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Title: The Effects of Meal Frequency on Plasma Lipids in Moderately Obese Humans

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The purpose of this investigation was to determine the effects of meal frequency on plasma lipids in moderately obese individuals consuming a weight maintenance diet. Four female and three male university students, who averaged 32 percent overweight, participated in the study. An initial five-day adjustment period established the energy requirements of the subjects. The first experimental period of 21 days provided the daily caloric intake in two meals; 20 percent in a morning meal and 80 percent in an evening meal. In the second experimental period, which was also 21 days long, the subjects consumed eight approximately isocaloric meals per day. The amounts and types of nutrients provided in each experimental period were similar.

Fasting plasma samples were obtained at intervals throughout the study and analyzed for cholesterol, nonesterified fatty acid (NEFA), and triglyceride concentrations. The mean cholesterol and NEFA

values were significantly greater in the two-meal than in the eight-meal period. There was no significant difference in triglyceride concentrations due to meal frequency.

Plasma samples taken during glucose tolerance tests given at the end of all three dietary periods were analyzed to determine the response of glucose, NEFA, and triglycerides to an oral glucose load. The rise in glucose concentration following glucose ingestion was greater after the two-meal period than it was following either the adjustment or the eight-meal period, but the results were not significant. There was no appreciable difference in the NEFA or triglyceride response, as determined by changes from fasting levels, at the end of the two experimental periods.

The results indicate that a decrease in meal frequency is associated with higher fasting concentrations of cholesterol and non-esterified fatty acids, and a tendency towards decreased glucose tolerance. Since obese individuals are prone to have elevated plasma lipids and decreased glucose tolerance, the cause and/or the control of such abnormalities may be associated with meal frequency.

The Effects of Meal Frequency on Plasma Lipids in Moderately Obese Humans

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INTRODUCTION

Excess body weight is a fairly common occurrence in Western society. This has caused much concern because obesity is associated with certain physiological disorders in lipid and carbohydrate metabolism such as hyperlipidemia and decreased glucose tolerance (Nestel and Goldrick, 1976). Obesity is also considered one of the risk factors in the development of coronary heart disease (Kannel et al., 1967), a leading cause of death in our society.

Stunkard et al. (1955) observed that some obese individuals habitually consume most of their daily food during one large evening meal. Population surveys have also shown that meal frequency is inversely related to obesity, as well as to hypercholesteremia and the occurrence of heart disease (Fábry and Tepperman, 1970). These studies indicate that meal frequency may be an independent contributing factor in the development of some of the abnormalities associated with obesity.

The effect of meal frequency on plasma lipids has been studied in a number of investigations on both obese and lean subjects. The results have not been conclusive but suggest that plasma lipids may be influenced by feeding frequency. Most of the studies using obese

subjects have been complicated by the fact that the subjects were also losing weight. Since weight reduction itself is known to alleviate some of the problems associated with obesity (Olefsky et al., 1974), there is a need to investigate the obese individual who is maintaining body weight. The purpose of this study, therefore, is to investigate the effects of meal frequency on the plasma lipids of obese individuals on a weight maintenance program.

REVIEW OF LITERATURE

Normal Plasma Values and Factors Affecting Them

Total Cholesterol

Most of the circulating cholesterol in the fasting state is derived from liver and intestinal synthesis and is transported as part of a lipoprotein complex. Approximately 70 percent is esterified to a fatty acid moiety with the remainder circulating as free cholesterol (Nestel, 1970). Physiologically, cholesterol serves a variety of functions in the body. Structurally, it is a necessary component of cell membranes and biochemically, it is a precursor of steriod hormones and bile acids (Sabine, 1977).

In health, the post-absorptive plasma cholesterol ranges between 144 and 290 mg/dl (Henry et al., 1974). Levels tend to increase gradually with age, reaching peak values in the fifth or sixth decade (Keys et al., 1950; Schaeffer, 1964). Population surveys indicate that the circulating cholesterol is generally lower in women than men (Schaeffer, 1964) and that it varies with the menstrual cycle (Oliver and Boyd, 1953). Day to day variations in plasma cholesterol have been found to be between 3 and 15 percent (Hollister et al., 1964).

Diet can have an effect on fasting cholesterol levels. The consumption of a diet high in fats, especially when those fats are

saturated, is associated with an increased plasma cholesterol concentration (Hardinge et al., 1962; Keys et al., 1965a). Dietary cholesterol will also exert an effect on the plasma cholesterol concentration (Key et al., 1965b). However, since intestinal absorption is low in the human, and the absorbed cholesterol tends to inhibit hepatic cholesterolgenesis, the effect is not extensive (Nestel, 1970).

Acute and chronic anxiety can cause increases in cholesterol levels (Jenkins, 1971). Studies on the effect of exercise are not conclusive, but tend to show that routine exercise will protect an individual from increasing cholesterol levels (Mann et al., 1969).

Nonesterified Fatty Acids

Nonesterified fatty acids (NEFA) are transported in the plasma bound to albumin. They appear in relatively small quantities and have a half-life in the plasma of only a few minutes (Ganong, 1977). Normal plasma levels in the fasting individual generally range from 450 - 900 μEq/L plasma; day to day variations range from 10 to 30 percent (Henry et al., 1974).

In the fasting state, NEFA provide the major source of available energy to most tissues of the body. The influx of NEFA into the plasma during the post-absorptive state is a result of the lipolytic breakdown of triglycerides stored in the adipose tissue (Heindel et al., 1975). The intracellular enzyme, hormone-sensitive lipase, catalyzes

the hydrolysis of stored triglycerides into glycerol and fatty acids which are then released into the circulation. The catecholamines, norepinephrine and epinephrine, and glucagon increase the activity of this enzyme by their action on cyclic AMP (Khoo et al., 1974). Insulin, on the other hand, decreases the activity of hormone-sensitive lipase (Butcher et al., 1968). The uptake of NEFA by the peripheral tissues is largely determined by their availability in the plasma. Therefore, the plasma levels are thought to be determined mainly by the rate of lipolysis (Masoro, 1977). Circulating NEFA are also taken up by the liver where they can be reesterified into triglycerides or oxidized to acetyl CoA. The latter serves as a precursor for the biosynthesis of new fatty acids, sterols, and ketone bodies, as well as a source of energy upon further oxidation (Robinson, 1964).

Triglycerides

The triglycerides (also called triacylglycerides) are transported in the plasma of the fasting individual as part of a very-low-density lipoprotein (VLDL) complex which is synthesized in the liver (Havel and Kane, 1975). Normal fasting levels range from 30 - 135 mg/dl of serum (Henry et al., 1974). Plasma levels tend to vary depending on the age, sex, and diet of an individual, the latter having the greatest effect. Males tend to have higher fasting triglyceride levels than females of the same age (Fletcher, 1968). Triglycerides are not

affected by the menstrual cycle (Henry et al., 1974), but do increase approximately 45 percent with the use of oral contraceptives (Hazzard et al., 1969).

In the fasting individual, the nonesterified fatty acids (NEFA) used in the synthesis of hepatic triglycerides are mainly derived from those circulating in the plasma (Basso and Havel, 1970). Since triglycerides do not normally accumulate in the liver, it is believed that they are released into the circulation as fast as they are synthesized. Diets high in carbohydrates, insulin, and ingestion of alcohol promote the synthesis and release of triglycerides from the liver (Harper, 1977). Also, an increased availability of NEFA to the liver stimulates triglyceride formation (Havel et al., 1970).

As the VLDL circulate in the plasma, their triglycerides are taken up and metabolized by the extraheptic tissues. Lipoprotein lipase, which is located in the capillary walls, catalyzes the hydrolysis of the VLDL triglycerides to glycerol and NEFA which can be used in cellular oxidation (Felts, 1975).

The Oral Glucose Tolerance Test

An oral glucose tolerance test is performed on an individual after an overnight fast. Initially, a fasting blood sample is drawn in which the glucose concentration normally ranges between 70 and 110 mg/dl plasma (Henry et al., 1974). The individual is then given a glucose

load ranging from 0.75 - 1.5 g/kg body weight. For the following 3-4 hours, blood is drawn at periodic intervals for analysis. In this way, the response of blood glucose to the glucose load can be measured. Typically, the glucose may rise from 90 mg/dl to 140 mg/dl within an hour and then return to normal levels within 2 1/2 hours (Berkow, 1977). Insulin secretion is stimulated by glucose ingestion and will also rise and fall during the course of the test (Yalow and Berson, 1960).

Plasma lipids also change during an oral glucose tolerance test. Nonesterified fatty acids (NEFA) characteristically decrease as the blood glucose and insulin increase and, conversely, rise again when the glucose decreases (Shafrir et al., 1965). This response is associated with inhibition of lipolysis by insulin and the resumption of lipolysis as insulin levels subside (Cahill, 1971). In the adipose cell, α-glycerophosphate derived from glucose provides a backbone for re-esterification of NEFA into triglycerides (Renold, 1965).

The information on the response of triglycerides to an oral glucose tolerance test is sparse. Nikkilä (1969) indicates that in man, triglycerides decrease after a sugar load and do not begin to rise for a period of up to nine hours. This is presumably due to the lowering of plasma NEFA which serve as precursors to triglycerides. It might be noted, however, that fasting levels of NEFA are regained in about four hours after glucose ingestion. Wadhwa et al. (1973) found that triglycerides increase for approximately one hour after an oral

glucose load and then gradually return to or below fasting levels within three hours. The stimulatory effect of insulin on triglyceride synthesis in the liver is thought to be the reason for this initial rise in plasma triglycerides.

Obesity

Obesity, or the excess of adipose tissue, is generally the result of an imbalance between energy consumption and expenditure. The excess energy is stored as triglycerides in the adipocyte which consequently enlarges. An increase in the number of fat cells can also be found in the obese individual, especially if adiposity occurred in childhood (Björntrop and Sjöstrom, 1971). Genetic prediposition, endocrine disorders, and environmental conditions can all contribute to the etiology of obesity, making any generalizations concerning metabolic disturbances found in obesity a complex task. Nevertheless, there are some abnormalities of lipid and carbohydrate metabolism which consistently occur in obesity and are also associated with cardiovascular disease (Kannel et al., 1967) and maturity-onset diabetes (Gastineau, 1972).

An increase in the concentration and turnover rate of circulating nonesterified fatty acids (NEFA) in obese individuals has been noted by many investigators (Dole, 1956; Opie and Walfish, 1963; Nestel and Whyte, 1968). Goldrick et al. (1970), and, more recently,

Arner et al. (1979) have observed that enlarged fat cells have increased lipolytic activity. Flatt (1972) pointed out that the increased mass of adipose tissue found in the obese individual could, by itself, account for the greater output of NEFA into the circulation. Early restoration of lipolysis after glucose ingestion has also been observed in obese individuals (Barter and Nestel, 1972). Hanley et al. (1967) reported that adiposity had only minor effects on the response of NEFA to a glucose load. They did, however, find a greater mean concentration of NEFA during a glucose tolerance test with increasing adiposity and attributed it to increased fasting levels. Nestel et al. (1978) noted a decrease in the clearance rate of NEFA from the plasma of obese subjects.

Triglyceride metabolism is also altered in obesity. Plasma levels of triglycerides and very-low-density lipoproteins often increase in obesity (Albrink and Meigs, 1965; Harlan et al., 1967). Most investigators believe that the high levels of circulating NEFA serve as precursors for hepatic triglyceride synthesis (Havel et al., 1970). However, in some obese subjects, as much as 75 percent of the newly secreted triglyceride-fatty acids may be derived from sources other than plasma NEFA. This would suggest that the splanchnic bed or the liver, itself, may provide much of the fatty acids necessary for triglyceride formation (Barter and Nestel, 1973). Research on the removal of triglycerides from the plasma in obesity

has produced inconclusive results (Nestel and Goldrick, 1976). Adipose tissue taken from obese individuals who are hyperinsulinemic displays an increased activity of lipoprotein lipase (Pykälistö et al., 1975). This, however, may not reflect the total ability to clear triglycerides from the plasma by all of the tissues (Forget et al., 1975).

Plasma levels of cholesterol have not been consistently correlated with adiposity. However, total body cholesterol and synthesis have been shown to increase in obesity (Miettinen, 1971). The excess cholesterol is presumably stored in the adipose tissue (Schreibman and Dell, 1975). Nestel and Goldrick (1976) suggested that the increased levels of NEFA available to the liver may provide the necessary acetyl precursors for increased sterol synthesis. In connection with this, Prigge and Grande (1973) found that in dogs fed a high fat diet, an increase in circulating fatty acids caused by a lipolytic agent was associated with an increased serum cholesterol concentration.

Insulin has received much attention as it relates to both carbohydrate and lipid metabolism. Obese individuals generally have hyperinsulinemia in the fasting state and following a glucose challenge (Karam et al., 1963; Bagdade et al., 1967; Kreisberg et al., 1967; Chiles and Tzagounis, 1970). Removal rates of glucose during a glucose tolerance test are either normal or reduced (Butterfield et al., 1965; Stunkard and Blumenthal, 1972) indicating an insulin resistance or insensitivity of peripheral tissues in obesity (Rabinowitz

and Zierler, 1962; Salans et al., 1968). Most (Harrison et al., 1976; Olefsky, 1976), but not all (Amatruda, 1975) studies of the enlarged adipocyte indicate that the hyperinsulinemia is associated with a decreased number of insulin receptor sites. Gavin et al. (1974) found that, in vitro, high concentrations of insulin led to decreased insulin receptors on cultured human lymphocytes, suggesting that insulin resistance in obesity may be secondary to the hypersecretion of insulin. Bar et al. (1976) found an inverse relationship between plasma insulin concentration and insulin receptors in obese humans. Randle and co-workers (1965) have postulated that the increased levels of NEFA in the obese may serve to inhibit enzymes necessary for the uptake and metabolism of glucose in peripheral tissues. The excess glucose would be available for further stimulation of insulin secretion thereby producing a state of hyperinsulinemia. It is somewhat paradoxical that elevated concentrations of NEFA would occur simultaneously with hyperinsulinemia since insulin inhibits lipolysis. This may, however, be another manifestation of insulin resistance.

Meal Frequency

The metabolic effects of meal frequency have been studied in a variety of experimental designs. Early animal studies, based on respiratory quotients, showed that an "adaptive hyperlipogenesis" occurred when rats learned to consume their daily ration of food within

a limited time period (Tepperman and Tepperman, 1964). This type of feeding regime was found to cause an increase in adipose tissue growth relative to controls which were permitted continuous access to food (Cohn et al., 1965; Leveille, 1970). A number of investigators have shown that key lipogenic enzymes increase in the adipose tissue of meal-fed rats (Hollifield and Parson, 1962; Leveille and Hanson, 1966; Chakrabarty and Leveille, 1968; Bray, 1972). Other adaptive responses include hypertrophy of the gastrointestinal tract (Feigenbaum et al., 1962; Tepperman and Tepperman, 1964) and increased insulin secretion in response to a glucose challenge (Wiley and Leveille, 1970). Glucose tolerance is generally increased in the meal-fed rat (Leveille and Chakrabarty, 1968). Fábry and Tepperman (1970) suggest that a "gorging" type meal pattern, which is commonly seen in some obese individuals (Stunkard et al., 1955), may contribute to obesity and certain metabolic abnormalities associated with obesity, such as hyperinsulinemia.

Epidemiological studies by Fábry and co-workers (Fábry et al., 1963; Hejda and Fábry, 1964; Fábry et al., 1968) indicate that meal frequency may be inversely related to the occurrence of a number of pathological conditions. In a group of 89 healthy men, aged 30-50 years, they found that the subjects with the fewest meals per day were more overweight, had thicker skinfolds, and a higher ratio of plasma cholesterol to phospholipid than those consuming many meals per day

(Hejda and Fábry, 1964). Two subsequent studies on men aged 60-64 years showed that as meal frequency decreased the body weight and serum cholesterol increased and glucose tolerance decreased. They found that the incidence of ischemic heart disease was significantly less among those people consuming five or more daily meals than those consuming three or less (Fábry et al., 1964, 1968). These investigators suggested that humans may go through some metabolic adaptations to a decreased meal frequency and that these adptations may be pathogenic when accompanied by a positive caloric balance which is commonly found in modern Western society (Fábry and Tepperman, 1970). More recently, Metzner et al. (1977) studied a group of 2000 men and women and found that adiposity, as measured by body weight and skinfold thickness, was inversely related to meal frequency, as determined by dietary recall.

The effect of meal frequency on fasting blood lipids has been studied in both experimental animals and humans. Cholesterol has been the primary lipid under study, although other lipids, including triglycerides and nonesterified fatty acids (NEFA), have also been looked at by some investigators. Both chickens and rabbits, allowed to consume a standard cholesterol-containing diet for only two hours each day, developed a two-fold increase in serum cholesterol and a four to seven-fold increase in atherosclerotic lesions when compared to ad libitum-fed controls, even though they consumed less food daily

(Cohn et al., 1959; Wells et al., 1962). Goplan et al. (1962) reported similar results in monkeys fed a semi-synthetic cholesterol-free diet. After eight weeks, the monkeys restricted to a one hour daily feeding showed 40 percent higher cholesterol levels than did those allowed free access to food 24 hours per day.

In a study with humans, Cohn's group found a slight but consistent decrease in serum cholesterol concentration when a group of adults ate six as opposed to three meals daily (Cohn, 1964). Another investigation showed that when the daily menu of some hyperlipemic patients was divided into multiple feedings, the serum cholesterol concentration tended to decline (Cohn, 1961). Other investigators have found similar results. Jagannathan et al. (1964) observed that consuming eight as compared to three meals per day enhanced the cholesterol-lowering effect of corn oil in 39 healthy males. However, there were no changes in fasting triglyceride concentration due to meal frequency. Gwinup et al. (1963) found a decrease in serum cholesterol and esterified fatty acids when a group of five normal males switched from eating one to ten meals daily. Increases in serum cholesterol were similarily recorded by Young et al. (1972) when a group of ten normal males consumed one as opposed to six meals per day. In their study, the fasting triglyceride concentration also tended to be higher on the one meal per day regime, but the results were not significant. Irwin and Feeley (1967) observed lower

cholesterol levels when a group of normal females ate three equal meals instead of two small and one large meal daily. Intermediate levels of cholesterol, however, were recorded when six meals per day were consumed. There were no differences in serum glycerides or total fatty acids attributable to the different meal frequencies. Wadhwa et al. (1973) compared the effects of eating eight small meals per day with eating one small and one large meal daily and observed no changes in fasting cholesterol, triglycerides, or NEFA due to the different regimes. Angel and Schwartz (1975) studied the effects of the Ramadan fast in seven Muslim males. This fast prohibits eating between sunrise and sunset; consequently there is a decrease in meal frequency. They found that the change in meal pattern had no effect on the fasting levels of cholesterol, glyceride glycerol, or NEFA.

Studies on the effect of meal frequency in obese individuals are mostly complicated by the fact that the subjects were also on a weight reducing diet. Both Bortz et al. (1966) and Finkelstein and Fryer (1971) found that changes in serum lipids were associated with weight loss and that altering the meal frequency had little or no effect. Young et al. (1971) did observe an increase in serum cholesterol when 11 moderately obese males on a hypocaloric diet consumed one as opposed to three or six meals per day.

Bortz et al. (1969) observed the effect of meal frequency on the diurnal NEFA levels in five males and found that the mean

concentration was higher when the subjects consumed a single meal per day. They postulated that the increased flux of NEFA to the liver may have initiated the mechanism for cholesterol and lipoprotein synthesis.

Responses to a glucose challenge have been studied by many investigators. Cohn et al. (1963) observed a decrease in the clearance rate of a glucose load when adult humans consumed three as compared to six meals daily. That glucose tolerance is diminished with decreased meal frequency is supported by other laboratory investigations (Nunes and Canham, 1963; Young et al., 1971; Pringle et al., 1976), as well as epidemiological studies (Fabry et al., 1964). Angel and Schwartz (1975), however, found no change in glucose tolerance with a decrease in meal frequency. It might be noted, though, that their subjects were consuming less food and had lower body weights when blood was drawn for the period of decreased meal frequency.

An excessive insulin response to a glucose challenge has been observed in both obese and lean subjects when the number of meals consumed daily was decreased (Wadhwa et al., 1973; Angel and Schwartz, 1975; Pringle et al., 1976). Young et al. (1972), on the other hand, found that meal frequency patterns had no significant effect on insulin concentrations during a glucose tolerance test.

The response of triglycerides and NEFA to an oral glucose load was observed by Wadhwa et al. (1973). In subjects adapted to a diet containing corn oil, there was a significantly greater increase in triglycerides after the ingestion of glucose when they were on a "gorging" as opposed to a "nibbling" feeding pattern. The response of NEFA was unaffected by meal frequency.

MATERIAL AND METHODS

The study was designed to compare the effects of meal frequency (two meals versus eight meals) on plasma lipids and glucose tolerance of obese individuals. The experimental plan was approved by the Human Subjects Committee of the University in accordance with guidelines of DHEW (1971).

Subjects

Three male and four female university students participated in the study. The primary criterion used in selecting subjects was that they be at least 25 percent above the average weight for age, height, and sex, as set by the Society of Actuaries (1959). However, due to difficulties in obtaining volunteers for the study, one subject who was 23.2 percent overweight was allowed to participate. All subjects were in general good health and free from any known disorders of carbohydrate or lipid metabolism. Volunteers were recruited through advertising on the University campus.

The subjects' ages ranged from 18 to 30 years with a mean of 21 years. Their weights ranged from 23.2 to 45.0 percent above the average weight for age, height, and sex, with a mean of 31.9 percent. None of the subjects were actively engaged in sports and an effort was made to ensure that their level of physical activity was maintained

throughout the duration of the study.

Dietary Treatment

The first dietary period was a five-day adjustment period during which the daily energy requirement for maintenance of body weight was established for each individual. Initial estimates of energy requirements were made from a three-day dietary record which each subject provided prior to the study. During the adjustment period, food was served in a three meal per day pattern. The two experimental periods were each 21 days in duration. In the first experimental period, the daily food was divided into one small and one large meal; this is called the two-meal period. In the second experimental period, the daily food was divided into eight small meals (the eight-meal period). The basic diet consumed during the experimental periods was constructed on the basis of the Exchange Lists for meal planning (American Diabetes Association, 1976) plus selected dessert items. This diet plan established the number of exchange units from each of the food categories on the Exchange List to be used each day. Individual differences in caloric requirements were accommodated by adding or subtracting exchange units from the basic diet. A four-day rotation plan was employed during both experimental periods. Although similar amounts and types of exchange units were used throughout the experiment, the specific foods fulfilling those exchange units varied

somewhat between the two experimental periods because foods that were appropriate for the large meal could not always be incorporated into small transportable meals. During the course of the study, the subjects were weighed every second day and small adjustments were made in the diet when necessary to maintain body weight. Every effort was made to maintain the subjects' weight throughout the study since weight reduction of itself can affect the plasma lipid concentrations (Olefsky et al., 1974).

During the first experimental period, the subjects consumed approximately 20 percent of the total daily allotment of calories between 7:00 and 8:00 o'clock A.M. and received the remaining 80 percent of their food during an evening meal which generally lasted from 5:30 to 7:00 o'clock P.M. If, on occasion, a subject was unable to complete the evening meal, a small portion (usually a dessert) was allowed to be taken home provided that it be eaten within two hours. In the second experimental period, the subjects consumed eight meals per day, all of which were approximately isocaloric. These meals were eaten about every two hours; the first one around 7:30 A.M. and the last one at approximately 10:00 P.M. The subjects were allowed free use of plain coffee, tea, and calorie-free beverages.

Meals were prepared in the kitchen of a family-type residence[1]

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[1] Kent House. 27 S.W. 26th St., Corvallis, Oregon.

which had been assigned to the study by the School of Home Economics. During the two-meal period, both meals were served in the dining room of the residence. During the eight-meal period, the subjects consumed the morning, noon, and evening meals at the residence and carried out interim feedings to be eaten on location. The planning, preparation, and service of all meals was shared by three graduate students in Foods and Nutrition.

Glucose Tolerance Test

At the end of each dietary period, glucose tolerance tests were given to the subjects after an overnight fast. Following the initial blood drawing, each subject received glucose (1 gm/kg) in the form of a grape sour drink.[2] The drink was consumed within ten minutes, and additional blood drawings occurred at 40, 80, 120, 180, and 240 minutes. During the four-hour period, the subjects remained calm and were allowed to engage in light activity, such as studying. A licensed medical technologist drew the blood from an antecubital vein into 10-ml heparinized Vacutainers. The blood was refrigerated immediately and centrifuged under refrigeration. Within 40 minutes after the drawing, the plasma was separated and stored in 2-ml screw-cap vials at -50°C. It was subsequently analyzed for glucose,

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[2] VanLab grape sour drink. VWR Scientific Inc.

nonesterified fatty acid, and triglyceride concentrations.

Interval Blood Samples

On the twelfth morning of each experimental period, fasting blood samples were drawn from each subject. Together with the fasting samples taken at each glucose tolerance test, a total of five fasting blood samples was available for the analysis of cholesterol, nonesterified fatty acid, and triglyceride concentrations. The method of drawing, handling, and storing of the blood samples taken during the interval time periods was the same as described for the glucose tolerance test. All five fasting blood samples were drawn at approximately the same time of day (7:00 o'clock A.M.) to obviate diurnal variations in plasma lipids. Also, the subjects were asked not to engage in physical activity, such as running, before the blood drawings, since excessive amounts of lactic acid have been known to interfere with the analysis of nonesterified fatty acids (Trout et al., 1960).

Analytical Methods

Total Cholesterol

Total plasma cholesterol was determined by the automated procedure described by Block et al. (1966). Duplicate extracts of 100 μl of plasma in isopropanol (1:20 dilution) were reacted with a preheated

color reagent containing ferric chloride, concentrated sulfuric acid, and glacial acetic acid. Absorbance of the colored product was recorded at 550 nm. Total cholesterol was calculated from a standard regression equation and reported in mg/dl plasma.

Nonesterified Fatty Acids

Plasma nonesterified fatty acids were determined by the procedure of Trout et al. (1960), which is a modification of the titration method of Dole (1956). After the fatty acids were extracted in a heptane/isopropanol/sulfuric acid solution, the heptane phase was washed with dilute sulfuric acid to remove any lactic acid. A microburette was used to titrate the fatty acids with a weak sodium hydroxide solution; Nile Blue served as an indicator. Results were determined by comparing the endpoint to that of a palmitic acid standard and are expressed as μEq/L plasma.

Triglycerides

Plasma triglycerides were analyzed by a modification of the semi-automated procedure of Royer and Ko (1968). Heptane was used as an extractant instead of nonane because of cost. Although Soloni (1971) reported that heptane was less efficient than nonane in excluding phosphoglycerides, heptane extraction has been used successfully by other investigators (Leklem, 1978). In the present study, the values

determined using a control standard[3] were excellent.

Two hundred microliters of plasma were mixed with dilute sulfuric acid and extracted in a heptane/isopropanol solution. Transesterification of the triglycerides with sodium hydroxide released the glycerol which was then oxidized to formaldehyde by periodate. Absorbance of the colored reaction product of formaldehyde and 2,4-pentanedione was recorded at 420 nm. The triglyceride concentration was calculated by regression analysis with triolein as the standard. Samples were analyzed in triplicate and the results expressed as mg/dl plasma.

Glucose[4]

Glucose was determined by an automated procedure based on the method of Hoffman (1937). This method takes advantage of the potassium ferricyanide-potassium ferrocyanide oxidation-reduction reaction.

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[3] Moni-Trol I Chemistry Control. DADE Division American Hospital Supply Corp.

[4] Glucose data was made available by C. Shirkey.

Statistical Treatment

An analysis of variance was used to determine any significant differences in lipid concentrations at fasting at the end of the three dietary periods. Paired *t* tests were also used to compare the fasting levels of lipids during the two experimental periods. In this case, the average of values taken on day 12 and day 21 for each period were taken as the value for the period.

Differences in the response of glucose, nonesterified fatty acids, and triglycerides during the three glucose tolerance tests were determined by analysis of variance. The concentrations as changes from fasting levels were used as opposed to the actual concentrations. A comparison of the three glucose tolerance tests was made at each of the blood drawings during the test.

RESULTS AND DISCUSSION

Weight and Dietary Intake

The body weights of the seven subjects at the end of each dietary period are presented in Table 1. Because there was an average weight loss of 1.4 kg during the five-day adjustment period, the weight at the end of the adjustment period was taken as the initial weight. An attempt was made to maintain the subjects' initial weight throughout the study, but there were some fluctuations. At the end of the first experimental period, the mean weight had decreased 1.5 kg but, by the end of the second experimental period, the average weight was only 0.5 kg below the initial weight. The mean maximum deviation from the initial weight was 2.4 percent during the experimental periods.

The mean daily intake of calories, protein, fat, and carbohydrate for each meal of both experimental periods is shown in Table 2. The analysis of nutrients was based on values from Handbook No. 456 (USDA, 1975). A more complete list of the average daily nutrients consumed by each subject appears in Table i of the Appendices. In most cases, the mean nutrient intake of each subject met or exceeded the Recommended Dietary Allowances (National Research Council, 1974). However, the mean iron intake of the four women was 1.3 and 2.5 mg below the recommended allowance of 18 mg during the two-meal and eight-meal periods, respectively. Also, two subjects, TH and CR,

TABLE 1. Description of Subjects and Weights at the End of Each Study Period.

Subject	Sex	Age	Height	Initial Weight		Weight During Study		
				Adjustment Period	Percent Overweight[a]	Two-meal Period	Eight-meal Period	Maximum Deviation[b]
		yr	cm	kg		kg	kg	%
BB	M	22	179	94.1	31.8	92.3	92.0	2.2
DC	M	30	184	100.2	23.2	101.8	102.3	2.0
TC	M	19	182	89.1	26.5	86.8	87.7	2.6
TH	F	18	179	87.5	35.6	86.8	85.9	1.8
DP	F	21	177	85.9	35.0	82.7	85.9	3.7
CR	F	18	177	79.1	26.1	76.8	80.0	2.9
MR	F	19	164	79.1	45.0	77.7	78.0	1.7
Mean		21	177	87.9	31.9	86.4	87.4	2.4
± SD		±4	±6	±7.6	±7.5	±8.7	±8.1	±0.7

[a] Based on average weight for age, height, and sex (Society of Actuaries, 1959).

[b] Deviation from initial weight.

TABLE 2. Mean Daily Dietary Intake During the Two Experimental Periods.

Experimental Period	Meal	Kilocalories	Protein	Fat	Carbohydrate
			gm	gm	gm
Two-meals	1	496	18.7	19.8	62.7
	2	2112	102.6	94.8	221.8
	Total	2608	121.3	114.6	284.5
Eight-meals	1	352	10.5	14.8	45.2
	2	379	18.3	19.9	32.2
	3	348	19.4	17.0	30.4
	4	335	3.4	12.1	55.4
	5	360	17.3	19.4	29.8
	6	301	22.4	9.5	32.9
	7	350	4.8	6.3	71.8
	8	362	15.9	19.8	31.9
	Total	2787	112.0	118.8	329.6

consumed slightly less than the recommended allowance of 1200 mg calcium for their age group (15-18 years). They were, however, almost in the next age group (19-22 years) for which the recommended calcium allowance is 800 mg.

During the two-meal period, the mean daily caloric intake was 2608 Kcal., ranging from 2356 to 2862 Kcal., depending on individual energy requirements. The evening meal provided, on the average, 81 percent of the daily food allotment. The proportion of calories provided by protein, fat, and carbohydrate was approximately 18, 39, and 43 percent, respectively.

In the eight-meal period, the daily food intake ranged from 2474 to 3108 Kcal. with a mean of 2787 Kcal. An increase in the caloric consumption during the second experimental period was necessary to maintain the weights of the subjects equal to their initial weights. Each of the eight meals during this period provided, on the average, 12.5 percent of the daily calories, ranging from 10.8 to 13.6 percent. The approximate portion of daily calories derived from protein, fat, and carbohydrate during the eight-meal period was 16, 38, and 47, respectively. The difference in carbohydrate intake between the two experimental periods would not be expected to appreciably affect the ability of the subjects to clear glucose during a glucose tolerance test (Brunzell et al., 1971).

The composition of dietary fat as measured by the

linoleic: saturated fatty acid ratio was approximately 0.4 during the two-meal period and 0.3 during the eight-meal period. Although an increase in the linoleic to saturated fatty acid ratio has been recommended as a lipid-lowering strategy, this slight change in ratio would probably have a negligible effect (Keys et al., 1965a).

Plasma Lipids at Fasting

The mean fasting concentrations of cholesterol, triglycerides, and nonesterified fatty acids (NEFA), determined at intervals throughout the study, are shown in Table 3. These values fall within the ranges reported for normal adults (Dole, 1956; Henry et al., 1974). The individual lipid values for each fasting plasma sample can be found in Tables ii, iii, and iv of the Appendices.

The plasma cholesterol increased during the two-meal period and returned to initial levels during the eight-meal period. The meal frequency effect was apparent by the twelfth day of each experimental period. A paired comparison between the averages of the two values recorded in each experimental period showed that mean cholesterol was significantly greater ($p < 0.05$) during the two-meal period than during the eight-meal period. That cholesterol concentrations tend to vary inversely with meal frequency is supported by epidemiological studies (Hejda and Fábry, 1964; Fábry et al., 1964), as well as in vivo studies on both obese (Young et al., 1971) and non-obese humans

TABLE 3. Plasma Lipids at Fasting (means ± S. D.).

	Cholesterol	Nonesterified Fatty Acids	Triglycerides
	mg/dl	μEq/L	mg/dl
Initial period	150 ± 27	629[a] ± 134	45 ± 8
Two-meal period			
Day 12	164 ± 25	447 ± 81	59 ± 26
Day 21	161 ± 22	451 ± 215	61 ± 22
Average[b]	162[c] ± 23	449[d] ± 112	60 ± 24
Eight-meal period			
Day 12	148 ± 33	405 ± 150	62 ± 15
Day 21	150 ± 26	395 ± 155	57 ± 15
Average[b]	149 ± 29	400 ± 118	60 ± 12

[a] Significantly greater than values at end of two-meal or eight-meal period ($p < 0.01$).

[b] Average of values determined on Day 12 and Day 21 of each experimental period.

[c] Significantly greater than the average value of the eight-meal period ($p < 0.05$).

[d] Significantly greater than the average value of the eight-meal period ($p < 0.005$).

(Cohn et al., 1963; Gwinup et al., 1963; Jagannathan et al., 1964; Young et al., 1972). The investigations of Wadhwa et al. (1973) and Angel and Schwartz (1975), however, found no relationship between meal frequency and cholesterol concentration in normal weight individuals. Bortz et al. (1966) and Finkelstein and Fryer (1971) reported that plasma cholesterol of obese subjects on hypocaloric diets did not vary with changes in meal frequency. It is possible that the effects of weight loss may have masked any effects due to meal frequency.

Although inter- and intra-subject variability was considerable, excessively high NEFA concentrations that have been reported for obese individuals (Dole, 1956; Opie and Walfish, 1963) were not seen in this study. Analysis of variance showed that the mean NEFA concentration at the end of the adjustment period was significantly higher ($p < 0.01$) than it was at the end of either experimental period. The higher initial NEFA values may have been due to the fact the subjects had not reached caloric balance at the end of the adjustment period. Bortz et al. (1966) also reported an increase in NEFA when obese subjects were given hypocaloric diets. This is justified by the greater physiological need to mobilize oxidizable fatty acids in response to a negative energy balance.

The NEFA concentrations responded differently to the two feeding frequencies. A paired comparison of the average of the two values

obtained for each experimental period showed that the NEFA were significantly higher ($p < 0.005$) during the two-meal than during the eight-meal period. This is contrary to the findings with some non-obese individuals. Neither Wadhwa et al. (1973) nor Angel and Schwartz (1975) found any changes in the fasting levels of NEFA of normal weight subjects that could be attributed to changes in meal frequency. The reason for this discrepancy is not clear but it would appear that the obese person has an exaggerated mobilization of depot fat when on a "gorging" type of feeding pattern.

The low initial plasma triglyceride concentrations may reflect the negative caloric balance during the adjustment period. Fasting triglyceride values were higher during the experimental periods but they did not differ appreciably between the two-meal and eight-meal periods. This is consistent with the findings of other investigators that the fasting levels of triglycerides did not change with changes in meal frequency (Irwin and Feeley, 1967; Young et al., 1972; Wadhwa et al., 1973). Young et al. (1971) found increased fasting triglyceride concentrations associated with decreased meal frequency in obese subjects on a hypocaloric diet, but the results were not significant by paired comparison.

It has been suggested that increased levels of circulating NEFA may initiate the mechanisms for increased hepatic synthesis of cholesterol and triglycerides (Nestel and Whyte, 1968; Havel et al.,

1970; Bortz, 1974). The results of our study support this, in that both NEFA and the cholesterol concentrations were greater during the two-meal than during the eight-meal period. Triglycerides, on the other hand, were not appreciably affected by the increased levels of NEFA, suggesting that other mechanisms, unaffected by meal frequency, are dominant in maintaining the concentration of triglycerides in the fasting state.

Glucose Tolerance Test

The mean concentrations of glucose, nonesterified fatty acid (NEFA) and triglycerides during the glucose tolerance test (GTT) given at the end of each dietary period are presented in Table 4. A more complete list of individual values during the glucose tolerance tests appears in Tables v, vi, and vii of the Appendices. The mean concentrations of each constituent as changes from fasting levels (Time zero) are also shown in Table 4 and depicted in Figure 1.

The concentration of plasma glucose throughout each GTT was within the normal range (Berkow, 1977). In all three GTT, plasma glucose reached a peak concentration at 40 minutes and decreased to near fasting levels within 120 minutes. This characteristic pattern of response depicts the sudden influx of glucose into the plasma which is followed by a decrease as the glucose is taken up by peripheral tissues. The maximum rise of glucose above fasting levels appeared

TABLE 4. Glucose, Nonesterified Fatty Acids (NEFA), and Triglyceride Concentrations of Plasma During Glucose Tolerance Tests (Means ± S. D.).

Period and Time	Glucose		NEFA		Triglycerides	
	Concentration	Change from Fasting	Concentration	Change from Fasting	Concentration	Change from Fasting
	mg/dl	mg/dl	μEq/L	μEq/L	mg/dl	mg/dl
Adjustment						
0'	100 ± 6		629 ± 134		45 ± 8	
40'	120 ± 28	20.0 ± 30.0	283 ± 106	-346 ± 163	44 ± 12	- 1.5 ± 4.8
80'	100 ± 31	- 0.3 ± 31.0	178 ± 64	-452[a] ± 115	36 ± 7	- 9.1 ± 5.6
120'	95 ± 27	- 4.4 ± 26.5	193 ± 85	-436[a] ± 150	38 ± 11	- 6.9 ± 6.5
180'	96 ± 9	- 3.4 ± 12.2	472 ± 134	-186 ± 111	39 ± 9	- 6.4 ± 5.5
240'	112 ± 6	13.3[b] ± 8.6	793 ± 90	147 ± 175	42 ± 13	- 4.7 ± 7.5
Two-meal						
0'	106 ± 8		451 ± 215		61 ± 22	
40'	168 ± 34	61.7 ± 33.5	263 ± 110	-188 ± 218	61 ± 20	0.1 ± 5.6
80'	114 ± 21	8.0 ± 21.9	186 ± 32	-265 ± 205	55 ± 24	- 5.9 ± 5.0
120'	89 ± 20	-16.9 ± 24.2	181 ± 15	-270 ± 216	50 ± 23	-10.8 ± 5.5
180'	102 ± 7	- 3.7 ± 13.5	354 ± 165	- 97 ± 191	45 ± 19	-15.7 ± 12.8
240'	102 ± 8	- 4.1 ± 12.2	540 ± 215	71 ± 239	50 ± 21	-10.3 ± 15.1
Eight-meal						
0'	100 ± 6		395 ± 155		57 ± 15	
40'	144 ± 36	44.1 ± 35.5	151 ± 41	-244 ± 129	63 ± 22	6.8 ± 12.1
80'	114 ± 9	14.0 ± 12.3	120 ± 42	-274 ± 159	60 ± 21	3.8[c] ± 9.3
120'	87 ± 13	-13.0 ± 15.6	148 ± 62	-246 ± 174	53 ± 18	- 3.9 ± 9.5
180'	100 ± 10	0.0 ± 9.6	305 ± 42	- 88 ± 151	48 ± 17	- 8.9 ± 5.1
240'	96 ± 6	- 1.9 ± 7.9	518 ± 154	124 ± 187	56 ± 16	- 0.8 ± 8.1

[a] Significantly less than values for two-meal or eight-meal period ($p < 0.05$).

[b] Significantly greater than values for two-meal or eight-meal period ($p < 0.005$).

[c] Significantly greater than values for adjustment or two-meal period ($p < 0.01$).

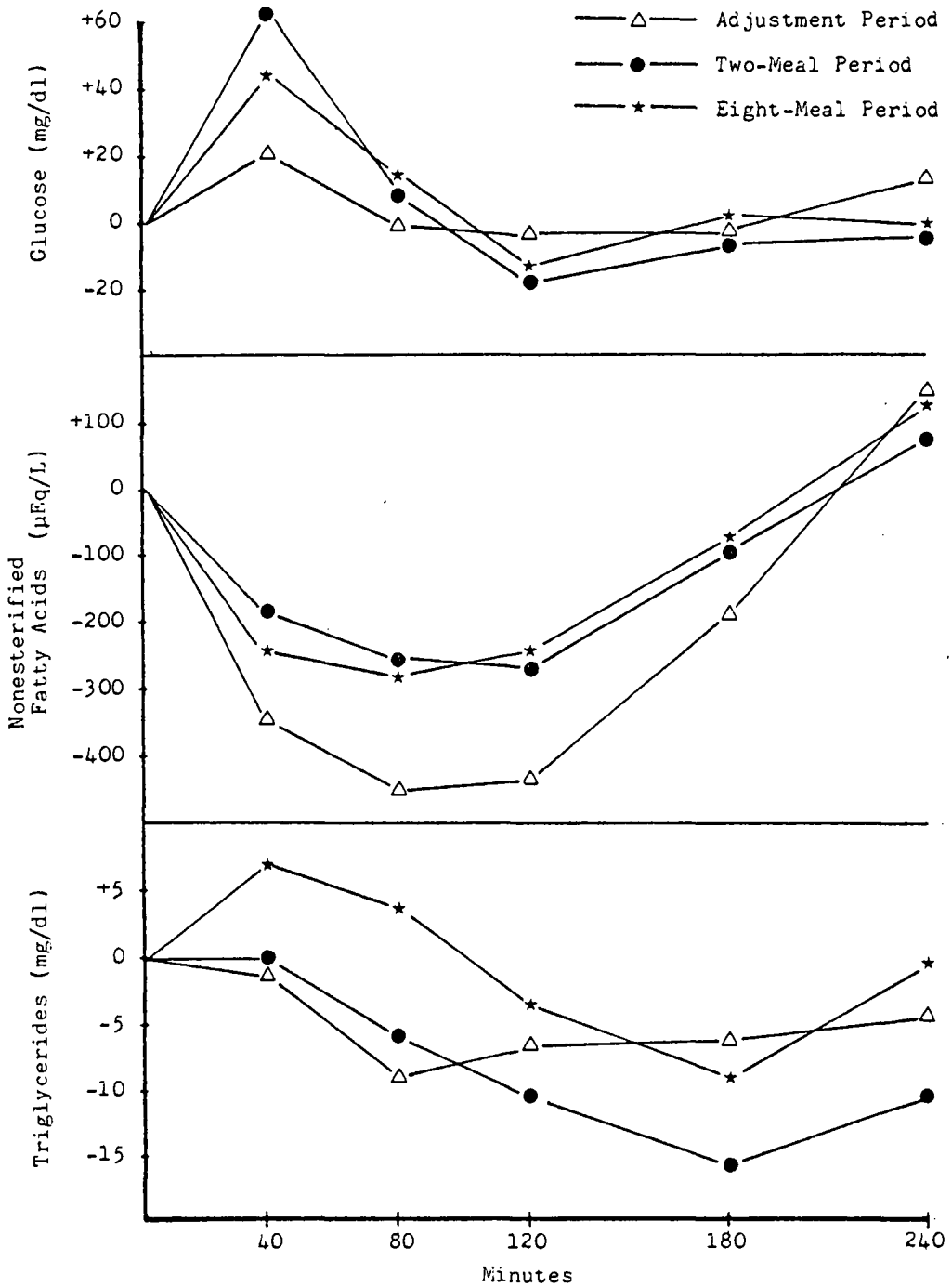

FIGURE 1. Plasma Values During Glucose Tolerance Tests as Changes From Fasting Levels.

to be slightly exaggerated in the GTT following the two-meal period as compared to either the adjustment or the eight-meal period. However, due to inter-subject variability, no significant differences could be demonstrated. That glucose tolerance is diminished with decreasing meal frequency is supported by a number of investigations (Nunes and Canham, 1963; Fábry et al., 1964; Young et al., 1971, 1972; Pringle et al., 1976).

The concentration of plasma NEFA during the three GTTs were within the normal range and followed a characteristic pattern (Shafrir et al., 1965). In all three GTTs, the concentration of NEFA decreased to a minimum level 80 to 120 minutes following glucose ingestion. The concentration then increased, reaching levels above fasting at 240 minutes. The mean decrease from fasting levels was significantly greater in the GTT following the adjustment period as compared to the two experimental periods at both the 80 and 120 minute blood drawings ($p < 0.05$, $p < 0.05$). The relevance of this is not certain, but may be due to the higher level of NEFA at fasting in this period. Meal frequency produced no significant differences in the response of NEFA to a glucose load as measured by the changes from fasting. Wadhwa et al. (1973) similarily found no effect of meal frequency on the response of NEFA to glucose ingestion. These results indicate that although a decrease in meal frequency may impair the utilization of glucose, the anti-lipolytic action of insulin is

unaltered. Hanley et al. (1967) found the same to be true when they compared the response of NEFA during a GTT in a group of subjects ranging from lean to obese. Although obese subjects tend to have decreased glucose tolerance (Butterfield et al., 1965) associated with insulin resistance (Karam et al., 1968; Rabinowitz, 1970), they found no abnormalities in the decrease of NEFA following a glucose load in the obese.

Randle et al. (1965) and Flatt and Blackburn (1974) have suggested that the glucose intolerance observed in obesity may be due, at least partially, to the elevated levels of NEFA commonly found in this condition. Key glycolytic enzymes appear to be inhibited by fatty acids (Randle et al., 1963; Lea and Weber, 1968). In light of this, it may be pertinent that there was higher overall concentration of NEFA during the GTT following the two-meal, as compared with the eight-meal period. The slightly higher concentration of NEFA may have partially inhibited the peripheral metabolism of glucose thereby decreasing the ability to clear glucose from the plasma.

The response of the plasma triglyceride concentration to a glucose load followed a somewhat similar pattern as reported by Wadhwa et al. (1973). The increase in triglycerides during the first hour after glucose ingestion as reported by Wadhwa et al. (1973) was not generally observed except for slight increases during the GTT following the eight-meal period. On the whole, there was not much

difference in the triglyceride response in the dietary periods. Statistical analysis of the triglyceride levels as net change from fasting levels indicated that the concentration at the 80 minute blood drawing of the GTT following the eight-meal period was significantly greater than it was during the other two GTT. This does not support the findings of Wadhwa et al. (1973) who reported a greater increase in triglyceride concentration after glucose ingestion in subjects consuming a "gorging" type diet. The results from our study indicate an initial increase in triglyceride concentrations during a GTT, and a tendency for the elevated levels to persist up to 80 minutes when food is consumed in frequent feeding. Although the changes in concentration were slight, they were not observed in the GTT following the adjustment or two-meal period.

SUMMARY

The effect of meal frequency on parameters of lipid metabolism was studied in seven moderately obese university students on a weight maintenance program. Following a short adjustment period were two experimental periods, each 21 days in length. In the first experimental period, the subjects consumed approximately 20 percent of the daily caloric intake in a morning meal, and 80 percent in an evening meal. During the second experimental period, the food was divided into eight approximately isocaloric meals per day. Similar amounts and types of nutrients were provided to the subjects throughout the study. The mean weights of the subjects did not vary more than 2.4 percent from their initial weights.

Fasting blood samples were drawn at intervals throughout the study and analyzed for cholesterol, nonesterified fatty acid, and triglyceride concentrations. Paired comparisons between the averages of two fasting samples from each experimental period showed that both cholesterol and nonesterified fatty acids were significantly higher during the two-meal than during the eight-meal period. The fasting concentration of nonesterified fatty acids was significantly higher at the end of the adjustment period than at the end of either the two-meal or eight-meal period. This was probably due to a negative caloric balance observed in the adjustment period. The level of

triglycerides in the fasting state did not change significantly throughout the study. Glucose tolerance tests were given to the subjects at the end of each of the three dietary periods and the plasma concentration of glucose, nonesterified fatty acids, and triglycerides was determined. The increase in plasma glucose concentration, as measured by the change from fasting levels, was exaggerated in the glucose tolerance test following the two-meal period, but the results were not significant. The changes in concentration of nonesterified fatty acids and triglycerides from fasting levels following glucose ingestion were not significantly different between experimental periods.

The results indicate that certain metabolic processes are altered in obese persons when most of the daily food is consumed during one meal. That the obese individual tends to consume food in this manner on a daily basis suggests that some of the metabolic abnormalities seen in obesity may be associated with a decreased frequency of food intake.

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APPENDICES

Table i. Average daily nutrient intake of subjects during the two experimental periods.

Subject	Kilocal.	Protein	Fat	Carbohydrate	Calcium	Iron	Vit. A	Thiamin	Riboflavin	Niacin	Vit. C
		gm	gm	gm	mg	mg	IU	mg	mg	mg	mg
Two-meal											
BB	2862	131.8	130.4	303.0	1548	17.5	6246	1.83	2.56	32.0	153
DC	2758	129.4	123.7	293.0	1495	17.2	6149	1.82	2.52	26.2	153
TC	2675	123.7	118.8	289.2	1308	17.2	5799	1.79	2.28	25.9	150
TH	2524	118.0	109.8	277.4	1141	16.7	5525	1.74	2.08	25.6	149
DP	2577	118.9	112.3	284.1	1152	17.1	5580	1.77	2.10	25.9	149
CR	2356	110.7	98.5	266.5	1044	16.1	5394	1.68	1.98	23.0	149
MR	2505	116.9	108.7	277.1	1105	16.7	5482	1.73	2.03	25.6	149
Mean	2608	121.3	114.6	284.5	1255	16.9	5739	1.77	2.22	26.3	150
± SD	170	7.4	10.6	12.1	199	0.5	338	0.05	0.24	2.7	2
Eight-meal											
BB	3108	127.1	130.0	371.2	1852	19.7	10820	1.94	2.91	24.0	248
DC	2865	112.3	119.4	348.6	1673	17.5	9866	1.76	2.74	20.6	241
TC	2985	123.2	122.1	361.4	1702	18.9	9871	1.88	2.83	23.0	241
TH	2673	106.5	117.4	309.4	1610	15.7	9596	1.54	2.64	18.7	196
DP	2730	107.3	117.6	323.2	1622	16.0	9866	1.63	2.66	19.2	241
CR	2474	100.3	108.3	284.7	1562	14.7	8436	1.48	2.55	17.4	193
MR	2673	106.5	117.4	309.4	1610	15.7	9596	1.54	2.64	18.7	196
Mean	2787	120.0	118.8	329.6	1662	16.9	9722	1.68	2.71	20.2	222
± SD	214	9.8	6.5	31.6	96	1.9	702	0.18	0.12	3.4	25

Table ii. Individual plasma cholesterol concentrations at fasting (mg/dl).

	Subjects								
	BB	DC	TC	TH	DP	CR	MR	Mean	± SD
Adjustment Period	144	109	143	193	172	134	155	150	27
Two-meal Period									
Day 12	148	166	167	160	215	138	151	164	25
Day 21	147	166	155	169	203	139	145	161	22
Eight-meal Period									
Day 12	130	140	128	149	217	156	115	148	33
Day 21	128	170	140	130	201	147	135	150	26

Table iii. Individual plasma nonesterified fatty acid (NEFA) concentrations at fasting (μEq/L).

	Subjects								
	BB	DC	TC	TH	DP	CR	MR	Mean	± SD
Adjustment Period	562	649	728	856	629	453	528	629	134
Two-meal Period									
Day 12	475	504	524	344	360	536	387	447	81
Day 21	419	289	823	674	345	243	363	451	215
Eight-meal Period									
Day 12	239	404	658	552	309	299	371	405	150
Day 21	625	243	535	471	260	388	241	395	155

Table iv. Individual plasma triglyceride concentrations at fasting (mg/dl).

	Subjects								
	BB	DC	TC	TH	DP	CR	MR	Mean	± SD
Adjustment Period	42	56	56	38	41	44	39	45	8
Two-meal Period									
Day 12	44	108	50	63	37	76	37	59	26
Day 21	45	90	50	74	35	86	44	61	22
Eight-meal Period									
Day 12	53	80	59	48	47	67	83	62	15
Day 21	35	74	60	74	45	48	60	57	15

Table v. Individual glucose concentrations during glucose tolerance tests (mg/dl).

Period and Time	Subjects BB	DC	TC	TH	DP	CR	MR	Mean ± SD
Adjustment								
0'	102	96	102	102	95	92	108	100 ± 6
40'	110	96	105	89	165	144	128	120 ± 28
80'	65	82	81	78	120	119	150	100 ± 31
120'	108	76	101	116	47	126	92	95 ± 27
180'	80	100	102	102	88	107	94	96 ± 9
240'	114	120	108	107	104	116	--	112 ± 6
Two-meal								
0'	94	96	110	104	114	110	115	106 ± 8
40'	175	141	163	192	210	108	186	168 ± 34
80'	124	98	73	120	126	134	124	114 ± 21
120'	91	98	105	86	48	110	87	89 ± 20
180'	102	116	102	100	95	102	100	102 ± 7
240'	104	99	102	102	89	116	102	102 ± 8
Eight-meal								
0'	95	97	99	105	108	92	102	100 ± 6
40'	134	102	150	100	196	176	149	144 ± 36
80'	104	132	112	113	107	118	110	114 ± 9
120'	87	79	76	90	70	99	106	87 ± 13
180'	91	117	102	104	103	88	93	100 ± 10
240'	93	102	106	104	92	94	94	96 ± 6

Table vi. Individual nonesterified fatty acid (NEFA) concentrations during glucose tolerance tests (μEq/L).

Period and Time	Subjects BB	DC	TC	TH	DP	CR	MR	Mean ± SD
Adjustment								
0'	562	649	728	856	629	453	528	629 ± 134
40'	158	399	439	211	213	322	239	283 ± 106
80'	195	161	204	278	99	207	100	178 ± 64
120'	216	356	165	143	237	119	118	193 ± 85
180'	235	494	444	634	552	--	475	472 ± 134
240'	891	759	636	859	817	797	--	793 ± 90
Two-meal								
0'	419	289	823	674	345	243	363	451 ± 215
40'	318	200	315	240	461	158	151	263 ± 110
80'	233	191	182	215	184	135	164	186 ± 32
120'	189	198	194	162	160	188	173	181 ± 15
180'	200	402	597	324	212	201	539	354 ± 165
240'	657	351	598	602	--	220	810	540 ± 215
Eight-meal								
0'	625	243	535	471	260	388	241	395 ± 155
40'	219	164	183	143	133	108	107	151 ± 41
80'	124	197	136	127	111	75	73	120 ± 42
120'	119	165	140	198	251	92	72	148 ± 62
180'	354	242	278	315	326	272	348	305 ± 42
240'	658	371	524	501	346	451	777	518 ± 154

Table vii. Individual triglyceride concentrations during glucose tolerance tests (mg/dl).

Period and Time	Subjects BB	DC	TC	TH	DP	CR	MR	Mean ± SD
Adjustment								
0'	42	56	56	38	41	44	39	45 ± 8
40'	35	58	61	30	41	40	40	44 ± 12
80'	32	43	42	26	29	43	38	36 ± 7
120'	31	59	42	32	28	43	34	38 ± 11
180'	32	58	41	36	34	36	35	39 ± 9
240'	32	66	46	35	37	35	--	42 ± 13
Two-meal								
0'	45	90	50	74	35	86	44	61 ± 22
40'	48	94	49	74	41	75	44	61 ± 20
80'	37	93	43	69	31	73	36	55 ± 24
120'	30	86	36	66	30	68	33	50 ± 23
180'	30	78	38	64	27	42	37	45 ± 19
240'	29	83	51	75	28	44	42	50 ± 21
Eight-meal								
0'	35	74	60	74	44	48	60	57 ± 15
40'	32	105	58	72	50	61	65	63 ± 22
80'	27	95	63	73	44	59	61	60 ± 21
120'	22	72	49	68	36	64	58	53 ± 18
180'	19	63	46	70	42	42	53	48 ± 17
240'	29	64	52	77	58	47	64	56 ± 16