) was greater in rats switched from meal-eating to *ad libitum* intake than in any of the other groups. There was no difference in fat accumulation among meal-fed, nibbling, and continuously *ad libitum* fed rats (Table 12). These results suggest that rats switched from a restricted food intake to *ad libitum* eating were more efficient in converting food energy to body fat than rats fed *ad libitum* throughout the experimental period.

Discussion

Addition of water to a dry diet has been suggested to have a growth promoting effect (Keane *et al.*, 1962; Hopkins and Steinke, 1976). It is possible that addition of water to the diet of force-fed rats may have contributed to the increased body fat of these rats relative to rats fed the dry diet *ad libitum* (Cohn and Joseph, 1959, 1963; Han, 1973). Consequently, in the first three experiments of this study we investigated the effects of feeding the diet in liquid form as compared to meal-feeding

the dry diet, force-feeding the liquid diet, or feeding the dry diet *ad libitum*. Meal-feeders require an initial adaptation to meal-feeding and food intake reaches only about 80% of that expected for *ad libitum* fed rats (Leveille, 1970). Force-fed rats in experiment 2 were also gradually adapted to the feeding pattern during the first week but, during the remainder of the experiment, they were pair-fed to the *ad libitum* fed group. In all three experiments, the liquid diet was pair-fed; consequently, several of the comparisons involved restricted food intake and others involved *ad libitum* intake.

When the rats were fed the diet in liquid form, the weight gain was not different from that of meal-fed or nibbling rats (expt. 1). Also, pair-feeding rats the liquid diet with a group of rats which had been fed *ad libitum* throughout the experiment did not change the weight gain. However, rats allowed to consume the diet in liquid form gained more weight than the force-fed rats or the rats which had been restricted for the first week and then fed *ad libitum* for 3 weeks (expt. 2). Thus it appears that feeding a high-carbohydrate diet in liquid form may increase body weight gain in some, but not all, situations.

Consumption of the liquid diet did not increase body fat content of the rats. Carcass protein and moisture

content of the liquid-fed rats were also similar to the meal-fed or nibbling counterparts. It appears very unlikely that the addition of water to the diet contributed to the increased body fat previously observed in force-fed rats (Cohn and Joseph, 1959, 1963; Han, 1973). In agreement with our previous report (Ozelci *et al.*, 1977), meal pattern did not influence body fat when pair-fed meal-eaters and nibblers were compared. Similarly, force-feeding did not increase body fat when control rats were pair-fed during the first week.

Since feeding the diet in liquid form did not result in greater fat content in these rats, we designed two more experiments to investigate the influence of meal pattern on body composition of rats. In these experiments, treatment and control rats were pair-fed and attempts were made to have the rats consume food at or near *ad libitum* intake throughout the experiments.

Rats were pair force-fed to *ad libitum* fed controls without an initial adaptation. In agreement with results obtained when both force-fed and control rats were fed restricted amounts of food during the first week (expt. 2), force-feeding did not influence body fat content. The design of these experiments differs from previous reports (Cohn and Joseph, 1955, 1959, 1963) in one aspect; Cohn and co-workers gave force-fed rats limited amounts of food

during the first week, whereas control rats consumed food *ad libitum* during this period. Our present results suggest that feeding pattern may have only a minimal influence on body fat accumulation in rats pair force-fed during the entire experiment.

In the last experiment, rats were fed two 1-hour meals for 2 weeks; at this time, the rats were consuming 20% less food than *ad libitum* fed controls. One group of rats was then switched from meal-eating to automated feeders which continuously delivered food to the cages. This switch in meal pattern did not result in an initial decrease in food intake as would have been observed if continuously-fed rats had been switched to meal-eating. Meal-fed rats did not accumulate more body fat than the pair-fed nibblers. This observation is in agreement with our previous results utilizing meal-fed rats and pair-fed nibblers (Ozelci *et al.*, 1977) as well as with the results of the present study with force-fed rats. Under the conditions of these experiments in which rats were pair-fed throughout the entire experiment, including the initial adaptation period, meal pattern did not influence body fat.

When previously trained meal-eaters were allowed to consume food *ad libitum*, their food intake equalled that of rats which had been fed *ad libitum* throughout the experiment. Under these conditions, rats switched from

meal-eating to *ad libitum* food intake gained more body fat than *ad libitum* fed rats, even though they did not consume more food than the *ad libitum* fed rats. These results are in agreement with a previous report (DeBont *et al.*, 1975) and suggest that a shift from one level of food intake to a higher level might cause a metabolic adaptation and increased food efficiency in rats. It remains to be established to what extent the initial food restriction utilized to adapt rats to force-feeding contributed to the increased body fat reported in force-fed rats.

CHAPTER IV

INFLUENCE OF INITIAL FOOD RESTRICTION ON SUBSEQUENT BODY WEIGHT GAIN AND BODY FAT ACCUMULATION IN RATS

Introduction

When the growth rate of animals or children has been retarded by food restriction, the rate of catch-up growth is faster after removal of the restriction than is the rate of growth in animals or children that have had continual access to adequate food (Wilson and Osbourn, 1960; Meyer and Clawson, 1964; Drew and Reid, 1975a, 1975b, 1975c; Robinson *et al.*, 1975; Levitski *et al.*, 1976; Szepesi and Vojnik, 1975; Szepesi *et al.*, 1975; Szepesi and Epstein, 1976; Ashworth, 1968; Miller and Wise, 1976). This compensatory growth has been reported by several investigators (Robinson *et al.*, 1975; Mendez, 1966) to represent the replacement of body fat which had been depleted during the restriction period, and by others (Wilson and Osbourn, 1960; Drew and Reid, 1975c) to increased protein and water accumulation.

Meal frequency has been suggested to alter body composition of rats (Cohn and Joseph, 1959, 1963; Han, 1973). Adaptation to an altered meal pattern usually involves an

initial restriction of food intake. Force-fed rats have been fed small amounts of food initially to prevent food shock (Cohn and Joseph, 1959, 1963; Han, 1973) and rats trained to eat meals ingest only a few grams of food daily when first placed on a regimen of one 2-hour meal per day (Leveille, 1970, 1975). These rats have been reported to have an increased body fat accumulation (Cohn and Joseph, 1959, 1963; Han, 1973) or an increased rate of body weight gain per gram food consumed (Leveille, 1970, 1975; DeBont *et al.*, 1975). However, when the same quantity of food was fed to both control and treatment groups by pair-feeding the nibblers to meal-eaters (Ozelci *et al.*, 1977, 1978) or by pair-feeding the force-fed group to *ad libitum* fed rats without an initial adaptation period (Ozelci *et al.*, 1978), we did not observe a difference in weight gain or fat gain between the two groups. Thus, we have postulated (Ozelci *et al.*, 1977, 1978) that the initial food restriction may be a factor contributing to the subsequent increased body fat or body weight gain of rats fed meals. In this study, we examined the influence of the initial food restriction utilized to adapt force-fed and meal-fed rats to their respective meal patterns on subsequent body weight and body fat changes in the rats.

Materials & Methods

Male Sprague-Dawley rats[1] weighing approximately 150 g initially were placed in individual stainless steel cages having raised wire floors. The rats had free access to water. Lights in the temperature-regulated room (22 ± 1°C) were on from 0700 to 1900 hours. Purified diets containing 20 parts casein or lactalbumin were prepared. Composition of the three diets used in these experiments is shown in Table 13. Diet 1 was used in all experiments except experiments 1 and 3. Diet 2 was used in experiment 1 where rats were force-fed. Lactalbumin was substituted for casein as it is more easily solubilized in water than casein. Diet 2 was cellulose-free and glucose and corn starch were utilized as sources of carbohydrate to duplicate the diet used in the force-feeding experiments of Cohn *et al.* (1959, 1963). Diet 3 was used in experiment 3 and was a high-fat diet prepared by substituting fat on an equal energy basis for all the carbohydrate in Diet 2 (about 77% of the energy in Diet 3 was derived from fat). Food intake was recorded each day and body weights were recorded twice weekly.

Rats were fed *ad libitum*, meal-fed, force-fed, pair-fed, or fed restricted amounts of food according to the experimental protocol. Meal-fed rats were allowed to eat between 0900 and 1100 hours daily. Force-fed rats were

TABLE 13

COMPOSITION OF THE DIETS

	Diet (g)		
	1	2	3
Casein	20.00	----	----
Lactalbumin	----	20.00	20.00
Basal[1]	9.9	9.9	9.9
Cellulose	4.0	----	----
Glucose	66.1	35.05	----
Corn starch	----	35.05	----
Hydrogenated vegetable shortening[2]	----	----	29.7

[1]The basal mix contained (in g/9.9 g): methionine, 0.3; vitamin mix, 0.4 (Yeh and Leveille, 1969); mineral mix, 4.0 (Leveille and O'Hea, 1967); choline chloride, 0.2; and corn oil, 5.0.

[2]Crisco^{R}

[3]Energy values used were: glucose = 3.64 kcal/g; Crisco^{R} = 8.59 kcal/g (Brambila and Hill, 1966).

administered food twice daily, at 0900 hours and again at 1600 hours. Pair-fed and restricted rats were fed at 0900 hours daily. The feeding pattern of the pair-fed rats was not controlled. When the rats were pair-fed to *ad libitum* fed rats, their feeding pattern approximately paralleled the feeding pattern of the *ad libitum* fed rats, that is, they consumed more than 50% of their food at night. When the rats were pair-fed to meal-eating rats, they consumed their food within 3 hours. Rats fed restricted amounts of food consumed their food within 2 hours of feeding.

The experimental protocol utilized in each experiment is presented in Table 14 and is explained in more detail in the Experimental Protocol and Results section. Rats were killed by decapitation after one week in each experiment, and after 2, 4, or 7 weeks according to the design of the experiment. Carcass weight represented the weight of the rat minus blood loss and minus weight of the stomach contents. Carcasses were autoclaved and blended with an equal weight of water. Duplicate aliquots were taken immediately after blending. Fat content of the carcasses was determined gravimetrically after chloroform: methanol (3:2 v/v) extraction.

TABLE 14

EXPERIMENTAL PROTOCOL

Exp	Diet	Treatment	Weeks 0-1	Weeks 2-4
1	2	(a) *Ad lib*	*Ad lib*	*Ad lib*
		(b) Force-fed	Restricted	Pair-fed to (a)
		(c) Pair-fed	Pair-fed to (b)	Pair-fed to (a)
2	1	(a) Meal-fed	Meal-fed	Meal-fed
		(b) Restricted, pair-fed	Restricted	Pair-fed to (a)
3	3	(a) *Ad lib*	*Ad lib*	*Ad lib*
		(b) Restricted, pair-fed	Restricted	Pair-fed to (b)
4[1]	1	(a) *Ad lib*	*Ad lib*	*Ad lib*
		(b) Restricted, pair-fed	Restricted	Pair-fed to (a)
5[2]	1	(a) *Ad lib*	*Ad lib*	*Ad lib*
		(b) Restricted 25%, pair-fed	Restricted 25%	Pair-fed to (a)
		(c) Restricted 50%, pair-fed	Restricted 50%	Pair-fed to (a)
		(d) Restricted 75%, pair-fed	Restricted 75%	Pair-fed to (a)
6	1	(a) *Ad lib*	*Ad lib*	*Ad lib*
		(b) Restricted 75%, pair-fed on intake basis	Restricted 75%	Pair-fed to (a) on intake basis
		(c) Restricted 75%, pair-fed on body weight basis	Restricted 75%	Pair-fed to (a) on body weight basis

[1]Rats in this experiment were also fed for an additional 3 weeks and killed.

[2]One-half the rats in the 2-4 week groups were killed at the end of Week 2; the remainder were killed at the end of Week 4.

Experimental Protocol & Results

Experiment 1. In previous force-feeding experiments (Cohn and Joseph, 1959, 1963; Han, 1973), rats were initially adapted to stomach tubing for a week by gradually increasing food intake. After the first week, these rats were pair-fed to the *ad libitum* eating rats. The first experiment included 3 groups of rats which were fed Diet 2 for 4 weeks (Table 14). The first group was allowed to eat *ad libitum* throughout the experiment. The second group was force-fed (at 0900 hours and 1600 hours daily) by gradually increasing food intake during the first week (6 g at day 1, to 18 g at day 7); these rats were then pair force-fed to the *ad libitum* fed rats for 3 more weeks. Another group was pair-fed (at 0900 hours daily) to the force-fed rats through the 4 week experiment. Rats were killed after 1 and 4 weeks and carcass fat was determined.

Food intake, weight gain, and carcass fat during the first week is shown in Table 15. Force-fed and pair-fed rats consumed, on average, 5 g less food per day than the *ad libitum* fed rats. The weight gain of the *ad libitum* fed rats during the first week was greater than weight gain of either the force-fed or the pair-fed rats. The force-fed rats gained less weight than the rats pair-fed the diet in dry form. The carcass weights followed the same trend. *Ad libitum* fed rats had more carcass fat at

TABLE 15

FOOD INTAKE, WEIGHT GAIN, CARCASS WEIGHT, AND CARCASS FAT OF RATS DURING THE FIRST WEEK OF EXP. 1-6[1]

Exp	Treatment	Food Intake (g/day)	Weight Gain (g)	Carcass Weight (g)	Carcass Fat (g)
1	*Ad lib*	$19.7 \pm 0.1_a$	$66 \pm 2_a$	$199 \pm 3_a$	$23.3 \pm 1.1_a$
	Force-fed	$14.9 \pm 0_b$	$21 \pm 1_b$	$156 \pm 2_b$	$16.0 \pm 0.8_b$
	Pair-fed	$14.9 \pm 0_b$	$30 \pm 1_c$	$165 \pm 2_c$	$16.5 \pm 0.9_b$
2	Meal-fed	$14.1 \pm 0.7_a$	$32 \pm 3_a$	$178 \pm 4_a$	$13.8 \pm 0.6_a$
	Restricted pair-fed	$9.6 \pm 1.7_b$	$4 \pm 2_b$	$146 \pm 4_b$	$9.8 \pm 0.5_b$
3	*Ad lib*	$14.4 \pm 0.7_a$	$63 \pm 2_a$	$214 \pm 3_a$	$34.4 \pm 1.8_a$
	Restricted pair-fed	$9.1 \pm 1.0_b$	$26 \pm 2_b$	$176 \pm 2_b$	$23.5 \pm 1.1_b$
4	*Ad lib*	$19.8 \pm 0.3_a$	$51 \pm 2_a$	$186 \pm 2_a$	$16.9 \pm 0.9_a$
	Restricted pair-fed	$14.0 \pm 0_b$	$16 \pm 1_b$	$155 \pm 1_b$	$10.6 \pm 0.5_b$
5	*Ad lib*	$19.1 \pm 0.3_a$	$47 \pm 2_a$	$193 \pm 2_a$	$19.1 \pm 1.5_a$
	25% Restricted pair-fed	$14.3 \pm 0_b$	$20 \pm 2_b$	$167 \pm 1_b$	$13.1 \pm 1.0_b$
	50% Restricted pair-fed	$9.6 \pm 0_c$	$0.4 \pm 2_c$	$147 \pm 2_c$	$9.2 \pm 0.9_b$
	75% Restricted pair-fed	$4.8 \pm 0_d$	$-29 \pm 2_d$	$121 \pm 2_d$	$3.1 \pm 0.3_c$
6	*Ad lib*	$21.0 \pm 0.3_a$	$49 \pm 1_a$	$188 \pm 1_a$	$13.4 \pm 0.5_a$
	75% Restricted pair-fed	$5.2 \pm 0_b$	$-37 \pm 1_b$	$109 \pm 1_b$	$2.1 \pm 0.2_b$

[1]Values represent mean ± SEM for 10-12 rats. Treatment means in the same column not sharing a common subscript letter differ significantly ($p < 0.05$).

the end of one week than did force-fed or pair-fed rats.

Between weeks 2 and 4, all three groups consumed approximately 22 g food per day. The pair-fed rats gained more weight than the force-fed groups, whereas there was no difference between the weight gain of pair-fed and *ad libitum* fed rats or between the force-fed and *ad libitum* fed rats (Figure 1). Fat gain during the last 3 weeks was greater in both force-fed and pair-fed rats than in the *ad libitum* fed rats. Force-fed rats and the rats which were pair-fed the diet in dry form gained similar amounts of body fat (Figure 1). Total body fat at the end of the 4 week experiment was greater ($p < 0.05$) in the force-fed rats (49.7 ± 2.2 g) than in *ad libitum* fed controls (40.8 ± 2.3 g), but the difference in fat content between *ad libitum* fed rats and the pair-fed rats (48.7 ± 1.8 g) was not significant.

Experiment 2. This experiment was conducted to determine whether meal-fed rats would exhibit compensatory gain following a restriction in food intake. Rats were fed 2 hours daily (0900 hours to 1100 hours) for 3 weeks prior to the experiment because meal-eating rats start with a food intake of only a few grams per day and increase their intake gradually (Leveille, 1970, 1975); an adaptation of about 10 to 15 days is necessary before meal-fed rats reach a plateau in food consumption. After 3 weeks, rats

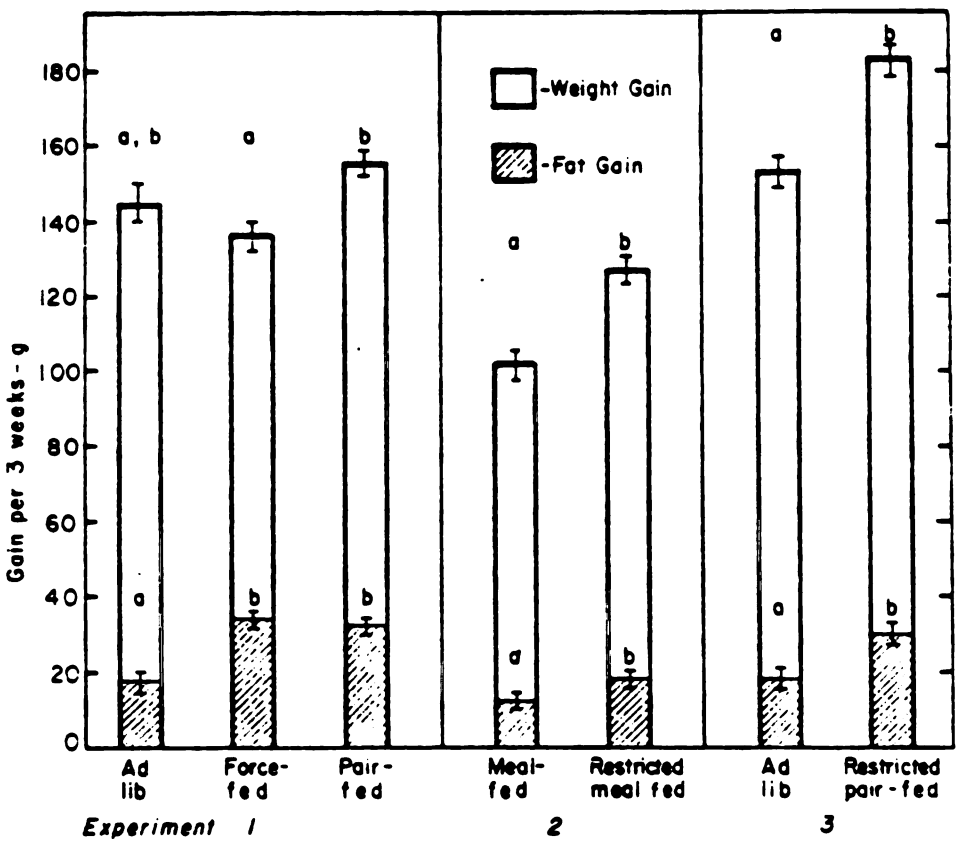

Fig. 1. Body weight gain and fat gain of rats during the last three weeks of Exp. 1, 2, and 3. Each bar represents the mean and SEM for 10-12 rats. Treatment means not sharing a common superscript letter differ significantly ($p < 0.05$).

were divided into two groups. One group continued to eat 2 hours daily for 4 weeks. The other group was restricted to 75% of the food intake of the meal-eaters for the first week; the rats were then pair-fed to the meal-eaters during the last 3 weeks (Table 14). Rats were killed after 1 and 4 weeks and carcass fat was determined.

During the first week, rats fed 2 hours daily consumed about 14 g food per day (approximately 75% of *ad libitum* intake) and the restricted rats were fed 9.6 g food per day (Table 15). Restricted meal-eaters gained only 4 g body weight during the first week; their rate of gain was significantly less than observed for the meal-eaters allowed access to food for two hours each day. Carcass weight and total carcass fat were also less in the restricted meal-eaters at the end of one week of restricted feeding (Table 15).

In the last 3 weeks of the experiment, meal-fed rats consumed 18 g food daily and the other group was pair-fed; thus, both groups consumed identical amounts of food. Figure 1 shows the body weight and fat gain of these rats during the last 3 weeks of the experiment. The restricted pair-fed rats gained significantly more body weight and fat than the meal-eaters during the last 3 weeks of the experiment. Final body fat content was similar ($p > 0.05$)

in the two groups: 25.4 ± 1.8 g for rats allowed food for 2 hours daily throughout the experiment; and 27.7 ± 0.9 g for rats restricted during the first week and pair-fed for the last 3 weeks.

Experiment 3. A carbohydrate-free, high-fat diet was fed *ad libitum* for 4 weeks or fed at 75% of *ad libitum* intake during the first week and pair-fed for the last 3 weeks (Table 14). Rats were killed after 1 and 4 weeks and carcass fat was analyzed. *Ad libitum* fed rats consumed about 14 g of food daily during the first week of the experiment; the restricted group consumed 9 g food daily (Table 15). The *ad libitum* fed rats gained more body weight and had heavier carcasses which contained more fat than the restricted rats at the end of the first week. During the last 3 weeks, both groups consumed 13.6 g food daily. The weight gain from weeks 2 to 4 was significantly greater in the group which had been restricted during the first week (Figure 1). Also, the rats restricted during the first week accumulated more body fat in the last 3 weeks than did the *ad libitum* fed rats. Thus, compensatory fat gain was evident in rats fed the high-fat diet as well as in rats fed the high-carbohydrate diet (experiments 1 and 2). Total body fat content at the end of 4 weeks did not differ between the two groups: 52.5 ± 3.3 g in the *ad libitum* fed rats; and

53.7 ± 3.1 g in rats restricted during the first week.

Experiment 4. Rats were killed after 1, 4, and 7 weeks to determine the duration of increased food efficiency in the restricted/re-fed rats. One group of rats was allowed to eat *ad libitum* for 7 weeks, the other group was restricted to 75% of *ad libitum* intake during the first week and pair-fed for the remaining 6 weeks (Table 14). The *ad libitum* fed group consumed 19.8 g food per day, and the restricted rats consumed 14 g food per day during the first week (Table 15). Again, *ad libitum* fed rats gained weight at a faster rate, their carcasses were heavier, and had significantly more fat at the end of 1 week than the restricted rats. Between weeks 2 and 4, both *ad libitum* and pair-fed rats consumed about 23.3 g food daily. The body weight gain during this period was greater in the rats which had been restricted during the first week (Figure 2). Also, as in experiments 1 to 3, the fat gain was greater in the restricted, pair-fed rats from weeks 2 to 4 (Figure 2) than in *ad libitum* fed rats.

During the last 3 weeks of the experiment (weeks 5-7), *ad libitum* and pair-fed rats consumed similar quantities of food per day: 26.6 g and 25.3 g, respectively. The increased food efficiency observed during the previous 3 weeks of pair-feeding was no longer apparent; both groups

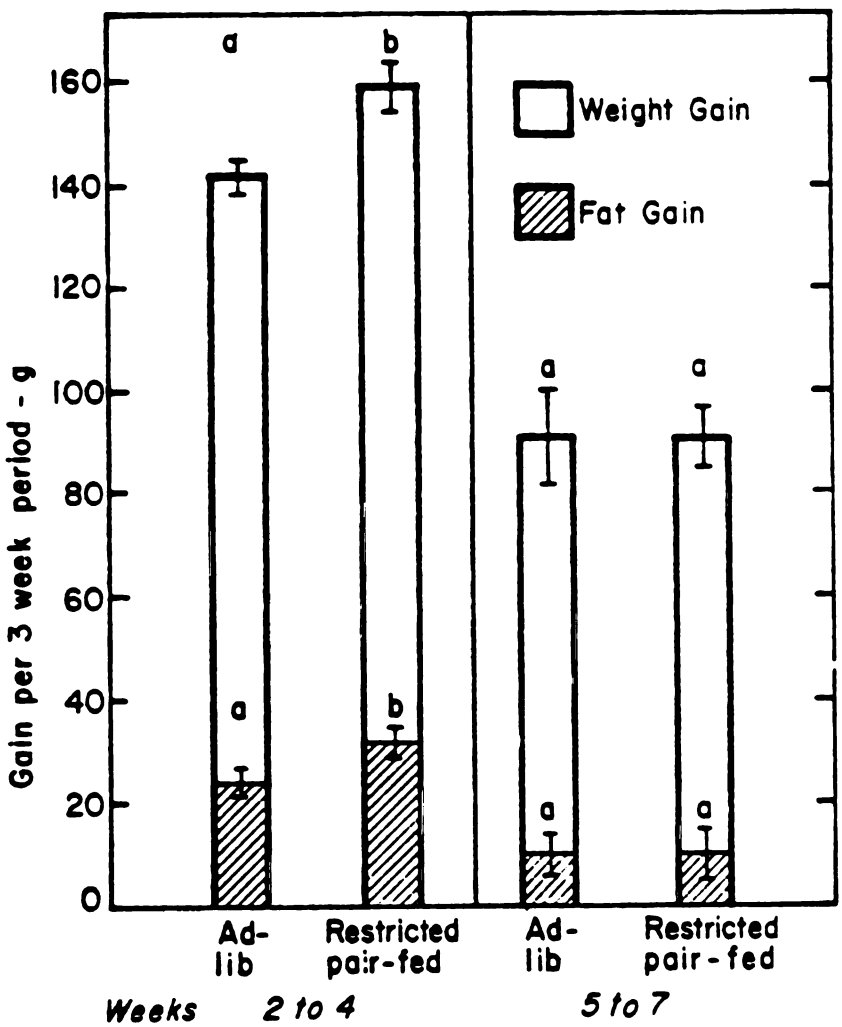

Fig. 2. Body weight gain and fat gain of rats during Weeks 2-4 and Weeks 5-7 in Exp. 4. Rats were fed *ad libitum* for 7 weeks or restricted to 75% of *ad libitum* intake during the first week and were pair-fed to the *ad libitum* fed rats for the last 6 weeks (Table 14). Each bar represents the mean and SEM for 10 rats. Treatment means not sharing a common superscript letter differ significantly ($p < 0.05$).

gained similar amounts of weight and body fat (Figure 2).

Experiment 5. This experiment was designed to evaluate the effect of the severity of the initial food restriction on subsequent body weight gain and body fat accumulation. Rats were fed *ad libitum* for 4 weeks or were restricted to 75%, 50%, or 25% of *ad libitum* intake during the first week, and pair-fed on a food-intake basis to *ad libitum* eating rats for the last 3 weeks (Table 14). In this experiment, rats were killed after 1, 2, and 4 weeks to obtain data on the time sequence of the compensatory gain.

During the first week, as in the other experiments of this study, *ad libitum* fed rats consumed about 19 g food daily and the rats restricted to 75%, 50%, and 25% of *ad libitum* intake were fed 14.3 g, 9.6 g, and 4.8 g food daily, respectively (Table 15). The weight gain, carcass weight, and carcass fat content of the *ad libitum* fed group were greater than for the other groups during this week; values for the restricted groups related to the degree of food restriction during the first week. After the first week, all the restricted rats were pair-fed to the *ad libitum* fed rats. All rats consumed about 22 g of food daily during the second week. Figure 3 shows the weight and fat gain during the second week and during the third to fourth weeks. Weight gain and fat gain were

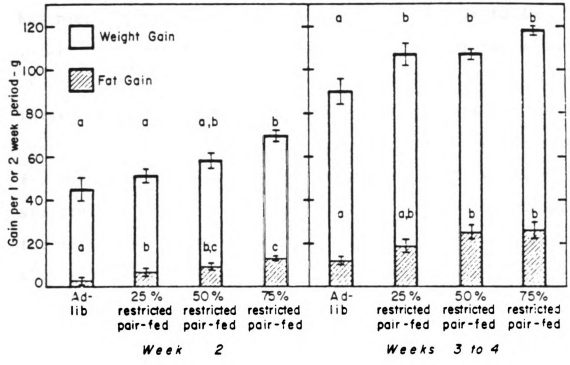

Fig. 3. Body weight gain and fat gain of rats during the 2nd week and during Weeks 3-4 in Exp. 5. Rats were fed *ad libitum* for 4 weeks or restricted to 75%, 50%, or 25% of *ad libitum* intake during the first week and were pair-fed to the *ad libitum* fed rats for the last 3 weeks (Table 14). Each bar represents the mean and SEM for 10 rats. Treatment means not sharing a common superscript letter differ significantly ($p < 0.05$).

greatest in rats that had been most severely restricted during the first week. The effects of the previous food restriction were evident during both the second and the third and fourth weeks of the study.

At the end of the 4 week experiment, the rats which had been restricted to 75% and 50% of *ad libitum* food intake during the first week weighed as much as the *ad libitum* fed rats; the rats restricted to 25% of *ad libitum* intake weighed less than the *ad libitum* fed rats but not less than the other restricted pair-fed rats. *Ad libitum* fed rats contained 31.6 ± 1.3 g fat; rats which were restricted to 75%, 50%, and 25% of *ad libitum* intake during the first week contained 39.2 ± 3.0 g, 43.1 ± 3.1 g, and 42.6 ± 3.5 g body fat, respectively. Body fat content of the rats restricted to 50% and 25% of *ad libitum* intake during the first week was greater than observed in rats fed *ad libitum*. These observations demonstrate that the catch-up growth was evident as early as the first week after refeeding and that the increased efficiency of converting the food energy to body fat gain was more pronounced in the more severely restricted rats.

Experiment 6. Rats refed *ad libitum* after a period of food restriction consume more food than rats fed *ad libitum* continuously (Wilson and Osbourn, 1960; Meyer and Clawson, 1964; Drew and Reid, 1975a, 1975b, 1975c;

Robinson *et al.*, 1975; Levitski *et al.*, 1976; Szepesi and Vojnik, 1975; Szepesi *et al.*, 1975; Szepesi and Epstein, 1976; Ashworth, 1968; Miller and Wise, 1976). In experiments 1 through 5, rats were pair-fed on a food intake basis to the control group. However, the restricted rats weighed less than the control rats after the initial week of food restriction; consequently, the restricted pair-fed rats consumed more food per gram body weight than did the control rats. In this experiment, one group of restricted rats was pair-fed on a food intake basis and the second group was pair-fed on a body weight basis (Table 14).

During the first week of the experiment, *ad libitum* fed rats consumed 21 g food per day and the restricted rats consumed an average of 5.2 g food per day (Table 15). As in experiment 5, *ad libitum* fed rats gained about 50 g body weight and the restricted group lost body weight during this period. The carcasses of the restricted rats weighed less and contained less fat at the end of the first week than did carcasses of the *ad libitum* controls (Table 15).

Rats pair-fed on a food intake basis grew faster than the *ad libitum* fed rats during each of the last three weeks. On the other hand, rats which were pair-fed on a body weight basis gained weight at a slower rate during the first and second weeks of refeeding, but gained weight faster during

the third week than the *ad libitum* fed rats. The total weight gain of the *ad libitum* fed rats and of the rats fed on the weight basis during the last 3 weeks was similar, whereas the rats which were pair-fed the same amount of food as the *ad libitum* groups gained more body weight than either of the other groups (Table 16). The total food intake per average body weight (weeks 2 to 4) was 18% greater in rats pair-fed on a food intake basis than in either *ad libitum* fed rats or in rats pair-fed on a body weight basis. Weight gain per gram of food intake during the last 3 weeks was greater in rats which had been restricted than in *ad libitum* fed rats (Table 16).

Body fat gain showed a similar trend to the weight gain; rats pair-fed on a food intake basis deposited more fat during the last three weeks than *ad libitum* fed rats. There was no difference in fat accumulation between rats pair-fed on a body weight basis and the *ad libitum* fed rats (Table 16).

Total 4 week food intake of the *ad libitum* fed rats was greater than either of the groups of rats that were restricted during the first week; food intake averaged 25 g daily in *ad libitum* fed rats, 20 g in rats pair-fed on a food intake basis, and 14 g in rats pair-fed on a body weight basis. Consequently, *ad libitum* fed rats gained more total weight during the 4 week experimental period

TABLE 16

WEIGHT GAIN, GAIN PER GRAM FOOD CONSUMED, FOOD INTAKE PER GRAM BODY WEIGHT, AND FAT GAIN IN RATS DURING A ONE-WEEK PERIOD IMMEDIATELY FOLLOWING ONE WEEK OF RESTRICTED (75%) FOOD INTAKE (EXP. 6)[1]

Treatment	Weight Gain (g)	Food Intake/ Body Weight[2]	Gain/ Food (g)	Fat Gain (g)
Ad libitum	$141 \pm 1_a$	$1.7 \pm 0.1_a$	$0.26 \pm 0.04_a$	$23.9 \pm 1_a$
Restricted 75%, pair-fed on intake basis	$186 \pm 1_b$	$2.0 \pm 0.2_b$	$0.37 \pm 0.04_b$	$35.4 \pm 1_b$
Restricted 75 %, pair-fed on body weight basis	$134 \pm 1_a$	$1.7 \pm 0.1_a$	$0.39 \pm 0.05_b$	$20.6 \pm 1_a$

[1]Values represent mean ± SEM for 10 rats. Treatment means in the same column not sharing a common subscript letter differ significantly ($p < 0.05$).

[2]Total food intake (g) during weeks 2-4 divided by the average body weight (g) of the rats during this period. Average body weight equalled body weight at 7, 14, 21, and 28 days divided by four.

than both restricted groups. The final body fat content of *ad libitum* fed and pair-fed (food intake basis) rats was similar (37.3 ± 0.9 g and 37.5 ± 0.9 g), but greater than that of rats fed on body weight basis (23.7 ± 0.7 g).

Discussion

In previous studies, rats re-fed after a fast or a period of restricted food intake were allowed to eat *ad libitum* and they consumed more food than rats fed *ad libitum* continuously (Szepesi, 1973; Szepesi and Vojnik, 1975; Szepesi *et al.*, 1975; Szepesi and Epstein, 1976). The restricted/re-fed rats also gained more weight and more body fat upon refeeding than *ad libitum* fed rats; however, it was not possible to separate the effects of increased food intake from possible effects of increased energy efficiency. By pair-feeding the rats during the refeeding period, we were able, in the present experiments, to eliminate the effect of greater food intake. Upon re-feeding, rats gained more body fat than the control rats. This compensation was apparent as early as the first week of refeeding and continued for an additional 2 weeks in rats that had been initially restricted to 50% or less of *ad libitum* intake.

The same compensatory fat gain occurred in rats pair-fed the dry diet as in rats force-fed twice daily (expt. 1), which suggests that the initial food restriction,

rather than force-feeding *per se* was a major causative factor in the increased body fat accumulation. Likewise, compensatory fat gain also occurred in rats restricted for 1 week and then pair-fed to meal-fed controls (expt. 2). The dietary carbohydrate to fat ratio did not influence the results; compensatory fat gain was evident when either a high-carbohydrate or a high-fat diet was fed.

Under the conditions of our present study, as well as in previous experiments in which meal-fed rats (Ozelci *et al.*, 1977, 1978), pigs (Romsos *et al.*, 1978a), and dogs (Romsos *et al.*, 1978b) were pair-fed throughout the entire experimental period, meal pattern did not influence body fat accumulation. Only when the alteration in meal pattern resulted in an initial food restriction in the treatment group, but not in the control group, did meal pattern influence body fat accumulation. We conclude that alterations in meal pattern have only a minimal influence on body fat accumulation provided treatment and control animals are pair-fed throughout the entire experiment.

The initial food restriction resulted in compensatory weight and fat gain when rats were subsequently pair-fed to control animals. These results are in agreement with numerous previous reports (Wilson and Osbourn, 1960;

Meyer and Clawson, 1964; Drew and Reid, 1975a, 1975b, 1975c; Robinson *et al.*, 1975; Levitski *et al.*, 1976; Szepesi and Vojnik, 1975; Szepesi *et al.*, 1975; Szepesi and Epstein, 1976; Ashworth, 1968; Miller and Wise, 1976; Widdowson and McCance, 1963). The compensatory gain could not be attributed to increased food intake above levels consumed by control rats because the rats were pair-fed. The severity of the initial food restriction influenced the magnitude of the subsequent compensatory gain (Meyer and Clawson, 1964; Szepesi and Epstein, 1976). Rats restricted to only 25% of *ad libitum* intake for the first week of the experiment gained more body weight and fat upon refeeding than did rats restricted to 75% of *ad libitum* intake for the initial week, although both groups of rats consumed the same amount of food upon refeeding. A detailed examination of the length of time during which compensatory gain was evident was not conducted; however, an increased accumulation of fat was detected during the first week of refeeding. Beyond 3 weeks of refeeding, compensatory gain was not evident in rats which had been restricted to 75% of *ad libitum* intake for 1 week.

In the majority of the present experiments, rats were pair-fed on a food-intake basis. Thus, rats that had been fed restricted amounts of food during the first week and consequently gained less weight than the control

rats, consumed more food per gram body weight upon re-feeding than did control rats. If it is assumed that the maintenance energy requirements of animals are related to body weight, then the rats re-fed on a food intake basis would have initially consumed more energy above maintenance than control rats because the restricted/re-fed rats weighed less than the control rats. The increased energy intake above maintenance could then have contributed to the compensatory gain.

To test the hypothesis that compensatory gain resulted from increased food intake per gram body weight, rats which had been fed restricted amounts of food for 1 week were subsequently pair-fed food on a body weight basis. The control rats weighed 200 g and the restricted rats weighed 114 g at the end of the first week; consequently, the rats pair-fed on a gram body weight basis initially consumed approximately 40% less energy per day than did the control rats. At the end of the experiment, these pair-fed rats still consumed 30% less energy per day than the control rats. Despite the reduced food intake per rat, the restricted rats re-fed on a body weight basis gained as much body weight and body fat during the re-feeding period as the *ad libitum* fed controls. Thus, these restricted rats, once re-fed, were more efficient than *ad libitum* fed controls in converting dietary energy

to body fat.

Several reports have suggested that the basal metabolic rate is decreased when food intake is restricted (Wilson and Osbourn, 1960; Meyer and Clawson, 1964; Stordy *et al.*, 1977). If the basal metabolic rate does not immediately increase when food intake is subsequently increased, the animals would have an increased portion of the energy consumed available for fat deposition. Alternatively, the restricted/re-fed animals might develop an increased efficiency to convert dietary energy to body fat.

One possible cause for the decreased basal metabolic rate and thus increased body fat might be a depression in thyroid function, with consequent diminished expenditure of energy. Hypothyroidism could result from at least one of two processes: (a) altered enzymatic pathways in the pituitary resulting in a slower rate of thyroid stimulating hormone formation and release; or (b) altered sensitivity of the hypothalamic-pituitary mechanisms for homeostasis of circulating thyroid hormone(s) (Cohn and Joseph, 1960). Another factor contributing to improved efficiency of re-fed animals might be decreased rates of protein turnover. Regardless of the exact mechanism(s) responsible for the observed compensatory fat gain, the possibility that such a phenomenon might occur when obese humans return to their "normal" eating habits following reduced food intake during weight reduction warrants further study.

SUMMARY AND CONCLUSIONS

Adaptive changes in body metabolism caused by eating habits have relatively recently attracted the interest of scientists. Studies from many parts of the world suggested that feeding frequency may alter metabolism of the body through direct or indirect mechanisms including enzymes and hormones. However, these effects might vary from species to species; even within the same species, different results may be obtained under different conditions. Human studies primarily include epidemiological experiments on some population groups which cannot be applied to all populations.

In the present studies, rats were used as experimental subjects. Several meal patterns were applied: meal-feeding for one or two hours per 24 or 48 hours; nibbling (pair feeding to meal-eaters with an automated feeding machine); force-feeding; and *ad libitum* feeding. The effect of the form of diet (liquid *versus* dry) on body weight and fat gain was also studied by feeding rats the diet mixed with an equal weight of water as compared to feeding the diet in dry form.

When the nibbling rats were pair-fed to meal-eaters with automated feeders, both groups gained the same amount of body weight. Similar results were obtained regardless of the composition of diet (10%, 20%, or 30% casein, high-carbohydrate diet, or 20% casein, high-fat diet) or whether rats were fed once per 24 or 48 hours. Meal-eating did not cause a depression in nitrogen retention and body protein or an increase in fat deposition.

In previous force-feeding studies (Cohn and Joseph, 1959, 1960, 1963, 1968; Han, 1973) where an increase in body fat accumulations was observed, water was added to the diet to be able to administer the food by tube. Feeding a liquid diet mixed with water did not influence body fat gain in our experiments. Also, pair-force-feeding rats to *ad libitum* fed control animals without an initial adaptation to food load did not increase body fat.

The final series of experiments was conducted to determine the role of initial food restriction inherent in meal-feeding and force-feeding experiments, on subsequent body weight and fat gain. When rats were re-fed the same amount of food as consumed by control rats, after restricted food intake for 1 week, re-fed rats gained more body weight and fat than control rats. This compensatory gain occurred regardless of the pattern of

feeding (force-feeding, meal-feeding, or *ad libitum* feeding), and regardless of the composition of the diet (high-carbohydrate or high-fat). These results suggest an increased efficiency of converting dietary energy to body fat in rats restricted in food intake and then pair-fed to control rats.

Mechanism(s) by which compensatory gain occurs cannot be readily explained. Pair-feeding the rats showed that compensatory gain was not the result of increased food intake above levels consumed by control rats. When the rats are pair-fed on a food intake basis, restricted rats weigh less and, upon refeeding, they consume more energy above maintenance levels than control rats. However, rats pair-fed on body weight basis still gained as much body fat during refeeding as the *ad libitum* fed controls, suggesting that these rats are more efficient in converting food energy to body fat than control rats. This increased efficiency results from a metabolic adaptation and might be caused by decreased basal metabolic rate.

There is evidence that obese humans gain more weight when they return to previous eating habits after a weight reduction diet. The applicability of the findings in the present studies to humans deserves further investigation.

FOOTNOTES

FOOTNOTES

Chapter II

[1]Male Sprague-Dawley rats were obtained from Spartan Research Animals, Inc., Haslett, Michigan.

Chapter III

[1]Male Sprague-Dawley rats were obtained as follows: Experiments 1-3 and 5 - Spartan Research Animals, Inc., Haslett, Michigan; Experiment 4 - Harlan Industries, Inc., Cumberland, Indiana.

[2]Glucostat, Worthington Biochemical Corp., Freehold, New Jersey.

[3]Tritium, New England Nuclear, 575 Albany Street, Boston, Massachusetts.

[4]Omnifluor, New England Nuclear, Pilot Chemicals Division, 575 Albany Street, Boston, Massachusetts.

[5]Feeding tube, size 8 French, C. R. Bard, Inc., Murray Hill, New Jersey.

Chapter IV

[1]Male Sprague-Dawley rats were obtained from Spartan Research Animals, Inc., Haslett, Michigan.

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