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Title: EFFECT OF SOURCE OF DIETARY CARBOHYDRATE ON PLASMA LIPIDS OF WOMEN

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The effect of source of dietary carbohydrate on the plasma lipid concentrations and glucose tolerance were studied. Three women received diets which contained 16%, 40%, and 44% of calories as protein, fat, and carbohydrate, respectively. During the control period of six days the carbohydrate was derived from mixed sources. In subsequent six-day periods, 80% of the carbohydrate was supplied alternately by sucrose (Sugar Diet) or by polysaccharides from natural sources (Complex Diet).

Total lipids, phospholipids, triglycerides, total and free cholesterol of the plasma were determined on the final day of each dietary period. The plasma concentrations of total lipids, phospholipids, and triglycerides were lowest following the Complex Diet. On the average, the Sugar Diet produced the same concentrations of these lipid fractions as did the Control Diet. No consistent changes in cholesterol could be attributed to dietary carbohydrate.

Glucose tolerance tests were performed at the end of each dietary period. In the two young subjects, glucose tolerance did not seem to be associated with the dietary treatment. The older subject showed impaired glucose tolerance after the diet containing complex carbohydrate.

# Effect of Source of Dietary Carbohydrate on Plasma Lipids of Women

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TABLE OF CONTENTS

## LIST OF TABLES



## LIST OF FIGURES

# LIST OF APPENDIX TABLES

# EFFECT OF SOURCE OF DIETARY CARBOHYDRATE ON PLASMA LIPIDS OF WOMEN

## INTRODUCTION

The various factors which influence the amounts and distribution of plasma lipids have been of intense interest because of the epidemiological association of hyperlipemia with degenerative heart disease. In recent years, it has become apparent that the amount and form of dietary carbohydrate may influence lipid patterns of the plasma. In general, long-term diets containing large amounts of sucrose, as contrasted to polysaccharides, resulted in high levels of plasma triglycerides of susceptible individuals (Kuo and Bassett, 1965). In the various studies of normal subjects, however, the lipid response to shifts in dietary carbohydrate has not been uniform. Several of the experimental regimens have included excessively large amounts of carbohydrate in forms which are rarely consumed by the average individual (Macdonald and Braithwaite, 1964). The lipid response also appears to be conditioned by the age and sex of the subject (Macdonald, 1966a).

It has also been shown that the efficiency of carbohydrate utilization, as measured by the glucose tolerance test, may be altered by the type of carbohydrate which has been consumed. After a high sucrose regimen the glucose tolerance was lowered (Cohen, 1967).

The present study was designed to provide a diet of natural

foods proportioned according to the typical American pattern. The effects of short term interchanges of sucrose and polysaccharides on the plasma lipid patterns and the glucose tolerance were assessed.

# REVIEW OF LITERATURE

## Plasma Lipids

### Lipids in the Plasma of Healthy Individuals

The main lipid components in the plasma are cholesterol, phospholipids, triglycerides, and free fatty acids. The plasma concentrations of these lipid components vary widely among healthy individuals. A daily spontaneous variation in the lipid concentration has also been observed in the same subject, but it tended to vary about a characteristic mean for that individual (Man and Gildea, 1937; Cromie et al., 1963).

Some representative data on plasma lipids of healthy men and women in the postabsorptive state appear in Table 1. The total lipids of plasma may range from 500 to 1000 mg/100 ml (Page et al., 1935). Most mean values of total lipids lie between 530 and 650 mg/100 ml of serum or plasma. Data on total lipids are comparable among laboratories because they are based on the dry weight of the lipid extract. Cholesterol values, on the other hand, may vary depending on the method of analysis. Adlersberg et al. (1956) observed a wide range of total cholesterol concentrations in the serum of healthy people--from 180 to 280 mg/100 ml. Although the concentration of total cholesterol varies among individuals, free cholesterol constitutes

Table 1. Lipids in plasma and serum of healthy individuals in postabsorptive state.

| Reference | Subjects | | | Sample | Total lipid | Cholesterol | | Phospholipid | Triglyceride | Non-esterified fatty acids |
|---|---|---|---|---|---|---|---|---|---|---|
| | Number | Sex | Age | | | Total | Free | | | |
| | | | | | mg/100 ml | mg/100 ml | mg/100 ml | mg/100 ml | mg/100 ml | mEq/L |
| Boyd, 1933 | 8 | F | 20-38 | Plasma | 589 | 162 | 47 | 195 | 153[a] | |
| Page et al., 1935 | 66 | M | 20-90 | Plasma | 519-951 | 170-294 | 65-99 | 110-252 | 88-362[a] | |
| Dole, 1956 | | | | Plasma | | | | | | 0.50-0.90 |
| Van Handel and Zilversmit, 1957 | 14 | M | Adult | Plasma | | | | | 79 (37-134) | |
| Hallgren et al., 1960 | 8 | M | Adult | Plasma | 588 | 224 | 69 | 197 | 76 | 19.0[b] |
| Lund, Sivertssen, and Godal, 1961 | 24 | F | 21-30 | Serum | 528 | 222 | | | | |
| | 30 | F | 31-40 | Serum | 505 | 227 | | | | |
| | 25 | F | 41-50 | Serum | 615 | 235 | | | | |
| | 22 | F | 51-60 | Serum | 649 | 273 | | | | |
| Svanborg and Svennerholm, 1961 | 62 | M | 16-35 | Plasma | 609 | 191 | 64 | 207 | 83 | 0.75 |
| | 29 | F | 16-35 | Plasma | 648 | 185 | 57 | 232 | 88 | 0.78 |
| Hallberg et al., 1966 | 39 | F | 23 | Plasma | | 219 | | 231 | 71 | |
| | 41 | F | 30 | Plasma | | 236 | | 248 | 70 | |
| | 33 | F | 40 | Plasma | | 260 | | 275 | 99 | |
| | 40 | F | 45 | Plasma | | 282 | | 276 | 89 | |
| | 83 | F | 50 | Plasma | | 309 | | 296 | 95 | |
| | 40 | F | 60 | Plasma | | 304 | | 291 | 120 | |
| Macdonald, 1967a | 5 | M | 21-28 | Serum | | 228 | 57 | 269 | 78 | |

[a] Estimated by difference.
[b] mg/100 ml.

25-30% of the total cholesterol in both sexes and in all age groups (Bloor, 1916; Adlersberg et al., 1956; Lund, Sivertssen and Godal, 1961). The range of phospholipids, as determined by Page et al. (1935), was 110 to 250 mg/100 ml.

The triglyceride content of plasma, reported by Boyd (1933) and Page et al. (1935), was 153.7 and 88-362 mg/100 ml, respectively. These values were obtained as the difference between total lipids and the other lipid components. The chemical method, used by Van Handel and Zilversmit (1957), Hallgren et al. (1960), Svanborg and Svennerholm (1961), and Macdonald (1967a), was based on the specific determination of glycerides in the lipid extract from which phospholipids had been removed. Concentrations of triglycerides, determined chemically, were considerably lower than those which were estimated by difference.

The concentration of non-esterified fatty acids, as reported by Dole (1956), was 0.5 - 0.9 mEq/L. The values reported for this lipid fraction are also dependent on the analytical method.

An inspection of Table 1 shows that slightly more than one-third of the plasma lipid is cholesterol ester and about one-tenth is free cholesterol. Phospholipid constitutes about one-third and triglyceride about one-fifth of the total lipid. Free fatty acids account for only 2-3% of total lipids (Luddy, Barford and Riemenschneider, 1958; Hallgren et al., 1960).

## Factors Affecting Plasma Lipid Concentrations

The plasma lipids may be modified by a variety of factors, such as age, sex, hormonal status, previous diet, body weight, race, heredity, environment, and some diseases. Among healthy subjects, the most noteworthy factors are age, sex, and diet.

Influence of age and sex on plasma lipids.- The effects of age and sex on lipid concentrations in plasma have been investigated extensively. The values are generally stated to be slightly higher in females than in males, but the difference is small (Kornerup, 1950). In both sexes, there is a tendency toward higher plasma lipid values in elderly than in young persons (Kornerup, 1950). Lund et al. (1961) reported that the total lipid content of serum tended to increase with age, reaching a maximum at about the age of 60, with no sex difference. Adlersberg et al. (1956) surveyed a group of healthy men and women. They reported that both the total cholesterol and phospholipid levels of the males remained constant from age 2 through 19, increased from age 20 through 33, and then remained constant to age 60. The cholesterol and phospholipids in the plasma of females stayed practically constant from age 2 through 32, then increased sharply through age 58. Keys et al. (1950a) stated that men of middle to old age had the highest serum cholesterol concentrations, younger men had the lowest values; serum cholesterol concentrations of very old

men were between those of the other two age groups. In a study of 360 females, reported by Hallberg et al. (1966), the cholesterol and phospholipid levels showed a continuous increase with age up to 60 years. Feldman, Benkel, and Nayak (1963) observed a highly significant increase in mean cholesterol and triglyceride levels of females over age 35. In men, the triglyceride level showed a continuous increase with age up to 46-55 years (Carlson, 1960), while in women the increase was more stepwise (Hallberg, et al., 1966). The latter investigators found that the triglyceride concentration was the same in 15, 23 and 30-year old women; it increased by 20% in the age group of 40-50 years. The triglyceride concentrations in plasma of 60 to 70 year old women were even higher. Free fatty acid concentrations did not vary with age (Feldman et al., 1963).

In females, the level of plasma cholesterol, phospholipid and triglyceride increased after menopause (Hallberg et al., 1967; Hallberg and Svanborg, 1967). Fluctuations in plasma lipids have also been observed during the female menstrual cycle. Oliver and Boyd (1953) found that there was a striking fall in plasma cholesterol ester and a less marked fall in phospholipid during ovulation, when estrogenic activity was maximal, and also immediately prior to menstruation. Okey and Boyden (1927) observed a decrease in plasma cholesterol during or within a few days of menstruation. No similar changes could be consistently demonstrated in the levels of fatty acids

and phospholipids. Svanborg and Vikrot (1967) could find no definite effect of the menstrual cycle upon any of the plasma lipid concentrations.

Although the estrogenic hormones have repeatedly been shown to have a hypocholesteremic effect (Boyd, 1962), the influence of progesterone on lipid metabolism is less well defined. Synthetic hormones, which mimic the combined effects of estrogen and progesterone, are in widespread use as oral contraceptives. Several investigators (Aurell, Cramér and Rybo, 1966; Wynn, Doar and Mills, 1966) have noted significant increases in serum concentrations of cholesterol, phospholipids and triglycerides of women who were receiving oral contraceptives. Elevated serum cholesterol is also noted in hypothroidism. Miettinen (1968) reported that the rapid reduction in serum cholesterol following administration of thyroid hormone to hypothyroid patients was due to enhanced catabolism of the sterol. Goolden, Gartside and Sanderson (1967) have shown that thyroid status in women was not changed by the use of oral contraceptives.

Influence of diet on plasma lipids. - The total energy value of a diet can affect the plasma lipid concentration. The postabsorptive free fatty acids of plasma were much greater in subjects on a low caloric regimen than in those on maintenance calories (Fairhurst and Waterhouse, 1963; Stormont and Waterhouse, 1963). On the other

hand, an excessive caloric intake, manifested by weight gain, may lead to an elevation in plasma triglycerides. Feldman et al. (1963), as well as Hallberg and Svanborg (1967) observed significantly higher triglycerides in the plasma of weight-gaining women than in plasma of weight-stable women. This effect of weight gain was also noted in men (Albrink, Meigs and Granoff, 1962). In females, neither cholesterol nor phospholipid concentrations appeared to be affected by weight gain (Hallberg and Svanborg, 1967). Albrink et al. (1962) reported a small increase in plasma cholesterol of males following weight gain.

The plasma lipid concentration is altered by the amount and type of dietary fat. The free fatty acids and cholesterol ester were elevated in the plasma of subjects who consumed 75% of their calories as fat (Stormont and Waterhouse, 1963). On this high fat diet triglyceride concentrations decreased. Diets high in saturated fatty acids, as in animal fats, tended to increase serum lipid concentrations (Ahrens et al., 1957). On the other hand, a diet rich in polyunsaturated fatty acids, as in corn oil, lowered the plasma concentration of triglycerides (Beveridge, Jagannathan and Connell, 1964), total cholesterol and phospholipids (Macdonald, 1967b). Kuo and Carson (1959) also observed reduced plasma lipids in response to the high polyunsaturated fatty acid intake.

In 1950, Keys et al. (1950b) found that the serum cholesterol

level was not significantly related to the cholesterol intake over a wide range (250-800 mg per day). If the dietary cholesterol level fell below a certain critical point, the serum cholesterol showed a marked decline. However, in 1965, Hegsted et al. observed a linear relationship between serum cholesterol concentrations and dietary cholesterol when the latter was varied between 200 and 700 mg/day.

The quantity of dietary protein was found to have no significant effect on plasma cholesterol concentrations in man (Keys and Anderson, 1957; Beveridge, Connell and Robinson, 1963). Hodges et al. (1967) reported that serum cholesterol concentrations were lower when vegetable protein was substituted for animal protein in the diet. The hypocholesteremic effect may have been due to the absence of cholesterol in plant sources of protein.

Diets of high carbohydrate content have been known to cause changes in plasma lipid concentrations. A diet in which 75% of the calories was provided by Dexin (a partially hydrolyzed starch) and fruit juice was found to increase fasting plasma triglycerides and decrease free fatty acids and cholesterol-esters of healthy individuals (Stormont and Waterhouse, 1963). A rice-rich diet gave a low serum phospholipid value (Kuo and Carson, 1959). Further studies by Waterhouse, Kemperman and Stormont (1964) showed that a high-carbohydrate regimen seemed to increase the rate of incorporation of free fatty acids into the plasma triglycerides. It was suggested that

this might be the factor which was responsible for the increased pool size of plasma triglycerides after high-carbohydrate feeding.

Groen (1967) noted that, when bread was substituted isocalorically for saturated fats in the diet, the serum cholesterol was decreased to as low a level as was reached by the use of polyunsaturated fats. The hypocholesteremic response occurred to a lesser degree when sucrose was substituted for saturated fat. Anderson (1967) also studied the effects of replacing various dietary fats with sucrose. In all cases, the sucrose feeding resulted in increased triglyceride concentrations.

Keys, Anderson and Grande (1960) compared the different effects of the American type diet with those of the Italian type diet in man. The former diet was relatively high in sucrose, skim milk, and meat; the latter diet contained large amounts of bread, fruit, vegetable, and leguminous seeds. Two levels of fat were used: 16% and 31% of total caloric intake. Regardless of the level of fat in the diet, the American type diet always produced a higher cholesterol and phospholipid response in the serum. Triglyceride concentrations were almost identical on the two kinds of diet. It was concluded that sucrose and milk sugar tended to produce higher serum cholesterol values than did equal calories of carbohydrate supplied as fruits, vegetable, and legumes.

The isocaloric replacement of dietary sucrose with complex

carbohydrate from cereal, vegetable, or leguminous sources has produced only small reductions in serum cholesterol when the exchange has involved less than 25% of the daily calories (Irwin, Taylor and Feeley, 1964; Grande, Anderson and Keys, 1965).

Another study of the effect of dietary carbohydrates on young men and women was reported by Antar and Ohlson (1965). They fed four males and four females, aged 20 to 25, a diet of 2500 Cal per day. Fat, protein and carbohydrate supplied 40%, 16%, and 44% of total calories respectively. Eighty percent of the carbohydrate was in the form of either simple sugar or starch. The duration of each diet was four weeks. The authors reported that total serum lipid, phospholipids and nonphospholipids all increased after the high sugar diet and decreased with the complex carbohydrate diet. The changes were less marked in women than in men. Of the serum lipids, the nonphospholipids showed the greatest increase following the sugar diet, the rise in total lipid was moderate, and the phospholipids increased least. These results suggested the lipemic effect of simple sugars.

A recent study by Hodges et al. (1967) showed that an interchange of dietary starch and sugar did not influence cholesterol or non-esterified fatty acid concentrations. Serum triglycerides, on the other hand, were very responsive to the source of carbohydrate; the mean triglycerides rose from 133 mg/100 ml on the starch diet

to 208 mg/100 ml on the sugar diet and declined to 88 mg/100 ml when the starch diet was resumed.

Macdonald and his coworkers have done a series of studies on groups of 5 to 7 normal men and women (Macdonald and Braithwaite, 1964; Macdonald, 1965; Macdonald, 1966a). These experiments involved a very drastic dietary exchange of carbohydrate, equivalent to 73% of the caloric intake and achieved by feeding 450 to 500 gm daily of raw maize starch or sucrose. There was practically no fat. The length of the dietary period was 25 days. The age of each group was 21 to 25, 54 to 68 and 21 to 41, respectively, for young women, old women, and men. The results of their studies were summarized in Table 2 (Macdonald and Braithwaite, 1964; Macdonald, 1965; Macdonald, 1966a).

Table 2. Changes in the concentration of serum lipid and its fractions on a diet low in fat and high in starch or sucrose as compared to the free choice diet.[a]

| Serum Lipid Fraction | Starch diet | | | Sucrose diet | | |
|---|---|---|---|---|---|---|
| | Male | Young Female | Old Female | Male | Young Female | Old Female |
| Total lipid | - | - | - | + | - | - |
| Sterol ester | - | - | - | 0 | 0 | - |
| Glyceride, free sterol and free fatty acids | 0 | 0 | + | + | - | + |
| Phospholipid | - | - | - | 0 | - | - |
| Cholesterol | - | - | - | + | - | - |

[a] 0: no change, +: increase, -: decrease.

The most interesting aspect of this work is the influence of sex on the effect of dietary carbohydrate. In this low fat diet containing a relative excess of sucrose, there were increased concentrations of free cholesterol and glycerides in men and old women, but decreased levels in young women. These findings are compatible with the greater incidence of coronary-artery disease in men and old women in the general population. When the sucrose in the diet was replaced by raw corn starch, the serum lipid concentration decreased in both sexes. The serum cholesterol changes in these experiments (58 mg% in men and 30 mg% in old women) were greater than those reported by others. This indicated that the changes of serum cholesterol concentration may be magnified by exaggerated dietary exchanges (Grande, 1967).

In a study of hyperglyceridemic and hypercholesteremic patients, Kuo and Bassett (1965) reported that when carbohydrate intake, which was about 45% of total calories, was supplied mainly by starch, all the plasma lipid concentrations, especially triglycerides, decreased. The same authors noted that sucrose aggravated hyperlipidemia in hyperglyceridemic patients and induced hyperglyceridemia in hypercholesteremic subjects. These data again indicated that sucrose could be a potent lipemic agent.

Anderson et al. (1963) observed that the isocaloric interchange of glucose, sucrose, and lactose, at approximate 30% of the total

calories, gave no significant change in serum cholesterol or phospholipids; an increase of triglycerides occurred when glucose was replaced by sucrose.

It has been noted that lipid changes are particularly marked when excessive carbohydrate is used in the diet. Wintz, Graff and Seedman (1964) reported that when men were fed a "synthetic diet" in which glucose provided 90% of daily calories, serum cholesterol decreased significantly. If 25% of the glucose was replaced by sucrose there was a marked elevation in serum cholesterol.

In 1966, Macdonald (1966b) studied the effects of fructose and glucose on the serum lipids of men, premenopausal and post-menopausal women, on a fat-free diet in the presence of starch. Dietary glucose, when compared with dietary fructose or starch, elevated the fasting phospholipids in men, but lowered them in women. The author postulated that androgen may have modified the glucose effect. In the same study, no significant difference in response to fructose or glucose was found in serum cholesterol. Serum cholesterol showed greater response to the changes of dietary fat than to the changes of dietary carbohydrate. Fructose feeding did, however, produce an increase in glycerides of men and post-menopausal women, and a decrease in glycerides of premenopausal women (Macdonald, 1966b). Estrogen or progesterone may have played a role in preventing the rise of fasting serum triglycerides associated with dietary fructose. The fructose effect on serum

triglycerides was similar to that observed with a sucrose diet (Macdonald and Braithwaite, 1964; Macdonald, 1965; Macdonald, 1966a). The author suggested that the fructose moiety may have been partially responsible for the lipid response to sucrose, as contrasted to starch. A further factor may have been the relatively slower rate of absorption of glucose derived from starch, due to the necessity for hydrolysis in the gut.

When fructose was fed instead of ground whole wheat to rats, the activities of glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and α-glycerophosphate dehydrogenase were increased (Fitch, Hill and Chaikoff, 1959; Fitch and Chaikoff, 1960). Thus, the fructose appeared to hasten the activity of the hexose monophosphate shunt and the formation of glycerol. A greater generation of NADPH from the hexose monophosphate pathway resulted. Both NADPH and glycerol are necessary for lipogenesis. Glucose had the same effects as fructose but to a lesser extent (Fitch et al., 1959; Fitch and Chaikoff, 1960). As a result, an increased lipogenesis can be predicted in association with fructose.

## Blood Glucose and Glucose Tolerance

The concentration of glucose in whole blood of healthy individuals in the postabsorptive state ranges from 80 to 120 mg/100 ml.

The wide variation in reported values is due partly to individual difference and partly to differences among analytical methods (Folin and Malmros, 1929; Benedict, 1931; Hoffman, 1937; Tustison, Bowen and Crampton, 1966). Somogyi (1931) reported that the glucose content of plasma was 10-15% higher than that of whole blood. According to Miller and Van Slyke (1936), the glucose concentration of capillary blood is about 10% higher than that of venous blood, even under fasting conditions.

The oral glucose tolerance test provides one means of assessing the efficiency of glucose utilization. When intestinal absorption is normal, the venous blood glucose concentration usually increases by about 150% in the first hour following the ingestion of 1.75 gm glucose per kg of body weight (Fajans and Conn, 1959). The subsequent decline to fasting levels by one and one-half to two hours reflects the uptake of glucose by tissues. Tustison et al. (1966) stated that the normal upper limits of glucose in venous plasma were 185 mg/100 ml at one hour and 140 mg/100 ml at two hours following the ingestion of 75 gm glucose. Arterial or capillary blood changes differ from those of venous blood in several aspects: the rise begins earlier and is more rapid, the peak is reached at 30 to 45 minutes instead of 30 to 60 minuts, the peak averages 30 mg/100 ml (20-70 mg/100 ml) higher than in venous blood, and the return to fasting level occurs between one and one-half and three hours (Cantarow and

Schepartz, 1962). When insulin is deficient, as in diabetes, the uptake by tissues is impaired and the blood glucose concentration remains high (Fajans and Conn, 1959). Elderly subjects frequently have impaired glucose tolerance. Streeten et al. (1965) showed that this was not simply due to retarded absorption nor to impaired insulin secretion from the pancreas. They presumed that it resulted from a higher level of insulin antagonist in the blood or the delayed uptake of glucose in the peripheral tissues.

Glucose metabolism is influenced by hormones other than insulin. Wynn and Doar (1966) reported that glucose tolerance was impaired as a result of the use of oral contraceptives. Mirsky and Broh-Kahn (1936) showed that thyroid hormone increased the uptake of glucose by tissues. Insulin secretion and glucose utilization, which had been impaired in hypothyroidism, were rapidly restored to normal by the administration of small doses of thyroid hormone (Elrick, Hlad and Arai, 1960; Malaisse, Malaisse-Lagae and McCraw, 1967).

Glucose tolerance may be affected by the composition of the diet and also by the number of meals consumed. Fabry et al. (1964) found that as the frequency of meals decreased, the oral glucose tolerance tended to diminish. Van Itallie and Shull (1957) studied four healthy subjects on a carbohydrate-free diet for three days and reported that oral glucose tolerance was impaired. This phenomenon

was shown to be associated with a decrease in glucose uptake by the extrahepatic tissues. Hales and Randle (1963) noted that in carbohydrate deprivation, more free fatty acids were released from glycerides. They suggested that this phenomenon might be the cause of insulin insensitivity and abnormalities of carbohydrate metabolism.

In 1964, Cohen and Teitelbaum reported that substitution of sucrose for dietary starch resulted in an impaired oral glucose tolerance in rats. The same result was also observed in man. Cohen (1967) fed a group of healthy men and women a low-fat diet in which 60% of the calories was supplied alternately by sucrose and by bread. The duration of each dietary period was five weeks. The average glucose tolerance curve on the high bread diet was significantly lower than that on the sucrose diet. The high sugar diet gave a slightly higher glucose tolerance curve than a high-fat "Western type" diet, but the difference was small. The responses varied among individual subjects. The author postulated that sucrose feeding, by producing a rapid influx of glucose into the circulation, constituted an acute and intermittent stimulus to the glucose storage system. The starch was digested slowly in the intestinal tract, and produced a less intense but more prolonged stimulation of that system. As a result, the starch diet gave a better adaptation to a glucose load. That reduced glucose tolerance may be related to hypertriglyceridemia has been suggested by the observation that both

conditions may be produced by sucrose feeding. Carlson and Wahlberg (1966) observed that the glucose tolerance curve was higher in hypertriglyceridemic males than in normal subjects. In females, the glucose tolerance curve was higher with increased levels of serum cholesterol. Whether or not these phenomena are related directly or indirectly is not clear at present time.

In a study of the effect of fructose feeding on glucose tolerance, Van Itallie and Shull (1957) fed at least 250 gm of fructose as the sole carbohydrate to a group of healthy subjects. Contrary to the findings in the rats (Hill, Baker and Chaikoff, 1954), fructose feeding did not impair the glucose tolerance in men. It increased the delivery of glucose to the periphery. The author stated that fructose might temporarily diminish the rate of glucose uptake by the liver, but this effect was obscured by the increased removal of glucose in the peripheral area.

# EXPERIMENTAL PROCEDURE

## Experimental Design

### Subjects

Three apparently healthy women served as subjects for this study. All of them were laboratory personnel. Subject S. V. was receiving thyroid hormone and oral contraceptives periodically. A description of the subjects is presented in Table 3.

Table 3. Age, weight, and caloric requirement of the subjects.

| Subject | Age | Weight | Height | Caloric Requirement |
|---|---|---|---|---|
| | yr | lb | in | Cal |
| E. Y. | 53 | 120 | 64 | 1600 |
| S. V. | 22 | 129 | 62 | 1800 |
| J. C. | 22 | 101 | 60 | 1600 |

The estimation of daily caloric requirement was based on the body weight and the experimental diet was designed to maintain constant body weight.

### Dietary Program

In all diets, the total caloric intake was derived 44% from carbohydrate, 40% from fat, and 16% from protein. There were

four dietary periods of six days each. In the initial period (Control Diet), the subjects chose their own foods to provide the designated distribution of calories. This period permitted the determination of the accuracy of the estimated caloric intake and minimized the difference in previous diets of subjects. During the control period, carbohydrate was derived from mixed sources. In the succeeding dietary periods, 80% of the carbohydrate was provided alternately as sucrose (Sugar Diet), or as bread, cereals, and potatoes (Complex Diet). The isocaloric interchange of carbohydrates constituted 35% of the daily caloric intake. The design of the diet study appears in Table 4.

Table 4. Design of diet study.

| Subject | Diet | | | |
|---|---|---|---|---|
| | Period 1 | Period 2 | Period 3 | Period 4 |
| E.Y. | Control | Complex | Sugar | Complex |
| S.V. | Control | Sugar | Complex | Sugar |
| J.C. | Control | Complex | Sugar | Complex |

The experimental diets were calculated according to the food exchange lists (Caso, 1950). The foods were divided into three meals plus an evening snack. The calculated composition of the experimental diets is shown in Table 5.

Table 5. Plans of experimental diets.

| | Complex | | | Sugar | | |
|---|---|---|---|---|---|---|
| | Protein | Fat | Carbohydrate | Protein | Fat | Carbohydrate |
| | g | g | g | g | g | g |
| 1600 Calories | | | | | | |
| Breakfast | 19 | 20 | 42 | 11 | 15 | 41 |
| Lunch | 21 | 20 | 48 | 22 | 20 | 48 |
| Dinner | 21 | 20 | 48 | 22 | 25 | 38 |
| Evening | 7 | 10 | 38 | 15 | 15 | 47 |
| | 68 | 70 | 176 | 70 | 75 | 174 |
| 1800 Calories | | | | | | |
| Breakfast | 20 | 25 | 49 | 22 | 25 | 52 |
| Lunch | 21 | 20 | 48 | 22 | 20 | 48 |
| Dinner | 21 | 20 | 48 | 22 | 25 | 43 |
| Evening | 12 | 15 | 52 | 8 | 10 | 52 |
| | 74 | 80 | 197 | 74 | 80 | 195 |

In the preparation of foods, meat and fish were purchased at one time, weighed, packaged, and stored for the whole program. Milk, bread, cereal, potatoes, margarine, and salad oil were of the same brands. There was freedom for each individual to choose her own vegetables and fruits. During the experimental diets, lunch was prepared together except on Sunday. The other meals were prepared by the subject herself. The amount of each foodstuff was weighed on a dietetic scale according to the weights of the exchange list (Caso, 1950). Menus for the 1600 Calorie diets appear in Tables 1 and 2 of the Appendix. On the first day of each diet period, a glucose tolerance test was performed (1.5 gm glucose per kg body

weight). Following the glucose tolerance test, only the milk and egg of the breakfast meal were consumed. On the Sugar Diet, 0.25 gm of methylcellulose was administered each meal for bulk.

## Methods of Blood Analysis

### Blood Sampling and Preparation

Samples of venous blood were drawn before breakfast on the last day of each dietary period. The blood was collected in 10 ml vacutainers which had been pretreated with 12 mg EDTA (Ethylenediaminetetraacetic acid). Ten milliliters of blood from each subject were centrifuged at 50 g for 10 minutes. About 2 ml of platelet-rich plasma were removed for clotting time tests (Vesecky, 1968). The remainder was centrifuged at 1300 g for 10 minutes. The plasma was removed and stored at -10°C.

### Total Lipid Extraction

Two milliliters of thawed plasma were extracted with chloroform:methanol, 2:1, V/V, according to the procedure of Smith (1965). The lipid extract was transferred to a tared one-milliliter volumetric flask and evaporated to dryness under nitrogen. The weight of the dried lipid extract was obtained and expressed as mg/100 ml plasma. The extract was redissolved in chloroform;

aliquots were taken for the analysis of the following lipid fractions.

## Free and Total Cholesterol Determination

Free and total cholesterol were determined essentially by the procedure described by Smith (1961). One hundred microliters of the total lipid extract were diluted to 1 ml with chloroform. Triplicate aliquots of 100 µl of the diluted specimen were used for the determination of free cholesterol. For total cholesterol analysis, triplicate aliquots of 20 µl of the diluted specimen were saponified and neutralized prior to determination of cholesterol. Cholesterol was then precipitated as the digitonide; the digitonide was purified, redissolved, and treated with the Liebermann-Burchard color reagent. For color development, the tubes were placed in a 28°C water bath in a dark cabinet for 20 minutes; the absorbance was measured exactly 45 minutes after the addition of the color reagent.

## Phospholipid Determination

Hawthorne, Smith, and Pescador's (1963) modification of the Lowry *et al.* (1954) microprocedure was used for the determination of lipid phosphorus. The method consists of the formation of phosphomolybdic acid which is then reduced by ascorbic acid to the molybdenum blue complex. The concentration of molydenum blue is measured photometrically. For determination of lipid phosphorus,

40 μl of the total lipid extract were diluted to 1 ml with chloroform. Triplicate 20-μl aliquots of the diluted sample were used. The phospholipid concentration was obtained by multiplying the lipid phosphorus by 25.

Triglyceride Determination

The triglyceride content of plasma was determined by an adaptation of the method of Van Handel and Zilversmit (1957) as modified by Van Handel (1961). Briefly, the method consists of the removal of phospholipids from the lipid extract, hydrolysis of the triglycerides to yield glycerol, oxidation of the glycerol to formaldehyde and formic acid, and photometric determination of formaldehyde by the chromotropic acid reaction. For this study, phospholipids were removed by adsorption onto silicic acid (Randrup, 1960; Mendelsohn and Antonis, 1961; Azarnoff, 1962). Baker Analyzed reagent grade silicic acid powder was activated overnight at 120°C and kept in a tightly stoppered bottle. One-half gram of activated silicic acid was slurried with chloroform in a 15 ml centrifuge tube. Two hundred microliters of lipid extract were added and the volume was made to 10 ml with chloroform. Tubes were shaken for 10 minutes and then centrifuged at 1300 g for 20 minutes. Three two-milliliter portions of the supernatant were analyzed for glycerol. The other modification of the Van Handel

and Zilversmit method was that the volumes of all tubes were equalized by the addition of redistilled water prior to absorbance measurements.

For cholesterol, phospholipids, and triglycerides, the optical density of the final compounds was measured by means of the Beckman Spectrophotometer Model D. U. set at appropriate wavelengths. The readings of the samples were compared with those of standards and the concentrations were expressed as mg/100 ml of plasma.

Glucose Tolerance Test

A glucose tolerance test was carried out on the first day after each dietary period. In the morning, a load of 1.5 gm of glucose per kg of body weight, administered as a 50% solution, was ingested after an over-night fast. Capillary (finger tip) blood samples were obtained immediately prior to glucose administration and at 30, 60, 90, and 120 minutes after the glucose feeding. Blood was collected in 1 ml tubes containing 0.3 mg of heparin. Plasma was separated immediately by centrifuging at 800 g for 20 minutes. Fifty or one hundred microliters of plasma were diluted to 1 ml with 0.9% saline solution. Glucose concentration was determined on the diluted

specimen by means of the Technical Auto Analyzer.[1] The procedure applied was a modification of the potassium ferricyanide micro-method of Hoffman (1937). The principle was based on the reduction of the yellow ferricyanide to the colorless ferrocyanide by glucose. The transmittance of the reduced solution was recorded automatically by the instrument. The concentration of plasma glucose was estimated from the standard curve and by applying appropriate dilution factors. Plasma glucose was expressed as mg/100 ml of plasma.

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[1] Technicon Corporation, Ardsley, New York.

# RESULTS AND DISCUSSION

## Total Lipids and Lipid Fractions of Plasma

The total lipids and different lipid fractions of plasma samples are shown in Table 6. Since there appeared to be no effect of order of treatment upon the lipid responses, the mean lipid concentrations following the diets were weighted in order to equalize the influence of the individual subjects upon the mean. To obtain the weighted mean value for a diet, each subject's responses to the diet were first averaged and the mean was computed from the averages. The concentrations of total lipids, triglycerides, and phospholipids observed in this study were comparable to the plasma lipid values of healthy subjects reported by the other investigators (Table 1). The values of cholesterol, however, were somewhat lower. This might be due to the individual character or the method used.

The plasma lipid concentrations of Subject E. Y. were consistently highest. This finding is in agreement with the results of previous studies. Higher plasma lipid concentrations in aged women were found by Hallberg et al. (1966). Lund et al. (1961) reported that the total lipid content of serum tended to increase with age. Adlersberg et al. (1956) observed increased cholesterol and phospholipid concentrations in the serum of older women. Feldman

Table 6. Total lipids and lipid fractions in plasma following experimental diets.

| Subject | Diet | Total Lipid | Phospholipid | Triglyceride | Cholesterol | | |
|---|---|---|---|---|---|---|---|
| | | | | | Total | Free | Free/Total |
| | | mg/100 ml | mg/100 ml | mg/100 ml | mg/100 ml | mg/100 ml | % |
| E.Y. | Control | 713 | 215 | 82 | 197 | 65 | 33 |
| | Complex | 638 | 202 | 54 | 194 | 60 | 31 |
| | Sugar | 719 | 241 | 67 | 211 | 63 | 30 |
| | Complex | 666 | 202 | 72 | 181 | 56 | 31 |
| S.V. | Control | 546 | 169 | 80 | 126 | 37 | 30 |
| | Sugar | 504 | 154 | 90 | 120 | 36 | 30 |
| | Complex | 510 | 146 | 75 | 122 | 36 | 29 |
| | Sugar | 557 | 178 | 92 | 134 | 38 | 29 |
| J.C. | Control | 587 | 192 | 71 | 141 | 45 | 32 |
| | Complex | 538 | 181 | 61 | 156 | 46 | 30 |
| | Sugar | 581 | 196 | 68 | 155 | 41 | 26 |
| | Complex | 498 | 172 | 57 | 128 | 34 | 26 |
| Weighted mean | Control | 615 | 192 | 78 | 155 | 49 | 32 |
| | Complex | 560 | 175 | 66 | 150 | 45 | 29 |
| | Sugar | 610 | 201 | 75 | 164 | 47 | 28 |

et al. (1963) reported that cholesterol and triglyceride concentrations increased in females over age 35. In the present study, the ratio of free cholesterol to total cholesterol was quite constant and did not vary with age. This again, corresponds to the observations of Bloor (1916), Adlersberg et al. (1956), and Lund et al. (1961).

In Subject S.V., the plasma lipid concentrations, especially cholesterol, were low. This did not agree with the finding of increased serum lipid concentrations after oral contraceptives (Aurell et al., 1966; Wynn et al., 1966). Since Subject S.V. also received thyroid hormone, the cholesterol-lowering effect of thyroid hormone (Miettinen, 1968) might have compensated for the cholesterol-increasing effect of the oral contraceptive.

Small modifications in the lipid concentrations of plasma were associated with the dietary treatment. In all but two cases, the total lipid, phospholipid, and triglyceride concentrations were lower after the Complex Diet than after either the Control or the Sugar Diet. The weighted mean values clearly reflect this trend. The Sugar Diet produced mean concentrations of total lipids, phospholipids, and triglycerides which were similar to those of the Control period in which the carbohydrate was derived from mixed sources. Only Subject S.V. showed an increase in triglycerides when sucrose replaced the Control Diet. The phospholipids of Subject E.Y. were higher after the Sugar Diet than after the Control period.

Both the free and the total cholesterol concentrations of the individual subjects fluctuated from period to period. No consistent changes could be attributed to the diets.

The decrease in plasma lipid fractions after the Complex Diet confirmed the observations of Antar and Ohlson (1965) who administered diets of similar proportions to young women for 28 day periods. These investigators also reported that the Sugar Diet produced higher total lipids, phospholipids, and nonphospholipids than did either the Complex or the Control Diets. It is possible that the lipid responses to the Sugar Diet in the present study would have exceeded those of the Control Diet if the dietary periods had been longer. The data presented in Table 6 do not correspond to the observations of Macdonald (1965, 1966a), who found that a diet containing 500 gm of either sucrose or starch produced decreases in total lipids, phospholipids, and cholesterol of young women and of postmenopausal women. The young women of Macdonald's study responded to the high sucrose diet with decreased plasma triglycerides; in older women, sucrose prompted an increase in triglycerides. In the present study, the plasma triglycerides of both younger women were higher after the Sugar Diet than after the Complex Diet; the response of the older subject was inconsistent.

## Glucose Tolerance Test

The glucose concentrations of capillary plasma before and after the oral glucose load are given in Table 7. The glucose tolerance curves of the individual subjects are shown in Figures 1, 2, and 3. The plasma glucose concentrations of all subjects in the postabsorptive state were in accordance with the concentrations of healthy individuals reported by the previous investigators (Somogyi, 1931).

The three subjects showed marked differences in their responses to oral glucose. The glucose tolerance curves of Subject E. Y. were consistently highest, regardless of the experimental diet. The glucose loads after the Complex Diet periods produced glucose concentrations of 252 and 232 mg/100 ml plasma. An excessive rise in absorptive blood sugar is seen in carbohydrate starvation (Van Itallie and Shull, 1957), in diabetes mellitus (Fajans and Conn, 1959), and in old age (Streeten et al., 1965). It seems unlikely that carbohydrate starvation, as such, caused the high glucose tolerance curves of this subject since the total carbohydrate intake was constant throughout the study and well above the 150 gm/day which was considered sufficient to produce normal glucose tolerance (Wilkerson, Butler and Francis, 1960). It also seems unlikely that diabetes mellitus exists in this subject. In diabetes,

Table 7. Plasma glucose concentration after oral glucose tolerance test.

| Subjects | Diet | Glucose | | | | |
|---|---|---|---|---|---|---|
| | | 0 min | 30 min | 40 min | 50 min | 120 min |
| | | mg/100 ml | mg/100 ml | mg/100 ml | mg/100 ml | mg/100 ml |
| E. Y. | Control | 94 | 201 | 186 | 154 | 86 |
| | Complex | 92 | 180 | 252 | 166 | 149 |
| | Sugar | 99 | 198 | 191 | 130 | 129 |
| | Complex | 93 | 222 | 232 | 195 | 155 |
| S. V. | Control | 72 | 134 | 135 | 121 | 104 |
| | Sugar | 76 | 124 | 129 | 99 | 97 |
| | Complex | 72 | 134 | 137 | 105 | 111[a] |
| | Sugar | 86 | 159 | 154 | 122 | 122 |
| J. C. | Control | 92 | 165 | 129 | 110 | 96 |
| | Complex | 84 | 166 | 149 | 133 | 112 |
| | Sugar | 88 | 163 | 161 | 130 | 110 |
| | Complex | 86 | 170 | 140 | 116 | 107 |
| Weighted mean | Control | 86 | 167 | 150 | 128 | 95 |
| | Complex | 83 | 168 | 174 | 137 | 124 |
| | Sugar | 88 | 167 | 164 | 124 | 116 |

[a]Badly hemolyzed.

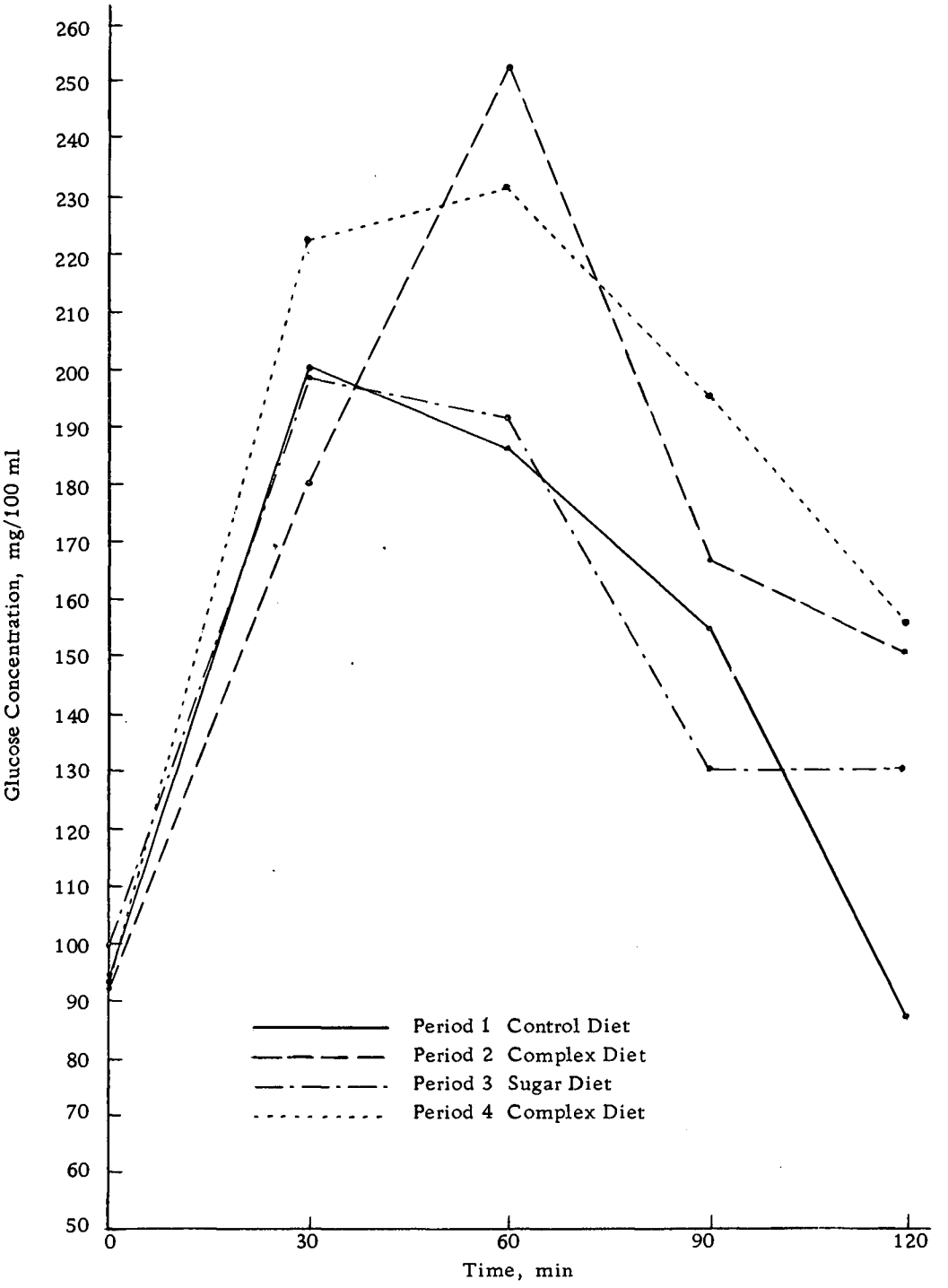


Figure 1. Glucose tolerance curves of Subject E. Y.

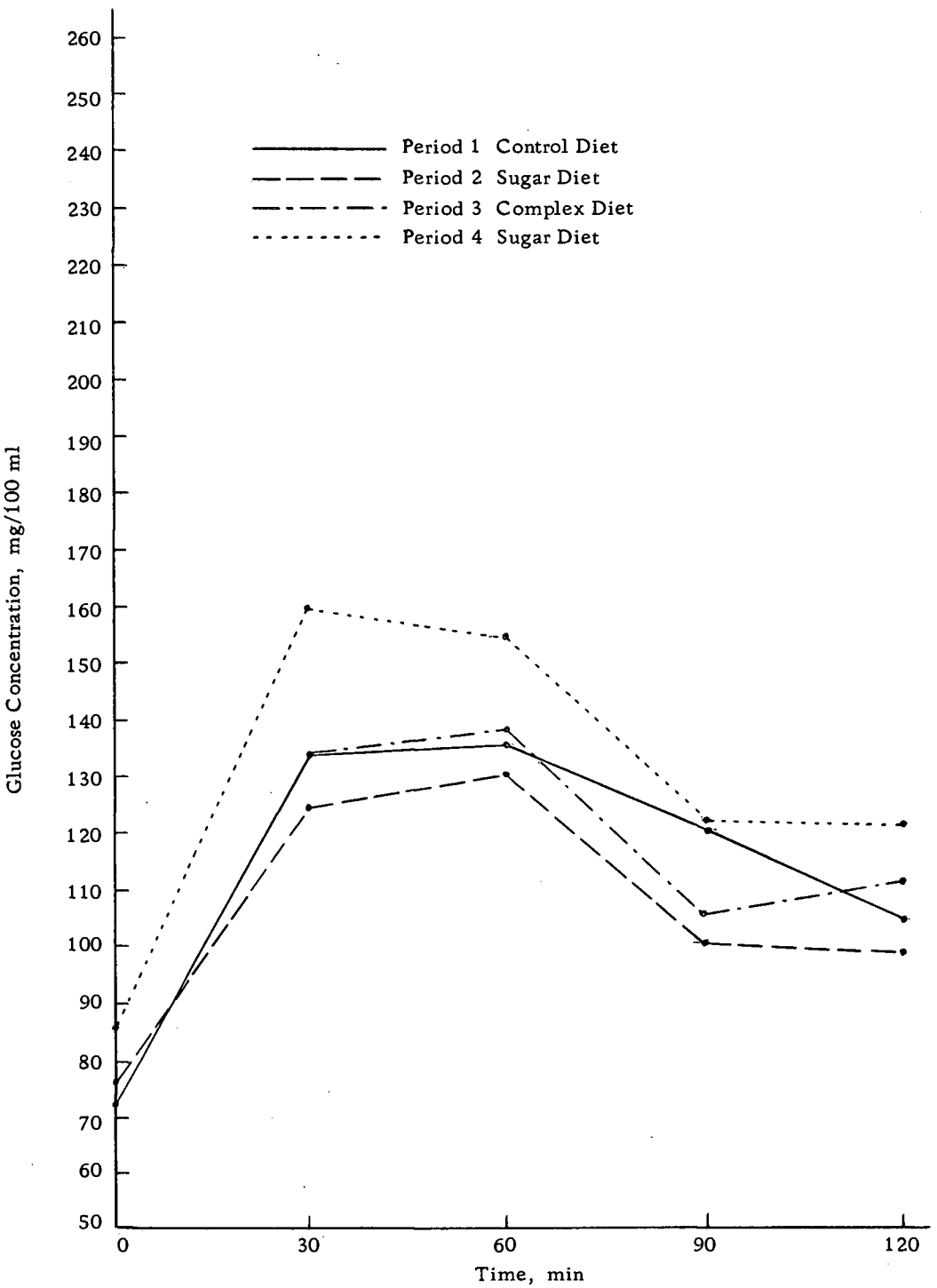


Figure 2. Glucose tolerance curves of Subject S. V.

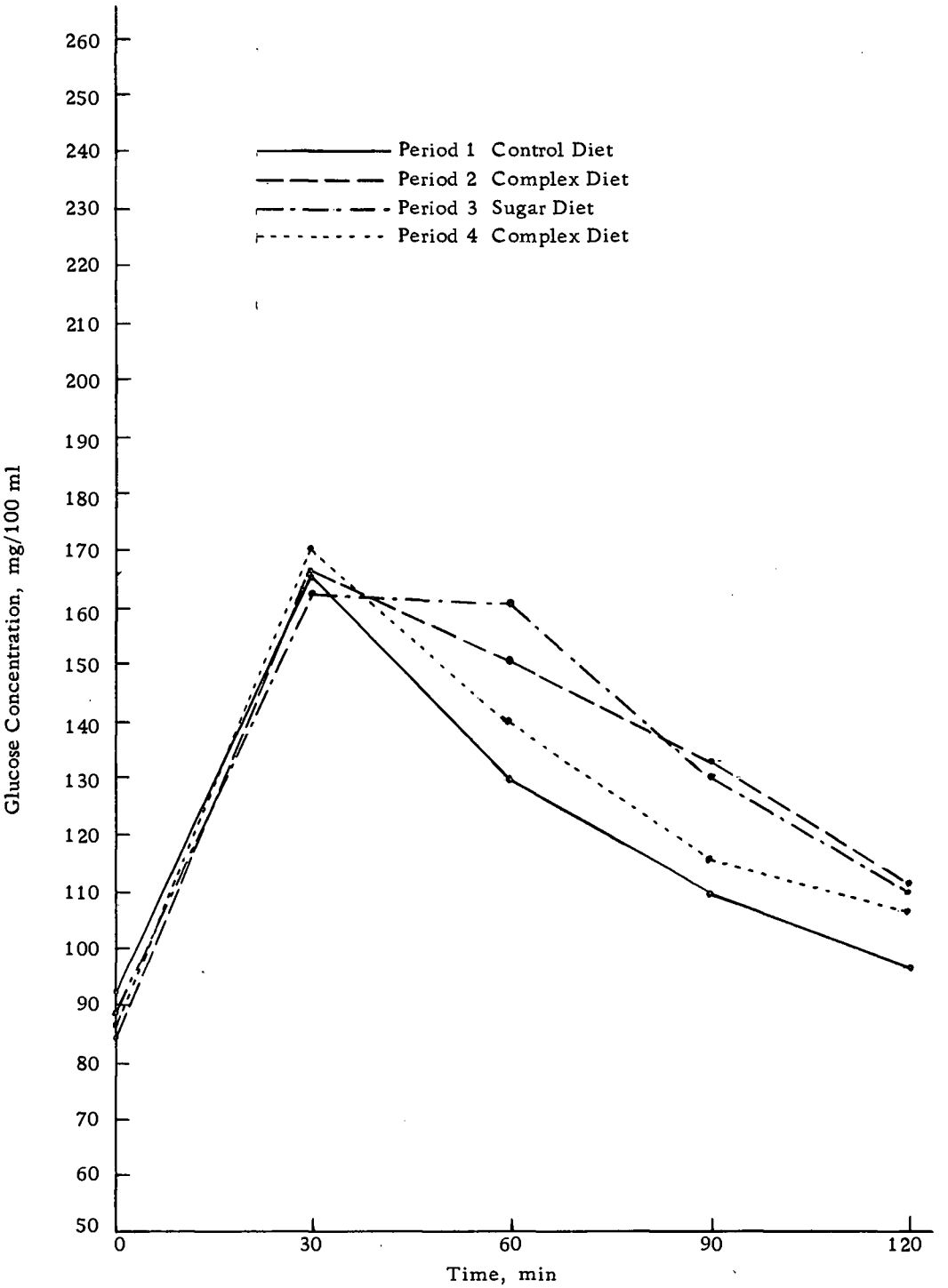


Figure 3. Glucose tolerance curves of Subject J. C.

the blood sugar rarely returns to fasting levels by the third hour. Although the plasma glucose was not routinely measured after the second hour in this study, Subject E. Y. experienced sweating and tremor following the glucose tolerance tests; her plasma glucose at the third hour following the test in Period 3 and 4 were 63 and 60 mg/100 ml, respectively. Therefore, it is probable that the characteristic high glucose tolerance curves were associated with a lag in peripheral uptake of glucose due to age (Streeten et al., 1965).

The glucose tolerance curves of Subject S. V. were generally more flat than those of the other subjects. This characteristic may have been associated with a rapid clearance of glucose by the tissues after receiving thyroid hormone (Mirsky and Broh-Kahn, 1936). The impairment in glucose tolerance after taking oral contraceptives was not found. There may have been an interaction of the effects of the two hormone medications.

The tolerance curves of Subject J. C. characteristically reached a peak at 30 minutes and declined thereafter.

The effects of the dietary regimens on the glucose tolerance curves differed with each subject. Other than a slightly delayed clearance of glucose from the blood after both the Sugar and the Complex Diets, the tolerance curves of Subject J. C. were remarkably uniform (Figure 3). The glucose tolerance of Subject S. V. after the

Complex Diet was not obviously different from that of the Control period. The two Sugar Diet periods produced quite different glucose tolerance curves (Figure 2). Tolerance was improved following the first Sugar Diet but decreased following the second period of sugar intake. It is possible that the reduced tolerance after Period 4 may have been due to a delayed effect of the intervening Complex Diet. The only subject who showed a pronounced and consistent effect of dietary carbohydrate upon glucose tolerance was Subject E. Y. (Figure 1). After each Complex Diet period, this subject showed abnormal glucose tolerance characterized by high plasma glucose concentrations, delayed clearance, and reactive hypoglycemia at the third hour.

These findings are not in accord with those of Cohen (1967) who noted impaired glucose tolerance following a high sucrose diet and improved tolerance after a high bread diet. They suggested that the bread, by delivering glucose slowly into the blood, may have maintained the glucose storage mechanism through prolonged stimulation of moderate intensity. The response of Subject E. Y. in the present study would suggest that adaptation to the Complex Diet rendered the subject less responsive to the sudden and intense stimulus of the glucose load. Sugar feeding, on the other hand, by producing a rapid influx of glucose into the circulation, may have maintained the ability of the pancreas to release insulin rapidly.

It is possible that, had the dietary periods been of longer duration, the intense stimulus of sucrose feeding might have exhausted the pancreatic response.

# SUMMARY

Two young women and one middle-aged woman consumed weighed diets in which protein, fat, and carbohydrate provided 16, 40, and 44% of the calories, respectively. There were four dietary periods of six days each. In the first period, carbohydrate was derived from mixed sources (Control Diet). During the second, third, and fourth periods, the subjects ingested 80% of the carbohydrate alternately as sucrose (Sugar Diet) or as polysaccharides (Complex Diet). Venous blood was drawn from fasting subjects on the last day of each dietary period; plasma concentrations of total lipids, phospholipids, triglycerides, total and free cholesterol were determined. Glucose tolerance tests were performed at the end of each dietary period.

The concentrations of plasma total lipids, phospholipids, and triglycerides were all in the normal range for healthy individuals. The values obtained for cholesterol were slightly lower. The older subject had consistently higher concentrations of all lipids in the plasma than did the younger women.

The total lipids, phospholipids, and triglycerides of plasma were lower after the Complex Diet than after either the Sugar Diet or the Control period. On the average, these lipid fractions were the same following the Sugar Diet as they had been on the Control Diet.

Two exceptions to this generalization were noted: the triglycerides of one young woman and the phospholipids of the older subject were higher after the Sugar Diet than after the Control Diet. No consistent changes in cholesterol concentrations could be attributed to diets.

The plasma glucose concentrations of all subjects in the post-absorptive state agreed with the concentrations reported for healthy individuals. Glucose tolerance curves of the older subject were consistently higher than those of the younger women. After the Complex Diet, the older subject showed impaired glucose tolerance. Glucose tolerance did not seem to be related to the dietary treatment in the two younger women.

# BIBLIOGRAPHY

Adlersberg, D. et al. 1956. Age, sex, serum lipids and coronary atherosclerosis. Journal of the American Medical Association 162:619-622.

Ahrens, E. H. et al. 1957. The influence of dietary fats on serum-lipid levels in man. The Lancet 2:943-953.

Albrink, M. J., J. W. Meigs and M. A. Granoff. 1962. Weight gain and serum triglycerides in normal men. New England Journal of Medicine 266:484-489.

Anderson, J. T. 1967. Dietary carbohydrate and serum triglycerides. American Journal of Clinical Nutrition 20:168-175.

Anderson, J. T. et al. 1963. Glucose, sucrose and lactose in the diet and blood lipids in man. Journal of Nutrition 79:349-359.

Antar, M. A. and M. A. Ohlson. 1965. Effect of simple and complex carbohydrate upon total lipids, nonphospholipids and different fractions of phospholipids of serum in young men and women. Journal of Nutrition 85:329-337.

Aurell, M., K. Cramér and G. Rybo. 1966. Serum lipids and lipoproteins during long-term administration of an oral contraceptive. The Lancet 1:291-293.

Azarnoff, D. L. 1962. Micromethod for the determination of serum lipids. Journal of Laboratory and Clinical Medicine 60:331-338.

Benedict, S. R. 1931. The analysis of whole blood. II. The determination of sugar and of saccharoids (non-fermentable copper-reducing substances). Journal of Biological Chemistry 92:141-159.

Beveridge, J. M. R., W. F. Connell and C. Robinson. 1963. Effect of the level of dietary protein with and without added cholesterol on plasma cholesterol levels in man. Journal of Nutrition 79: 289-295.

Beveridge, J. M. R., S. N. Jagannathan and W. F. Connell. 1964. The effect of type and amount of dietary fat on the level of plasma triglycerides in human subjects in the postabsorptive state. Canadian Journal of Biochemistry and Physiology 42: 999-1003.

Bloor, W. B. 1916. The distribution of the lipids ("fat") in human blood. Journal of Biological Chemistry 25:577-599.

Boyd, E. M. 1933. A differential lipid analysis of blood plasma in normal young women by microoxidative methods. Journal of Biological Chemistry 101:323-336.

Boyd, G. S. 1962. Effect of linoleate and estrogen on cholesterol metabolism. Federation Proceedings 21:86-92.

Cantarow, A. and B. Schepartz. 1962. Biochemistry. Philadelphia, Saunders. 938 p.

Carlson, L. A. 1960. Serum lipids in normal men. Acta Medica Scandinavica 167:377-397.

Carlson, L. A. and F. Wahlberg. 1966. Serum lipids, intravenous glucose tolerance and their interrelation studied in ischaemic cardiovascular disease. Acta Medica Scandinavica 180:307-315.

Caso, E. K. 1950. Calculation of diabetic diets. Journal of the American Dietetic Association 26:575-583.

Cohen, A. M. 1967. Effect of dietary carbohydrate on the glucose tolerance curve in the normal and the carbohydrate-induced hyperlipemic subjects. American Journal of Clinical Nutrition 20:126-130.

Cohen, A. M. and A. Teitelbaum. 1964. Effect of dietary sucrose and starch on oral glucose tolerance and insulin-like activity. American Journal of Physiology 206:105-108.

Cromie, J. B. et al. 1963. Studies in serum lipids; with special reference to spontaneous variations and the effect of short-term dietary changes. Circulation 27:360-365.

Dole, V. P. 1956. A relation between non-esterified fatty acids in plasma and the metabolism of glucose. Journal of Clinical Investigation 35:150-154.

Elrick, H., C. J. Hlad and Y. Arai. 1960. Influence of thyroid function on carbohydrate metabolism and a new method for assessing response to insulin. Journal of Clinical Endocrinology and Metabolism 21:387-400.

Fabry, P. et al. 1964. The frequency of meals; its relation to overweight, hypercholesterolemia, and decreased glucose-tolerance. The Lancet 2:614-615.

Fairhurst, B. J. and C. Waterhouse. 1963. Effect of previous dietary intake on the fatty acid composition of the plasma cholesterol esters. American Journal of Clinical Nutrition 13:92-97.

Fajans, S. S. and J. W. Conn. 1959. The early recognition of diabetes mellitus. Annals of the New York Academy of Sciences 82:208-218.

Feldman, E. B., P. Benkel and R. V. Nayak. 1963. Physiologic factors influencing circulating triglyceride concentration in women. Age, weight-gain, and ovarian function. Journal of Laboratory and Clinical Medicine 62:437-448.

Fitch, W. M. and I. L. Chaikoff. 1960. Extent and patterns of adaptation of enzyme activities in livers of normal rats fed diets high in glucose and fructose. Journal of Biological Chemistry 235:554-557.

Fitch, W. M., R. Hill and I. L. Chaikoff. 1959. The effect of fructose feeding on glucolytic enzyme activities of the normal rat liver. Journal of Biological Chemistry 234:1048-1051.

Folin, O. and H. Malmros. 1929. Blood sugar and fermentable blood sugar as determined by different methods. Journal of Biological Chemistry 83:121-127.

Goolden, A. W. G., J. M. Gartside and C. Sanderson. 1967. Thyroid status in pregnancy and in women taking oral contraceptives. The Lancet 1:12-15.

Grande, F. 1967. Dietary carbohydrates and serum cholesterol. American Journal of Clinical Nutrition 20:176-184.

Grande, F., J. T. Anderson and A. Keys. 1965. Effect of carbohydrates of leguminous seeds, wheat and potatoes on serum cholesterol concentration in man. Journal of Nutrition 86:313-317.

Groen, J. T. 1967. Effect of bread in the diet on serum cholesterol. American Journal of Clinical Nutrition 20:191-197.

Hales, C. N. and P. J. Randle. 1963. Effects of low-carbohydrate diet and diabetes mellitus on plasma concentrations of glucose, non-esterified fatty acids, and insulin during oral glucose-tolerance tests. The Lancet 1:790-794.

Hallberg, L. and A. Svanborg. 1967. Cholesterol, phospholipids and triglycerides in plasma in 50 year-old women. Influence of menopause, body-weight, skinfold thickness, weight-gain and diet in a random population samples. Acta Medica Scandinavica 181:185-194.

Hallberg, L. et al. 1966. Plasma lipids in women. Variation in cholesterol, phospholipids and triglycerides at different ages in a random population sample. Acta Medica Scandinavica 180:697-707.

_____ 1967. Individual plasma phospholipids in women. A comparison of menstruating and menopausal 48 year-old women. Acta Medica Scandinavica 181:143-146.

Hallgren, B. O. et al. 1960. Gas chromatographic analysis of the fatty acid composition of the plasma lipids in normal and diabetic subjects. Journal of Clinical Investigation 39:1424-1434.

Hawthorne, B. E., E. Smith and J. O. Pescador. 1963. Free and total cholesterol in human blood fractions. Journal of Nutrition 81:241-248.

Hegsted, D. M., et al. 1965. Quantitative effects of dietary fat on serum cholesterol in man. American Journal of Clinical Nutrition 17:281-295.

Hill, R., N. Baker and I. L. Chaikoff. 1954. Altered metabolic patterns induced in the normal rat by feeding an adequate diet containing fructose as sole carbohydrate. Journal of Biological Chemistry 209:705-716.

Hodges, R. E. et al. 1967. Dietary carbohydrates and low cholesterol diets: effects on serum lipids of man. American Journal of Clinical Nutrition 20:198-208.

Hoffman, W. S. 1937. A rapid photoelectric method for the determination of glucose in blood and urine. Journal of Biological Chemistry 120:51-55.

Irwin, M. I., D. D. Taylor and R. M. Feeley. 1964. Serum lipid levels, fat, nitrogen and mineral metabolism of young men associated with kind of dietary carbohydrate. Journal of Nutrition 82:338-342.

Keys, A. and J. A. Anderson. 1957. Dietary protein and the serum cholesterol level in man. American Journal of Clinical Nutrition 5:29-34.

Keys, A., T. Anderson and F. Grande. 1960. Diet-type (fats constant) and blood lipids in man. Journal of Nutrition 70:257-266.

Keys, A. et al. 1950a. The concentration of cholesterol in the blood serum of normal man and its relation to age. Journal of Clinical Investigation 29:1347-1353.

_____ 1950b. The relation in man between cholesterol levels in the diet and in the blood. Science 112:79-81.

Kornerup, V. 1950. Concentrations of cholesterol, total fat and phospholipid in serum of normal man. Report of a study with special reference to sex, age and constitutional type. Archives of Internal Medicine 85:398-415.

Kuo, P. T. and D. R. Bassett. 1965. Dietary sugar in the production of hyperglyceridemia. Annals of Internal Medicine 62:1199-1212.

Kuo, P. T. and J. C. Carson. 1959. Dietary fats and the diurnal serum triglyceride levels in man. Journal of Clinical Investigation 38:1384-1393.

Lowry, O. H. et al. 1954. The quantitative histochemistry of brain. I. Chemical method. Journal of Biological Chemistry 207:1-17.

Luddy, F. E., R. A. Barford and R. W. Riemenschneider. 1958. Fatty acid composition of component lipids from human plasma and atheromas. Journal of Biological Chemistry 232:843-851.

Lund, J. C., E. Sivertssen and H. C. Godal. 1961. Studies on serum lipids. I. Healthy individuals. Acta Medica Scandinavica 169:623-627.

Macdonald, I. 1965. The lipid response of young women to dietary carbohydrate. American Journal of Clinical Nutrition 16:458-463.

_____ 1966a. The lipid response of postmenopausal women to dietary carbohydrate. American Journal of Clinical Nutrition 18:86-90.

_____ 1966b. Influence of fructose and glucose on serum lipid levels in men and pre- and post-menopausal women. American Journal of Clinical Nutrition 18:369-372.

_____ 1967a. Dietary carbohydrates in normolipemia. American Journal of Clinical Nutrition 20:185-190.

_____ 1967b. Interrelationship between the influences of dietary carbohydrates and fats on fasting serum lipids. American Journal of Clinical Nutrition 20:345-351.

Macdonald, I. and D. M. Braithwaite. 1964. The influence of dietary carbohydrates on the lipid pattern in serum and in adipose tissue. Clinical Science 27:23-30.

Malaisse, W. J., F. Malaisse-Lagae and E. F. McCraw. 1967. Effects of thyroid function upon insulin secretion. Diabetes 16:643-646.

Man, E. B. and E. F. Gildea. 1937. Variations in lipemia of normal subjects. Journal of Biological Chemistry 119:769-780.

Mendelsohn, D. and A. Antonis. 1961. A fluorimetric micro glycerol method and its application to the determination of serum triglycerides. Journal of Lipid Research 2:45-50.

Miettinen, T. A. 1968. Mechanism of serum cholesterol reduction by thyroid hormones in hypothyroidism. Journal of Laboratory and Clinical Medicine 71:537-547.

Miller, B. F. and D. D. Van Slyke. 1936. A direct microtitration method for blood sugar. Journal of Biological Chemistry 114: 583-595.

Mirsky, I. A. and R. H. Broh-Kahn. 1936. The effect of experimental hyperthyroidism on carbohydrate metabolism. American Journal of Physiology 117:6-12.

Okey, R. and R. E. Boyden. 1927. Studies of the metabolism of women. III. Variations in the lipid content of blood in relation to the menstrual cycle. Journal of Biological Chemistry 72:261-281.

Oliver, M. F. and G. S. Boyd. 1953. Changes in the plasma lipids during the menstrual cycle. Clinical Science 12:217-222.

Page, I. H. et al. 1935. Plasma lipids of normal men at different ages. Journal of Biological Chemistry 111:613-639.

Randrup, A. 1960. A specific and reasonably accurate method for routine determination of plasma triglyceride. Scandinavian Journal of Clinical and Laboratory Investigation 12:1-9.

Smith, E. 1961. Concentrations of free and total cholesterol in human blood fractions. Master's thesis. Corvallis, Oregon State University. 73 numb. leaves.

_____ 1965. Lipids in human blood fractions. Ph. D. thesis. Corvallis, Oregon State University. 120 numb. leaves.

Somogyi, M. 1931. Note on the distribution of blood sugar. Journal of Biological Chemistry 90:731-735.

Stormont, J. M. and C. Waterhouse. 1963. Effect of variations in previous diet on fasting plasma lipids. Journal of Laboratory and Clinical Medicine 61:826-831.

Streeten, D. H. P. et al. 1965. Reduced glucose tolerance in elderly human subjects. Diabetes 14:579-583.

Svanborg, A. and L. Svennerholm. 1961. Plasma total lipid, cholesterol, triglycerides, phospholipids and free fatty acids in a healthy Scandinavian population. Acta Medica Scandinavica 169:43-49.

Svanborg, A. and O. Vikrot. 1967. Plasma lipids during the menstrual cycle. Acta Medica Scandinavica 181:93-96.

Tustison, W. A., A. J. Bowen and J. H. Crampton. 1966. Clinical interpretation of plasma glucose values. Diabetes 15: 775-777.

Van Handel, E. 1961. Suggested modifications of the micro determination of triglycerides. Clinical Chemistry 7:249-251.

Van Handel, E. and D. B. Zilversmit. 1957. Micromethod for the direct determination of serum triglycerides. Journal of Laboratory and Clinical Medicine 50:152-157.

Van Itallie, T. B. and K. H. Shull. 1957. Effect of fructose feeding on glucose tolerance in man. Journal of Laboratory and Clinical Medicine 50:391-399.

Vesecky, S. A. 1968. The influence of dietary carbohydrate on blood phospholipids. Master's thesis. Corvallis, Oregon State University. 52 numb. leaves.

Waterhouse, C., J. H. Kemperman and J. M. Stormont. 1964. Alterations in triglyceride metabolism as produced by dietary change. Journal of Laboratory and Clinical Medicine 63:605-620.

Wilkerson, H. L. C., F. K. Butler and J. O'S. Francis. 1960. The effect of prior carbohydrate intake on the oral glucose tolerance test. Diabetes 9:386-391.

Winitz, M., J. Graff and D. A. Seedman. 1964. Effect of dietary carbohydrate on serum cholesterol levels. Archives of Biochemistry and Biophysics 108:576-579.

Wynn, V. and J. W. H. Doar. 1966. Some effects of oral contraceptives on carbohydrate metabolism. The Lancet 2:715-719.

Wynn, V., J. W. H. Doar and G. L. Mills. 1966. Some effects of oral contraceptives on serum-lipid and lipoprotein levels. The Lancet 2:720-723.

# APPENDIX

Appendix Table 1. Daily menu plan for Complex Diet (1600 Calories).

| Day | Breakfast | grams | Lunch | grams | Dinner | grams | Evening | grams |
|---|---|---|---|---|---|---|---|---|
| 1 | Glucose Tolerance Test | | Ham | 60 | Cooked Fish | 60 | Milk, whole | 120 |
| | (1.5 g/kg body wt.) | | Tossed Salad | (1 serv.)[b] | Vegetable | (1 serv.) | Fruit | (1 serv.)[b] |
| | Milk, whole | 240 | Cooked Macaroni | 100 | Cooked Potato | 100 | Bread | 37.5 |
| | Egg | 50 | Bread | 50 | Bread | 50 | Fat | 5 |
| | | | Fat[a] | 10 | Fat | 10 | | |
| 2 | Puffed Wheat | 20 | Cottage Cheese | 90 | Cooked Hamburger | 60 | Milk, whole | 120 |
| | Milk, whole | 240 | Tomato & Lettuce | (1 serv.) | Vegetable | (1 serv.) | Fruit | (1 serv.) |
| | Egg | 50 | Crackers | 20 | Cooked Rice | 100 | Bread | 37.5 |
| | Bread | 25 | Bread | 50 | Bread | 50 | Fat | 5 |
| | Fat | 5 | Fat | 10 | Fat | 10 | | |
| 3 | Puffed Rice | 20 | Turkey | 60 | Cooked Pork Chop | 60 | Milk, whole | 120 |
| | Milk, whole | 240 | Celery & Lettuce | (1 serv.) | Vegetable | (1 serv.) | Fruit | (1 serv.) |
| | Egg | 50 | Bread | 75 | Mashed Potato | 100 | Bread | 37.5 |
| | Bread | 25 | Fat | 10 | Bread | 50 | Fat | 5 |
| | Fat | 5 | | | Fat | 10 | | |
| 4 | Shredded Wheat | 20 | Ham | 60 | Cooked Hamburger | 60 | Milk, whole | 120 |
| | Milk, whole | 240 | Tomato & Lettuce | (1 serv.) | Vegetable | (1 serv.) | Fruit | (1 serv.) |
| | Egg | 50 | Cooked Potato | 100 | Cooked Macaroni | 100 | Bread | 37.5 |
| | Bread | 25 | Bread | 50 | Bread | 50 | Fat | 5 |
| | Fat | 5 | Fat | 10 | Fat | 10 | | |
| 5 | Puffed Wheat | 20 | Tunafish | 60 | Cooked Beefsteak | 60 | Milk, whole | 120 |
| | Milk, whole | 240 | Celery & Lettuce | (1 serv.) | Vegetable | (1 serv.) | Fruit | (1 serv.) |
| | Egg | 50 | Bread | 75 | Mashed Potato | 100 | Bread | 37.5 |
| | Bread | 25 | Fat | 10 | Bread | 50 | Fat | 5 |
| | Fat | 5 | | | Fat | 10 | | |
| 6 | Puffed Rice | 20 | Roast Beef | 60 | Turkey | 60 | Milk, whole | 120 |
| | Milk, whole | 240 | Tomato & Lettuce | (1 serv.) | Vegetable | (1 serv.) | Fruit | (1 serv.) |
| | Egg | 50 | Cooked Potato | 100 | Cooked Rice | 100 | Bread | 37.5 |
| | Bread | 25 | Bread | 50 | Bread | 50 | Fat | 5 |
| | Fat | 5 | Fat | 10 | Fat | 10 | | |

[a] Fat was supplied by margarine, oil, or mayonnaise.

[b] Weights of individual fruits and vegetables were according to exchange list figures (Caso, 1950).

Appendix Table 2. Daily menu plan for Sugar Diet (1600 Calories).[a]

| Day | Breakfast | grams | Lunch | grams | Dinner | grams | Evening | grams |
|---|---|---|---|---|---|---|---|---|
| 1 | Glucose Tolerance Test | | Ham | 90 | Cooked Fish | 90 | Milk, whole | 240 |
| | (1.5 g/kg body wt.) | | Tossed Salad | (1 serv.)[b] | Vegetable | (1 serv.) | Sugar | 35 |
| | Milk, whole | 120 | Fruit | (1 serv.)[b] | Sugar | 35 | | |
| | Egg | 50 | Sugar | 35 | Fat | 10 | | |
| | | | Fat[c] | 5 | | | | |
| 2 | Milk, whole | 120 | Cottage Cheese | 135 | Cooked Hamburger | 90 | Milk, whole | 240 |
| | Egg | 50 | Tomato & Lettuce | (1 serv.) | Vegetable | (1 serv.) | Sugar | 35 |
| | Sugar | 35 | Fruit | (1 serv.) | Sugar | 35 | | |
| | Fat | 5 | Sugar | 35 | Fat | 10 | | |
| | | | Fat | 5 | | | | |
| 3 | Milk, whole | 120 | Turkey | 90 | Cooked Pork Chop | 90 | Milk, whole | 240 |
| | Egg | 50 | Celery & Lettuce | (1 serv.) | Vegetable | (1 serv.) | Sugar | 35 |
| | Sugar | 35 | Fruit | (1 serv.) | Sugar | 35 | | |
| | Fat | 5 | Sugar | 35 | Fat | 10 | | |
| | | | Fat | 5 | | | | |
| 4 | Milk, whole | 120 | Ham | 90 | Cooked Hamburger | 90 | Milk, whole | 240 |
| | Egg | 50 | Tomato & Lettuce | (1 serv.) | Vegetable | (1 serv.) | Sugar | 35 |
| | Sugar | 35 | Fruit | (1 serv.) | Sugar | 35 | | |
| | Fat | 5 | Sugar | 35 | Fat | 10 | | |
| | | | Fat | 5 | | | | |
| 5 | Milk, whole | 120 | Tunafish | 90 | Cooked Beefsteak | 90 | Milk, whole | 240 |
| | Egg | 50 | Celery & Lettuce | (1 serv.) | Vegetable | (1 serv.) | Sugar | 35 |
| | Sugar | 35 | Fruit | (1 serv.) | Sugar | 35 | | |
| | Fat | 5 | Sugar | 35 | Fat | 10 | | |
| | | | Fat | 5 | | | | |
| 6 | Milk, whole | 120 | Roast Beef | 90 | Turkey | 90 | Milk, whole | 240 |
| | Egg | 50 | Tomato & Lettuce | (1 serv.) | Vegetable | (1 serv.) | Sugar | 35 |
| | Sugar | 35 | Fruit | (1 serv.) | Sugar | 35 | | |
| | Fat | 5 | Sugar | 35 | Fat | 10 | | |
| | | | Fat | 5 | | | | |

[a] 0.25 g methyl-cellulose with each meal.

[b] Weights of individual fruits and vegetables were according to exchange list figures (Caso, 1950).

[c] Fat was supplied by margarine, oil, or mayonnaise.