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Title: THE INFLUENCE OF DIETARY CARBOHYDRATE ON BLOOD PHOSPHOLIPIDS

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The effect of source of dietary carboyhydrate upon the concentration and distribution of phospholipids in the fractions of human blood was studied. Three healthy women received diets which contained 16% of the calories as protein, 40% as fat, and 44% as carbohydrate. During the four dietary periods of six days each, 80% of the carbohydrate was supplied alternately by sucrose (Sugar Diet) or by polysaccharides from natural sources (Complex Diet). Blood samples were drawn before breakfast on the final day of each dietary period.

The total lipid, lipid phosphorus, and distribution of phospholipids were determined in erythrocytes, leukocytes, platelets, and plasma. Clotting time of the platelet-rich plasma of the subjects was determined. In periods of this length, no consistent changes in the blood lipids could be detected. The Sugar Diet appeared to increase the proportion of phosphatidyl choline and decrease the

proportion of sphingomyelin slightly in the plasma and platelets. The phosphatidyl choline fraction of the leukocyte phospholipids decreased slightly after the Sugar Diet.

No relationship was observed between in vitro coagulation time and the distribution of phospholipids in the blood fractions. One subject did demonstrate a marked increase in coagulation time after the diet containing complex carbohydrate.

The Influence of Dietary Carbohydrate On Blood Phospholipids

by

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INTRODUCTION

It has long been known that animals convert a part of their dietary carbohydrate to body fat. Recent studies suggest that certain forms of carbohydrate may be more effective than others in the synthesis of lipids. Of particular interest has been the epidemiological association of high levels of dietary sucrose with human cardiovascular disease and lipemia.

Both qualitative and quantitative changes in plasma lipids have been observed when complex dietary carbohydrate was replaced by sucrose. Two of these changes are particularly noteworthy. First, a diet in which sucrose provided most of the calories was shown to result in an increase in the plasma triglycerides. This observation is of general interest because of the association of hyperglyceridemia with ischemic heart disease. Secondly, the substitution of sucrose for complex carbohydrate in the diet produced a change in the pattern of plasma phospholipids and an acceleration of blood coagulation. The participation of phospholipids in the clotting process has already been established, with respect to platelets.

The lipid concentrations of blood plasma are constantly changing as a result of intestinal absorption, tissue uptake, and tissue

release. In contrast, the lipid patterns of the formed elements of blood show little variation under ordinary circumstances. Very little is known about the influence of dietary carbohydrate on the concentrations of lipids in erythrocytes, leukocytes, or platelets. In view of these facts, it seemed of interest to study the interrelationships among the following three factors: dietary carbohydrates, blood phospholipids, and plasma clotting time. The present investigation was undertaken as a pilot study to explore these areas.

REVIEW OF LITERATURE[1]

Total Lipids and Phospholipids in Blood Fractions

The lipid composition of human serum and plasma has been reported by many investigators and the lipids of human erythrocytes have been well characterized. Relatively less information is available concerning the lipid components of human leukocytes and platelets, probably because it is difficult to isolate these fractions in quantities suitable for analysis.

Total lipids and phospholipids in plasma. Despite the general similarities between serum and plasma, minor differences in the lipid composition of the two fluids have been observed. Vikrot (1964) reported that the LLec fraction of serum was 10% higher than that of plasma; he attributed this phenomenon to the enzymatic hydrolysis of PC during the period of clot formation. He also suggested that the phospholipid pattern of serum may differ from that of plasma because some phosphatides are released from the platelets during coagulation. In contrast, Gjone, Berry, and Turner (1959) observed no differences in the phospholipid content of serum and

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[1] The following abbreviations will be used throughout this paper: CL: cardiolipin, LLec: lysolecithin, PA: phosphatidic acid, PC: phosphatidyl choline, PE: phosphatidyl ethanolamine, PI: phosphatidyl inositol, PS: phosphatidyl serine, Sph: sphingomyelin, TLC: thin layer chromatography.

plasma from the same subjects. Furthermore, they did not find a change in LLec concentration of serum samples as a result of storage for one week at 4°C. Vogel, Zieve, and Carleton (1962) reported that the phospholipid content of plasma was the same as that of serum except when sodium fluoride was used as the anti-coagulant for plasma preparation. They observed that repeated freezing and thawing of serum resulted in the conversion of part of the PC to LLec. Some representative values for total lipids and phospholipids of plasma and serum from human subjects in the post-absorptive state appear in Table 1.

The lipid content of postabsorptive human plasma is approximately 600-725 mg/100 ml. The total lipid is usually determined gravimetrically after extraction with either ethanol-ether or chloroform-methanol. Sperry and Brand (1955) noted that the two solvent systems extracted equivalent amounts of total lipid and lipid phosphorus. These investigators, without differentiating between serum and plasma, reported an average concentration of 729 mg total lipid per 100 ml. In a study of 91 healthy Swedish subjects, aged 16-35 years, Svanborg and Svennerholm (1961) found mean plasma lipid concentrations of 609.7 mg/100 ml for men and 648.1 mg/100 ml for women. Smith (1965) analyzed serum samples from a group of 16 men and women, ranging in age from 23 to 66 years. She reported 603 mg total lipid per 100 ml serum as the mean value

Table 1. Normal fasting values for plasma (or serum) lipids and phospholipids calculated from the data of other investigators.

Reference	Material	Separation Method	Subjects	Total Lipid	Lipid P	Distribution of Lipid Phosphorus						
						Sph	PC	LLec	PE	PS	PI	Other
			N	mg/100ml	mg/100ml	%	%	%	%	%	%	%
Sperry & Brand, 1955	Plasma & Serum	Direct	16 - 17	729	9.16							
Svanborg & Svennerholm, 1961	Plasma	Direct	62 Male 29 Fem.	609.7 648.1	8.3 9.3							
	Serum	Column & Paper				22.8	←— 73.8 —→		2.6	0.8	--	--
Smith, 1965	Serum	Direct	16	603	7.48							
Hack, 1947	Plasma	Direct	2		8.87	15.4	78.3	--	←— 6.3 —→		--	--
Axelrod et al., 1953	Plasma	Direct	2		8.06	←————	92.3	————→	4.6	5.4	--	--
Gjone et al., 1959	Serum	Column	8		9.84	←— 84.7 —→		8.9	←— 6.4 —→		--	--
Marinetti et al., 1959	Plasma	Column & Paper	2			20.8	67.8	9.2[a]	2.0	--	[a]	--
Phillips, 1960	Serum	Column	15		9.98	19.0	68.2	7.7	←— 5.0 —→		--	--
Vogel et al., 1962	Serum	Paper	10		11.04	22.6	66.4	9.5	3.0	--	--	--
Betschart, 1966	Serum	TLC	8			19	63[b]	3	trace	[b]	--	16
Doizaki & Zieve, 1963	Serum	TLC	7		11.53	21.9	64.8	10.8	2.6	--	--	--
Hallberg et al., 1967	Plasma	TLC	40		10.84	22.59	69.08	5.38	2.95	--	--	--
Robinson & Phillips, 1963	Serum	TLC	3			14.7	65.0	14.5	5.8	--	--	--
Vikrot, 1964	Plasma	TLC	9		8.75	21.2	69.9	5.9	3.0	--	--	--

[a] LLec and PI reported as LLec.

[b] PC and PS reported as PC.

(range 455-913 mg).

Phospholipids represent about one-third of the total lipids in plasma or serum. The weight of phospholipids is usually considered to be 25 times the analyzed weight of lipid phosphorus. Svanborg and Svennerholm (1961) reported that, on the basis of fatty acid analyses, the factor of 25 was reasonably accurate. They obtained mean phospholipid concentrations of 207.8 mg/100 ml plasma for men and 232.4 mg/100 ml plasma for women (8.3 and 9.3 mg phosphorus, respectively). According to these data the plasma phospholipids accounted for 34-36% of the total lipids. Smith (1965) found that phospholipids comprised 31% of the total lipids of serum; this proportion corresponds to a concentration of 7.48 mg lipid phosphorus per 100 ml. A similar proportion may be calculated from the data of Sperry and Brand (1955), although the absolute values for total lipid and lipid phosphorus were higher.

The major constituents (94-98%) of the plasma phospholipids are the choline-containing components: PC, Sph, and LLec. Smaller amounts of PE, and occasionally PI and PS, have been observed (Table 1). In earlier studies (Axelrod, Reichenthal, and Brodie, 1953; Hack, 1947), only the major groups of phospholipids were reported. Later, with the development of chromatographic techniques, it became possible to separate the groups into several constituents. Silicic acid columns (Gjone et al., 1959; Marinetti et al., 1959;

Phillips, 1960; Svanborg and Svennerholm, 1961), silicic acid-impregnated paper (Marinetti et al., 1959; Vogel et al., 1962), and thin layer chromatograms (Betschart, 1966; Doizaki and Zieve, 1963; Hallberg et al., 1967; Robinson and Phillips, 1963; Vikrot, 1964) have been employed to effect the separation of the phosphatides. There was good agreement among values obtained by the different separation methods. PC accounted for 65-78% of the lipid phosphorus, Sph 15-23%, LLec 3-14%, PE 2-6%, and PS 0-5% (Table 1).

Total lipids and phospholipids in erythrocytes. The concentration of lipids in erythrocytes is approximately 475-600 mg/100 ml packed cells (Table 2). Values within this range have been obtained by several workers (Gottfried, 1967; Karaca and Stefanini, 1966; Reed et al., 1960; Smith, 1965; Ways and Hanahan, 1964) who used similar methods of analysis. Karaca and Stefanini (1966) reported that the lipid content of red cells was 1.26% of the dry weight. Calculations from their data indicate that 1 ml packed red cells was equivalent to 0.40-0.41 mg dry solids.

According to Smith (1965), 61% of the red cell lipid was phospholipid. Similar percentages may be computed from the data of other investigators (Table 2) who reported lipid phosphorus concentrations ranging from 11.5 to 14.4 mg/100 ml packed cells. These values correspond to 287.5-360.0 mg phospholipid per 100 ml (lipid P x 25). Karaca and Stefanini (1966) found that lipid phosphorus

Table 2. Normal fasting values for erythrocyte lipids and phospholipids calculated from the data of other investigators.

Reference	Separation Method	Subjects	Total Lipid	Lipid P	Distribution of Lipid Phosphorus							
					Sph	PC	LLec	PE	PS	PI	CL	Other
		N	mg/100ml	mg/100ml	%	%	%	%	%	%	%	%
Gottfried, 1967	TLC	7	526	12.6	26.0	29.2	--	29.3	←14.2→		0.9	0.4
Karaca & Stefanini, 1966	Paper	10	507 (1.26)[a]	12.3 (0.03)[a]	18.6	29.6	3.8	25.6	20.0	7.5	--	--
Reed et al., 1960	Paper	20	604	13.7	22.0	30.0	2.5	24.6	14.9	4.0	--	2.0
Smith, 1966	Direct	16	555	13.5								
Ways & Hanahan, 1964	Column	18	476	11.5	23.8	29.5	--	25.7	15.0	2.2	--	3.3
Betschart, 1966	TLC	8			31	38[b]	3	25	[b]	--	--	4
Phillips & Roome, 1959	Column	6		14.4	22.8	32.9	1.9	←42.5→			--	--

[a] Percent of dry weight.

[b] PC and PS reported as PC.

constituted 0.03% of the dry weight of the erythrocyte.

As in the analysis of plasma phosphatides, several methods of separation have been used to characterize the red cell phospholipids. The methods include silicic acid-impregnated paper, thin layer chromatography, and column chromatography. Except for Sph, only minor differences appeared among the values obtained by the various methods (Table 2). The major constituents of the phospholipid fraction were: PC 29-33%, PE 25-31%, PS 15-20%, and Sph 19-31%. Low concentrations of PI, LLec, and CL were reported by some, but not all, investigators.

Total lipids and phospholipids in leukocytes. In an early study, Boyd (1933) observed that the lipid content of the "buffy coat" ranged from 4.57 to 11.68 mg/g, wet weight. More recently, an average concentration of 30.51 mg lipid per gram of packed white cells was found by Smith (1965). Gottfried (1967) calculated the lipid concentration to be 1.56×10^{-8} mg per cell. Data concerning the lipid content of leukocytes are assembed in Table 3.

Approximately two thirds of the white cell lipid components reported by Smith (1965) were phospholipid. Gottfried (1967), and also Boyd (1933), obtained somewhat lower ratios of phospholipid to total lipid--45% and 47%, respectively. The major phospholipids in white cells are: PC 40-46%, PE 18-31%, and Sph 7-18%. Smaller quantities of PI, PS, LLec, PA, and CL have been identified

Table 3. Normal fasting values for leukocyte lipids and phospholipids, calculated from the data of other investigators.

Reference	Separation Method	Subject	Total Lipid	Lipid P	Distribution of Lipid Phosphorus								
					Sph	PC	LLec	PE	PS	PI	CL	PA	Other
		N	mg/g wet	mg/g wet	%	%	%	%	%	%	%	%	%
Boyd, 1933	Direct	8	17.10	0.32									
Smith, 1965	Direct	16	30.51	0.81									
Gottfried, 1967	TLC	4	(1.56×10^{-8})[a]	(2.79×10^{-13})[a]	12.2	39.4	--	30.8	14.2[b]	[b]	2.5	--	0.9
Betschart, 1966	TLC	8			18	55[c]	5	18	[c]		--	--	5
Firkin and Williams, 1961[d]	Column & Paper	5			10	42	--	29	9	6	--	4	--

[a] mg/cell.

[b] PS and PI reported as PS.

[c] PC and PS reported as PC.

[d] Leukemic subjects.

(Betshcart, 1966; Firkin and Williams, 1961; Gottfried, 1967). The distribution of lipid phosphorus in leukemic cells (Firkin and Williams, 1961) was similar to that found by Gottfried (1967) in normal cells.

Total lipids and phospholipids in platelets. Several conventions have been used for reporting the lipid content of platelets. Smith (1965) found a concentration of 25.87 mg/g, wet weight. On a dry weight basis, approximately 25% of the platelets has been shown to be lipid (Karaca and Stefanini, 1966; Woodside and Kocholaty, 1960). Karaca and Stefanini (1966) noted that this figure was equivalent to 38.7 mg total lipid per milliliter, packed volume.

Values reported for lipid phosphorus and the distribution of phosphorus among phospholipids are shown in Table 4. The phospholipids represent about 60-70% of the lipid material. Approximately 0.6% of the dry weight of platelets was lipid phosphorus (Karaca and Stefanini, 1966; Woodside and Kocholaty, 1960). The phospholipids contained about 28-44% PC, 20-24% PE, 17-19% Sph, and 8-19% PS (Betschart, 1966; Iacono, Zellner and Malott, 1966; Troup et al., 1960). Small amounts of PI and LLec were identified by some, but not all, investigators.

Factors Which May Influence Lipid and Phospholipid Concentrations

Age and sex. According to Lindholm (1966), who studied

Table 4. Normal fasting values for platelet lipids and phospholipids calculated from the data of other investigators.

Reference	Separation Method	Subject	Total Lipid	Lipid P	Distribution of Lipid Phosphorus						
					Sph	PC	LLec	PE	PS	PI	Other
		N			%	%	%	%	%	%	%
Smith, 1965	Direct	16	25.87 mg/g wet	0.66 mg/g wet							
Karaca & Stefanini, 1966	Column & Paper	--	38.7 mg/ml 25.94% dry wt.	0.834 mg/ml 0.56% dry wt.	17.71	28.15	3.63	24.38	19.01	7.11	--
Woodside & Kocholaty, 1960	Column	5	24.7% dry wt.	0.64% dry wt.							
Iacono et al., 1966	--	24			19	32	--	20	14	10	12
Betschart, 1966	TLC	8			17	52[a]	5	21	[a]	--	5
Troup et al., 1960	Paper & Column	10			17.8	43.8	--	23.3	8.2	6.9	--

[a]PS and PC reported as PC.

nearly 200 Swedish men and women between the ages of 20 and 91, the serum lipids of both men and women increased gradually from age 20 to age 50. After that time the lipid concentrations remained relatively constant in men but continued to increase with age in women. As a result, the sex-related differences in serum lipids after age 50 were statistically significant. The phospholipids followed a pattern similar to that of the total lipids. In a study of 1200 males and females between the ages of two and seventy-seven years, Adlersberg et al. (1956) also found that the phospholipid concentration of serum was influenced by age and sex. In males the phospholipid levels were constant until age 20, increased gradually until age 33, and finally remained constant between ages 33 and 60. In females the pattern was noticeably different; the phospholipid concentrations did not change until age 33; they subsequently increased steadily through age 58. Hallberg et al. (1966, 1967) compared the plasma phospholipids of two groups of Swedish women, aged 23 and 48 years. Although the phospholipid concentrations were greater in the older women, the distribution of individual phospholipids was the same in the two groups.

No differences in the content or distribution of lipids in erythrocytes could be attributed to sex or age, according to a study by Reed et al. (1960). The subjects included at least one male and one female in each age decade from 20 to 70 years. The authors

stated that comparable concentrations had been observed in erythrocytes of children aged six to twelve years.

In a study of 24 healthy subjects between the ages of 23 and 66 years, Iacono et al. (1966) observed a relationship between platelet lipids and age. Concentrations of total lipid ranged from 34% of dry weight for the younger subjects to 18% for the older subjects. The amount of phospholipid, as percentage of total lipid, and the distribution of individual phospholipids were not affected by age.

Dietary carbohydrate. The effect of dietary components upon the concentration of blood lipids in humans has been the subject of numerous investigations. It appears that protein, when consumed in amounts sufficient to meet normal daily needs, does not greatly influence lipid metabolism (Macdonald, 1966c). Similarly, Keys (1967) stated that variations of protein, vitamins, and minerals, within the range of natural diets, have not been shown to affect serum cholesterol levels. On the other hand, the amounts and types of dietary lipid may modify the lipid constituents of the blood. It was observed, for example, that serum cholesterol concentrations rose when the amount of cholesterol in the diet was increased (Anderson et al., 1962). Large amounts of saturated fatty acids in the diet also produced higher serum cholesterol concentrations, while polyunsaturated fatty acids had the opposite effect (Anderson, Keys, and Grande, 1957). Lowering the amount of total fat in the diet has been shown to

cause a decrease in serum cholesterol concentration (Anderson et al., 1957).

The blood lipids are also affected by changes in the amounts and types of dietary carbohydrate. Antonis and Bersohn (1961) found that a diet which was low in fat and high in carbohydrate induced a hypertriglyceridemia which persisted for several months. Anderson et al. (1963) reported that subjects who received sucrose in place of part of their dietary fat responded with an increase in serum triglycerides and a moderate decrease in serum cholesterol.

The response of normolipemic subjects to isocaloric interchanges between sugar and starch has been studied more recently. Macdonald and Braithwaite (1964) fed a low-fat diet which provided 500 g/day of either sucrose or raw maize starch to young men for 25-day periods. The sucrose diet produced higher serum concentrations of total lipids, triglycerides, and cholesterol, and the starch diet effected a decrease in total lipids, phospholipids, and cholesterol. A similar response was not observed in women: both forms of carbohydrate induced a decrease in total lipids, phospholipids, and cholesterol in the serum of young women (Macdonald, 1965) and of postmenopausal women (Macdonald, 1966b). The concentration of glycerides in the serum of young women was lowered by the sucrose diet. In the older women the same fraction was increased by both regimens. Antar and Ohlson (1965) confirmed the lipid-elevating

effect of sucrose in young men. Serum phospholipids and total lipids rose when diets containing 80% of the carbohydrate and 35% of the total calories as sucrose were fed to eight young men and women for 28 days. A decrease of similar magnitude was observed when starch was substituted isocalorically for the sugar in the diets. Both changes were less pronounced in the women than in the men. On the other hand, Irwin et al. (1964) were unable to establish a relationship between the concentration of blood phospholipid or cholesterol and consumption of either rice or sucrose.

Antar and Ohlson (1965) found that feeding sugar to young men and women effected a rise in the PE and Sph fractions of serum phospholipids. These changes were countered by a decrease in the PC:total phospholipid ratio. The opposite trend was observed when starch was substituted isocalorically for sugar.

The response of hyperlipemic subjects to the manipulation of dietary carbohydrate was similar to that of normal individuals, but even more pronounced. Serum and plasma triglyceride concentrations increased with the consumption of sucrose and decreased when sugar was replaced by starch (Kaufmann et al., 1966; Kuo et al., 1967). Cholesterol and phospholipid levels changed similarly. Rifkind et al. (1966) reported that a temporary depression of serum cholesterol and phospholipids was induced by sugar restriction, but levels rose again within ten weeks of the beginning of the study.

Some workers believe that the fructose portion of the sucrose molecule may be responsible for the elevation of blood lipids. Kaufmann et al. (1967) observed that triglyceride levels in hypertriglyceridemic subjects were greatest when fructose, rather than glucose, sucrose, or starch was fed. The most extreme responses were observed in the individuals with the highest concentration of blood lipids. Macdonald (1966a) reported that dietary fructose appeared to be associated with elevated triglyceride concentrations in the serum of normolipemic individuals.

Phospholipids and Blood Coagulation

It is generally recognized that platelet phospholipids have a role in thrombus formation, but the mechanism of their action has not been completely resolved. Marcus (1966) has recently summarized some of the current concepts of the function of lipids in blood coagulation. The platelet phospholipids, along with calcium and certain plasma proteins, participate in the formation of a prothrombin activator. The fact that not all phosphatides are equally effective in accelerating clot formation (Grisdale and Okany, 1965; Marcus and Spaet, 1958; Rouser and Schloredt, 1958; Rouser, White and Schloredt, 1958; Woodside, Therriault and Kocholaty, 1964) may be the result of differences in molecular configuration, surface charge, or particle size (Marcus, 1966). Rouser et al.

(1958) proposed that the presence of a proton donor group may be necessary for a phosphatide to possess coagulant activity. The existence of both polar and nonpolar areas in the phosphatide molecule may be responsible for the formation of large proteolipid micelles which could function in clot development (Marcus, 1966). The formation of such complexes may occur more readily with phosphatides which contain unsaturated fatty acids; Rouser and Schloredt (1958) noted that the clot-promoting activity of certain phosphatides appeared to increase with the degree of unsaturation of the constituent fatty acids.

In vitro coagulation tests have been employed in an attempt to identify the phosphatides which are most influential in clot formation. According to Marcus and Spaet (1958) PS gave as much clotting activity as the entire crude lipid extract from platelets, although the potency of PS decreased at high concentrations. The thromboplastic activity of PS has been confirmed by other investigators (Marcus and Zucker-Franklin, 1965; Troup et al., 1961; Woodside et al., 1964). PE has also been reported to be active in clot promotion (Rouser and Schloredt, 1958; Rouser et al., 1958; Silver, 1965; Woodside et al., 1964). Grisdale and Okany (1965) observed that PS had no thromboplastic activity unless either PE or lysophosphatidyl ethanolamine was added to it. It was found by Troup et al. (1961) that PC markedly enhanced the thromboplastic activity of PS. They also noted

that PE alone did not accelerate clot formation but it did show activity when combined with either PC or Sph. Therriault, Nichols and Jensen (1958) also found that combinations of PC and PS possessed thromboplastic activity even though the individual components did not.

Increased PE levels and decreased PC levels in the serum of human subjects have been associated with shorter in vitro coagulation times (Antar, Ohlson and Hodges, 1964). Changes in proportions of PI, LLec, and Sph had little effect on thrombus formation.

EXPERIMENTAL PROCEDURE

Design of Experiment

Subjects. Three women served as subjects for this study. All were laboratory personnel who appeared to be healthy and were of average weight for height. A description of the subjects is given in Table 5. Subject III was receiving thyroid and estrogenic hormones regularly. Subject I was treated briefly with an antibiotic for an infection not connected with the study.

Table 5. Age, height, weight, and caloric requirement for subjects participating in diet study.

Subject	Age yr	Height in	Weight lb	Caloric Requirement kcal
I	53	64	120	1600
II	22	60	101	1600
III	22	62	129	1800

The caloric requirement of the subjects was estimated on the basis of body size. Diets were then designed with the goal of maintaining usual body weight; subjects were weighed weekly.

Diet. All diets provided approximately 16% of the calories as protein, 40% as fat, and 44% as carbohydrate. A

pre-experimental diet (Control Diet) containing both simple and complex sources of carbohydrate was given initially to all subjects to determine if estimates of caloric requirement were accurate, and to minimize differences among previous diets of the subjects. The experimental diets provided 80% of the carbohydrate as either sucrose (Sugar Diet) or as bread, cereal, crackers, and potatoes (Complex Diet). The experimental diet plans, shown in Table 6, were calculated according to the food exchange lists (Caso, 1950). Nutrients were distributed among three meals and an evening snack.

Table 6. Experimental diet plans.

	Complex			Sugar		
	Pro. g	Fat g	Carb. g	Pro. g	Fat g	Carb. 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

There were four dietary periods, six days in length. Each subject served as her own control by receiving both experimental diets, repeating the diet of Period 2 during Period 4 (Table 7).

Table 7. Design of diet study.

Subject	Diet			
	Period 1	Period 2	Period 3	Period 4
I	Control	Complex	Sugar	Complex
II	Control	Complex	Sugar	Complex
III	Control	Sugar	Complex	Sugar

Although each subject prepared her own diet, most of the foodstuffs were purchased from the same source. Meat and fish were purchased in advance, weighed, packaged and frozen. They were prepared without additional fat. The same brands of bread, cereal products, potato flakes, margarine, and salad oil were used. All eggs were hard cooked. Freedom of choice was allowed in the selection of fruits and green vegetables. Size of servings was determined by using the exchange list figures and foods were weighed on a dietetic scale. A tablet containing 0.25 g methylcellulose was administered with each meal of the Sugar Diet. Menus for experimental diets are shown in Appendix Tables I and II.

A glucose tolerance test to be reported in another study was

performed on the first morning after each dietary period. Following these tests a breakfast of milk and egg was eaten.

Methods of Blood Analysis

Collection and preparation of blood samples. Blood samples were collected in 10 ml vacutainers which had been previously treated with 12 mg EDTA (ethylenediaminetetraacetic acid). Approximately 45 ml of blood were drawn from each subject before breakfast on the last day of each dietary period. One vacutainer of blood from each subject was reserved for clotting time tests and plasma analyses. This blood was centrifuged at 50 g for 10 minutes. Approximately two milliliters of platelet-rich plasma were removed for clotting time tests and the remainder was centrifuged at 1300 g for 10 minutes. The plasma was drawn off and stored at -10°C.

The remainder of the whole blood was transferred to centrifuge tubes and 10% Triton (oxyethylated tertiary octyl phenol formaldehyde polymer) solution was added in the amount of 11% of the volume of blood. Platelets, white cells, and red cells were separated by differential centrifugation according to the procedure of Smith (1965). Isolated cells were weighed. The following day they were lyophilized, weighed, and stored at -10°C.

Clotting time. Clotting time of fresh platelet-rich plasma

was determined according to the Howell method described by Davidsohn and Wells (1962). According to this procedure, the clotting time is the time required for plasma samples to coagulate following the restoration of calcium. The endpoint was taken when the tubes could be inverted. Siliconized glassware was not used.

Extraction of lipids. The extraction procedure of Reed et al. (1960) was adapted for use with plasma and cells. All solvents were purchased as reagent grade and spectroanalyzed.

One milliliter of thawed plasma was pipetted into a 35-ml graduated centrifuge tube. To it were added 5 ml of methanol followed by 5 ml chloroform. The contents of the tube were agitated after each addition. The tube was placed in a water bath at 62 $\pm$ 2°C for 2 minutes, removed, and allowed to stand at room temperature for 8 minutes. The mixture was centrifuged at 2800 rpm (1300 g) for 10 minutes. The supernatant was decanted into another 35-ml centrifuge tube for evaporation under a stream of nitrogen. The extraction was repeated three times using 5 ml of methanol and 5 ml of chloroform each time. Supernatants were combined and evaporation was continued 15 minutes after the sample appeared to be dry.

The dried residue from the supernatants was reextracted three times with 3-ml amounts of chloroform. The chloroform extract was filtered through a fritted glass filter and evaporated to a small volume which was transferred quantitatively to a 2-ml tared

volumetric flask. The extract was evaporated to dryness under nitrogen, then held in a desiccator until a constant weight was obtained. Dried extracts were stored at -10°C.

Lyophilized erythrocytes were reconstituted and diluted prior to extraction. Approximately 0.12 g lyophilized red cells were weighed and transferred to a 35-ml graduated centrifuge tube. One milliliter of redistilled water was added without agitation. The tube was capped with parafilm and placed in the refrigerator overnight. The erythrocyte lipids were extracted according to the procedure used for plasma, except that heat was not applied. The final extract was collected in a 2-ml volumetric flask, dried, weighed, and stored as described above.

In order to obtain maximal extraction of lipids from lyophilized white cells and platelets, it was necessary to rupture the cells. The sample, weighing about 1.5 to 6 mg, was placed in a tissue grinder with at least 15 μl redistilled water per milligram dry cells (minimum amount, 50 μl). It was ground for 5 minutes, capped with parafilm, frozen for a minimum of 3-4 hours, and thawed. This procedure was repeated twice. One-half milliliter each of methanol and chloroform were added to the wet cells. The mixture was ground for 5 minutes and quantitatively transferred to a 35-ml centrifuge tube with the aid of 1 ml each of methanol and chloroform. The sample was centrifuged at 2800 rpm (1300 g) for 10 minutes. The

supernatant was decanted into another centrifuge tube and evaporated under nitrogen.

The precipitate was extracted three additional times. Each time the residue was mixed with 1 ml each of methanol and chloroform, warmed in a water bath at 62 $\pm$ 2°C for 5 minutes, held at room temperature for an additional 5 minutes, and centrifuged as above. The supernatants were combined and evaporated to dryness.

The dried residue from the supernatants was reextracted with three 3-ml aliquots of chloroform, filtered through a fritted glass filter, evaporated to a small volume, and transferred to a tared 1-ml volumetric flask. The extract was dried, weighed, and stored as described above.

Separation of phospholipids. The phospholipid fractions were isolated by thin layer chromatography of the total lipid extracts. Chromatography jars were fully-lined with filter paper. At least six runs could be performed without a change of liner if the freshly-lined tanks were first equilibrated with the solvent system and then dried before use. The solvent system chosen for developing the chromatograms consisted of chloroform:methanol:glacial acetic acid:water in the proportions 70:20:6:3 v/v/v/v. This system was devised to allow clear resolution of six phosphatides from a lipid mixture without prior removal of non-phospholipids.

Glass plates[2], 5 x 20 cm, precoated with Silica Gel H to a thickness of 250 μ were activated for 10 minutes at 110° C. The dried lipid extracts, which had been stored in volumetric flasks, were diluted to volume with chloroform. Lipid extracts, in amounts corresponding to 1.5-2 μg phosphorus were applied to the chromatoplates by means of a gas-tight Hamilton syringe (100 μl in 1 μl). The amounts chosen for separation on triplicate plates were 50 μl lipid extract from plasma, 100 μl extract from red cells, and 200 μl extract from platelets or white cells. In order to assess the extent of recovery, three 25-μl aliquots of each extract were applied to a plate but were not chromatographed. The plate had been previously exposed to the solvent.

The atmosphere of the chromatography jars was equilibrated for one hour with 99 ml of the solvent system prior to introduction of the plates. The solvent was allowed to ascend to 16 cm above the point of application. Plates were air dried and the phosphatides were visualized in iodine vapor. Spots were outlined, identified by comparison with position of standards, and collected with a spot collector.[2]

Phosphatides were eluted from the silicic acid with 10 ml methanol. Eluates were evaporated in a water bath until nearly dry

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[2] Brinkmann Instruments Inc., Westbury, N. Y.

and transferred, with rinsing, to 7 x 70 mm tubes for phosphorus determination. Evaporation was completed under a stream of nitrogen in the presence of warm air.

Phosphorus determination. The concentration of phospholipids was measured in terms of the amount of lipid phosphorus found in the samples (phospholipid = lipid P x 25). Phosphorus analyses were done by the procedure of Hawthorne, Smith, and Pescador (1963) which was adapted from the method of Lowry et al. (1954). The method is based on the photometric determination of molybdenum blue produced by the reduction of phosphomolybdic acid with ascorbic acid. The only modification in the procedure was the addition of approximately 50 µl of 30% peroxide since the perchloric acid-sulfuric acid mixture did not completely oxidize all organic materials. The samples were returned to the 165°C oven for an additional 3 hours before treatment with color reagent.

Lipid phosphorus was estimated by comparing samples to standards and multiplying by appropriate dilution factors. Values for samples eluted from TLC plates were corrected for silica blank readings. Total lipid phosphorus was expressed as µg P/ml plasma or µg P/mg dry weight of cells.

RESULTS AND DISCUSSION

Reliability of Data

The results of the lipid extractions were not completely satisfactory. The total lipid concentrations of four plasma samples and some of the platelet and leukocyte samples were much greater than any comparable observed values. The excessive weights may be attributed to accidental contamination of the chloroform which was used in extraction. It was discovered that the chloroform, which had been stored in a polyethylene wash bottle, left a greasy residue upon evaporation. Apparently, in the heated environment, some of the plastic material had dissolved in the solvent. Although no platelet or leukocyte samples were available for reextraction, it was possible to obtain an estimate of the total lipids in the plasma samples. Aliquots of the plasma samples had been extracted by Chiu (1968) who used chloroform:methanol, 2:1 v/v, as the extraction solvent. It had been established that the two extraction procedures gave comparable weights for plasma lipids. Therefore, the total lipid values of Chiu have been substituted in the tabular results for the unreliable plasma values.

The solvent system employed for TLC successfully separated six phosphatides which appeared on the plate in ascending order as follows: LLec, Sph, PS, PC, PE, and PA. Preliminary

experiments had shown that nonphospholipids were concentrated in the solvent front. Small amounts of unidentified phosphorus were found at the solvent front and at the point of application. Although standard solutions of PA and CL had the same rate of migration, the spot found in this position was identified as PA on the basis of its characteristic shape. The R_f values of the PS and PI standard solutions were the same but the spot in this position was assumed to be PS. In preliminary studies of the phospholipids in plasma and cells no PI was detected by ammoniacal silver nitrate spray. The average recovery of phosphorus from the plates was 74% of the unseparated samples. Much of the unrecovered phosphorus was found in the unstained areas between the spots. The individual phosphatide fractions are reported as percentage of recovered lipid phosphorus, in accordance with the custom of other investigators.

Total Lipids and Phospholipids of Plasma

The total lipid, lipid phosphorus, and phospholipid fractions of the plasma samples are shown in Table 8. Highest concentrations of lipid and lipid phosphorus were consistently found in the plasma of Subject I. This observation is in agreement with the reports of Adlersberg et al. (1956), Hallberg et al. (1966), and Lindholm (1966), who reported that plasma total lipids and phospholipids of women generally increase with age. Dietary treatment did not appear

Table 8. Total lipids and distribution of phospholipids in plasma following experimental diets.

Subject	Diet	Total Lipid	Lipid P	Distribution of Lipid P							
				Sph	PC	LLec	PE	PS	PA	Unidentified S. F.[a]	Or.[b]
		mg/100ml	mg/100ml	%	%	%	%	%	%	%	%
I	Control	741	9.0	20	67	10	3	-	-	0	0
	Complex	761	9.1	20	64	8	1	3	2	2	1
	Sugar	719[c]	7.6	17	69	13	-	-	-	1	0
	Complex	666[c]	8.1	23	53	19	2	-	-	1	1
	Average	722	8.4	20	63	12	2	1	0	1	0
II	Control	596	6.4	19	61	18	-	-	-	1	1
	Complex	574	6.5	13	73	12	-	-	-	2	0
	Sugar	580[c]	7.8	20	64	12	-	-	-	1	3
	Complex	617	6.0	14	62	17	-	-	-	1	5
	Average	592	6.7	16	65	15	-	-	-	1	2
III	Control	502	4.2	17	64	18	-	-	-	0	0
	Sugar	540	5.3	15	71	12	-	-	-	1	1
	Complex	510[c]	5.1	18	66	13	-	-	-	1	2
	Sugar	550	6.7	16	69	14	-	-	-	0	1
	Average	526	5.3	16	68	14	-	-	-	0	1
Average	Control	613	6.5	19	64	15	1	0	0	0	0
	Complex	626	7.0	18	64	14	1	0	0	1	2
	Sugar	597	6.8	17	68	13	0	0	0	1	1
	All	613	6.8	18	65	14	0	0	0	1	1

[a] Solvent front

[b] Origin

[c] Data of Chiu

to influence the total lipid or lipid phosphorus in any consistent manner. The response to diet varied within and among subjects but the treatment averages were remarkably similar. It should be noted that the total lipids are comprised of various individual fractions which may change independently (Macdonald, 1966c) , and a change in any one fraction may offset an opposite change in another. Nevertheless, Antar and Ohlson (1965) did observe an increase in both total lipids and phospholipids of the plasma when diets similar to the Sugar Diet of this experiment were fed to young men and women for 28 days. They also noted the opposite effect of a regimen comparable to the Complex Diet. The dietary periods of the current study may have been too short to produce noticeable changes.

The major phospholipids identified in the plasma were PC, Sph, and LLec. This confirmed the findings of other investigators (Table 1). Slight differences in the distribution of the phosphatides were observed among subjects: PE, PS, and PA were identified in samples from Subject I, but not from the other two subjects; the PC fraction was slightly lower and the Sph fraction slightly higher in the plasma of Subject I. Hallberg et al. (1967) did not find a difference in phosphatide distribution with age. The dietary treatment did not greatly influence the distribution of plasma phospholipids. There was a slight decrease in the average Sph fraction and a slight increase in the PC fraction following the Sugar Diet. This

observation is not in accord with the report of Antar and Ohlson (1965) that diets high in sugar increased the proportions of PE and Sph and decreased the proportions of PC. Again, the differences in results from the two experiments may be explained by the differences in length of the dietary periods.

Total Lipids and Phospholipids of Erythrocytes

The total lipid and lipid phosphorus content of lyophilized erythrocytes, presented in Table 9, agree well with the data reported by Karaca and Stefanini (1966). The total lipid concentration was notably higher in the red cells of Subject II. This appeared to be caused by a response to the Complex Diet, but no dietary effect was observed in the red cells of the other subjects. The lipid phosphorus content of erythrocytes was quite similar for all subjects and showed no consistent relationship to dietary treatments.

The chief phosphatides found in the red cells were Sph and PC. The PE and PS fractions were considerably smaller than those reported by other investigators (Table 2). It is possible that the methods used in this study did not completely extract the cephalins. Slight differences in the distribution of the phosphatides were noted among subjects: there was a smaller proportion of PC in the erythrocytes of Subject II and a greater percentage of PE in the cells of Subject III. The average distribution of phospholipids in the

Table 9. Total lipids and distribution of phospholipids in erythrocytes following experimental diets.

Subject	Diet	Total Lipid	Lipid P	Distribution of Lipid P							
				Sph	PC	LLec	PE	PS	PA	Unidentified S. F.[a]	Or.[b]
		% dry wt.	% dry wt.	%	%	%	%	%	%	%	%
I	Control	2.2	0.042	33	29	7	6	10	2	3	9
	Complex	1.7	0.032	38	30	8	2	6	2	2	12
	Sugar	2.4	0.038	28	36	7	5	8	2	1	12
	Complex	2.1	0.039	31	35	5	6	7	10	1	6
	Average	2.1	0.038	32	32	7	5	8	4	2	10
II	Control	2.1	0.031	43	13	8	5	10	3	1	18
	Complex	3.5	0.057	28	25	7	5	9	2	0	24
	Sugar	2.5	0.032	23	31	9	8	10	5	1	13
	Complex	4.9	0.038	33	28	10	5	10	4	1	10
	Average	3.2	0.040	32	24	8	6	10	4	1	16
III	Control	2.2	0.036	29	39	3	11	9	2	1	6
	Sugar	2.0	0.036	29	30	4	12	12	5	2	6
	Complex	1.9	0.042	33	30	5	11	11	4	1	6
	Sugar	1.5	0.026	33	29	8	5	8	-	3	13
	Average	1.9	0.035	31	32	5	10	10	3	2	8
Average	Control	2.2	0.036	35	27	6	7	10	2	1	11
	Complex	2.8	0.042	31	30	7	6	9	4	1	12
	Sugar	2.1	0.033	28	32	7	8	10	3	2	11
	All	2.4	0.038	31	31	7	7	9	3	1	11

[a] Solvent front

[b] Origin

erythrocytes was similar following both experimental diets.

Total Lipids and Phospholipids of Leukocytes

The total lipid, lipid phosphorus, and phospholipid fractions of lyophilized leukocytes are reported in Table 10. Total lipids comprised 35-40% of the dry weight of the white cells. Variable results were obtained for the lipid phosphorus; on an average, the phosphorus constituted 0.29% of the dry weight of cells. The consumption of different types of carbohydrate did not appear to influence the lipid phosphorus concentration of the leukocytes.

The major phospholipids identified in white cells were PC, PE, PS, and Sph. The average proportions of these phosphatides are in accord with the observations of other investigators (Table 3). The distribution of lipid phosphorus appeared to be unaffected by age and only slightly influenced by diet. A reduction in the PC fraction occurred after the Sugar Diet; an increase in the PS fraction followed the Complex Diet.

Total Lipids and Phospholipids of Platelets

Lyophilized platelets contained, on the average, 34% total lipids and 0.34% lipid phosphorus. The data for platelets are tabulated in Table 11. Karaca and Stefanini (1966) had found 26% total lipids and 0.56% lipid phosphorus in lyophilized platelets. In the

Table 10. Total lipids and distribution of phospholipids in leukocytes following experimental diets.

Subject	Diet	Total Lipid	Lipid P	Distribution of Lipid P							
				Sph	PC	LLec	PE	P5	PA	Unidentified S. F. [a]	Or. [b]
		% dry wt.	% dry wt.	%	%	%	%	%	%	%	%
I	Control	47	[c]	11	40	4	14	17	-	11	4
	Complex	36	0.23	11	38	6	20	18	-	4	4
	5ugar	31	0.28	23	36	5	16	15	-	4	2
	Complex	38	0.33	17	42	3	16	13	-	5	4
	Average	38	0.28	16	39	4	16	16	-	6	4
II	Control	[c]	0.37	21	43	5	7	13	-	1	10
	Complex	27	0.23	11	40	2	22	17	-	5	3
	Sugar	[c]	0.38	17	43	4	16	15	-	4	2
	Complex	22	0.16	18	43	4	15	14	-	4	2
	Average		0.28	17	42	4	15	15	-	4	4
III	Control	[c]	0.17	14	42	6	9	13	-	13	3
	5ugar	58	0.40	20	35	2	24	13	2	3	1
	Complex	[c]	0.42	11	35	5	17	22	-	9	1
	Sugar	[c]	0.19	2	28	9	13	17	-	27	4
	Average		0.30	12	35	6	16	16	0	13	2
Average	Control		0.27	15	42	5	10	14	-	8	6
	Complex		0.27	14	40	4	18	17	-	5	3
	Sugar		0.31	16	36	5	17	15	0	10	2
	All		0.29	15	39	5	16	16	0	8	3

[a] Solvent front

[b] Origin

[c] Unreliable data

Table 11. Total lipids and distribution of phospholipids in platelets following experimental diets.

Subject	Diet	Total Lipid	Lipid P	Distribution of Lipid P							
				Sph	PC	LLec	PE	PS	PA	Unidentified S. F.[a]	Or.[b]
		% dry wt.	% dry wt.	%	%	%	%	%	%	%	%
I	Control	16	0.18	19	37	6	17	14	-	2	4
	Complex	29	0.16	19	38	6	11	14	-	9	3
	Sugar	29	0.39	16	41	3	21	15	-	3	2
	Complex	46	0.42	15	44	3	17	16	-	3	2
	Average	30	0.29	17	40	4	16	15	-	4	3
II	Control	55	0.25	15	39	4[d]	15	16	-	12	[d]
	Complex	[c]	0.16	20	28	4	16	8	-	18	6
	Sugar	[c]	[c]	15	42	5	16	17	-	3	2
	Complex	[c]	0.47[c]	15	44	4	14	20	-	2	1
	Average	-	0.29	16	38	4	15	15	-	9	3
III	Control	24	0.15	12	37	2	18	20	2	3	4
	Sugar	40	0.26	10	40	5	9	13	8	10	5
	Complex	40	0.45	22	40	3	19	12	-	2	1
	Sugar	26	0.43	22	40	3	18	14	-	1	2
	Average	32	0.32	16	39	3	16	15	2	4	3
Average	Control	-	0.19	15	37	4	17	17	1	6	3
	Complex	-	0.33	18	39	4	15	14	0	7	3
	Sugar	-	0.36	16	41	4	16	15	2	4	3
	All	-	0.30	17	39	4	16	15	1	6	3

[a]Solvent front

[b]Origin

[c]Unreliable data

[d]LLec and Or reported as LLec

present study, platelet lipids varied among subjects but, in contrast to the report of Iacono et al. (1966), no relationship between age and lipid concentration was observed.

The lipid phosphorus of platelets was distributed as PC, PE, PS, Sph, and LLec in proportions similar to those observed by other workers (Table 4). The PC fraction of the platelet phospholipids was slightly greater after the Sugar Diet than after the Complex Diet; the Sph fraction was slightly smaller.

Clotting Time of Platelet-Rich Plasma

The data from the clotting time experiments are shown in Table 12. Subject II demonstrated a marked increase in coagulation time after the Complex Diet periods. No consistent relationships between dietary treatment and clotting time of plasma could be found for the other two subjects. The clotting time did not appear to be influenced by the lipid concentration or by the phospholipid distribution in any of the blood fractions.

Effects of Dietary Carbohydrate

The form of dietary carbohydrate produced no definite modification in the total lipid or lipid phosphorus content of plasma or cells. Plasma triglycerides of two of the subjects showed a small increase following the Sugar Diet (Chiu, 1968). No consistent

Table 12. Coagulation time, in seconds, of platelet-rich plasma following experimental diets.

Subject	Diet			
	Control	Complex	Sugar	Average
I	147.5	129.2[a] 174.0[b]	131.0	145.4
II	143.3	204.0[a] 207.0[b]	140.0	173.6
III	147.5	137.0	160.8[a] 136.0[b]	145.3
Average	146.2	170.2	142.0	

[a]Period 2

[b]Period 4

changes were found in plasma cholesterol (Chiu, 1968). The Sugar Diet, compared to the Complex Diet, induced slightly greater proportions of PC and slightly smaller proportions of Sph in plasma and platelets. A decrease in the PC fraction of leukocyte phospholipids followed the Sugar Diet.

It has been shown (Marks, Gellhorn, and Kidson, 1960), that circulating leukocytes and platelets, but not mature erythrocytes, synthesize neutral lipids and phospholipids from acetate within a period of hours. Within a very short time, the newly-synthesized lipids appeared in the plasma, neutral lipids being exchanged more rapidly than phospholipids. Therefore, it would be expected that some changes in plasma and cell lipids would have been seen if the source of dietary carbohydrate were responsible for an altered lipid synthesis. The failure to observe pronounced changes in total lipids or phospholipids as a result of the high sugar diet may be due, in part, to the short duration of the regimen in this study. Marked elevations in total lipids and triglycerides of plasma (Macdonald and Braithwaite, 1964), as well as shifts in the proportions of phospholipids (Antar and Ohlson, 1965), have occurred when high sugar diets have been consumed for periods of several weeks. It would appear that any changes in lipid metabolism, induced by sugar consumption, must involve a shift in metabolic pathways and an adjustment of enzyme systems, perhaps as a result of hormonal stimuli.

The results of the present study suggest that such adaptations may require more than a week to become established.

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APPENDIX

Table 1. Daily menu plan for Complex Diet (1800 Calories).

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

Table I. (Continued)

Day	Breakfast		Lunch		Dinner		Evening	
		grams		grams		grams		grams
6	Puffed Rice	25	Roast Beef	60	Turkey	60	Milk	240
	Milk	240	Tomato & Lettuce	(1 serv.)	Vegetable	(1 serv.)	Fruit	(1 serv.)
	Egg	50	Cooked Potato	100	Cooked Rice	100	Bread	50
	Bread	25	Bread	50	Bread	50	Fat	5
	Fat	10	Fat	10	Fat	10		

[a] Weights of individual fruits according to exchange list figures (Caso, 1950)

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

Table II. Daily menu plan for Sugar Diet (1800 Calories).[a]

Day	Breakfast	grams	Lunch	grams	Dinner	grams	Evening	grams
1	Glucose Tolerance Test		Ham	90	Cooked Fish	90	Milk	240
	(1.5 g/kg body wt.)		Tossed Salad	(1 serv.)[b]	Vegetable	(1 serv.)	Sugar	40
	Milk	240	Fruit	(1 serv.)	Sugar	40		
	Egg	50	Sugar	35	Fat[c]	10		
			Fat	5				
2	Milk	240	Cottage Cheese	135	Cooked Hamburger	90	Milk	240
	Egg	50	Tomato & Lettuce	(1 serv.)	Vegetable	(1 serv.)	Sugar	40
	Sugar	40	Fruit	(1 serv.)	Sugar	40		
	Fat	5	Sugar	35	Fat	10		
			Fat	5				
3	Milk	240	Turkey	90	Cooked Pork Chop	90	Milk	240
	Egg	50	Celery & Lettuce	(1 serv.)	Vegetable	(1 serv.)	Sugar	40
	Sugar	40	Fruit	(1 serv.)	Sugar	40		
	Fat	5	Sugar	35	Fat	10		
			Fat	5				
4	Milk	240	Ham	90	Cooked Hamburger	90	Milk	240
	Egg	50	Tomato & Lettuce	(1 serv.)	Vegetable	(1 serv.)	Sugar	40
	Sugar	40	Fruit	(1 serv.)	Sugar	40		
	Fat	5	Sugar	35	Fat	10		
			Fat	5				
5	Milk	240	Tunafish	90	Cooked Beefsteak	90	Milk	240
	Egg	50	Celery & Lettuce	(1 serv.)	Vegetable	(1 serv.)	Sugar	40
	Sugar	40	Fruit	(1 serv.)	Sugar	40		
	Fat	5	Sugar	35	Fat	10		
			Fat	5				

Table II. (Continued)

Day	Breakfast		Lunch		Dinner		Evening	
		grams		grams		grams		grams
6	Milk	240	Roast Beef	90	Turkey	90	Milk	240
	Egg	50	Tomato & Lettuce	(1 serv.)	Vegetable	(1 serv.)	Sugar	40
	Sugar	40	Fruit	(1 serv.)	Sugar	40		
	Fat	5	Sugar	35	Fat	10		
			Fat	5				

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

[b] Weights of individual fruits according to exchange list figures (Cas6, 1950).

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