Diabetologia 13, 7–11 (1977)

Diabetologia

The Influence of Age on the Development of Hypertriglyceridaemia and Hypercholesterolaemia in Genetically Diabetic Mice *

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Summary. The effect of spontaneous diabetes on plasma lipids during the natural course of the disease was studied in genetically diabetic mice (C57BL/KsJ-db/db). Hyperlipidaemia developed uniformly in all mice studied and was found to be a characteristic part of the diabetic syndrome, as compared to normal littermates. The hyperlipidaemia was characterized by a marked rise in plasma triglyceride levels with age and severity of the disease, increasing from 120 ± 6 mg/dl at 5 weeks of age to 400 ± 91 mg/dl at 19 weeks of age. In addition, db/db mice were observed to be hypercholesterolaemic as compared to age-matched normal littermates. The plasma cholesterol levels of diabetic mice were elevated early in the disease, as compared to control mice (200 ± 6 vs. 130 ± 7 mg/dl, respectively), and the mean level remained elevated throughout the period of observation.

Key words: C57BL/KsJ-db/db, genetically diabetic mice, hypertriglyceridaemia, hypercholesterolaemia, plasma triglycerides, plasma cholesterol, plasma glucose, plasma insulin.

The mutation, diabetes (db) is inherited in C57BL/KsJ mice as a unit autosomal recessive gene with complete penetrance [2, 4], and all mice homozygous for the db gene develop diabetes spontaneously. Although there are variations in the age of onset and severity of the disease in individual mice, the diabetic syndrome appears to develop biphasically [2, 4]. The early phase is characterized by obesity, hyperinsulinaemia (4–8 × normal), inappropriate hyperglycaemia and, histologically, a reduction in the population of granulated B-cells in the Islets of Langerhans. Other documented abnormalities during the early stage of the syndrome include glycosuria, increased systemic glucose oxidation, increased lipogenesis, and increased hepatic gluconeogenesis. Consistent with the enhanced glucose oxidation and hyperinsulinaemia are the increased activities of several insulindependent glycolytic and pentose phosphate shunt enzymes. Furthermore, the levels of several key hepatic gluconeogenic enzymes (glucose 6-phosphatase, fructose 1,6-diphosphatase, pyruvate carboxylase, phosphoenolpyruvate carboxykinase) are greatly elevated [1]. In this regard, it was shown by Laube et al. [6], using the isolated perfused pancreas, and more recently by Stearns [8] in the intact animal, that genetic diabetic mice are also hyperglucagonaemic.

The second stage of the disease is characterized by weight loss, near normal levels of circulating immunoreactive insulin, extensive degranulation of the B-cells, decreased peripheral glucose utilization, decreased lipogenesis and a continued high rate of hepatic gluconeogenesis. The decrease in glucose oxidation, coupled with the increased production of glucose, produces a continual stress on the B-cell, resulting in B-cell exhaustion and severe hyperglycaemia. These phenomena suggest severe insulin resistance, and the final result is lethal diabetes at six to eight months of age [2, 4].

Although a number of unique characteristics of the diabetic syndrome in db mice have been studied in detail, little has been reported regarding changes in plasma lipid levels during the natural course of the disease. The purpose of this paper is to characterize the plasma lipid profile of the db/db mouse with increasing age, and to correlate these changes with

* This work was supported by a grant from the USPHS-NIAMDD-AM15,100

changes in plasma glucose and insulin, which are documented parameters of the severity of the disease.

Materials and Methods

Homozygous diabetic (db/db) mice and nondiabetic (+/+) control littermates of the C57BL/KsJ strain were purchased from The Jackson Laboratory, Bar Harbor, Maine. All mice were 5 to 7 weeks of age at the time of arrival. Diabetic mice were housed in individual stainless steel wire mesh cages and nondiabetic littermates were housed with no more than six mice per cage. All animals had free access to tap water and were fed mouse chow ad libitum (Purina Formulab Chow, Ralston Purina Co., St. Louis, Missouri). The progression of the diabetic syndrome with age was followed by measuring plasma glucose, insulin, triglyceride, and cholesterol. Body weight was determined at weekly intervals.

Blood samples were obtained at the same time each day (10 a.m.) by puncture of the retro-orbital venous plexus [7] using a thin-walled heparinized haematocrit capillary tube (Sherwood Medical Industries, St. Louis, Missouri); the plasma was collected by centrifugation for five minutes in a haematocrit centrifuge. After separation, the plasma was extruded on to parafilm which permitted the formation of a compact bubble of plasma, from which microliter samples were drawn for analysis. Samples of plasma for the determination of insulin, triglyceride, and cholesterol, when not analyzed immediately, were stored at −20° C until the time of assay not more than one week later.

The nonfasting plasma glucose concentrations were determined immediately in duplicate on 10 µl aliquots of plasma, using the Beckman Glucose Analyzer which employs the enzyme glucose oxidase and is based on the rate of oxygen utilization [5].

Plasma immunoreactive insulin (IRI) of diabetic and control mice was determined in duplicate on 50 µl aliquots of plasma using a radioimmunoassay kit purchased from Amersham-Searle Corporation, Amersham, England. This employs the double antibody method of Hales and Randle [3].

Plasma triglyceride levels were determined in duplicate on 50 µl samples of plasma using the DADE Tri-25 kit (DADE Division, American Hospital Supply Corporation, Miami, Florida).

Total plasma cholesterol was determined in duplicate on 50 µl samples of plasma using the HYCEL Cholesterol kit (Hycel, Inc., Houston, Texas). The methodology is based on the Liebermann-Burchard reaction for determining total cholesterol without prior extraction or precipitation of the plasma. The absorbance of the coloured reaction product is measured at 625 nm and the cholesterol concentration of unknowns is calculated by comparison with HYCEL Cholesterol Standard (200 mg/dl cholesterol in glacial acetic acid).

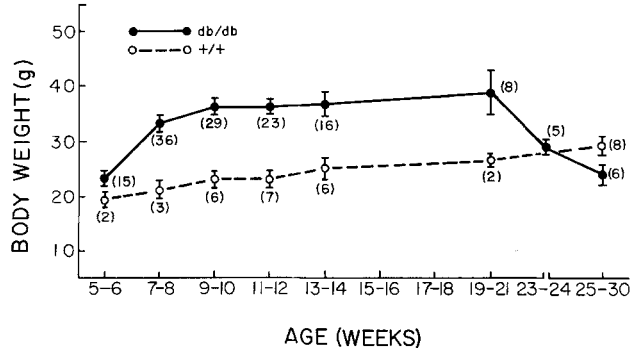

Fig. 1. Change in body weight with age in genetically diabetic (db/db) and normal (+/+) mice. Each point represents the MEAN ± SEM. The numbers in parentheses indicate the number of mice for each point

Results

The characteristics of body weight, plasma glucose, plasma IRI, and plasma triglyceride and cholesterol of diabetic mice as compared to non-diabetic littermates are described below, and document the progressive severity of the diabetic syndrome with increasing age.

Body Weight

The mean body weight of diabetic and normal mice as a function of increasing age is outlined in Figure 1. Diabetic mice were significantly heavier than control animals at 5–6 weeks of age (23 g vs. 19 g, respectively) and their body weight increased rapidly, reaching a mean of 36 g by 9 weeks of age. It was not unusual for some diabetic mice to attain body weights of 45–50 g between 12–15 weeks of age. In contrast, the mean body weight of normal littermates increased gradually with age reaching a relatively stable plateau of 26–28 g at 13–14 weeks of age. Between 20–30 weeks of age diabetic mice gradually lost weight. This onset of weight loss is characteristic of the terminal stage of the diabetic syndrome, and most mice succumbed to the disease by 6–8 months of age.

Plasma Glucose

The mean nonfasting plasma glucose level of diabetic mice was similar to that of nondiabetic control mice at

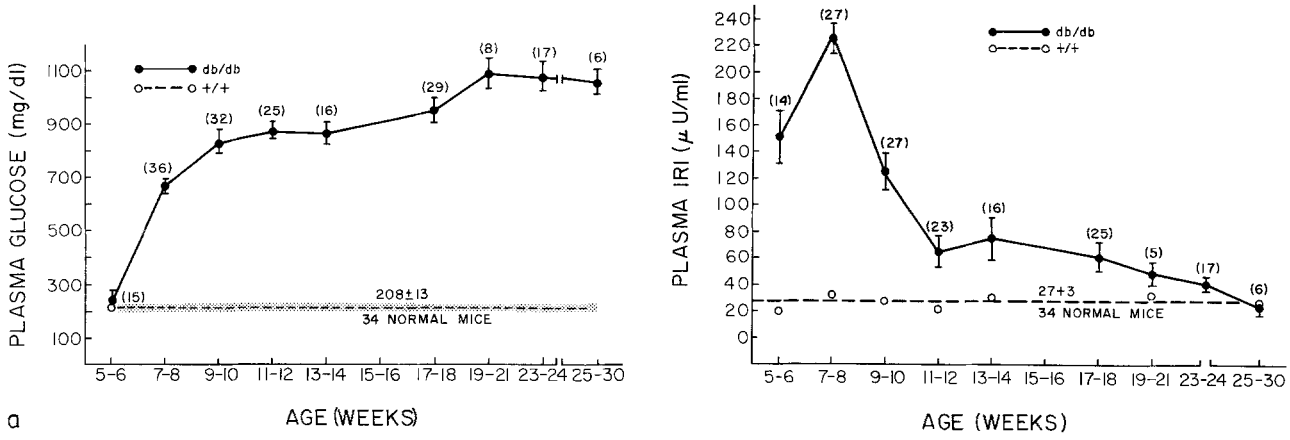

Fig. 2. a Plasma glucose concentration in ad libitum fed genetically diabetic (db/db) and normal (+/+) mice as a function of age. Each point represents the MEAN ± SEM of observations on (n) mice. The overall mean plasma glucose concentration of normal mice is shown for comparison. **b** Plasma immunoreactive insulin (IRI) concentration in ad libitum fed genetically diabetic (db/db) and normal (+/+) mice as a function of age. Each point represents the MEAN ± SEM of observations on (n) mice. The overall mean plasma IRI of normal mice is shown for comparison

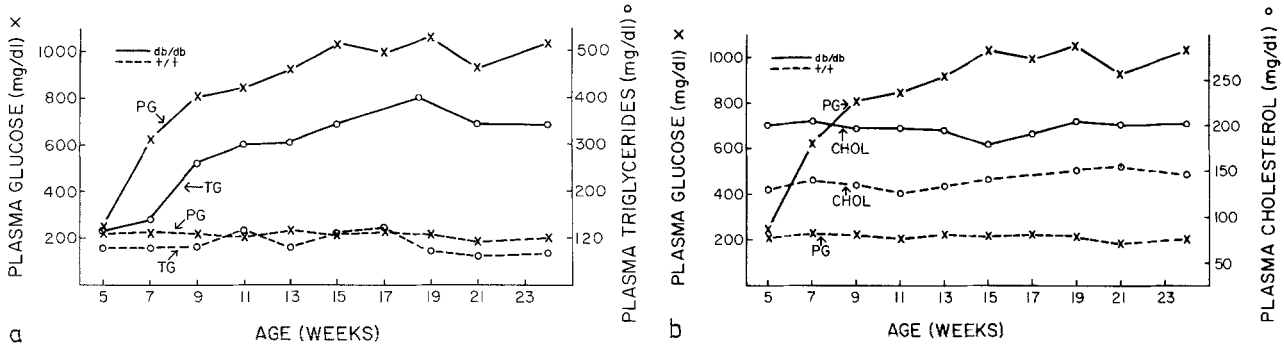

Fig. 3. a Comparison of nonfasting plasma triglyceride (TG) concentration in genetically diabetic mice versus normal littermates as a function of age and plasma glucose concentration. Each point represents the MEAN of 6 mice at each age. **b** Comparison of nonfasting plasma cholesterol concentration in genetically diabetic mice versus normal littermates as a function of age and plasma glucose concentration. Each point represents the MEAN of 6 mice at each age

5–6 weeks of age (Fig. 2a). However, there was a rapid increase in glycaemia, so that by 7–8 weeks of age plasma glucose had reached an uncontrollable level of greater than 600 mg/dl, and by 9–10 weeks of age was 800 mg/dl. Subsequently, there was a slow, but progressive, increase in hyperglycaemia until approximately 19 weeks of age, at which time the glucose level stabilized in the 1000–1100 mg/dl range. In contrast, the plasma glucose level of normal littermates remained stable over the same period of observation, with a mean plasma glucose level of 208 ± 13 mg/dl from 5–30 weeks of age.

Plasma Insulin

Figure 2b shows the mean plasma immunoreactive insulin (IRI) levels of diabetic versus normal littermate controls as a function of increasing age. The observed trend in diabetic mice was one of high circulating plasma IRI levels early in the disease (stage 1), with the levels gradually decreasing to normal or subnormal as the animal entered stage 2. The mean plasma IRI level of 5–6 week old diabetic mice was 7–8 times higher than that of control littermates (153 ± 19 vs. 20 ± 3 μU/ml). The hyperinsulinaemia reached a peak of 226 ± 11 μU/ml at 7–8 weeks of age, beyond which there was a rapid decline until approximately 12 weeks of age. At 11–12 weeks of age, diabetic mice had a mean IRI level of 65 ± 11 μU/ml, approximately three times the amount of hormone present in the normal mouse (22 ± 3 μU/ml). Subsequent to 12 weeks of age, as the severity of the disease increased, there was a gradual decline in plasma insulin, although some degree of hyperinsulinaemia was always present prior to the terminal phase of the syndrome. Between 24–30 weeks of age,

plasma IRI approached normal levels, and the mean insulin level of diabetic mice (24 ± 2 μU/ml) was comparable to that measured in normal mice (27 ± 3 μU/ml). In contrast, the mean plasma IRI level of normal mice remained essentially unchanged during the entire period of observation (27 ± 3 μU/ml).

Plasma Triglycerides and Cholesterol

The mean nonfasting plasma triglyceride and cholesterol levels of genetically diabetic mice versus nondiabetic littermates as a function of age and plasma glucose levels are summarized in Figure 3 a, b, respectively. At 5 weeks of age, the plasma triglyceride level of diabetic mice was only moderately elevated as compared to normal mice (120 ± 6 vs. 84 ± 4 mg/dl). Subsequently, the mean plasma triglyceride level increased progressively with age, rising from 120 ± 6 mg/dl at 5 weeks of age to a peak of 400 ± 91 mg/dl at 19 weeks of age, after which the mean level declined to approximately 350 mg/dl and was essentially unchanged during the remaining period of observation. In contrast, nondiabetic control mice showed significantly lower levels of plasma triglycerides which remained relatively constant throughout the 19 week period of observation. The overall mean plasma triglyceride level of the diabetic group during the 19 week period of study was significantly greater than that of the control animals (305 ± 38 vs. 88 ± 7 mg/dl, $P < 0.01$).

Genetic diabetic mice were hypercholesterolaemic as early as 5 weeks of age as compared to nondiabetic control animals (200 ± 6 vs. 130 ± 7 mg/dl, respectively), and the mean plasma cholesterol level was significantly elevated throughout the 19 weeks of observation (Fig. 3b). The overall mean plasma cholesterol level of the diabetic group was 197 ± 11 mg/dl as compared to 138 ± 8 mg/dl for the control animals. In contrast with the observed rise in plasma triglycerides that occurs with age and increasing plasma glucose in diabetic mice, plasma cholesterol levels did not increase with age or with increasing plasma glucose, but was elevated early (at 5 weeks) and remained high throughout the period of observation.

The overall mean plasma glucose concentration for the 19 week period was 914 ± 43 mg/dl for the diabetic group and 211 ± 8 for the control mice, and the mean body weight for each group was 43 and 25 gm, respectively.

Discussion

Since the initial discovery of the mutant diabetic mouse, several manifestations of the syndrome have been characterized [1, 2, 4]. The data presented herein are in agreement with earlier observations and extend the characterization of the diabetic syndrome to include two additional parameters not previously described. These include the changes in plasma triglyceride and cholesterol levels that occur with increasing age and severity of the disease.

Mutant diabetic mice were shown to uniformly develop hyperlipidaemia, unlike nondiabetic littermates. The hyperlipidaemia was charaterized by a marked rise in plasma triglycerides between the ages of 5–24 weeks. The data obtained also show that db mice are hypercholesterolaemic compared with age-matched normal littermates. Plasma triglycerides were approximately normal in the young animal (5–6 weeks), but the plasma cholesterol level was elevated early in the disease and remained significantly higher than normal throughout the period of study.

The observation that the plasma triglyceride level was only moderately elevated at 5–8 weeks of age when hyperinsulinaemia was present, and that the level increased rapidly as the plasma insulin level declined, suggests the possible involvement of the insulin-sensitive adipose tissue lipolytic and lipogenic enzymes, triglyceride lipase and lipoprotein lipase.

Furthermore, the observation that the progressive increase in plasma triglyceride level with age was associated with a parallel increase in the plasma glucose concentration is of interest. The severe hyperglycaemia in these animals is presumably due to impaired peripheral utilization of glucose, as well as increased endogenous hepatic synthesis of glucose. Since the liver is relatively permeable to glucose in the absence of insulin, as compared to adipose tissue and muscle, the possibility exists that as the diabetes becomes progressively more severe the excess circulating glucose is taken up by the liver and used as substrate for hepatic lipogenesis. The FFA synthesized in the liver would then be esterified to make triglycerides, packaged into very low density lipoprotein (VLDL), and secreted into the circulation.

Thus, the diabetic mouse may have an imbalance between production of glycerides by the liver and their removal from the blood by adipose tissue. The present data do not, however, permit one to conclude whether enhanced lipogenesis or decreased disposal of triglycerides is responsible for the observed hypertriglyceridaemia. Measurements of lipid synthesis or disposal during various stages of the disease would yield information concerning mechanisms possibly involved in the pathogenesis of the increasing hypertriglyceridaemia and whether one mechanism might predominate during the hyperinsulinaemic phase of the syndrome and another during the later stages.

In summary, the characteristics of hypertriglyceridaemia and hypercholesterolaemia were shown to be a part of the spontaneous diabetic syndrome in db mice.

References

1. Chang, A.Y., Schneider, D.I.: Abnormalities in hepatic enzyme activities during development of diabetes in db mice. Diabetologia **6**, 274–278 (1970)
2. Coleman, D.L., Hummel, K.P.: Studies with the mutation, diabetes, in the mouse. Diabetologia **3**, 238–248 (1967)
3. Hales, C.N., Randle, P.J.: Immunoassay of insulin with insulin antibody precipitate. Biochem. J. **88**, 137–146 (1963)
4. Hummel, K.P., Coleman, D.L.: The mutation, diabetes, in the mouse. Science **153**, 1127–1128 (1966)
5. Kadish, A.H., Little, R.L., Sternberg, J.C.: A new and rapid method for the determination of glucose by measurement of rate of oxygen consumption. Clin. Chem. **14**, 116 (1968)
6. Laube, H., Fussgänger, R.D., Maier, V., Pfeiffer, E.F.: Hyperglucagonemia of the isolated perfused pancreas of diabetic mice (db/db). Diabetologia **9**, 400–402 (1973)
7. Riley, V.: Adaptation of orbital bleeding technique to rapid serial blood studies. Proc. Soc. exp. Biol. (N.Y.) **104**, 751–754 (1960)
8. Stearns, S.: Doctoral dissertation, State University of New York, Upstate Medial Center, Department of Anatomy, Syracuse, New York 1976

Received: June 21, 1976, and in revised form: September 27, 1976

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