The Effect of Acute Insulin Deficiency in the Rat on Adipose Tissue Lipolytic Activity and Plasma Lipids

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Recently lipoprotein lipase activity of rat adipose tissue has been compared with tributyrinase activity* of the same tissue.[1] In contrast to the tributyrinase activity, lipoprotein lipase activity increased markedly when insulin was administered to diabetic rats. This response to insulin administration, and the apparent role of lipoprotein lipase in plasma clearing[2,3] prompted the present study. The rapidity with which adipose tissue lipoprotein lipase activity decreases after cessation of insulin administration to diabetic rats was compared with changes in tributyrinase activity. These enzymatic activities were related to the plasma triglyceride and fatty acid levels at the same time intervals. Blood glucose was determined in addition to the ability of the excised diaphragm to remove glucose from an incubation medium.

METHODS

Preparation and handling of animals: Rats weighing 75 to 135 gm. were fasted for forty-eight hours and then given intravenously alloxan monohydrate, 40 mg. per kilogram of body weight. At the end of two weeks, blood sugars of each of the surviving diabetic rats were found to be greater than 350 mg. per 100 ml. Protamine Zinc Insulin† was then administered subcutaneously in a dosage of 2 units per 100 gm. of body weight per day for two weeks. After this, Crystalline Zinc Insulin, 1 unit per 100 gm. of body weight, was administered every eight hours for four doses.

The fourth and final dose of Crystalline Insulin was given with 0.250 gm. of glucose subcutaneously. The rats were allowed to eat ad libitum and were given no further medications. The fifty-nine rats included in this study were sacrificed at intervals between one and seventy-two hours after the final injection of insulin. At predetermined times each rat was selected randomly and decapitated.* The blood was collected in tubes containing oxalate. The diaphragms were immediately excised and placed in Krebs-Ringer bicarbonate buffer at a *p*H of 7.4, after which the epididymal fat pads were removed and placed in twenty volumes of acetone.

Extraction and determination of lipolytic activity: The epididymal fat was desiccated and defatted in the manner described by Korn.[2] The lipolytic activity was extracted, assayed, and expressed as the microequivalents (μEq.) of fatty acid released per milligram of protein per hour as previously described.[1]

Blood glucose and lipid determination: An unpublished glucose oxidase method developed in this laboratory by Dr. Y. D. Halsey was used to determine the glucose content of 0.1 or 0.2 ml. aliquots of blood which were deproteinized with barium hydroxide and zinc sulfate.[4] Three milliliters of glucose oxidase reagent† was added to 1 ml. of the protein-free filtrate, mixed, and heated at 36°C. for two hours. One milliliter of 0.7 N hydrochloric acid was then added and mixed immediately. The resultant color, which is stable at room temperature for two hours, was read at 405 mμ. Standards containing 20 to 100 μg. of glucose were

*Tributyrinase activity is an extractable adipose tissue enzymatic activity demonstrated by its ability to catalyze the in vitro hydrolysis of tributyrin.

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*The time elapsed after the final insulin injection and the number of rats included at that time were as follows: 1 hour, 11 rats; 5 hours, 8 rats; 7 hours, 6 rats; 10 hours, 6 rats; 12 hours, 8 rats; 14 hours, 3 rats; 24 hours, 8 rats; 32 hours, 2 rats; 48 hours, 6 rats; 72 hours, 1 rat. Due to the high mortality during the last twenty-four hours only one rat was studied at seventy-two hours.

†250 milliliters of glucose oxidase reagent contained 12.5 mg. of glucose oxidase (crude Sigma, purified), 2.5 mg. of horse-radish peroxidase (C. F. Boehringer und Sohns, Mannheim, Germany), 2 ml. of a 1 per cent solution of o-dianisidine (Eastman Practical, recrystallized from methanol-water) in methanol, and sufficient phosphate buffer to yield a final concentration of 0.1 M potassium phosphate, *p*H 7.0. The purity of the glucose oxidase and the age of the peroxidase determine the amount of each to be used in making the reagent which is stable for several days in the refrigerator.

run concomitantly. The above conditions have been established as optimal for amounts of glucose up to 150 μg. per assay system, with an average difference between duplicates of two parts per 700.

Fatty acids were determined in 0.5 or 1.0 ml. aliquots of plasma by the method of Dole.[5] Plasma triglycerides were determined in the following manner. The lipid content was extracted from 0.5 or 1.0 ml. aliquots of plasma by means of the two-phase system described by Freeman et al.[6] and transferred in hexane to a silicic acid column.*

Preparation and elution of the columns was carried out as described by Barron and Hanahan.[7] The organic solvents were evaporated from an aliquot representing 0.01 to 0.02 of the triglyceride fraction. The saponification procedure of Van Handel and Zilversmit[8] has been modified in this laboratory so that 0.1 ml. of 1 N alcoholic KOH is used for the hydrolytic reaction and 0.2 ml. of 1 N H_2SO_4 is added subsequently. Triolein standards containing 0.01 to 0.08 mg. were processed simultaneously. The glycerol resulting from the saponification reaction was quantitated in the manner described by Korn.[2]

Glucose uptake by the excised diaphragm: An estimate of residual insulin was made by incubating the excised diaphragm in Krebs-Ringer bicarbonate buffer which contained 10 μmoles of glucose per liter. The glucose content of the medium before and after incubation was determined by the glucose oxidase method. The incubations and calculations were carried out as reported by Spiro et al.[9]

RESULTS

Figure 1 is a composite of the data obtained from the fifty-nine diabetic rats included in the study. After the cessation of all insulin injections, lipoprotein lipase activity decreased rapidly while tributyrinase activity changed very little. Plasma fatty acids and blood glucose rose rapidly to a plateau. In contrast to this, the plasma triglycerides rose less rapidly but more steadily. Glucose uptake by the excised diaphragm decreased significantly ($p<0.001$) in five hours. No further significant changes were noted.

When the logarithms of lipoprotein lipase activity and tributyrinase activity as dependent variables are related to the logarithm of time as the independent variable, the regression is a straight line function for each enzyme (figure 2). The mean values as plotted in figure 2 represent different numbers of animals at each time interval. This has been taken into consideration in the calculated regression lines which are weighted according to the number of observations at a particular time. Thus, the one-hour value which includes eleven animals is given considerably more weight than the seventy-two-hour value which includes only one animal. The calculated slopes are —0.290 for lipoprotein lipase activity and —0.041 for tributyrinase activity. The probability that these are significantly different slopes is high ($p<0.005$). Analysis of the calculated regression line for lipoprotein lipase activity reveals that between one and twelve hours the extractable lipolytic activity had decreased by one half.

Although there is no correlation of plasma lipids and blood glucose levels with tributyrinase activity, a significant inverse (negative) correlation does exist with lipoprotein lipase activity. Table 1 lists the correlation

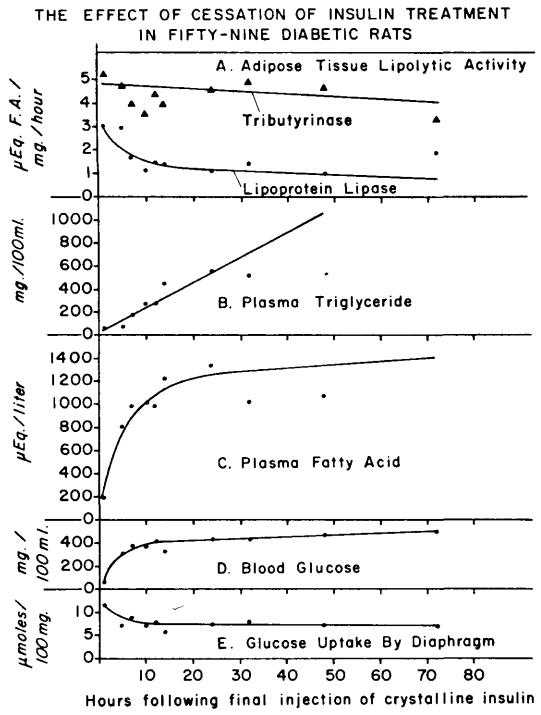

FIG. 1. Fifty-nine alloxan diabetic rats were treated for two weeks with Protamine Zinc Insulin, 2 units per 100 gm. body weight per day, and then with Crystalline Zinc Insulin, 1 unit per 100 gm. body weight every eight hours for four doses. They were sacrificed in groups between one and seventy-two hours after the final injection of Crystalline Insulin. Average values are plotted for each time interval studied. The forty-eight- and seventy-two-hour values for plasma triglyceride rose above the limits of the composite graph. They were 1,234 mg. per 100 ml. as the average for the six rats sacrificed at forty-eight hours and 4,400 mg. per 100 ml. for the one rat surviving until seventy-two hours. Similarly, the seventy-two-hour value for plasma fatty acids was 2,508 μEq. per liter.

*Silicic acid was packed to a height of 5 cm. in columns 0.5 cm. in diameter. The capacity of such columns is 8 to 10 mg. of lipid.

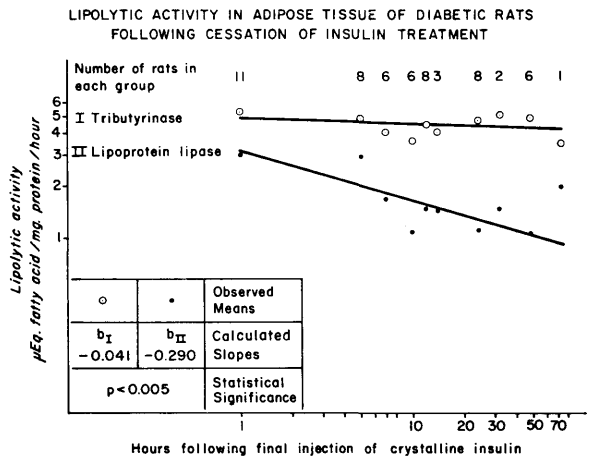

FIG. 2. The mean values for lipolytic activity were plotted at the selected time intervals. Weighted regression slopes were calculated with the logarithms of the lipolytic activity used as the dependent variable and the logarithms of time as the independent variable. The statistical significance of the difference between the calculated slopes was determined by the *t* test.

coefficients as calculated from the logarithms of lipoprotein lipase activity, plasma lipids, and blood glucose.

DISCUSSION

The relationship between plasma and adipose tissue triglyceride can be divided into three phases for the purpose of this discussion; namely, disappearance of triglyceride from the plasma, intracellular metabolism of triglyceride, and release of the fatty acid constituents from adipose tissue. Current knowledge of adipose tissue metabolism has been outlined in detail in several recent reviews[10,11] and will be mentioned here only as necessary to the discussion of the experimental results.

Hydrolysis of plasma triglyceride occurs prior to the incorporation of its constituent fatty acids into tissue triglyceride[10] and is known to be catalyzed by the postheparin "clearing factor," lipoprotein lipase.[2,3] Adipose tissue is capable of removing fatty acids[12–15] and triglycerides[12,17–19] from plasma in vivo and from incubation media. Thus it appears that although hydrolysis is an essential reaction it does not necessarily have to occur in the extracellular compartment. If the fatty acids are removed as the triglyceride ester, hydrolysis occurs within the cell, presumably as a result of the action of lipoprotein lipase.[17]

TABLE 1

Statistical correlation of plasma lipids and blood glucose with adipose tissue lipoprotein lipase activity

	r	t	p
Triglyceride	−0.63	6.13	<0.001
Fatty acids	−0.58	5.32	<0.001
Glucose	−0.56	5.11	<0.001

r = Correlation coefficient.
t = Value obtained by t test.
p = Statistical significance of the correlation.

In support of the hypothesis that lipoprotein lipase plays an important role in the assimilation of plasma triglyceride by adipose tissue are the following observations: the presence in adipose tissue of lipoprotein lipase[2] which is liberated in vitro by heparin,[20] the presence of high levels of tissue lipoprotein lipase activity[20,21] under nutritional conditions known to be associated with increased uptake of triglyceride by the tissue[12,17,19] and the apparent relationship between lipoprotein lipase activity and in vitro uptake of triglyceride by adipose tissue.[17,22]

From the foregoing discussion it would be expected that a deficiency in lipoprotein lipase would result in a decreased plasma clearance, followed by hypertriglyceridemia. In fact, a decrease in postheparin plasma lipoprotein lipase activity has been shown to be associated with essential familial hyperlipemia.[23]

The regression of lipoprotein lipase activity with time (figure 2) demonstrates a dependence of adipose tissue lipoprotein lipase activity on insulin but does not distinguish between the mere presence of insulin and an increased glucose or protein metabolism as facilitated by insulin. Other investigators[20] have shown that adipose tissue from fed rats contains more lipoprotein lipase activity than tissue from fasted rats. It is very likely that a common factor, active glucose metabolism, is responsible for the increased lipoprotein lipase activity in fed normal rats and in insulin-treated diabetic rats.

The rapidity with which adipose tissue lipoprotein lipase activity decreases after the cessation of insulin is worthy of note. A decrease in liver glycogen content and fatty acid synthesis as well as a rise in glucose-6-phosphatase activity have previously been noted in diabetic rats handled in a manner similar to that employed here.[9] While a direct temporal comparison cannot be made between the two experiments, it appears that the decrease in lipoprotein lipase activity of adipose tissue is as rapid as the alterations in liver glycogen content, fatty acid synthesis, and glucose-6-phosphatase activity which follow the cessation of insulin.

The decrease in lipoprotein lipase activity occurred concomitantly with a rise in plasma triglycerides, suggesting that a deficiency of lipoprotein lipase activity may contribute significantly to the elevated plasma triglycerides of uncontrolled diabetes in rats.

Triglyceride, the major component of adipose tissue,

has been shown to be in a dynamic equilibrium.[24,25] This equilibrium is characterized by the following two components: (a) esterification of fatty acids with α-glycerophosphate to form triglyceride and (b) hydrolysis of triglyceride to yield fatty acids and glycerol. Alteration in the rate of either of these reactions will upset the homeostatic balance, causing a net increase in either synthesis or degradation of tissue triglyceride.

The synthesis of triglyceride from fatty acids has been accomplished in vitro with homogenates of rat adipose tissue.[26] The dependence of this reaction upon active glucose metabolism is indicated by its requirement of α-glycerophosphate. Thus it would be expected that in uncontrolled diabetes the esterification reaction is decreased. Indeed, glucose and insulin have been shown to stimulate esterification.[25]

Hydrolysis of tissue triglyceride, presumably the result of a specific lipase,[27] increases the tissue pool of fatty acid.[28] Release of fatty acid from the adipose tissue pool has been attributed to decreased esterification[25] of tissue fatty acids and increased hydrolysis[29] of tissue triglyceride.

Adipose tissue tributyrinase activity which remained relatively constant in the experiments described in this paper provides an interesting comparison with lipoprotein lipase activity. Its physiological significance is not known. One possibility, however, is that in vitro hydrolysis of tributyrin is a manifestation of an intracellular lipase which has as its in vivo function the hydrolysis of tissue triglyceride. Persistent lipolytic activity concomitant with the decrease in esterification, which might be expected with uncontrolled diabetics, would upset the homeostatic balance between synthesis and degradation of adipose tissue triglyceride. This could then lead to an accumulation and release of fatty acids from adipose tissue, and may be the sequence of events which produces the elevated plasma fatty acids seen in uncontrolled diabetes.

SUMMARY

In fifty-nine insulin treated alloxan-diabetic rats, adipose tissue lipoprotein lipase and tributyrinase activities were determined between one and seventy-two hours after the cessation of insulin injections. Blood glucose, plasma fatty acids, and plasma triglycerides were measured simultaneously along with the in vitro uptake of glucose by the excised diaphragm. It was shown that lipoprotein lipase activity decreased rapidly after cessation of insulin, in contrast to the relatively small change in tributyrinase activity. The rapid change in lipoprotein lipase activity exhibits an inverse correlation with blood glucose, plasma triglycerides, and fatty acids. The relationship of adipose tissue lipolytic enzymes to diabetes mellitus is discussed. It is proposed that a deficiency in lipoprotein lipase activity may contribute significantly to the elevated triglycerides of uncontrolled diabetes mellitus.

SUMMARIO IN INTERLINGUA

Le Effecto Exercite per Carentia Acute de Insulina in Rattos Super le Activitate Lipolytic de Tissu Adipose e Super le Lipidos del Plasma

In cinquanta-novem alloxano-diabetic rattos tractate con insulina, le activitate de tributyrinase e lipase de lipoproteina del tissu adipose esseva determinate inter un e septanta-duo horas post le cessation del injectiones de insulina. Le glucosa del sanguine, le acidos grasse del plasma, e le triglyceridos del plasma esseva mesurate in simultaneitate con le acceptation in vitro de glucosa per le excisionate diaphragma. Esseva monstrate que le activitate de lipase de lipoproteina declinava rapidemente post le suspension del administration de insulina, per contrasto con le relativemente micre alteration in le activitate de tributyrinase. Le rapide alteration del activitate de lipase de lipoproteina exhibi un correlation inverse con le glucosa del sanguine, le triglyceridos del plasma, e le acidos grasse. Le relation inter le enzymas lipolytic de tissu adipose e diabete mellite es discutite. Es proponite le these que un deficientia in lipase de lipoproteina contribue significativemente al elevation del triglyceridos de non-stabilisate diabete mellite.

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Partial Hydrogenation and Serum Cholesterol

The bewilderment of the housewife confronted in recent months by promotion for products based upon fats and oils may be reduced in part by interpretation for the layman of a well-planned and well-executed study on the influence of partially hydrogenated dietary fats on serum cholesterol concentrations in man. D. E. McOsker, F. K. Mattson, H. B. Sweringen, and A. M. Kligman (JAMA *180*:380, 1962) have made a statistical evaluation of data collected in a thirty-two week study on thirty-six healthy male prisoners ranging in age from twenty-five to forty-four years (median thirty years) fed various liquid formula diets each containing one of several fats with iodine numbers ranging from 114 to 32. They concluded that partially hydrogenated vegetable fats appear to be no diffferent from unhydrogenated fats in their effect on serum cholesterol concentrations in man.

Although the fats used in this study cover a major portion of the compositional range of hydrogenated vegetable oils consumed in the diet, the authors have carefully pointed out that the entire range was not covered. Thus, some questions about hydrogenated fats remain for both the scientist and the housewife. The test sheds light on the effect of *trans* isomers and adds further data on the effect of mean unsaturation as measured by iodine value, polyunsaturated and saturated fatty acid content, and the ratio of these two fatty acids. However, like other studies of its type, it does not answer all of the questions with regard to the effect of these factors. These investigations do provide interesting information which undoubtedly is already providing foundations for additional studies suggested by remaining gaps in present knowledge.

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