Serum Lipids in Diabetic and Nondiabetic Rats

Effect of Varying Lipid Diets

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Since the pre-insulin era, treatment of diabetes by a high fat diet has been an accepted therapy. Little question exists as to the efficacy of this diet in aiding diabetic control. However, as diabetics live longer in our present insulin age, more are succumbing to arteriosclerotic vascular disease. Since considerable evidence exists relating high blood lipids to the development of arteriosclerosis, the efficiency with which a diabetic animal metabolizes excess fat becomes a pertinent question.

Early work with diabetic dogs revealed an abnormally high lipemia in those fed high fat diets.[1] And recent work in humans showed a decreased tolerance for ingested cholesterol by diabetics.[2]

The following investigation was performed in order to determine the tolerance of the uncontrolled diabetic rat to ingested cholesterol, phospholipid and fatty acid.

METHODS

Male rats of the Wistar strain weighing 200 to 250 gm. were divided into control and diabetic groups. Half the number of animals in each group were placed on a diet containing 47 per cent cocoanut oil, 16 per cent cholesterol, 10 per cent phospholipid (from dried egg yolks), 26 per cent powdered Rockland Mouse Diet and added supplements of Vitamins B and C and Calcium Gluconate. The control normal and diabetic animals were fed the Rockland Mouse Diet with similar supplements.

We recognize that previous investigators have fed more physiologic quantities of cholesterol in their high fat diet. However, because of our interest in producing vascular lesions and because of the difficulties in previous attempts to do so in rats, we decided to use the noted high dosages of cholesterol. On this diet the animals gained as much as or more weight than did the control or diabetic animals on the normal diet. The rats were weighed weekly. Table 1 is a summary of the variations in weights in each group.

TABLE 1
Changes in rats' weights per week (grams)

	Control diet	Lipid diet
Normal	+ 5 to +15	+10 to +20
Diabetic	−20 to + 5	− 5 to +15

It was our chief objective to determine whether the diabetic animals on a high lipid intake suffered any abnormality in their tolerance of lipids. Although analysis of fasting sera may have resulted in more uniform results, lipemia is most prominent postprandially. Thus, if any abnormal lipid tolerance were to be detected, it would be more obvious after eating. In view of these facts, the experiment was conducted on nonfasting rats permitted to eat their respective diets as desired. The animals' food cups were kept full at all times and blood was drawn always at the same time of day, between 9:30 and 10:30 a.m.

Diabetes was induced by three subcutaneous injections of 60 mg. of alloxan on successive days when a nonfasting blood sugar usually revealed a blood glucose of 200 mg. per 100 ml. or more. If necessary, a fourth injection was given.

Diabetic animals were maintained for twelve to sixteen weeks before their serum lipids were examined. Nonfasting blood sugars were obtained every two weeks. Those showing blood sugars below 190 mg. per 100 ml. were treated with alloxan again until their blood glucose reached 200 mg. per 100 ml. About two-fifths of the diabetic animals required additional alloxan treatment.

Blood for lipid determination was not taken from a diabetic animal appearing terminally ill or from one that had its alloxan less than seven days previously. It was desirable that animals with only mild or moderate diabetes be used lest terminal shock or dehydration become factors causing important alteration of the animals' serum lipid.

Blood was taken from animals maintained on a high lipid diet for ten to twelve weeks. About three milliliters were obtained for analysis by cardiac puncture of the nembutal-anesthetized rat. Serum total cholesterol,[3] total fatty acids,[4] lipid phosphorus[5, 8] and a final blood sugar[6]

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were determined.

All analyses were made in duplicate. Recoveries and standards of cholesterol, palmitic acid and inorganic phosphorus were used to control the determinations of the respective blood lipid components. A determination was considered valid, if the following criteria were fulfilled for each series:

1. Duplicates within 2 per cent.
2. Duplicates on known cholesterol solution between 98 and 102 mg. for 100 mg. per 100 ml.
3. Duplicates on known inorganic phosphorus solution between 9.8 and 10.2 mg. for 10 mg. per 100 ml.
4. Duplicates on a known palmitic acid solution between 39.0 and 41.0 mEq. for 40.0 mEq/L.
5. Recoveries from sera of cholesterol (97 to 102 mg.), inorganic phosphorus (9.8 to 10.2) and palmitic acid (39.0 to 41.0 mEq.) were required after the above noted quantities of the respective lipids had been added.

RESULTS

One may observe from table 2 that the normal animals on a high lipid diet (group 3) have significantly elevated serum lipids. The values for the serum lipids in this group are significantly* elevated over those serum values found in the rats of group 1 (nondiabetic on normal diets). In the latter group serum total cholesterol was 47.1 mg. per 100 ml., total fatty acids 10.7 mEq/L., and lipid phosphorus 4.3 mg. per 100 ml. These values compare with the respective values in group 3 of 85.4 mg., 18.7 mEq., and 5.4 mg.

The diabetic animals on a control diet (group 2) may be seen from the table to have significant elevations only in the serum fatty acid component. This value, 22.3 mEq/L., may be compared with 10.7 mEq. in the serum of the control rats in group 1.

Upon comparing the serum lipids in normal rats, groups 1 and 3, and diabetic rats, groups 2 and 4, the increment in each serum lipid component from groups 1 to 3 and from groups 2 to 4 reveals that normal rats on a high lipid diet (group 3) have an average increase in serum lipids over the respective values in the normal rats on a control diet (group 1) of 38.3 mg. of cholesterol per 100 ml. of serum, 8.0 mEq. of fatty acid per liter of serum, and 1.1 mg. of lipid phosphorus per 100 ml. of rat serum. These values compare with the respective increases in the serum lipid components from group 2 (the diabetic on control diet) to group 4 (the diabetic on a high lipid diet) of 75.1 mg. of cholesterol, 6.4 mEq. of fatty acid, and 2.9 mg. of lipid phosphorus. *The serum elevations of cholesterol and of lipid phosphorus were significantly higher in the diabetic group.* However, the increase in serum fatty acids was approximately the same in both groups.

An additional observation was that the sera of the diabetic animals on a high lipid diet (group 4) always were lipemic. The normal animals on a control diet never revealed lipemic sera while group 2 and 3 animals had lipemic sera only 20 per cent of the time. Furthermore, the degree of lipemia was far more intense in group 4.

DISCUSSION

It is a well established fact that ingestion of fat results in a rise in serum lipids. Our data are in agreement, for both the normal and diabetic rats when fed a high fat diet (groups 3 and 4) revealed marked elevation of their serum lipids above the respective values in the groups fed control diets (groups 1 and 2).

The present data from the diabetic rats (group 2) reveal that only the total serum fatty acids were elevated significantly above those values in normal rats. The serum cholesterol and phospholipid showed no change. These facts differ from our previous findings in diabetic rats on a normal diet.[8] However, the previously reported study dealt with rats acutely diabetic, that is, for two

* All calculations for statistical significance are based on formulae from R. A. Fisher's text.[7]

TABLE 2

The serum lipids and blood glucose of intact and alloxan-diabetic rats maintained on control and high-fat diets

				Serum lipids, average values with S.D.				
Group number	Diet	Animal condition	Number in series	Total cholesterol mg./100 ml.	Total fatty acids mEq/L.	Lipid phosphorus mg./100 ml.	Lipemia % of animals	Blood glucose (final) mg./100 ml.
1	Control	Normal	13	47.1(±12.5)	10.7(±3.1)	4.3(±0.43)	0	99±17
2	Control	Diabetic	13	50.4(± 7.3)	22.3(±9.0)*	4.8(±0.25)	23	287±90
3	Lipid	Normal	12	85.4(±12.0)*	18.7(±5.5)*	5.4(±1.12)*	17	120±27
4	Lipid	Diabetic	15	125.5(±31.0)†	28.7(±8.0)	7.7(±1.51)†	100	329±79

*Elevation from group 1 is mathematically significant (p=less than 0.01).
†Elevation from group 2 is mathematically significant (p=less than 0.01).

to ten weeks. The fact that the rats in the present study had suffered diabetes for a prolonged period suggests that their metabolism had greater opportunity to adjust, that the severity of the diabetes ameliorated, or that the fat deposits had become depleted. The explanation for persistence of the elevation in the fatty acid component in the diabetic group on control diet (group 2) may be found in Gurin's work.[9] He showed that lack of insulin prevents incorporation of the short chain fatty acids into the normal metabolic channels and therefore results in accumulation of the shorter chain acids as fat deposits became depleted.

Upon comparing the *elevation* in serum lipids in nondiabetic animals fed a high fat diet with the rise in serum lipids in diabetic animals fed a similar diet, significant differences are uncovered in the cholesterol and phospholipid components of the two groups. The increase in fatty acids is about the same. The rise in the total cholesterol of the diabetic rats (group 4) is almost double that of the nondiabetic group on a similar diet (group 3). Whether this defect in capacity to utilize cholesterol by the diabetic rat may have significance in the development of arteriosclerosis in these animals cannot be answered at this time. The experimental production of arteriosclerotic lesions in the rat is a notoriously difficult procedure. But, certainly in view of the accelerated development of arteriosclerosis in the diabetic human, and in view of the relationship of cholesterol feeding and resultant arteriosclerosis in the rabbit, it is interesting to speculate that perhaps a decreased tolerance for cholesterol in the diabetic accentuates the arteriosclerosis. This possibility becomes more real if one realizes that recent studies suggest that the bulk of cholesterol deposition in experimental arteriosclerosis is from exogenous sources.[10, 11]

Why do the diabetic animals suffer a decreased tolerance for ingested cholesterol? Is the (alloxan-diabetic) rat's liver defective or is its cholesterol excretory mechanism somehow impaired so that exogenous cholesterol cannot be metabolized at the usual rate? The probability is that the surplus exogenous fat in the form of fatty acids and phospholipids fed to these animals is broken down and resynthesized to cholesterol. For, in accordance with Brady and Gurin's work, the diabetic liver can no longer convert acetate to the long chain fatty acid, but can synthesize cholesterol at the usual rate. Thus, the diabetic animals on a high fat diet (group 4) suffered a rise in serum cholesterol not only because they were fed cholesterol but also because they were fed other fats. These fats could be broken down and resynthesized to cholesterol but no longer into fatty acids. Therefore, since the usual channels for resynthesis of longer chain fatty acids from the two carbon fragments were blocked because of insulin lack, the formation of cholesterol was facilitated.

The additional finding of a *greater* increment in the serum phospholipid in the diabetic animals (groups 2 to 4) when compared to the increase in the control rats (groups 1 to 3) can be explained similarly. The liver, small intestines and kidneys of animals with no insulin have a higher than normal phospholipid synthesis.[12] In view of this fact, we can understand that the diabetic rats fed abnormal quantities of cephalin and fatty acids have an increase in their serum phospholipid not only because of the administered cephalin but also (in contrast to the nondiabetic) because they are reforming larger quantities of degraded fatty acid fragments to phospholipid.

The observation that the diabetic rats on a high lipid intake (group 4) was the only group to provide sera which was consistently lipemic is only a corroboration of the fact that this group yielded sera with the highest absolute values for total fatty acids. This finding is in agreement with turbidometric studies done on normal and diabetic postprandial, human sera.[13]

SUMMARY

1. Sera of control and alloxan-diabetic rats on normal and high lipid diets were analyzed for total cholesterol, lipid phosphorus and total fatty acids.

2. Sera of nonfasting control rats on a high lipid intake revealed significant elevations above the control group on normal diet in all three lipid components. The sera of diabetic rats on a high lipid diet showed mathematically significant elevations over their controls only in the cholesterol and phospholipid components.

3. Rats, diabetic for more than twelve weeks, and on a normal diet, revealed significant elevations only in the serum fatty acids. Serum cholesterol and lipid phosphorus were not elevated.

4. Comparison of the *increases* in serum lipids between the diabetic (group 2 and 4) and the nondiabetic (group 1 and 3) groups revealed that the diabetic animals on a high lipid intake sustained significantly higher *elevations* in the cholesterol and phospholipid components.

5. The metabolic mechanisms which are thought to facilitate abnormal increases in serum cholesterol and phospholipid while maintaining a "brake" on the increase in the serum fatty acid in the diabetic animals on a high lipid diet are discussed.

SUMMARIO IN INTERLINGUA

Lipidos Seral in Diabetic e Nondiabetic Rattos a Dietas Normal e Ric in Lipidos

1. Seros ab rattos de controlo e ab rattos con diabete

alloxanogene, mantenite con dietas normal e con dietas ric in lipidos, esseva analysate pro cholesterol total, phosphoro lipidic, e acidos grasse total.

2. Seros ab nonjejun rattos de controlo con un ingestion ric in lipidos revelava significative elevationes supra le gruppo de controlo con dietas normal, relative a omne tres componentes lipidic. Le seros de rattos diabetic con un dieta ric in lipidos monstrava mathematicamente significative elevationes supra le controlos solmente in relation a lor componentes cholesterolic e phospholipidic.

3. Rattos que esseva diabetic pro plus que 12 septimanas e que recipeva dietas normal revelava significative elevationes solmente in le acidos grasse del sero. Cholesterol e phosphoro lipidic in le sero non esseva elevate.

4. Le comparation del augmentos in lipidos seral in (1) le gruppos diabetic e (2) le gruppos nondiabetic revelava que le animales con un plus alte ingestion lipidic experientiava significativemente plus alte elevationes del componentes cholesterolic e phospholipidic.

5. Le mechanismos metabolic que es possibilemente involvite in facilitar anormal augmentos del cholesterol e del phospholipidos del sero durante que illos mantene un influentia restringente super le augmento del acidos grasse del sero in animales diabetic que recipe un dieta ric in lipidos es discutite.

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Social Attitude Toward Invention

Today we have thousands of scientists for every one (in the modern sense) that existed a century ago. But the vast majority of today's scientists—often the most competent—are engaged not in invention of fundamentally new principles, processes, or things, but in development and in various essential forms of technical service. Our society often lures even the scientist of highest integrity away from his dreams to become a well-paid cog in organized research. Business is aware of this difficulty and tries to compensate by generous contributions to academic training, but it is questionable whether this accomplishes anything more than enlargement of the pool of talent from which to recruit its technical staff.

The present social attitude toward invention in the United States is one of complete acceptance of any sort of development announced by a responsible agency. In general, public skepticism is a thing of the past. But this represents less change than it seems, for a century ago there were few responsible agencies in the modern sense. New things were promoted largely by impecunious individuals who would inspire about as little public confidence today as they did then. Respect is given today not to individual technologists (as a rule) but to the institutions they represent. In one sense we seem to be drifting backward toward the attitude of the seventeenth century for at no time in history has conformity been more important than it is today. And this applies to both sides of the iron curtain. In Russia it is dangerous not to conform. Here it is economically inadvisable, and most of us want to make a living.

Eugene Ayres in *American Scientist* 40: 521-40, October 1955.