Age-related Changes in Hepatic Glycogen Metabolism in the Genetically Diabetic (*db/db*) Mouse

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SUMMARY

Hepatic glycogen metabolism was investigated in genetically diabetic C57BL/KsJ-*db/db* mice during their development. Initially, the development of obesity, hyperglycemia, hyperinsulinemia, and hyperglucagonemia in these mice was examined, which illustrated that the diabetes progressed normally. Little difference in hepatic glycogen concentrations was observed, averaging approximately 50 and 60 mg/g liver in diabetic (*db/db*) and control heterozygote (*db/+*) mice, respectively. Glycogen synthase activity (total and *a*-form) was significantly elevated by 5 wk in the diabetic mice relative to controls and reached maximum levels (twofold higher than controls) around 8–9 wk. This activity then slowly declined during the rest of the 15-wk period examined. Both phosphorylase *a* and total phosphorylase activities were also elevated by 5 wk, reaching levels twofold higher than controls. These activities did not decline at the end of this 15-wk period, but instead continued to slowly increase. Glycogen synthase *a* activity showed a positive correlation ($r = 0.54$, $N = 144$) with circulating levels of insulin, and a similar correlation was seen for phosphorylase *a* activity and plasma glucagon levels ($r = 0.64$, $N = 72$). Protein kinase and phosphoprotein phosphatase activities were also measured, but no differences were detected between diabetic and control mice. This longitudinal study clarifies some of the changes in hepatic glycogen metabolism that occur during the progression of diabetes in the *db/db* mouse and indicates a role for circulating insulin and glucagon concentrations on the steady-state activities of glycogen synthase and phosphorylase, respectively. DIABETES 1985; 34:395–402.

The effects of type I (insulin-dependent) diabetes on hepatic glycogen metabolism are well characterized. In streptozocin- or alloxan-induced diabetic animals, it has been observed that glycogen concentrations, glycogen synthase *a*, and phosphorylase (*a*-form and total) activities are all decreased and can be returned to normal levels by insulin supplementation.[1-4] It is not known whether these changes in enzyme activity are due to changes in the amount of enzymic protein and/or changes in the activities of other regulatory enzymes involved in the phosphorylation-dephosphorylation of these two enzymes. Little is known about the effects of type II (non-insulin-dependent) diabetes on glycogen metabolism. However, they might be expected to differ from those seen in type I diabetes, since the obesity, normo- or hyperinsulinemia, and the severe insulin resistance seen in type II diabetes are not usually associated with type I diabetes.

The genetically diabetic C57BL/KsJ-*db/db* mouse, first described by Hummel et al.,[5] is characterized by obesity, hyperglycemia, and an early, transient hyperinsulinemia and is thus a model of type II diabetes.[5-7] This animal model provides a tool to examine (1) the metabolic abnormalities (including alterations in glycogen metabolism) associated with type II diabetes and (2) the role of hormones in metabolic regulation, since plasma insulin and glucagon levels are altered during the development of diabetes in these mice. Liver glycogen has been measured by several laboratories and, while some report 2–3-fold higher concentrations in the *db/db* mouse,[6,8] others report no differences existing.[9] Discrepancies also exist in the reported changes in glycogen synthase activity, although elevations in phosphorylase activity is a consistent observation.[8,9] No information is available on the various kinase and phosphatase activities in these mice that regulate the interconversion between active and inactive forms of glycogen synthase and phosphorylase.

Part of the reason for these discrepancies may be a result of comparing data of animals at different stages in diabetes development. Because of the rapidly changing endocrine status in this mouse during the first few months, sampling at regular intervals may be critical to our full understanding of the changes occurring. In this study, we attempted to examine more thoroughly the glycogen metabolism in *db/db* mice by studying mice 2–15 wk of age at 1-wk intervals. The

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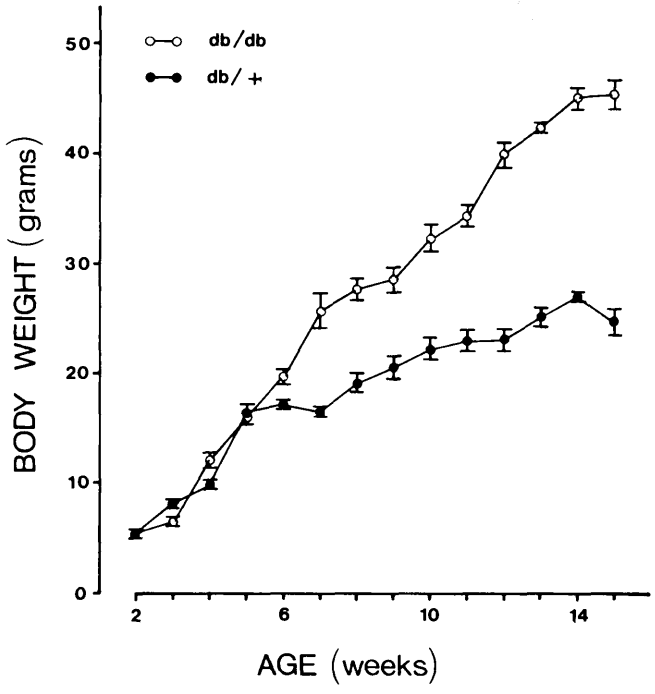

FIGURE 1. Body weights of diabetic (*db*/*db*) and control heterozygote (*db*/+) mice. Values shown are the mean ± SEM of eight mice.

population was first characterized with respect to body weight, plasma glucose, insulin and glucagon, and liver glycogen. The various enzyme activities involved in hepatic glycogen metabolism were then measured, including those catalyzing phosphorylation-dephosphorylation of glycogen synthase and phosphorylase.

MATERIALS AND METHODS

Crystalline rabbit skeletal muscle phosphorylase *b* was prepared as described by Fischer and Krebs.[10] Rabbit skeletal muscle phosphorylase kinase was prepared according to Hayakawa et al.[11] [^{32}P]phosphorylase *a* was prepared from phosphorylase *b* using [γ-^{32}P]ATP, Mg^{2+}, and phosphorylase kinase according to Krebs et al.[12] The preparation of phosphorylated histone has been previously described.[13] [γ-^{32}P]ATP, [^{14}C]glucose-1-phosphate, UDP-[^{14}C]-glucose, monoiodinated [^{125}I]glucagon, and Aquasol-2 for liquid scintillation counting were obtained from New England Nuclear (Lachine, Quebec, Canada). Trasylol was obtained from Boehringer Ingelheim (Burlington, Ontario, Canada). UDP-glucose, glucose-1-phosphate, type III rabbit liver glycogen, AMP, 2-(N-morpholino) ethane sulfonic acid (Mes), type II-A histone, and cyclic AMP were supplied by Sigma Chemical Co. (St. Louis, Missouri). All other chemicals were of reagent grade.

Treatment of animals. A breeding colony of C57BL/KsJ-*db*/+ mice was obtained from Jackson Laboratory (Bar Harbor, Maine). Heterozygote (*db*/+) mice were used as controls in this study. Diabetic offspring 4 wk of age and older were distinguishable by their obvious obesity, but at younger ages, diabetic and heterozygote mice were identified by their difference in basal body temperature[14] using a YSI 524 × 25-gauge temperature probe (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio). Although both male and female mice were used in this study, data from male and female mice were frequently compared to ensure that no significant differences existed between the genders that might influence the data. The mice were fed a pelleted rodent chow (77% carbohydrate, 16% protein, and 7% fat) ad libitum and kept under a constant 12-h light–12-h dark cycle.

All samples were collected between 0900 and 1000 h. Blood samples from ether-anesthetized mice in NaEDTA and Trasylol (500 KIU) were obtained by cardiac puncture except in the 2- and 3-wk-old mice, where collection was by decapitation. Immediately after blood collection, the livers were excised and frozen in liquid nitrogen. The livers were pulverized and stored at −80°C. The blood samples were chilled on ice and centrifuged at 3000 × *g* for 10 min. The plasma was stored at −80°C until used.

Enzyme assays. Liver homogenates used in the various enzyme assays were prepared as described previously.[2] Phosphorylase *a* and total phosphorylase (*a* + *b*) activities were determined by the method of Tan and Nuttall.[15] Glycogen synthase activity was determined by the method of Thomas et al.[16] by measuring the incorporation of [^{14}C]glucose from UDP-[^{14}C]glucose into glycogen. The radioactive glycogen was precipitated on Whatman 31ET paper (2 × 2 cm), washed, and counted for radioactivity as described previously.[2] Protein kinase activity was assayed by following the incorporation of ^{32}P from [γ-^{32}P]ATP into type II-A histone as described by Reimann et al.[17] Phosphorylase kinase activity was determined by measuring the amount of phosphorylase *a* formed from phosphorylase *b* by a

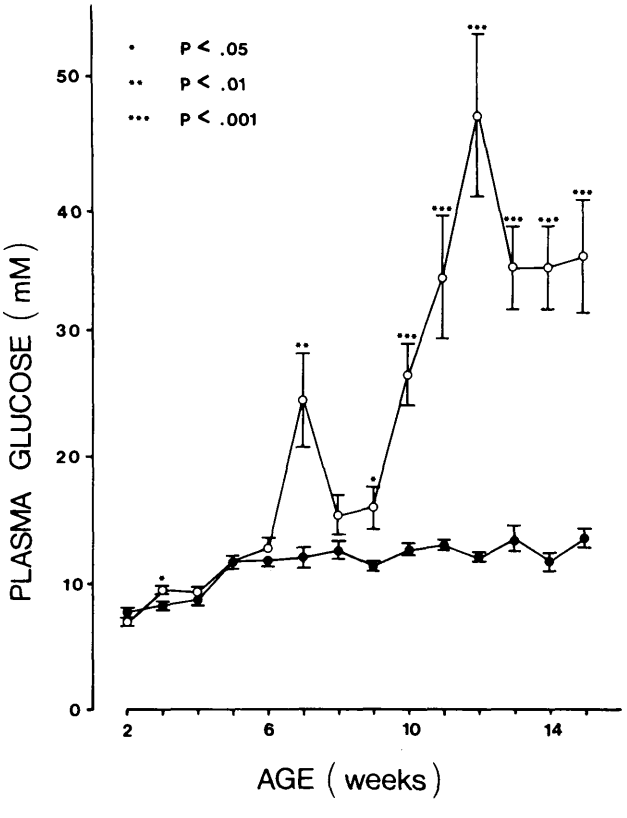

FIGURE 2. Plasma glucose concentrations in diabetic (○——○) and control heterozygote (●——●) mice. Values shown are the mean ± SEM of eight mice.

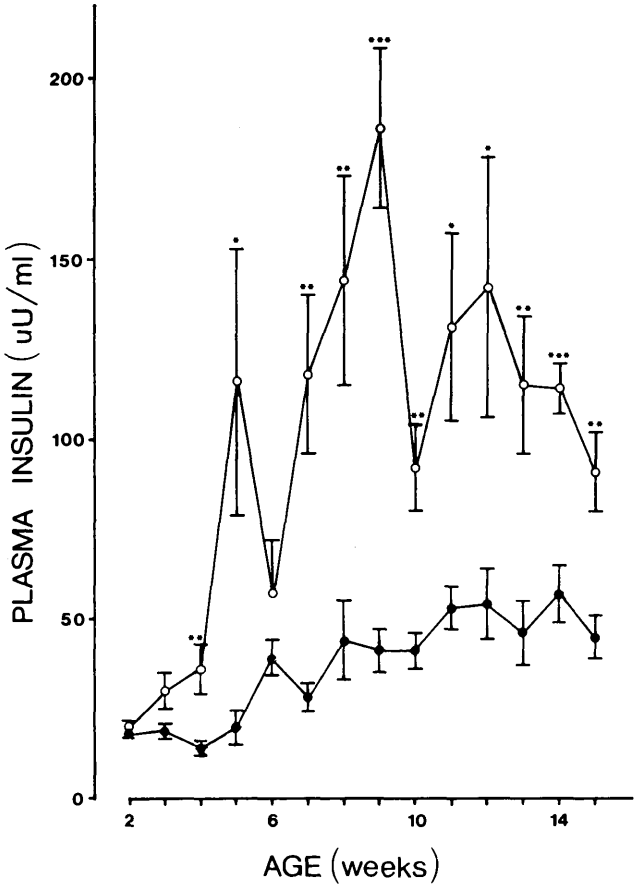

FIGURE 3. Plasma insulin concentrations in diabetic (○——○) and control heterozygote (●——●) mice. Values shown are the mean ± SEM of eight mice. *P < 0.05, **P < 0.01, and ***P < 0.001.

modification[18] of the method originally described by Krebs et al.[19] The phosphorylase *a* formed was measured in the direction of glycogen synthesis according to the method of Cori et al.[20] Phosphoprotein phosphatase activity was determined by using ^{32}P-labeled phosphorylase *a* and phosphorylated histone as substrates as described previously.[13] Glycogen synthase phosphatase activity was measured as described by Gilboe and Nuttall.[21]

Analytic methods. Liver glycogen was determined by the method of Lo et al.[22] Plasma glucose was measured by the glucose-oxidase method using a diagnostic kit from Sigma. Plasma insulin was measured using the double-antibody method[23] using a kit from Cambridge Medical Diagnostics, Inc. (Billerica, Massachusetts). Porcine insulin was used as the standard. Plasma glucagon was measured by the method of Brockman.[24] Glucagon antiserum was Mann's GP26 (guinea pig antiserum), which was a gift from Dr. B. Laarveld (Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Saskatchewan). This antiserum gives values similar to the 30K antiserum.[25] Protein determination was performed by the method of Lowry et al.[26] using bovine serum albumin as the standard.

Data analysis. Statistical analysis was performed by the Student's *t*-test. Regression analysis was used to determine the correlation coefficients and the regression lines in Figure 7.

TABLE 1
Plasma immunoreactive glucagon (IRG) levels in control heterozygote (*db*/+) and diabetic (*db*/*db*) mice

Age (wk)	Type	N	IRG* (pg/ml)	P-value
3–4	*db*/+	15	168 ± 23	
	db/*db*	6	191 ± 26	NS
7–9	*db*/+	19	195 ± 19	
	db/*db*	7	299 ± 30	<0.01
12–13	*db*/+	13	139 ± 15	
	db/*db*	12	415 ± 39	<0.001

*Values are the mean ± SEM.

RESULTS

It appeared from the literature that there was a significant degree of heterogeneity in these genetically diabetic mice. It was essential, therefore, to initially characterize our mouse population by measuring body weight, plasma glucose, and insulin and glucagon levels. Figure 1 shows the body weights of the mice used in our study. Although the diabetic mice had noticeably increased fat deposition at 4 wk, this was not reflected in a larger body weight until 7 wk. Eventually, the diabetic mice attained weights 1.6-fold greater than control mice.

A noticeable hyperglycemia was present in the diabetic mice by 7 wk (Figure 2) and eventually reached values of approximately 35 mM compared with 12 mM in controls. The pattern for insulin levels in the diabetic mice (Figure 3) was

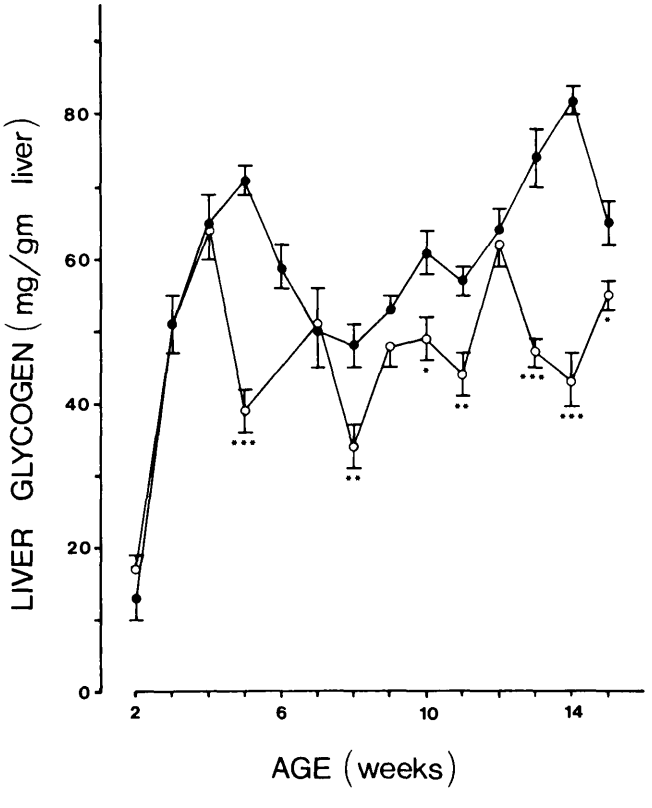

FIGURE 4. Liver glycogen content in diabetic (○——○) and control heterozygote (●——●) mice. Values shown are the mean ± SEM of eight mice. *P < 0.05, **P < 0.01, and ***P < 0.001.

similar to those reported previously,[5-7] with a marked transient hyperinsulinemia peaking around 8–9 wk. Interestingly, the insulin levels did not fall to control values by 15 wk, but remained significantly elevated. Glucagon (IRG) levels were measured in three age groups representative of different phases of the diabetes: ages 3–4 wk (early diabetes, insulin levels near normal), ages 7–9 wk (peak of hyperinsulinemia), and ages 12–13 wk (insulin beginning to decline). Table 1 shows that no difference in plasma IRG was noticeable between 3–4-wk diabetic and control mice, but by 7–9 wk a significant hyperglucagonemia was present in the diabetic mice that increased even further by 12–13 wk.

The liver glycogen concentrations in the mutant diabetic mouse have been measured in several laboratories with wide-ranging results. Results from this study (Figure 4) indicate that the levels in diabetic mice were slightly lower than in controls. Control values generally averaged 50–60 mg/g liver, while diabetic mice averaged 40–50 mg/g liver. Similar relative values were obtained when glycogen concentrations were calculated per milligram protein, since protein content in the livers of these control and diabetic mice were determined not to be significantly different (data not shown).

Glycogen synthase activity was measured in the absence (glycogen synthase *a* activity) and presence (total activity) of glucose-6-phosphate. As shown in Figure 5A, the active *a*-form activity remained relatively constant during the time measured in the control mice at a value of 0.20 units, whereas in diabetic mice, the activity increased to values twofold higher at 8 wk and then gradually dropped to near control values. The total activity (Figure 5B) showed a similar pattern except that the activity in diabetic mice did not drop to control values by 15 wk but was still significantly elevated. Although the total activity and active form activity changed over the 15-wk period in the diabetic mice, the activity ratio of glycogen synthase *a* activity to total synthase activity was constant over the time period and was unchanged from controls at a value of 0.20–0.25.

The activity of phosphorylase *a* (Figure 6A) was elevated in diabetic mice by 5 wk and remained approximately twofold higher relative to controls. The pattern for total activity was similar (Figure 6B), with diabetic values being 1.5–2-fold greater than controls. As was the case for glycogen synthase, the ratio of the active form to total activity was similar in control and diabetic mice (0.50–0.60) and remained constant throughout the period examined.

Insulin and glucagon are known to play important roles in the regulation of hepatic glycogenesis and glycogenolysis, respectively.[27-31] It was of interest, therefore, to see if there was a correlation between the circulating hormone concentrations and the activities of the key regulatory enzymes for the above-mentioned metabolic processes. There was a positive correlation ($r = 0.54$, $P < 0.001$) between glycogen synthase *a* activity and the level of circulating plasma insulin (Figure 7A). A similar correlation was obtained between total glycogen synthase activity and plasma insulin (results not shown). In Figure 7B, a positive correlation is also shown to exist between phosphorylase *a* activity and plasma glucagon concentrations ($r = 0.64$, $P < 0.001$). The relationship between total phosphorylase activity and plasma glucagon was also similar (results not shown).

Because elevations in the active forms of glycogen synthase and phosphorylase were observed in the diabetic mouse, the enzyme activities that regulate the interconversion between active and inactive forms were measured. For these studies, the three groups of mice described above (3–4 wk, 7–9 wk, and 12–13 wk) were selected. No difference in protein kinase activity (measured in the absence and presence of 2×10^{-6} M cyclic AMP), phosphorylase phosphatase, synthase phosphatase, or histone phosphatase ac-

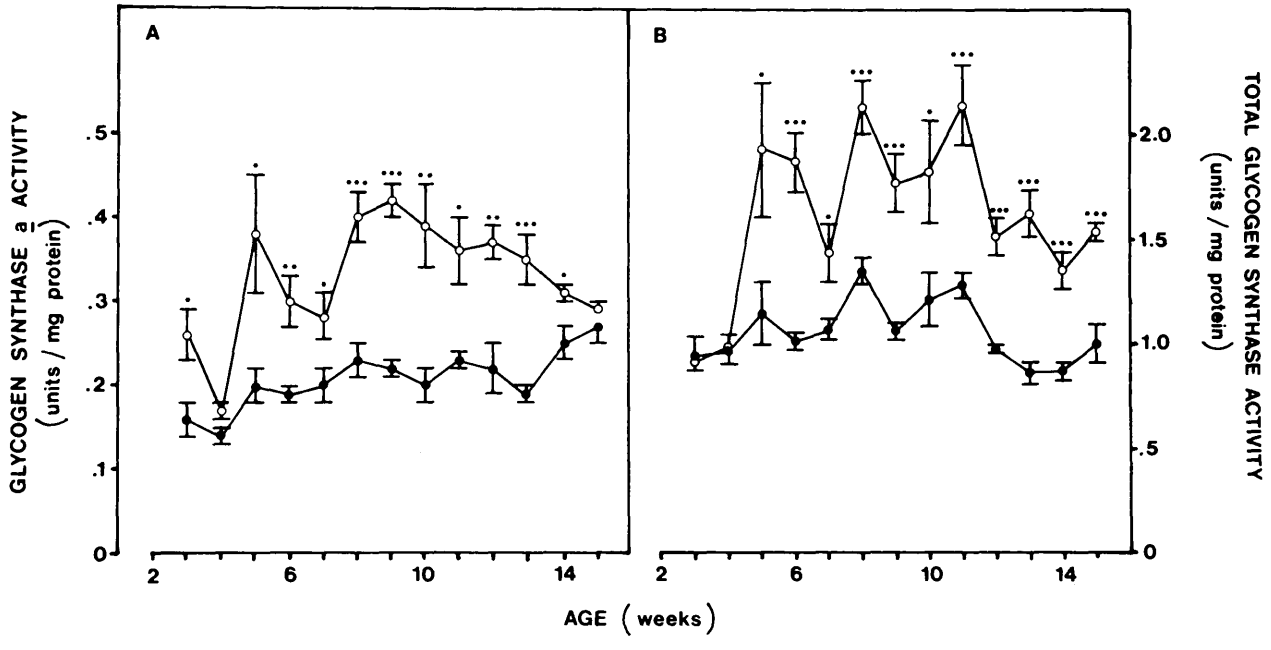

FIGURE 5. Glycogen synthase activity in diabetic (○——○) and control heterozygote (●——●) mice livers. (A) Glycogen synthase *a* activity measured in the absence of glucose-6-phosphate. (B) Total glycogen synthase activity measured in the presence of 10 mM glucose-6-phosphate. Values shown are the mean ± SEM of eight mice. *P < 0.05, **P < 0.01, and ***P < 0.001.

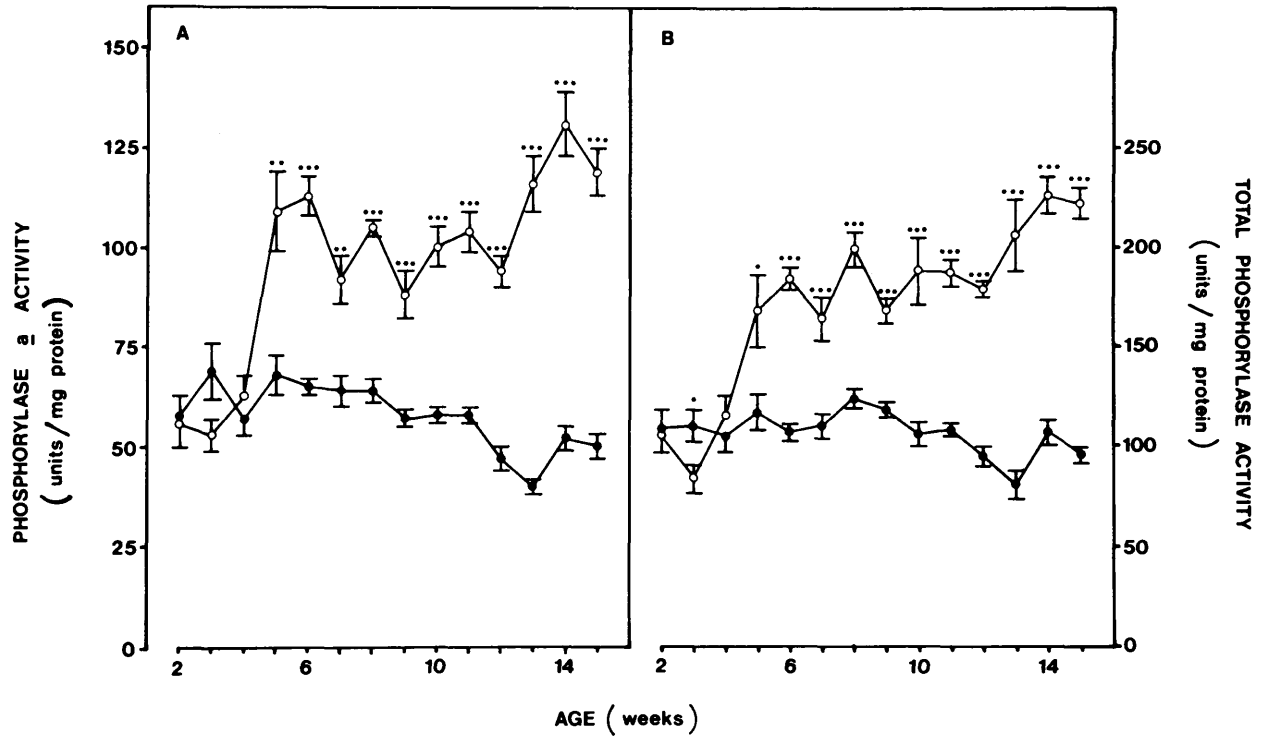

FIGURE 6. Phosphorylase activity in diabetic (○——○) and control heterozygote (●——●) mice livers. (A) Phosphorylase *a* activity. (B) Total phosphorylase activity. Values shown are the mean ± SEM of eight mice. *P < 0.05, **P < 0.01, and ***P < 0.001.

tivity was observed in diabetic mice relative to controls (Table 2). Phosphorylase kinase activity was significantly elevated in diabetic mice relative to controls in the 12–13-wk age group only (Table 2).

DISCUSSION

The lack of knowledge about the changes in hepatic glycogen metabolism in non-insulin-dependent (type II) diabetes has been due primarily to the lack of a good animal model. However, the discovery of the diabetic mutation in an inbred mouse strain (C57BL/Ks) by Hummel and coworkers[5] solved this problem. This mutant mouse displays obesity, hyperglycemia, and hyperinsulinemia[5–7] and is thus a suitable model for type II diabetes. Little progress has been made in understanding hepatic glycogen metabolism in the genetically diabetic mouse and this is in part due to the controversial nature of the results reported so far, especially those concerning liver glycogen concentrations and glycogen synthase activities.[6,8,9] Therefore, this developmental study was designed to more thoroughly examine the changes that occur in hepatic glycogen metabolism during the progression of diabetes in this mouse.

The initial characterization of these mice confirmed that the development of obesity, hyperglycemia, and hyperinsulinemia (Figures 1–3) resembled that of other populations of these diabetic mice[5–7] with the exception that the plasma insulin levels in the diabetic mice were still elevated at 15 wk (Figure 3). This may be due to heterogeneity in the development of diabetes in these mice, which would alter the stress placed on the pancreatic insulin supply early in the disease, as suggested by Coleman and Hummel.[6] However, the relative pattern of hyperinsulinemia, peaking at 8–9 wk and then declining, was observed in the present study. The elevated circulating glucagon concentration in the diabetic mice by 7–9 wk is also in agreement with previous determinations.[9]

The hyperphagia of these diabetic mice[5] might be expected to increase their liver glycogen levels. Several studies have reported glycogen levels 2–3-fold higher in diabetic mice relative to controls;[6,8] however, Stearns and Benzo[9] reported similar glycogen stores in both groups of animals. Interestingly, in every study, the levels reported for diabetic mice are very similar; it is the levels in the control mice that vary. For example, Coleman and Hummel[6] reported glycogen levels in diabetic and control mice to be 51 and 29 mg/g liver, respectively, whereas, in the present study, values obtained were approximately 50 and 60 mg/g liver, respectively. It is possible that, due to the hyperphagia, the diurnal rhythm of hepatic glycogen in diabetic mice is different from that in controls, suggesting that differences in killing time could be responsible for the discrepancies. This possibility is currently under investigation.

Glycogen synthase exists in two forms (*a* and *b*), and only synthase *a* (the less phosphorylated form) is thought to be active in vivo.[32] Glycogen synthase is activated by glucose and insulin[4,30,33–35] and, therefore, it is not surprising that its activity was elevated in the hyperglycemic, hyperinsulinemic diabetic mice (Figure 5). Both total glycogen synthase and glycogen synthase *a* activity were elevated by 5 wk and the activities appear generally to parallel the plasma insulin concentrations of the diabetic mice (Figure 3). Figure 7A further illustrates a correlation between glycogen synthase *a* activity and circulating insulin (r = 0.54) and, although not shown, a correlation between total glycogen synthase activity and

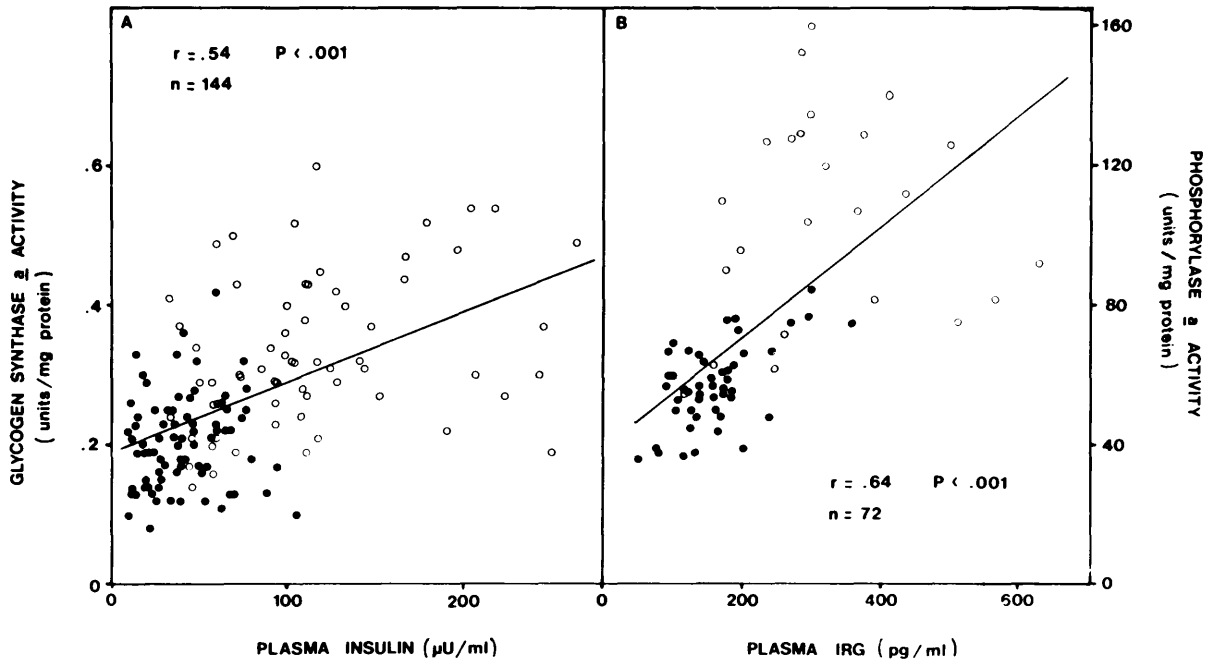

FIGURE 7. (A) Correlation between plasma insulin concentrations (from Figure 3) and glycogen synthase *a* activities (from Figure 5A) in diabetic (○) and control heterozygote (●) mice. (B) Correlation between plasma immunoreactive glucagon concentrations (from Table 1) and phosphorylase *a* activities (from Figure 6A) in diabetic (○) and control heterozygote (●) mice.

insulin was also observed. Because of insulin's role in the activation of glycogen synthase and its regulation of hepatic protein synthesis,[36-38] the increases in both total and *a*-form activity may be partly due to the hyperinsulinemia. However, other factors such as glucose,[39] glucocorticoids,[40] epinephrine and glucagon,[41] and thyroid hormones[42] might act in concert with insulin to provide complete control of glycogen synthase activity. Insulin may, in fact, modulate the degree to which glycogen synthase is affected by these various stimuli. Although with the assays employed no changes in kinase or phosphatase activities were detected that might account for the increased glycogen synthase *a* activity, it is possible that utilization of assays measuring specific glycogen synthase kinases and phosphatases would detect some correlating changes. A strong possibility exists that, due to mass action, the elevated glycogen synthase *a* activity observed in the diabetic mice is due only to the increase in total enzyme. These possibilities are also under investigation.

The elevated phosphorylase *a* activity (Figure 6A) may be due in part to the hyperglucagonemia in these diabetic mice. However, it is questionable whether or not the cyclic AMP cascade system is stimulated in these mice, since adenylate cyclase activity,[43] cyclic AMP levels,[43] and cyclic AMP-dependent protein kinase activity (Table 2) are not elevated relative to controls. Also, phosphorylase kinase activity is elevated only in 12–13-wk diabetic mice (Table 2), well after a rise in phosphorylase *a* activity is observed (Figure 6). It may be that phosphorylase *a* activity is being altered by a glucagon-stimulated, calcium-mediated mechanism[44] or by

TABLE 2
Kinase and phosphatase activities in heterozygote and diabetic mice

Age (wk)	Type	N	Protein kinase −cAMP	Protein kinase +cAMP	Phosphorylase kinase	Phosphorylase phosphatase	Histone phosphatase	Glycogen synthase phosphatase
3–4	*db*/+	8	19 ± 1	62 ± 1	0.26 ± 0.01	938 ± 85	186 ± 19	2.3 ± 0.5
	db/*db*	8	27 ± 2†	67 ± 5	0.23 ± 0.01	835 ± 77	182 ± 16	2.1 ± 0.5
7–9	*db*/+	10	21 ± 3	68 ± 5	0.40 ± 0.05	799 ± 64	177 ± 15	1.7 ± 0.4
	db/*db*	10	25 ± 2	73 ± 4	0.37 ± 0.01	682 ± 50	169 ± 16	1.5 ± 0.2
12–13	*db*/+	11	21 ± 2	69 ± 5	0.26 ± 0.02	706 ± 75	160 ± 14	2.0 ± 0.7
	db/*db*	11	28 ± 2*	75 ± 5	0.44 ± 0.02‡	591 ± 39	135 ± 7	1.5 ± 0.3

Details for preparation of homogenates and methods for enzyme assays are given under MATERIALS AND METHODS. One unit of protein kinase activity is defined as the amount of enzyme that incorporates 1 nmol of ^{32}Pi from [γ-^{32}P]ATP into histone/min. One unit of phosphorylase kinase activity is that amount of enzyme that converts 1 unit of phosphorylase *b* into phosphorylase *a*/min. One unit of phosphorylase phosphatase and histone phosphatase is that amount of enzyme that releases 1 pmol of ^{32}Pi from ^{32}P-labeled substrates/min. Glycogen synthase phosphatase activity is expressed as the number of units of glycogen synthase *a* generated per 10 min. All enzyme activities are expressed per milligram protein of liver homogenate used.
*$P < 0.05$, †$P < 0.01$, and ‡$P < 0.001$.

other hormones (epinephrine, vasopressin) that produce a calcium signal.[45] The increase in total phosphorylase activity could be due to an increase in enzyme protein, which in turn could be a result of the hyperglucagonemia. Glucagon and cyclic AMP have been shown to affect the rate of synthesis or degradation of a number of enzymes, including phosphoenolpyruvate carboxykinase[46] and glucose-6-phosphate dehydrogenase.[47] Therefore, glucagon may alter enzyme activity by affecting the total enzyme protein present in the cell.

An interesting phenomenon was observed in the diabetic mice at 5 wk of age. At this point, a sharp rise in plasma insulin concentration (Figure 3), glycogen synthase activity (Figure 5), and phosphorylase activity (Figure 6) occurred. Although the reason for these sudden changes is at present not known, it may signify the onset of the metabolic abnormalities that result from the primary defect in the diabetic mice.

This study provides further characterization of the diabetic mouse, an important animal model of type II diabetes. It has been clearly shown that both glycogen synthase and phosphorylase activities are increased in the livers of these mice and the data suggest that the steady-state activities of glycogen synthase and phosphorylase are correlated with the circulating concentrations of insulin and glucagon, respectively. This is also the first report of the various kinase and phosphatase activities associated with glycogen metabolism in the *db/db* mouse. Future studies will focus on the mechanism by which the glycogen synthase and phosphorylase activities are increased in this diabetic mouse.

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REFERENCES

[1] Steiner, D. F., Rauda, V., and Williams, R. H.: Effects of insulin, glucagon, and glucocorticoids upon hepatic glycogen synthesis from uridine diphosphate glucose. J. Biol. Chem. 1961; 236:299–304.

[2] Khandelwal, R. L., Zinman, S. M., and Zebrowski, E. J.: The effect of streptozotocin-induced diabetes and of insulin supplementation on glycogen metabolism in rat liver. Biochem. J. 1977; 168:541–48.

[3] Gold, A. H.: The effect of diabetes and insulin on liver glycogen synthetase activation. J. Biol. Chem. 1970; 245:903–905.

[4] Miller, T. B., Jr., and Larner, J.: Mechanism of control of hepatic glycogenesis by insulin. J. Biol. Chem. 1973; 248:4383–88.

[5] Hummel, K. P., Dickie, M. M., and Coleman, D. L.: Diabetes, a new mutation in the mouse. Science 1966; 153:1127–28.

[6] Coleman, D. L., and Hummel, K. P.: Studies with the mutation, diabetes, in the mouse. Diabetologia 1967; 3:238–48.

[7] Wyse, B. M., and Dulin, W. E.: The influence of age and dietary conditions on diabetes in the *db* mouse. Diabetologia 1970; 6:268–73.

[8] Chan, T. M., Young, K. M., Hutson, N. J., Brumley, F. T., and Exton, J. H.: Hepatic metabolism of genetically diabetic (*db/db*) mice. I. Carbohydrate metabolism. Am. J. Physiol. 1975; 229:1702–12.

[9] Stearns, S. B., and Benzo, C. A.: Structural and chemical alterations associated with hepatic glycogen metabolism in genetically diabetic (*db*) and in streptozotocin-induced diabetic mice. Lab. Invest. 1977; 37:180–87.

[10] Fischer, E. H., and Krebs, E. G.: The isolation and characterization of rabbit skeletal muscle phosphorylase *b*. J. Biol. Chem. 1958; 231:65–71.

[11] Hayakawa, T., Perkins, J. P., Walsh, D. A., and Krebs, E.: Physicochemical properties of rabbit skeletal muscle phosphorylase kinase. Biochemistry 1973; 12:567–73.

[12] Krebs, E. G., Kent, A. B., and Fischer, E. H.: The muscle phosphorylase *b* kinase reaction. J. Biol. Chem. 1958; 231:73–78.

[13] Khandelwal, R. L., Vandenheede, J. R., and Krebs, E. G.: Purification, properties and substrate specificities of phosphoprotein phosphatase(s) from rabbit liver. J. Biol. Chem. 1976; 251:4850–58.

[14] Yen, T. T., Fuller, R. W., and Pearson, D. V.: The response of "obese" (*ob/ob*) and "diabetic" (*db/db*) mice to treatments that influence body temperature. Comp. Biochem. Physiol. 1974; 9A:377–85.

[15] Tan, A. W. H., and Nuttall, F. Q.: Characteristics of the dephosphorylated form of phosphorylase from rat liver and measurement of its activity in crude liver preparations. Biochim. Biophys. Acta 1975; 410:45–60.

[16] Thomas, J. A., Schlender, K. K., and Larner, J.: Specificity of activation of glycogen synthase I from skeletal muscle and heart. Biochim. Biophys. Acta 1973; 293:84–93.

[17] Reimann, E. M., Walsh, D. A., and Krebs, E. G.: Purification and properties of rabbit skeletal muscle adenosine 3',5'-monophosphate-dependent protein kinase. J. Biol. Chem. 1971; 246:1986–95.

[18] Brostrom, C. O., Hunkeler, F. L., and Krebs, E. G.: The regulation of skeletal muscle phosphorylase kinase by Ca^{2+}. J. Biol. Chem. 1971; 246:1961–67.

[19] Krebs, E. G., Love, D. S., Bratvold, G. E., Trayser, K. A., Meyer, W. L., and Fischer, E. H.: Purification and properties of rabbit skeletal muscle phosphorylase *b* kinase. Biochemistry 1964; 3:1022–33.

[20] Cori, C. F., Cori, G. T., and Green, A. A.: Crystalline muscle phosphorylase. III. Kinetics. J. Biol. Chem. 1943; 151:39–55.

[21] Gilboe, D. P., and Nuttall, F. Q.: Stimulation of liver glycogen particle synthase D phosphatase activity by caffeine, AMP, and glucose-6-phosphate. Arch. Biochem. Biophys. 1982; 219:179–85.

[22] Lo, S., Russell, J. C., and Taylor, A. W.: Determination of glycogen in small tissue samples. J. Appl. Physiol. 1970; 28:234–36.

[23] Morgan, C. R., and Lazarow, A. A.: Immunoassay of insulin: two antibody system. Diabetes 1963; 12:115–26.

[24] Brockman, R. P.: Effect of somatostatin suppression of glucagon secretion on glucose production in sheep. Can. J. Physiol. Pharmacol. 1979; 57:848–52.

[25] Brockman, R. P., Manns, J. G., and Bergman, E. N.: Quantitative aspects of secretion and hepatic removal of glucagon in sheep. Can. J. Physiol. Pharmacol. 1976; 54:666–70.

[26] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 1951; 193:265–75.

[27] Bishop, J. S., and Larner, J.: Rapid activation-inactivation of liver uridine diphosphate glucose-glycogen transferase and phosphorylase by insulin and glucagon in vivo. J. Biol. Chem. 1967; 242:1354–56.

[28] Beynen, A. C., and Geelen, M. J. H.: Control of glycogen metabolism by insulin in isolated hepatocytes. Horm. Metab. Res. 1981; 13:376–78.

[29] Storer, G. B., Topping, D. L., and Trimble, R. P.: Direct stimulation by glucose and insulin of glycogen synthesis in perfused rat liver. FEBS Lett. 1981; 136:135–37.

[30] Margolis, R. N., and Curnow, R. T.: The role of insulin and glucocorticoids in the regulation of hepatic glycogen metabolism: effect of fasting, refeeding, and adrenalectomy. Endocrinology 1983; 113:2113–19.

[31] Studer, R. K., Snowdowne, K. W., and Borle, A. B.: Regulation of hepatic glycogenolysis by glucagon in male and female rats. J. Biol. Chem. 1984; 259:3596–604.

[32] Mersmann, H. J., and Segal, H. L.: An on-off mechanism for liver glycogen synthetase activity. Proc. Natl. Acad. Sci. USA 1967; 58:1688–95.

[33] Ciudad, C. J., Bosch, F., and Guinovart, J. J.: Insulin activation of basal hepatic glycogen synthase. FEBS Lett. 1981; 129:123–26.

[34] Gilboe, D. P., and Nuttall, F. Q.: Direct glucose stimulation of glycogen synthase phosphatase activity in a liver glycogen particle preparation. Arch. Biochem. Biophys. 1984; 228:587–91.

[35] Witters, L. A., and Avruch, J.: Insulin regulation of hepatic glycogen synthase and phosphorylase. Biochemistry 1978; 17:406–10.

[36] Steiner, D. F.: Insulin and the regulation of hepatic biosynthetic activity. *In* Vitamins and Hormones. Vol. 24. Harris, R. S., Wool, I. G., and Lorame, J. A., Eds. New York, Academic Press, 1966:1.

[37] Poli, A., Gordan, P. B., Schwarze, P. E., Grinde, B., and Seglen, P. O.: Effects of insulin and anchorage on hepatocytic protein metabolism and amino acid transport. J. Cell. Sci. 1981; 48:1–18.

[38] Goldfine, I. D., Purrello, F., Clawson, G. A., and Vigneri, R.: Insulin binding sites on the nuclear envelope: potential relationships to mRNA metabolism. J. Cell. Biochem. 1982; 20:29–30.

[39] Nuttall, F. Q., Theen, J. W., Niewoehner, C., and Gilboe, D. P.: Response of liver glycogen synthase and phosphorylase to in vivo glucose analogues. Am. J. Physiol. 1983; 245:E521–27.

[40] Mersmann, H. J., and Segal, H. L.: Glucocorticoid control of the liver glycogen synthetase-activating system. J. Biol. Chem. 1969; 244:1701–704.

[41] Bosch, F., Ciudad, C. J., and Guinovart, J. J.: Different effects of glucagon and epinephrine on the kinetic properties of liver glycogen synthase. FEBS Lett. 1983; 151:76–78.

[42] Malbon, C. C., and Campbell, R.: Thyroid hormones regulate hepatic glycogen synthase. Endocrinology 1984; 115:681–86.

[43] Levilliers, J., Pairault, J., Lecot, F., Tournemolle, A., and Laudat, M. H.: Adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate: levels and cyclase activities in liver and adipose tissue from diabetic mice (*db*/*db*). Eur. J. Biochem. 1978; 88:323–30.

[44] Kimura, S., Matsumoto, T., Tada, R., Ogata, E., and Abe, K.: Inhibition by calcium channel blockers of the glycogenolytic effect of glucagon in perfused rat liver. Acta Endocrinol. 1982; 99:559–66.

[45] Blackmore, P. F., Dehaye, J.-P., and Exton, J. H.: Studies on α-adrenergic activation of hepatic glucose output. J. Biol. Chem. 1979; 254:6945–50.

[46] Schudt, C.: Regulation of phosphoenolpyruvate carboxykinase by glucagon and glucocorticoids in primary cultures of rat hepatocytes. Biochim. Biophys. Acta 1980; 628:277–85.

[47] Rudack, D., David, B., and Holten, D.: Regulation of rat liver glucose-6-phosphate dehydrogenase levels by adenosine 3',5'-monophosphate. J. Biol. Chem. 1971; 246:7823–24.