Hyperlipemia and Diabetes Mellitus

Edwin L. Bierman, M.D., Jose A. P. Amaral, M.D., and Benjamin H. Belknap, M.D., Seattle

SUMMARY

Untreated alloxan diabetic rats developed marked hyperlipemia only when their diet contained fat. Diabetes had no effect on plasma triglyceride levels of rats fed a fat-free diet. This result, coupled with prior observations, suggests that hyperlipemia in alloxan diabetes in the rat, and perhaps in diabetes mellitus in man, is related to the intake of dietary fat. DIABETES *15*:675-79, September, 1966.

Marked hyperlipemia, accompanied by lipemia retinalis and eruptive xanthomata, occasionally is associated with diabetes in man. The appearance of milky plasma, with its high concentration of large, light-scattering, triglyceride-rich fat particles, is rare,[1] and when it occurs is associated with chronic hyperglycemia and glycosuria, but minimal ketoacidosis,[2-5] in contrast to the usual mild rise of plasma triglyceride associated with the severe ketoacidosis of acute insulin deficiency.[4,6]

The source of the marked excess triglyceride in plasma and the mechanism of its accumulation are unknown. Preliminary characterization[7] of fat particles in the plasma of a few of these patients has indicated a predominance of "primary" particles derived from dietary fat, rather than an accumulation of endogenous triglyceride.[5] Therefore, to assess the role of dietary fat in the pathogenesis of diabetic hyperlipemia, the present study was performed on an experimental model of the chronic diabetic state—the untreated alloxan diabetic rat. Results indicate that marked hyperlipemia develops only in the presence of dietary fat.

METHODS

Male Sprague Dawley rats, weighing approximately 250 gm., were divided into control and diabetic groups. Animals in each group, housed in individual cages with free access to food and water, were fed either a fat-free diet containing mainly casein, sucrose, and cellulose ("Fat-Free" Test Diet; Nutritional Biochemicals Corp., Cleveland) or a diet containing 40 per cent of the total calories as fat in the form of Purina Chow (Ralston Purina Co., St. Louis) supplemented with corn oil, 20 gm./100 gm. chow (table 1). Both diets contained standard vitamin and mineral supplements.

Diabetes was produced by the injection of a freshly prepared aqueous solution of alloxan monohydrate, 40 mg./kg. body weight, into the tail vein. Survivors who failed to demonstrate glycosuria within two weeks after alloxan administration were removed from the study. No insulin was administered. Therefore, those rats with glycosuria who survived alloxanization remained in the untreated diabetic state for the duration of the experiment.

Blood for analysis was obtained periodically from the tail vein of animals lightly anesthetized with ether, and at the conclusion of the experiment in twelve to sixteen weeks, by cardiac puncture. Food was withdrawn four to five hours prior to blood sampling. Blood glucose was determined by the glucose oxidase method[8] and plasma triglyceride by modifications of the Carlson method.[7,9] Analysis of fat particle composition was performed after starch block electrophoretic separation[7] of pooled lipemic plasma obtained from the fat-fed diabetic group at the end of the experiment.

RESULTS

All diabetic rats had glycosuria. The mean blood sugar in both diabetic groups exceeded 250 mg. per 100 ml. with no significant difference attributable to diet.

A comparison of changes in body weight among the four groups of rats shows that the control groups, whether on the 40 per cent fat or the fat-free diet, continued to gain weight (figure 1). After an initial lag in weight gain in the fat-free group, attributable to

TABLE 1

Diet	Grams per 100 gm.			Per cent total calories		
	Protein	Fat	Carbohydrate	Protein	Fat	Carbohydrate
40 per cent fat	19	20	48	17	41	42
Fat-free	21	—	75	22	—	78

From the Department of Medicine, University of Washington School of Medicine and Veterans Administration Hospital, Seattle, Washington.

Dr. Amaral's present address is: University of Recife School of Medicine, Recife, Brazil.

adaptation to a powdered diet, there was no significant difference in weight between the two diet groups. All alloxan diabetic animals failed to gain weight normally. Again, the difference in diet produced no significant difference in weight.

Marked hyperlipemia was produced only in fat-fed diabetic rats (figure 2). Their plasma triglyceride level

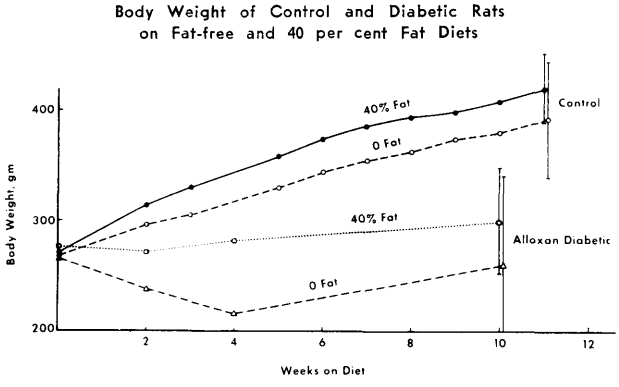

FIGURE 1

(439 ± 523 mg. per 100 ml.*) at the end of the experiment was significantly higher ($p < .02$) than that observed in control rats fed the identical 40 per cent fat diet (59 ± 19 mg. per 100 ml.). This difference was apparent by four to six weeks (table 2). In contrast, diabetes had no effect on plasma triglyceride levels of rats fed the fat-free diet, since triglyceride levels of the alloxan diabetic group were virtually identical ($p > .50$) to those of the nondiabetic controls.

Since milky plasma was produced only in the fat-fed diabetics, fat particles could be obtained for analysis only from this group. The composition of these fat particles (5 per cent sterol, 3 per cent phospholipid, and 92 per cent triglyceride) was comparable to the composition of plasma fat particles derived from dietary fat in normal rats (6 per cent sterol, 8 per cent phospholipid, and 86 per cent triglyceride)[5] and man.[10-11]

*Mean ± standard deviation.

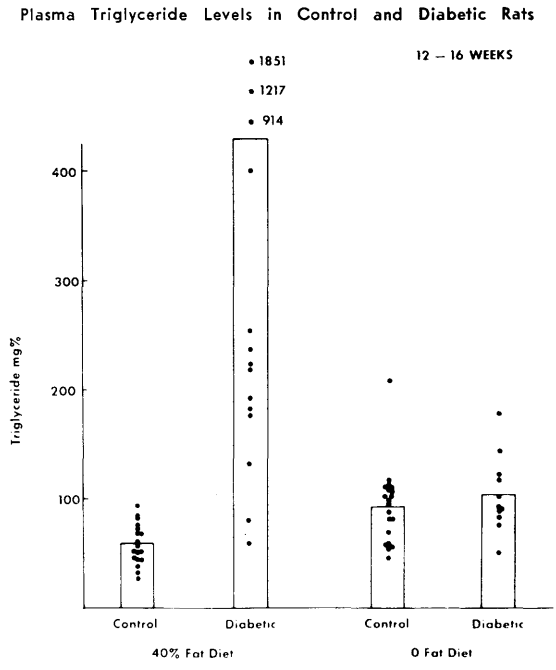

FIGURE 2

A difference in triglyceride levels was also observed between control groups. Normal rats fed the fat-free (high carbohydrate) diet had significantly higher triglyceride levels (94 ± 36 mg. per 100 ml.) than normal animals fed the 40 per cent fat diet (59 ± 19 mg. per 100 ml.) ($p < .001$).

The degree of hyperlipemia in the fat-fed diabetic group was not correlated with blood glucose levels ($r = -0.15$; $p > .60$) (figure 3), but was inversely correlated with weight gain ($r = -0.63$; $p < .02$).

DISCUSSION

These results indicate that hyperlipemia in chronic alloxan diabetes is dependent on the presence of dietary

TABLE 2

Weeks after alloxan	Diet	Group	Number of rats	Plasma triglyceride mg. per cent Range	Mean±S.D.*
4-6	40 per cent fat	Control	12	24-99	61± 20
		Diabetic	13	43-2,204	369±558
	0 fat	Control	13	56-153	101± 29
		Diabetic	12	30-211	113± 58
12-16	40 per cent fat	Control	18	26-94	59± 19
		Diabetic	14	60-1,851	439±523
	0 fat	Control	20	46-210	94± 36
		Diabetic	11	51-179	105± 35

*Standard deviation

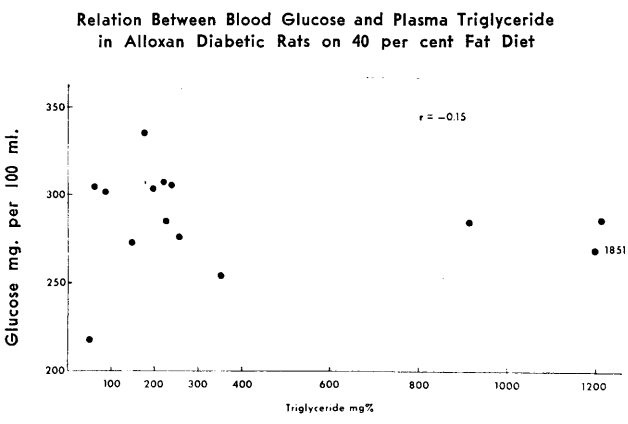

FIGURE 3

fat. When fat is excluded from the diet, diabetic and normal rats have similar triglyceride levels. Presumably, the hyperlipemia previously reported in alloxan diabetic rats, rabbits and dogs fed ordinary stock diets[12-15] or fat-enriched diets[16-17] is related to the fat content of the diet. Similarly, hyperlipemia has been observed in fed pancreatectomized rats,[18] dogs[19] and baboons.[20]

A decrease in adipose tissue lipoprotein lipase activity has been demonstrated in acute alloxan diabetes in rats[15,21-22] which appears to correlate with increased plasma triglyceride levels.[15] Similarly, a decrease in plasma lipolytic activity has been reported in alloxan diabetic and pancreatectomized rats[23] and dogs.[24] If lipoprotein lipase activity is important in the assimilation of circulating particulate triglyceride of dietary origin, then decreased activity might be expected to contribute to hyperlipemia. In accord with this formulation, a relationship between decreased post-heparin plasma lipolytic activity and hyperlipemia has been observed in other hyperlipemic states in man,[25-26] related to intake of dietary fat.

Analysis of plasma fat particle composition in this study suggests that they are "primary" particles derived from dietary fat.[7] Thus this result is also consistent with the concept that a fault in the handling of dietary fat is involved in the pathogenesis of hyperlipemia associated with chronic insulin insufficiency. Attempts to study the removal of particulate fat from plasma in the diabetic state in other species have yielded inconclusive results. With the use of a radioactive triglyceride-plasma complex, thought to be a physiological tracer for plasma particulate fat of dietary origin,[27-28] insulin deprivation had no effect on triglyceride clearance in diabetic subjects.[29] Studies of the clearance of artificial fat emulsions from plasma, shown to be impaired in diabetic animals deprived of insulin,[24,30] is of questionable relevance, since the mode of removal of these emulsions differs from that of native particulate fat.[31-33]

An elevation of plasma triglyceride has been observed in fasting rats within twelve to eighteen hours after total pancreatectomy.[34,35] Mobilization of free fatty acids (FFA) in this acute insulin deficiency state might be expected to result in some increase in circulating plasma triglyceride levels in the absence of dietary fat, since chronic mobilization of FFA can lead to a rise in plasma triglyceride.[36] The frequent mild elevation of plasma triglyceride levels observed in human acute diabetic ketoacidosis[4,6] is consistent with this concept. However, in untreated alloxan diabetic rats, plasma FFA levels are not elevated.[37] Furthermore, interruption of FFA mobilization with nicotinic acid[37] or by hypophysectomy[18] does not reduce the hyperlipemia of diabetic rats fed fat-containing diets.

Although it is possible that accumulation of dietary triglyceride in plasma is partly related t elevated endogenous triglyceride levels,[38] these levels attained by diabetic rats maintained on the fat-free diet could not be distinguished from the normal response to the same diet. The elevated plasma triglyceride levels observed in all rats fed the fat-free, high carbohydrate diet confirms previous observations in the rat[17,39] and appears to be similar to the effect of high carbohydrate diets on plasma triglyceride levels in man, whether normal[40] or insulin-treated diabetic subjects.[9]

Results in this study, extrapolated to man and coupled with prior observation,[2,3,7,41] suggest that there may be two distinct types of gross hyperlipemia associated with the human diabetic state. One variety appears to be associated with chronic, partial insulin deficiency. Diabetics who develop severe hyperlipemia generally are young, and have symptoms of insulin insufficiency for many months,[3] while remaining free of marked ketoacidosis.[2-3] The hyperlipemic manifestations are readily reversible with the addition of insulin. An abnormality in the handling of dietary fat appears to be involved in the pathogenesis of this form of diabetic hyperlipemia.

The other type appears to be more frequent and is associated with the normal, or even excessive, plasma insulin characteristic of the mild, nonketosis prone, adult diabetic.[41-42] Typically, this variety of hyperlipemia is accentuated on fat-free high-carbohydrate diets. In this situation, circulating fat particles are distinct from those found in plasma during fat absorption,[7] and appear to be derived from triglyceride-rich lipoproteins synthesized in the liver. Dietary fat, when isocalorically substituted

for carbohydrate, produces decreased plasma triglyceride levels. Post-heparin lipolytic activity is normal. This variety of hyperlipemia has been termed "carbohydrate-induced." The syndrome always includes some demonstrable abnormality of carbohydrate tolerance,[7,41-42] albeit minimal in many instances, and may be associated with premature vascular disease.[43-44] The addition of insulin has little or no effect on plasma triglyceride levels. Dietary fat clearly has no role in the pathogenesis of this form of hyperlipemia, in contrast to its apparent role in the development of hyperlipemia associated with chronic insulin deficiency, as exemplified in the present study.

Thus, one type of diabetic hyperlipemia could be considered to be "fat-induced," analogous to the hyperlipemia associated with myxedema,[45] and to primary "fat-induced" hyperlipemia.[25] Further support for this concept awaits additional studies in man.

ACKNOWLEDGMENT

This study was supported in part by U.S. Public Health Service Grant AM 066770, Training Grant TI AM 5020 and Research Career Development Award 1 K3 AM 28, 167-01 (Dr. Bierman).

The authors are grateful for the skilled technical assistance of Eileen M. Severns.

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Digestibility of High-Amylose Corn Starches

Because of the industrial importance of amylose, an intensive breeding program has been undertaken to develop corn yielding starch high in content of this carbohydrate. Normal corn starch contains about 25 per cent of amylose, whereas some of the new hybrids have 60 to 70 per cent. These grains could also find their way into feedstuffs, particularly if agronomic characteristics are desirable or if production exceeds industrial needs. Although varietal differences in corn have been reported with respect to content of protein, fat, niacin, and pantothenic acid (*Nutrition Reviews* 3:26, 1945; 8:241, 1950; *11*:50, 1953; *12*:110, 139, 1954), relatively little is known concerning the influence of genetic selection on availability of nutrients from this important crop.

Because of their interest in the structure of starch granules, R. M. Sandstedt, D. Strahan, S. Ueda and R. C. Abbot (*Cereal Chem.* 39:123, 1962) were led to study the susceptibility of raw, high-amylose corn starch to enzymatic degradation with pancreatin in vitro. For comparative purposes, in vitro digestibility of other common cereal and root starches was also measured.

The widest variation in digestibility was found among the various maize starches, but digestibility was not related to amylose content per se. For example, starches intermediate in amylose content (36 to 47 per cent) were as readily digestible as was waxy corn starch containing less than 5 per cent of amylose. Corn starch samples containing greater amounts of amylose (48 per cent and above) showed markedly reduced digestibility. Further evidence of the nonassociation of digestibility with amylose content was revealed by comparisons among root starches: although potato and cassava starch both contain about 20 per cent of amylose, the former was only 5 per cent digested but the latter was 53 to 58 per cent digested in the standard twenty-hour period.

Analysis of the data relative to genetic composition demonstrated that poor digestibility was associated with the presence of the *ae* gene. The *du* and su_2 genes were associated with intermediate amylose content and a high degree of susceptibility to the action of amylase. The homozygous su_2 sample was the most digestible of the known genetic strains and that homozygous for

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