Effect of Low Sugar Intake upon Blood Lipids and Insulin Levels of Hyperlipemic Subjects[1] (35144)

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The feeding of excess carbohydrate to some (but not all) patients with marked hyper-pre-beta lipoproteinemia [Frederickson Type IV, (1)] further elevates their plasma concentration of pre-beta, or S_f 20–400 lipoproteins (2, 3) and concomitantly elevates their plasma triglyceride (TG) level (4, 5). More recently some observers (6) have suspected that it is the simple, not the complex sugars, which cause these elevations. Many of these hypertriglyceridemic patients, besides possessing a carbohydrate-sensitive lipoprotein metabolism, also appear to possess a carbohydrate-sensitive insulin metabolism, in that they exhibit an exaggerated insulin response to a glucose load (7, 8). Similar effects are rarely seen when young normotriglyceridemic subjects of either sex are given large amounts of either simple or complex sugars (9). Thus it is possible that the ingestion of carbohydrate by carbohydrate-sensitive hypertriglyceridemic patients may aggravate an already existing abnormality which, initially, may not have been caused by carbohydrate ingestion.

In view of this possibility, we thought it important to study a group of otherwise healthy subjects who exhibited only a moderate hypertriglyceridemia, of possible relatively recent origin. We were especially interested in determining what effect, if any, a severe restriction in dietary simple sugars would exert upon their plasma concentrations of cholesterol, pre-beta lipoprotein, TG, and upon their insulin response to a glucose load. The results of this study are given below.

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Methods. A. Selection of subjects. We selected 6 healthy firefighter officers (av. age: 45 years) whom we had observed previously (10) and who exhibited a moderate pre- and postprandial hypertriglyceridemia and a prominent pre-beta lipoprotein band according to the criteria of Lees and Hatch (11). None of the six, however, had shown either an abnormal fasting or 2-hr postprandial plasma glucose level (10). Finally 3 of the 6 subjects were judged by us to exhibit a moderate form of Behavior Pattern A[2]. The remaining three subjects were less well defined in that they exhibited elements of both Behavior Pattern Types A and B.

For control subjects we selected 10 men of similar age who also were apparently healthy and who exhibited the completely developed form of Behavior Pattern Type B (12). Men with this behavior pattern were chosen for controls because we believe, for reasons previously discussed (13), that the serum lipid levels usually observed in Type B subjects should be considered as relatively "ideal" normal values. Five of these 10 controls also were firefighter officers and the remaining 5 were accountants or clerks.

Data concerning the age, height, weight, and reported amount of physical activity were obtained from all 16 subjects. Table I shows the mean values of these general characteristics.

[2] Behavior Pattern A is a complex of certain personality attributes and mannerisms suggesting the presence of an incessant and excessive struggle against the exigencies of time or against the competitive efforts of other persons. Individuals exhibiting this type of behavior pattern have been found (12) to be more prone to occurrence of clinical coronary heart disease than persons exhibiting a converse sort of Behavior Pattern (Type B).

TABLE I. The General Characteristics of the Hyperlipemic and Normolipemic Subjects.

	Hyperlipemic subjects (6)	Normolipemic subjects (10)
Age (years)		
Mean	45	45
Range	(39–51)	(29–54)
SEM	±1.7	±1.9
Height (in.)		
Mean	72[a]	69
Range	(69–75)	(68–71)
SEM	±0.9	±0.5
Wt (lb)		
Mean	227[a]	170
Range	(190–300)	(155–183)
SEM	±15	±2.7
% Ideal weight		
Mean	+23	+14
Range	(7–55)	(6–24)
SEM	±6.4	±2.2
Exercise (hr/week)		
Mean	4.3	4.1
Range	(0–14)	(0–20)
SEM	±2.5	±1.9

[a] The mean of the hyperlipemic subjects is significantly greater ($p < 0.01$) than that of the control normolipemic subjects.

B. Dietary changes. Each of the 6 hypertriglyceridemic and the 10 control subjects was asked to record his daily intake of food and beverages for 7 consecutive days. These food diaries were given to a dietitian (Evelyn Piercy) who calculated the average daily total caloric intake as well as the intake of protein, fat (saturated and polyunsaturated), carbohydrate (simple and complex sugars), chlolesterol, and alcohol.

Several weeks later each of the six hypertriglyceridemic subjects was instructed to avoid consumption of simple sugars by eliminating from his diet not only table sugar but also all pastries, jellies, preserves, honey, syrups, beer, ale, soft drinks, wine, milk, ice creams, fruits (fresh or canned), white bread, and certain vegetables (e.g., beets, green peas, and sweet potatoes). The subjects ingested this sugar-poor diet for 60 days.

C. Serum cholesterol and lipid studies. A preliminary "triglyceride tolerance test" was done on the 6 hypertriglyceridemic and the 10 control subjects prior to the beginning of the sugar-poor diet. Thus all 16 subjects fasted for 13 hr preceding 8:00 a.m. of the test day, at which time they ingested a liquid meal containing 85 g of fat (13). We obtained blood samples before, and then 4, and 9 hr after the ingestion of the test meal. The fasting samples were analyzed for cholesterol according to the method of Martinek (14).

The pre-beta lipoprotein content of these same fasting samples were obtained as follows: One μl of the serum was subjected to electrophoresis in a thin agarose gel film (Analytical Chemists, Inc.) for 30 min at 120 V, 15 mA. After 10 min in acetic acid solution (10%), the lipoproteins on the gel were heat fixed and dried for about 20 min in a convection oven until they were solid. They then were stained in a working solution of Fat Red 7B for 60 min at room temperature. This solution was freshly prepared for each run by mixing 5.0 vol of stock stain solution (0.95 g of Fat Red 7B/1000 ml of alcohol, (J. T. Baker Co. reagent grade) with 1.5 vol of deionized water. The final pH of the solution was adjusted to 11.0–11.5 with a few drops of 1 *N* NaOH and then filtered through Whatman No. 42 filter paper. After staining, the excess dye was removed with a rinsing solution consisting of 5 vol of reagent grade alcohol and 3.0 vol of deionized water. Finally the agarose film was air-dried and scanned in a densitometer. The results are expressed as percentage of pre-beta lipoproteins in the total stainable unstaurated lipids. The postprandial samples were analyzed only for their TG content by the method of Van Handel and Zilversmit (15).

The triglyceride tolerance test was repeated twice more in the six hypertriglyceridemic subjects, first after they had been ingesting the special diet for 30, and again after 60 days.

D. Glucose tolerance and plasma insulin studies. All 16 subjects, before the initial glucose tolerance and insulin study, were asked to and did, eat daily approximately 75 g of sugar in the form of Hershey bars, for 2 days, in addition to their regular diet. Then, after an overnight fast, each was given 100 g of glucose dissolved in cold water and lemon juice. Blood samples were obtained before

TABLE II. The Regular and Special Daily Dietary Intake of Hyperlipemic Subjects.

Food intake	Regular diet[a]		Special diet[a]
	Hyperlipemic subjects (6)	Normolipemic subjects (10)	Hyperlipemic subjects (6)
Total calories			
Mean	2375	2509	1716[c]
Range	(2070–2971)	(1751–3695)	(1257–2302)
SEM	±162	±180	±137
Protein			
Mean	405	425	352
Range	(252–524)	(360–643)	(258–449)
SEM	±33	±31	±26
Total fat			
Mean	932	1000	781
Range	(666–1611)	(603–1546)	(541–1191)
SEM	±135	±95	±98
% of total calories			
Mean	38.7	39.8	44.9
Range	(32.2–54.2)	(31.3–45.7)	(39.6–51.5)
SEM	±1.8	±1.3	±1.5
Saturated fat			
Mean	763	841	630
Range	(594–1368)	(495–1378)	(364–1028)
SEM	±110	±77	±83
Unsaturated fat			
Mean	169	159	151
Range	(45–306)	(54–369)	(103–232)
SEM	±42	±28	±19
% of total calories			
Mean	7.1	6.3	9.0
Range	(2.1–11.0)	(3.1–12.7)	(5.8–14.1)
SEM	±1.5	±1.1	±1.4
Total CHO			
Mean	695[b]	935	412[c]
Range	(508–888)	(649–1184)	(277–523)
SEM	±66	±52	±43
% of total calories			
Mean	29.2[b]	37.4	24.8
Range	(19.2–38)	(24.7–47.3)	(16.6–36.4)
SEM	±1.8	±2.5	±8.8
Polysaccharides			
Mean	462	633	317
Range	(276–696)	(432–932)	(167–429)
SEM	±73	±45	±42
Simple sugars			
Mean	233	302	96[c]
Range	(96–332)	(98–536)	(29–118)
SEM	±44	±32	±13
% of total calories			
Mean	9.8	12	5.5[c]
Range	(4.6–15.5)	(3.9–18.8)	(2.3–6.8)
SEM	±1.8	±1.9	±0.6

TABLE II (continued)

Food intake	Regular diet[a]		Special diet[a]
	Hyperlipemic subjects (6)	Normolipemic subjects (10)	Hyperlipemic subjects (6)
Alcohol			
Mean	343[b]	149	171
Range	(0–516)	(0–594)	(0–408)
SEM	±66	±58	±57
Cholesterol (mg)			
Mean	488	552	543
Range	(380–764)	(375–773)	(255–852)
SEM	±57	±52	±85

[a] All values denote calories except those describing cholesterol intake.

[b] Mean is significantly different ($p<0.02$) from the corresponding value found in control normolipemic subjects.

[c] Mean is significantly less ($p<0.01$) than the corresponding value found in these same subjects while ingesting regular diet.

and 30, 60, and 120 min after the sugar had been taken. These samples were analyzed for glucose according to the method of Marks[3] (16) and for insulin by the immunoassay method of Hales and Randle[4] (17).

The glucose and insulin study was repeated in the 6 hypertriglyceridemic subjects after they had been on the sugar-poor diet for 30 and 60 days. After these 2 studies were done, the subjects continued to ingest their usual sugar-poor diet plus 75 g of sugar/day for an additional 2 days and then the same study was repeated.

Results. A. Comparison of regular and sugar-poor diets consumed by the hyperlipemic subjects. As Table II indicates, the average caloric intake as well as the intake of protein, fat (both saturated and unsaturated), and cholesterol of the 6 hyperlipemic subjects was approximately the same as that of the 10 control Type B subjects. The hypertriglyceridemic subjects, however, appeared to ingest significantly less total carbohydrate and more alcohol than the control subjects.

The 6 hyperlipemic subjects ingested significantly less total calories per day (see Table II) while taking the sugar-poor diet. These same subjects also drastically reduced their consumption of simple sugars (from 233 to 96 calories/day). Although a fall in total fat consumption occurred, the percentage of the total caloric intake ingested as fat increased significantly because of the drastic reduction in intake of sugars. The subjects lost an average of 9 pounds at the end of the first 4 weeks of the sugar-poor diet. However they lost an average of only 3 additional pounds at the end of the second 4-week period.

B. Effect of the sugar-poor diet on serum cholesterol, pre-beta lipoproteins and "triglyceride tolerance" test. The average serum cholesterol (265 mg/100 ml) of the 6 hyperlipemic subjects did not change significantly (see Table III) after 60 days of eating the sugar-poor diet.

The elevated average serum pre-beta lipoprotein concentration (34% of total unsaturated lipids) of the 6 hyperlipemic subjects decreased to a normal value (17.4%) after being on the sugar-poor diet for 30 days (see Table III). No further decrease however took place after an additional 30 days of this diet.

The average fasting serum triglyceride level (253 mg/100 ml) of the 6 experimental subjects which, prior to the special diet, was significantly greater than that (60 mg/100 ml) of the control subjects, dropped (see Table III) to an average level of 129 mg/100

[3] Reagents were supplied as "Biochemicatest" by C. F. Boehringer & Soehne.

[4] Reagents were supplied by Amersham Ltd., Buckinghamshire, England.

TABLE III. The Plasma Cholesterol and Pre-beta Lipoprotein Concentrations and the Triglyceride, Glucose, and Insulin Tolerance Curves of Hyperlipemic Subjects on Regular and on Special Diet.

	Plasma cholesterol (mg/100 ml)	Pre-beta lipoprotein conc (% of total stainable unsat. lipids)	Plasma triglyceride (mg/100 ml)			Glucose tolerance (mg/100 ml)				Plasma insulin (μ-units/ml) before and after oral glucose, 100 g					
				After fat meal			After glucose, 100 g								
			Fasting	4 hr	9 hr	Fasting	30 min	60 min	120 min	Fasting	30 min	60 min	120 min	Peak	Secretion /2 hr[c]
I. Regular diet															
A. Hyperlipemic subjects (6)															
Mean	265[d]	34.0[d]	253[d]	353[d]	185[e]	91	155	143	91	17	126	160[d]	40	180[d]	208[d]
Range	(249–301)	(27–50)	(145–425)	(229–471)	(83–388)	(84–100)	(96–194)	(87–190)	(50–186)	(13–26)	(81–243)	(109–194)	(15–99)	(109–243)	(185–237)
SD	±24.0	±9.0	±101	±92.	±97	±8.0	±37	±35	±46	±4.0	±53	±32	±29	±39	±18
SEM	±9.0	±3.0	±41.0	±37	±38	±3.0	±14	±13	±18	±2.0	±22	±13	±12	±16	±7.0
B. Control normolipemic subjects (10)															
Mean	220	16.4	60	107	65	90	139	127	104	22	85	89	65	97	148
Range	(162–262)	(6.7–22.7)	(38–106)	(74–137)	(42–133)	(77–97)	(104–176)	(97–188)	(83–139)	(12–33)	(46–188)	(53–164)	(30–116)	(53–188)	(91–234)
SD	±30.0	±7.0	±21	±19	±32	±6.0	±25	±35	±21	±6.0	±36	±34	±23	±42	±47
SEM	±9.0	±2.0	±7.0	±6.0	±10	±2.0	±8.0	±10	±7.0	±2.0	±11	±11	±7.0	±13	±15
II. Special diet[a]															
After 30 days															
Mean	296	17.4[e]	129[e]	252	190	80	160	170	102	17	147	212	131[e]	220	301[e]
Range	(248–359)	(9.5–27.6)	(46–275)	(112–405)	(75–262)	(54–90)	(124–209)	(129–220)	(74–149)	(7.0–33)	(33–375)	(120–360)	(51–300)	(120–375)	(145–608)
SD	±38	±8.0	±77	±99	±116	±13	±29	±34	±30	±10	±116	±73	±96	±77	±152
SEM	±14	±3.0	±31	±39	±48	±6.0	±13	±13	±11	±4.0	±49	±30	±39	±31	±25
After 60 days															
Mean	279	19.6[d]	108[e]	223[e]	126	93	161	169	114	28	159	228[e]	137[e]	228	318[e]
Range	(234–334)	(14–27.6)	(82–142)	(172–263)	(75–212)	(83–108)	(136–204)	(90–221)	(80–137)	(13–62)	(48–358)	(158–356)	(58–257)	(158–358)	(204–574)
SD	±39	±8	±19	±33	±46	±9.0	±31	±52	±24	±16	±104	±65	±88	±88	±129
SEM	±15	±3.0	±8.0	±14	±19	±3.0	±13	±19	±9.0	±7.0	±43	±27	±36	±36	±53
After 62 days[b]															
Mean	—	—	—	—	—	100	170	153	104	44[e]	160	209	144[e]	224	319[e]
Range						(82–108)	(140–219)	(100–185)	(68–165)	(8–96)	(108–23)	(52–355)	(26–216)	(132–355)	(120–396)
SD						±9.0	±32	±32	±37	±28	±39	±90	±71	±76	±102
SEM						±4.0	±12	±120	±13	±12	±16	±37	±29	±28	±38

[a] Only the hyperlipemic subjects ingested the special diet.

[b] The hyperlipemic subjects ingested 75 g of extra sucrose daily for 2 days prior to the third glucose tolerance test.

[c] Mean insulin secretion/2 hr calculated according to the formula of Welborn *et al.* (Lancet 1, p. 280, 1966).

[d] Mean is significantly different ($p<0.01$) from corresponding value found in control normolipemic subjects.

[e] Mean is significantly different ($p<0.01$ or $p<0.02$) from corresponding value found in same subjects while ingesting regular diet.

ml after they had ingested the sugar-poor diet for 30 days. This level decreased somewhat more after an additional 30 days had elapsed, to reach a value almost within the upper range of normal as calculated from the values observed in the 10 control normolipemic subjects (*i.e.*, the average mean of these latter subjects plus 2 × SD).

The average 4-hr postprandial serum triglyceride level (252 mg/100 ml) of the experimental subjects dropped somewhat (see Table III) 30 days after beginning the special diet but a significant fall ($p < 0.01$) occurred in these subjects only after they had ingested the sugar-poor diet for 60 days. However, their 4-hr level at this latter time still was significantly greater than that of the 10 control subjects (on their usual diets).

No significant decrease occurred in the average 9-hour post-prandial serum triglyceride level of the 6 experimental subjects either 30 or 60 days after they had begun to ingest the sugar-poor diet.

C. *Effect of the special diet on glucose tolerance and plasma insulin levels.* No essential change (see Table III) was observed in the average, or in the individual, fasting and postprandial blood glucose values of the 6 hyperlipemic subjects either 30 or 60 days after ingesting the sugar-poor diet. Likewise, the results of the fourth glucose tolerance test (preceded by the ingestion of supplementary glucose) were approximately the same as those observed in the preceding three tests.

Following the ingestion of 100 g of glucose, the 6 hyperlipemic subjects showed a significantly higher average plasma insulin level than the controls (see Table III). Employing 157 μ-units/ml as the maximal normal value for the 60-min postprandial level (*i.e.*, the mean value of the control subjects plus 2 × SD), 5 of the 6 hyperlipemic subjects exhibited at 60-min postprandial insulin content above normal. The one subject who did not exhibit this hyperinsulinemic response was the same subject whose 2-hr postprandial glucose was 186 mg/100 ml. As might be expected, the average peak insulin values and the average insulin secretion/2 hr were similarly elevated in the hyperlipemic subjects.

Ingestion of the sugar-poor diet for either 30 or 60 days did not normalize the average increased postprandial plasma insulin levels of the hyperlipemic subjects. Actually, their average 1- and 2-hr postprandial serum insulin levels significantly increased (see Table III) both after 30 and 60 days of the sugar-poor diet.

Discussion. Current interest in carbohydrate-induced (or perhaps more correctly stated, carbohydrate-aggravated) hypertriglyceridemia is partially sustained by the fact that many, if not the majority, of coronary patients exhibit this type of lipemia (6, 18) regardless of whether an abnormal glucose tolerance is present or not. In the present study, a drastic restriction in the ingestion of simple sugars was accompanied by a moderate weight loss, particularly during the first 4 weeks. These dietary changes led to a relatively rapid normalization of the previously elevated serum pre-beta lipoprotein levels and also a modest diminution of the hypertriglyceridemia. Similar changes in these lipid fractions following reduction in the intake of simple sugars also have been observed by previous investigators (19, 20). Since no significant weight loss occurred in some of these studies, it seems likely that despite the moderate weight loss which occurred in our subjects, it was the reduced intake of simple sugars that was chiefly responsible for the lipid changes which took place. This belief is supported by the fact that after the first month of the diet, very little weight loss occurred in our subjects but the serum lipid levels decreased even further.

Despite the serum lipoprotein and triglyceride reductions effected by the sugar-poor diet, no significant change took place in the average serum cholesterol levels of the 6 experimental subjects. Rifkind *et al.* (20) also noted that, although improvement in the serum lipids of their subjects occurred after a reduced ingestion of simple sugars, their serum cholesterol levels remained relatively unaffected. Stare (21) as well as Szanto and Yudkin (22) also did not observe any alteration in the serum cholesterol of their patients following manipulation of their carbohydrate intake. Indeed only Kuo and Bassett (19) have reported a significant reduction in

the serum cholesterol of hyperlipemic patients following reduction in the intake of simple sugars. However, their study indicates that they not only reduced the sugar content of their subjects' diets, but also substituted polyunsaturated for saturated fats.

The present study also clearly indicates that the dietary changes, regardless of their effect upon certain serum lipid values, had no effect in normalizing a previous hyperinsulinemic response to a glucose load. As Table III suggests, the hyperinsulinemic responses of our subjects worsened during the ingestion of the sugar-poor diet. In this same connection it is of interest that while Szanto and Yudkin (22) were able to increase the insulin level of some of their subjects by administering excess sucrose, they were not able to diminish the serum insulin level of any of their subjects by restricting sucrose intake.

Summary. The intake of simple sugars of 6 moderately hypertriglyceridemic, hypercholesterolemic subjects was reduced for 60 days. This restriction led to a moderate weight loss, a normalization of their previously elevated pre-beta lipoprotein levels and a moderate decrease in their pre- and postprandial hypertriglyceridemia. The elevated serum cholesterol and pre- and postprandial serum insulin levels were not normalized by the dietary change.

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