MEDICAL PROGRESS

DIETARY FATS, CARBOHYDRATES AND ATHEROSCLEROTIC VASCULAR DISEASE (Concluded)*

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Most of the studies so far reported have been carried out with subjects whose levels of serum total cholesterol and, where reported, of triglycerides were well below those usually considered hyperlipidemic. So-called hyperlipidemias, for which Fredrickson et al.[45] have provided a useful classification, in effect are usually applied to persons representing the upper 5 per cent or 10 per cent of the general population (Fig. 1 and Table 1). The widespread prevalence of atherosclerosis and its clinical complications in developed societies and a broader view of blood lipid distributions in various populations, including the age-related increase in American society, suggest that most middle-aged American men have hypercholesterolemia and probably hypertriglyceridemia as well. If one is to think in terms of dietary changes with the reasonable idea of preventing or retarding atherosclerotic vascular disease, there is no reason at all to restrict such efforts to a small segment of a susceptible population.

On the other hand, there is evidence that patients with the Type 4 abnormality[45] – carbohydrate-inducible hypertriglyceridemia – do show an exaggerated response to changes in the absolute quantity as well as to the type of dietary carbohydrate.

Kuo and Bassett[46] have reported on the levels of serum lipids and the fatty acid composition of the major lipid moieties in 5 middle-aged subjects with hyperlipidemia and atherosclerosis in response to dietary changes made isocalorically with simple sugar and starch. Neither the self-selected comparison diets nor the experimental diets of high sugar or high starch taken for four to six weeks were described in detail. These subjects were apparently maintained on diets providing about 30 per cent of the daily calories from fats. The sources and the nature of the carbohydrates were not mentioned, except for the fact that simple sugar, which comprised 180 to 205 gm. per day on self-selected diets, was increased to 200 to 240 gm. during the period on high sugar. The source of starch and the types of simple sugars (monosaccharides and disaccharides) were not mentioned. Levels of serum cholesterol, which ranged from 250 to 450 mg. per 100 ml. on self-selected diets, were increased in 1 and unaffected in 4 subjects on the diets containing sugar, but were slightly decreased in all subjects on diets high in starch. During the experimental periods of four to six weeks, serum triglycerides were markedly increased on the high sugar and decreased by the high starch – both changes in relation to the self-selected diet. Moreover, the distribution of triglycerides and fatty acids in cholesterol esters showed changes on diets high in sugar compatible with endogenous lipogenesis (increased palmitic, palmitoleic and oleic acid and decreased linoleic acid) but were uninfluenced by the diets high in starch. This is odd, since the diets high in sugar were very similar to the self-selected diet, whereas the starch diet was quite different.

Kuo[47] described similar studies in more detail. He reported that in patients who had severe hypertriglyceridemia, either sucrose or starch elevated the serum triglycerides when a high level of carbohydrate calories was fed. Starch, however, was relatively less hyperlipemic. On the other hand, he too was unable to raise the serum triglyceride levels in young men in whom the daily sucrose intake was approximately doubled. Limited studies comparing a fructose with a sucrose diet led Kuo to conclude that fructose has a "low lipemic effect as compared to sucrose."

Kaufmann et al.[48] reported on the blood lipid responses of 4 patients with hypertriglyceridemia induced by carbohydrates. Their self-selected diets (whose composition was not described in detail) were, under close supervision, changed to diets very low in fat (3 to 12 per cent of total calories) and high in starch, sucrose or glucose for different periods (ten to forty days). Both the sucrose and the glucose diets led to an increase in serum triglycerides; however, during periods on high starch diets, triglycerides were unchanged or lower than they were on the self-selected diets. The total serum cholesterol in general paralleled the triglyceride changes. In the 2 patients in whom both sucrose

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and glucose were compared, the response of the serum triglycerides was the same although the response of serum cholesterol was smaller on glucose.

Thus, these studies on subjects with gross hypertriglyceridemia, although possibly confirming the previously described carbohydrate effects, have raised other issues. Kaufmann's sucrose-glucose comparisons and Kuo's studies with fructose may cast some doubt on the idea usually advanced that fructose yielded by the hydrolysis of sucrose is responsible for the hypertriglyceridemia. Since in Kaufmann's subjects the serum triglycerides did not rise further on the diet low in fat, and high in starch, one sees a further discrepancy when considering the report of Antonis and Bersohn[25] on European patients put on a "Bantu" type of diet that was also high in starch.

These types of studies, descriptive, clinical and epidemiologic investigations, have advanced the understanding of the role of diet and serum lipids in atherosclerotic vascular disease about as far as possible. Clearly needed now are longitudinal clinical trials designed to show whether or not a group of individuals who have achieved a reduction in serum cholesterol through dietary manipulation will also manifest a reduced risk of clinical atherosclerotic vascular disease. To carry out such a study beginning with healthy, middle-aged men, properly randomized into treatment and control groups, and followed up for the development of coronary heart disease, is a truly formidable undertaking. The nature of these problems is outlined in the preliminary report of the National Diet-Heart Study group.[49]

Nevertheless, the most recent report from the Anti-Coronary Club in New York City[50] does suggest a significant reduction in the incidence of coronary heart disease in a group of men whose average level of serum cholesterol was reduced by 12 per cent on a diet restricted in saturated fats and cholesterol while increased in polyunsaturated fats.

Another approach has been used in Oslo by Leren,[51] who has reported a five-year-follow-up study of 412 male survivors, thirty to sixty-seven years of age, of a documented myocardial infarction. These men were randomly assigned to a dietary treatment and a control group. Goals of the dietary instruction were similar to those in the New York City study.[50] The serum cholesterol level was reduced by 17 per cent in the treatment group, an effect maintained over the five years of the study. Over the five-year period of observation, 34 of the treatment group had 43 new myocardial infarctions (10 fatal); 54 of the control group had 64 new infarctions (23 fatal). Furthermore, 10 of 75 patients in the dietary group who were initially without angina pectoris subsequently manifested this syndrome; the rate was 29 of 79 in the control group. If confirmed by other studies, this report represents a signal advance in the ability to control the major cause of disability and death in contemporary Western societies.

Dietary Regulation of Serum Lipids in Laboratory Animals

Elevation of the level of serum cholesterol produces vascular lesions of varying similarity to human atherosclerosis in nearly all animal species that have been studied. Several recent reviews are available on the manipulations, including dietary, that have been used to initiate and to accelerate the development of such vascular lesions.[52,53] For the most part, unfortunately, most of these studies have dealt with diets containing large amounts of cholesterol, amounts far greater, in proportion to size, than those consumed by man. Much less is known of the influence of various kinds of fatty acids in diets incorporating little or no exogenous cholesterol. The question may be raised whether studies done with diets heavily supplemented with dietary cholesterol, or the effects of various dietary modifications of such diets, can be meaningfully interpreted in terms of human nutrition. In our opinion, most of the studies reported with such diets have little significance, especially those done with rats, whose response in serum cholesterol to dietary fat and cholesterol is markedly different from that of man.[54] Effort is needed to identify species whose response in serum lipids to dietary modifications is similar to that seen in man.

A variety of studies on the effects of dietary carbohydrate on the serum lipid levels in animals have been reported. Portman et al.[55] found that the biliary excretion of bile acids in rats was substantially higher when the animals received crude diets or diets containing starch than when the dietary carbohydrate was sucrose. In a later study,[56] in which the diet contained 5 per cent cholesterol, the serum cholesterol was found to be 124 mg. per 100 ml. in the animals fed sugar, as compared with 106 mg. when starch was supplied. When cholic acid was added to this diet, the differences were accentuated. For example, in 1 experiment the sucrose diet yielded levels of serum cholesterol at 378 mg., and the starch diet levels of 257 mg. per 100 ml. When sucrose, glucose, and fructose were compared, levels of serum cholesterol were 414, 361 and 411 mg. per 100 ml. respectively. The level obtained when glucose was fed, however, was not significantly lower in this case. In diets of this kind the addition of sulfasuxidine did not affect the levels of serum cholesterol obtained with the diet containing sucrose, but when it was added to the starch diet, the level of serum cholesterol rose. Dietary influence on the intestinal flora was therefore suggested.

Guggenheim et al.[57] also found that the dietary carbohydrate had little effect upon serum or liver cholesterol in rats unless the diet was made hypercholesterolemic by the addition of cholesterol and cholic acid. Under these conditions, they found that

glucose resulted in lower levels of serum cholesterol (166 mg. per 100 ml.) than sucrose or starch (258 and 232 mg. per 100 ml. respectively). Levels of liver cholesterol were somewhat lower when sucrose was fed than when the other two diets were given.

Grant and Fahrenbach[58] fed chicks diets in which the carbohydrate was either sucrose or glucose. Serum cholesterol levels were not different unless the diet was heavily supplemented with cholesterol, but then the sucrose diet was more hypercholesterolemic than the glucose diet. Similar results were obtained with rabbits, and generally similar results with chicks were reported by Kritchevsky et al.,[59] and the differences persisted when germ-free chickens were tested.

Macdonald and Roberts[60] reported that male rats given diets containing sucrose for twelve weeks had substantially higher levels of total serum lipids (350 mg. per 100 ml.) than animals given either glucose or fructose. Female rats, on the other hand, had similar levels of serum lipids on all 3 diets. In the male rats the levels of serum cholesterol were slightly higher on the sucrose diet, but, again, the dietary carbohydrate had no effect on levels of serum cholesterol in females. The authors report that when the animals were fed small amounts of carbohydrate labeled with radioactive carbon (^{14}C), conversion to serum lipid was higher in animals fed the sucrose and fructose diets than in animals fed glucose. Christophe and Mayer[61] had previously shown that incorporation of labeled acetate into fatty acids in the liver was markedly influenced by the type of dietary carbohydrate. Under comparable conditions, approximately three times as many counts were found in the fatty acids of the animals fed fructose as in those fed glucose. On the other hand, animals receiving a commercial rat food (Purina Rat Chow) incorporated only 25 per cent as much label in fatty acids as those receiving the purified diet, even though the total content of protein, fat and carbohydrate was approximately the same. Whether this difference represents a carbohydrate effect is unclear.

Nath et al.[62] gave rats diets containing cholesterol and cholic acid. Substitution of wheat flour for sucrose lowered the serum cholesterol level, but sucrose, dextrin, and glucose yielded essentially the same degree of hypercholesterolemia.

It is readily apparent that under certain conditions in the rat, the type of carbohydrate in the diet can be shown to affect the level of serum cholesterol. Generally speaking, these effects appear to be minimal, unless the diet is capable of producing a substantial degree of hypercholesterolemia. It is significant that even under these conditions, – that is, with a diet containing cholesterol and cholic acid, – Fillios et al.[63] found that the difference in the rat between sucrose and starch diets, which was substantial after three or four weeks, decreased with time. The difference was not statistically significant after six weeks on the diets, and had disappeared at twelve and seventeen weeks. The same study showed that the level of protein in the diet is an important factor in determining the levels of serum cholesterol in the rat, as has long been known.[64]

An evaluation of the significance of these findings in animals in relation to the problem of hypercholesterolemia and atherosclerosis in man does not seem possible at present. For reasons that are not clear, diets given to rats are traditionally low in fat. Thus, the response of rats, as of man, to diets high in sugar and low in fat may be thought to be of limited significance since such diets are rarely consumed by man. In addition, the question arises whether the hypercholesterolemic rat is sufficiently similar to hypercholesterolemic man to serve as a useful model. It has been demonstrated[54] that the response of such animals to variations in the kind of dietary fat has little similarity to that seen in man. When a wide variety of fats were tested in such animals, the highest levels of serum cholesterol were found with olive oil, and it appears that monounsaturated fatty acids tend to elevate levels of serum cholesterol above those seen with either more saturated or less saturated oils. This is contrary to all data available on man. In view of the fact that Antonis and Bersohn[25] found that the adaptation of their subjects to diets high in carbohydrate required a substantial time, and Fillios et al.[63] have provided evidence that similar adaptation may occur in rats, most of the data on laboratory animals obtained with relatively short experimental periods may represent a temporary adjustment to the diets given. Finally, it should be borne in mind that the diets administered to these animals, in addition to being low in fat and very high in carbohydrate, also represent maximal changes in the dietary carbohydrate – all starch or all sugar. These diets should have the greatest metabolic effects but probably have limited significance when compared with the more moderate changes possible under ordinary conditions in diets for man.

It has long been recognized that glucose and fructose are metabolized by somewhat different routes and that tissues vary in their ability to use the 2 substances. Although there is now abundant evidence of metabolic adaptation to diets in which the dietary carbohydrate is different, most of the nutritional and biochemical literature considers carbohydrate as simply carbohydrate, irrespective of source, and the extent to which the source of dietary carbohydrate might have affected the many experimental results is unknown. On the other hand, when the effects of specific carbohydrates have been investigated, the investigators for obvious reasons have usually compared diets in which all the carbohydrate is from one source. The effects observed are thus presumably maximal. Under practical conditions, the major source of fructose will be from sucrose mixed with other carbohydrate sources yielding primarily glucose upon hydrolysis.

It is thus uncertain how far the findings may be extrapolated.

Cori[65] showed as early as 1926 that fructose formed glycogen more rapidly in the liver than other sugars. Bollman and Mann[66] reported that, in the dog deprived of both liver and gastrointestinal tract, fructose did not alleviate hypoglycemia but that it was partially effective when only the liver was removed. Thus, the gastrointestinal tract has the ability to convert fructose to glucose, and this conversion has also been shown to occur in isolated gut loops. The extent to which it may occur under usual dietary conditions in man or animals is unknown and is presumably variable. In the studies of Kiyasu and Chaikoff,[67] in which portal blood was collected from gut loops of rats and guinea pigs after labeled fructose was placed in the loops, about 10 per cent of the absorbed fructose appeared as glucose in the rats, but nearly 70 per cent in the guinea pigs. Furthermore, substantial amounts of the label, from 2 to 60 per cent, appeared as lactate, the only other labeled compound found. It is said that in man about one sixth of ingested fructose is converted to glucose in the intestinal mucosa. In contrast to most other important dietary sugars, fructose enters the bloodstream by passive diffusion rather than by active transport.

Fructose is metabolized very slowly by skeletal muscle, but rapidly by the liver, gastrointestinal tract and adipose tissue. There appears to be substantial agreement on the major qualitative aspects of fructose metabolism but considerable doubt about the quantitative aspects. Fructose enters the cells of liver and adipose tissue freely and is not dependent on insulin. The fructose is then phosphorylated by a specific fructokinase to yield fructose-1-phosphate, which can be directly cleaved to dihydroxyacetone phosphate and glyceraldehyde. Apparently, it is uncertain whether this is a specific aldolase, but, if it is not, the aldolase present has a substantially higher affinity for the fructose-1-phosphate than for fructose-1,6-phosphate formed in the usual glycolytic breakdown of glucose. The glyceraldehyde can then be converted to the 3-phosphate by a specific kinase in liver. Kattermann et al.[68] observed substantial accumulations of D-glycerate in the livers of animals injected with fructose. Since the entrance of glucose into metabolism is apparently self-regulating owing to modifying effects of glucose-6-phosphate, the more rapid entrance of fructose and formation of glycerol and triose phosphates from fructose may be the important difference and may favor lipogenesis. Once the trioses are formed, there is presumably no difference in the metabolism of glucose and fructose. Synthesis of glucose and glycogen can proceed by the usual channels. Since gluconeogenesis requires the hydrolysis of fructose-1,6-phosphate and glucose-6-phosphate, particular attention has been paid in some studies to the phosphatases accomplishing these tasks.

Much of the pertinent material on fructose metabolism has been reviewed by Krane.[69] The scheme outlined above is believed to describe the major pathway of metabolism, although other possibilities exist. Fructose is apparently metabolized normally by diabetic patients,[70] but one judges that the differences between sucrose and glucose (or glucose precursors) have not been thought to be clinically significant. Baker et al.[71] found that acetate to fatty acid synthesis, was markedly impaired in diabetic rats on glucose diets but not in animals given fructose. Animals on the diets high in fructose (58 per cent of the diet) had an impaired glucose tolerance, and the livers had a decreased ability to oxidize glucose.[72] Cohen and Teitelbaum[73] also observed impaired glucose tolerance in rats fed sucrose as compared with starch, and the extent of impairment was apparently related to the amount of sucrose fed. Some impairment was observed when only 33 per cent of the carbohydrate was sucrose, an amount similar to that in many diets of man, but only after a hundred days on the diet. The authors suggest that rapid flooding of the system with easily absorbable sugars may eventually impair the insulin response. Uram et al.[74] had previously indicated the possible presence of a "hypoglycemic" substance in starch. Whether these studies are complicated by such a factor as chromium, a deficiency of which apparently produces diabetes[75] and which may be implicated in some types of diabetes in man,[76] remains to be seen.

Freedland and Harper[77] demonstrated a marked rise in glucose-6-phosphatase in the livers of animals when glucose or dextrin in the diet was replaced by protein, fat or a carbohydrate that did not yield only glucose on hydrolysis. This "adaptation" was transient on diets high in protein and fat but persisted when the dietary carbohydrate was sucrose or fructose. The changes in dietary carbohydrate had lesser or no effects upon fructose-1,6-diphosphatase. Carroll[78] also found liver glucose-6-phosphatase to be lower in animals receiving glucose or starch than in those fed sucrose or fructose. Liver nitrogen, liver glycogen and fructose-1,6-phosphatase were elevated by sucrose feeding. The findings were modified, however, and partially dependent upon the kind of fat included in the diet. Glucose-6-phosphatase was generally higher on diets containing hydrogenated coconut oil than when the fat was corn oil, regardless of the type of dietary carbohydrate. In later work[79] the proportions of carbohydrate and fat in the diet were varied. As might be expected, the response of enzymes to changes in dietary carbohydrate was less marked when diets were high in fat, and the changes induced by the kind of fat were more important.

It is recognized that glucose-6-phosphatase and fructose-1,6-phosphatase are important in gluconeogenesis; yet it is clear from recent work that the control mechanisms in enzyme actions are indeed

complicated, and it appears that only limited conclusions are justified from measures of enzyme levels in simple systems. The modifying effects of various intermediates in glycolysis and the citric acid cycle upon these systems have been discussed by a variety of authors. Recent reviews by Atkinson[80] and Wood[81] evaluate the current knowledge in this field.

LACTOSE

As indicated above, there is little evidence in man that lactose has specific effects upon cholesterol or lipid metabolism. McGandy and his associates[39] found relatively high levels of serum cholesterol in the group receiving a diet high in lactose when coconut oil supplied the dietary fat, but not when olive or safflower oil was fed. This suggests a possible dietary carbohydrate-oil interaction that requires confirmation. Anderson et al.[35] did not identify a specific lactose effect in their studies. The data available from animal experiments, however, indicate lactose effects distinct from other sugars. Wells and Cooper[82] and Nath et al.[62] provided evidence that in rats, diets high in lactose increased absorption of cholesterol. The animals fed lactose and cholesterol had higher levels of cholesterol in the liver than animals receiving a similar diet containing sucrose, and fecal cholesterol was lower. Labeled cholesterol recovered from the thoracic lymph duct after the administration of a single dose of cholesterol-4-^{14}C was measured.[83] In animals given the 40 per cent lactose diet the mean lymph volume per twenty-four hours was 128 ml., as compared with 71 ml. in animals fed sucrose. Approximately twice as much total sterol and three times as much labeled cholesterol were recovered from the animals fed lactose. Collection of bile from rats receiving sucrose and lactose by cannulation of the duct showed a substantial increase in bile acid excretion in animals fed lactose. The authors suggest that lactose feeding may affect the conversion of cholesterol to coprostanol, thus influencing the amount of absorbable cholesterol, or that it directly influences bile acid formation and thus increases cholesterol absorption. Hepatic cholesterol synthesis,[84] estimated by injection of acetate or mevalonate labeled with ^{14}C, the animals being killed one hour later, was found to be substantially lower in the animals fed lactose.

In studies on rabbits, a diet containing 30 per cent lactose and 0.35 per cent cholesterol produced higher levels of serum cholesterol and more atherosclerotic lesions than the sucrose diet given to the control animals.[85] With variation in the level of dietary cholesterol, the difference between diets containing sucrose and lactose was much more marked when the cholesterol content was low (0.2 or 0.35 per cent) than when it was higher (0.5 per cent). The inclusion of succinylsulfathiazole in the diet[84] raised the level of serum cholesterol and the atherosclerosis score, particularly of the animals receiving sucrose.

Tomarelli et al.[86] gave rats diets in which the proportions of carbohydrate, fat and protein approximated those found in milk. The animals receiving lactose ate less food, grew at a reduced rate and stored less body fat than animals fed glucose, sucrose, dextrin or a glucose-galactose mixture. Since animals receiving the glucose diet stored much more fat than the animals fed lactose, even when their food intake was limited to allow equal weight gains, and since differences in body fat persisted after a forty-two-week feeding period when weight had leveled off, the effect did not appear to be attributable to differences in food intake. Animals fed lactose or other poorly digested carbohydrates (sorbitol, cellobiose or raw potato starch) all had enlarged ceca and low content of carcass fat; the limited fat deposition was thought to be related to events in the gastrointestinal tract.

A substantial literature exists on the effects of lactose on calcium absorption.[87,88] In general, it has been thought that the slow digestion of lactose may change the pH of the lower gut or make metabolizable sugar available at lower segments of the gut[89] and thus influence calcium absorption. However, not all the evidence favors such explanations.[90] Charley and Saltman[91] have reported evidence of a calcium-lactose chelate that may have a role. The enhanced absorption from lactose in rats is not limited to calcium. Test doses of radioactive barium, strontium, magnesium and radium were also found to be absorbed to a greater extent.[92]

CONCLUSIONS

The major evidence today suggests only one avenue by which diet may affect the development and progression of atherosclerosis. This is by influencing the levels of serum lipids, especially serum cholesterol, though this may take place by means of different biochemical mechanisms not yet understood.

There can be no doubt that levels of serum cholesterol can be substantially modified by manipulation of the fat and cholesterol of the diet. We conclude, on the basis of epidemiologic, experimental and clinical evidence, that a lowering of the proportion of dietary saturated fatty acids, increasing the proportion of polyunsaturated acids and reducing the level of dietary cholesterol are the dietary changes most likely to be of benefit. The solution here, in our opinion, is a responsibility and opportunity for the food industry – namely, the manufacture of many common foods with characteristics that will lessen the development of atherosclerosis. This is possible today, and only awaits leadership from the food industry.

Limited evidence from studies on man as well as from researches on laboratory animals show a slightly significant role for the kind and amount of dietary carbohydrate in the regulation of serum lip-

ids. These effects are somewhat more pronounced when diets low in fat are consumed. Since diets low in fat and high in sugar are rarely taken, we conclude that the practical significance of differences in dietary carbohydrate is minimal in comparison to those related to dietary fat and cholesterol.

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