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Characterization of Diabetes in the Chinese Hamster

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Summary. Diabetes in Chinese hamster was initially detected by qualitative tests for urine glucose. The disease was characterized by quantitating urine glucose, glucose tolerance tests and measurement of fasting and nonfasting blood sugar, blood ketones, plasma free fatty acids (FFA), plasma insulin, pancreatic insulin and fasting levels of liver glycogen. In addition, basal levels of glucose utilization by diaphragms and epididymal adipose tissue and the response of these tissues to insulin was measured. Those animals requiring insulin received their last injection 24 hours prior to study. Glucosuria varied from 51 to 1600 mg/24 hours. Diabetics had significantly decreased tolerance to a 250 mg/kg glucose load. The response varied considerably in diabetics but was uniform in the nondiabetics. Diabetics had mean fasting liver glycogen levels of 3.1 ± 1.0 compared with 0.4 ± 0.7 percent of fresh liver weight for nondiabetics. Severely ketotic, diabetic Chinese hamsters had significantly elevated FFA and ketone levels and significantly lower plasma and pancreatic insulin levels but mild diabetics did not differ from controls with respect to these parameters. All Chinese hamsters had high plasma FFA levels (nondiabetics 1800 μE/l, severe diabetics 2800 μE/l). Fasting and nonfasting FFA levels did not differ in mild diabetic and nondiabetic animals. Muscle and adipose tissues from diabetic hamsters had basal rates similar to nondiabetics and had similar responses to insulin. Hamsters maintained on insulin had greatly elevated immunoreactive insulin levels in their plasma, which persisted for 26 days. — The data suggest that severely diabetic hamsters may have a decreased ability of the pancreas to secrete insulin in response to a glucose stimulus. The observations that plasma insulin levels are normal in mild diabetics but that these animals have glucosuria, hyperglycemia and abnormal glucose tolerance suggest that mild diabetes in the Chinese hamster may involve interference with insulin action and/or increased hepatic glucose output.

Caractérisation du diabète du hamster chinois.

Résumé. Nous avons dépisté le diabète chez le hamster chinois par des dosages qualitatifs de la glucosurie. Nous avons ensuite caractérisé chaque cas par la mesure quantitative du glucose urinaire, par des surcharges au glucose et par le dosage de la glycémie à jeun et non à jeun, des corps cétoniques, des acides gras libres (AGL) plasmatiques, de l'insuline plasmatique et pancréatique, et des taux hépatiques de glycogène. En outre, nous avons mesuré l'utilisation du glucose par le diaphragme et le tissu adipeux épididymaire, avec et sans stimulation par l'insuline. Les animaux nécessitant l'administration d'insuline ont reçu leur dernière injection au moins 24 heures avant chaque étude. Les taux de glucose dans l'urine ont varié entre 51 et 1600 mg par 24 heures. Le test de surcharge au glucose s'est révélé nettement anormal chez les animaux diabétiques, la surcharge étant de 250 mg de glucose par kg de poids. Chez les diabétiques, la glycémie après surcharge est fortement variable, contrairement à l'uniformité de la réponse observée chez les non-diabétiques. Le glycogène hépatique à jeun est de 3, $1 \pm 1{,}0\%$ pour les diabétiques, et de $0{,}4 \pm 0{,}7\%$ de poids frais d'organes pour les non-diabétiques. Les taux plasmatiques des AGL et des corps cétoniques sont manifestement élevés chez les hamsters chinois diabétiques et cétosiques alors que le contenu en insuline du plasma et du pancréas est abaissé de façon marquée. Ceci n'a pas été observé chez les diabétiques moins sévères qui se rapprochent des non-diabétiques. Les AGL plasmatiques sont élevés chez tous les hamsters chinois (moyenne pour les non-diabétiques 1800 μE/l, pour les diabétiques sévères 2800 μE/l) Les taux d'AGL sont les mêmes à jeun et non à jeun chez les animaux non-diabétiques ou souffrant d'un diabète léger. L'utilisation du glucose par le muscle et le tissu adipeux du hamster diabétique ne diffère pas significativement de celle des tissus non-diabétiques et leur stimulation par l'insuline est semblable. L'insuline immunoréactive des hamsters traités à l'insuline reste élevée dans le plasma pendant plus de 26 jours après la dernière injection. — Ces observations semblent indiquer que le pancréas des hamsters chinois diabétiques sécrète plus difficilement de l'insuline en réponse à une stimulation par le glucose. Les taux normaux d'insuline plasmatique chez les hamsters avec un diabète léger, mais présentant pourtant une nette glucosurie, une hyperglycémie et une réponse anormale à la surcharge en glucose, suggèrent l'existence d'une interférence avec l'action normale de l'insuline et peut-être une augmentation de la production de glucose par le foie.

Beschreibung des Diabetes mellitus beim chinesischen Hamster.

Zusammenfassung. Beim chinesischen Hamster wurde der Diabetes durch qualitative Urinuntersuchungen auf Glucose aufgespürt. Es wurde dann jeder Fall näher charakterisiert durch quantitative Uringlucosebestimmungen, Glucosebelastungsproben und Messungen von Nüchtern- und Nichtnüchternwerten für Blutzucker, Blutketonkörper, freie Fettsäuren (FFS) und Insulin im Plasma, von Insulin im Pankreas, und von Nüchternwerten für Leberglykogen. Außerdem wurde der Glucoseverbrauch des Zwerchfells und des epididymalen Fettgewebes sowie mit, als auch ohne Insulin gemessen. Diejenigen Tiere, die Insulin erforderten, erhielten ihre letzte Injektion 24 Stunden vor dem Versuch. Glucosurie schwankte zwischen 51 und 1600 mg/24 Std. Bei einer Belastung von 250 mg/kg wiesen die diabetischen Tiere eine wesentlich verminderte Glucosetoleranz auf. Sie sprachen sehr unterschiedlich auf die Glucosebelastung an, während die nichtdiabetischen Tiere in ihrer Reaktion einheitlich waren. — Im Vergleich zu den nichtdiabetischen Hamstern, wo die Mittelwerte für Nüchternleberglykogen bei 0.4 ± 0.7 Prozent des Frischlebergewichtes lagen, hatten die diabetischen Tiere einen Glykogengehalt der Leber von 3.1 ± 1.0 Prozent. — Chinesische Hamster mit schwerem Diabetes haben deutlich erhöhte FFS-Werte und Ketonkörper, sowie einen wesentlich niedrigeren Plasma- und Pankreasinsulinspiegel. Bei Tieren mit leichter Diabetes hingegen sind diese Untersuchungen, im Vergleich zu den Kontrolltieren, unverändert. Alle chinesischen Hamster haben hohe Plasma-FFS-Werte (nichtdiabetische 1800 μE/L, schwer diabetische 2800 μE/L). Nüchtern- und Nichtnüchternwerte für FSS sind beim leicht diabetischen oder gesunden Tier etwa gleich hoch. Der Glucoseabbau durch Muskel- und Fettgewebe des diabetischen und nichtdiabetischen Hamsters unterscheidet sich nicht sehr, und beide Gruppen sprechen ungefähr gleich stark auf Insulinstimulierung an. Das im Plasma der insulinbehandelten Tiere vorkommende immunreaktive Insulin

ist noch 26 Tage nach der letzten Injektion nachweisbar. — Diese Ergebnisse lassen vermuten, daß der Pankreas des diabetischen Hamsters nur schwerlich in der Lage ist, nach einer Glucosestimulierung Insulin auszuschütten. Die Beobachtung, daß der Plasma-Insulinspiegel im leichten Diabetes normal ist, diese Tiere jedoch Glucosurie, Hyperglykämie und abnormale Glucosetoleranz aufweisen, führt zu der Annahme, daß ein leichter Diabetes beim chinesischen Hamster eine Beeinträchtigung der Insulinwirkung mit sich bringen, und Ursache einer vermehrten Glucoseproduktion durch die Leber sein kann.

Key-words: Spontaneous diabetes, Chinese Hamster, Cricetulus griseus, Insulin in Plasma, Insulin in pancreas, Pancreas, Free fatty acids, Detection of diabetes, Muscle metabolism, Adipose tissue metabolism, Liver glycogen.

It has become increasingly evident that glucosuria and hyperglycemia are late rather than initial manifestations of diabetes mellitus [9, 29, 7, 47, 51]. Since human diabetes is inherited [45, 46], the unknown basic defect of diabetes might be expected to occur at conception if the proper genotype is present.

Although considerable effort has been expended in attempts to define the initial lesions of prediabetes [6, 15], studies of prediabetes in man is difficult. Consequently, a laboratory animal with the same characteristics of diabetes in man is needed for the study of prediabetes. The Chinese hamster was considered because it satisfied many of these needs: 1. it becomes diabetic as defined by classical definition [34]; 2. the disease is inherited [35]; 3. selective breeding is possible; 4. variations in severity of onset and drug responses are similar to variations occurring in man [20]; 5. environment can be controlled and 6. terminal studies can be done as needed.

Although considerable information on diabetes in Chinese hamsters is available [60], it was important to confirm and extend these earlier observations. The purposes of this investigation were to evaluate various biochemical and physiological parameters of the diabetic and nondiabetic hamster in order to define diabetes in this animal and to compare the lesions of frank chemical diabetes of the Chinese hamster with those occurring in spontaneous or induced diabetes in other animals and diabetes in man. Comparisons of this nature may indicate which laboratory animal has diabetes similar to that of man and therefore which is the best tool to use for extensive studies designed to define the basic lesions of diabetes. It was reasoned that if abnormalities were not measurable in the frankly diabetic hamster it would be unlikely that they could be detected in prediabetics. If changes could be found in prediabetic Chinese hamster, then these findings could be used as guide lines for future studies in prediabetic man.

Methods

Animals. Animals were selected for study from the Upjohn colony of Chinese hamsters, which has been described previously by GERRITSEN and DULIN [20]. Diabetic animals were selected on the basis of a consistent Tes-Tape^R value of 4+. This initial qualitative test for urine glucose was done by expressing the urine from the animal directly onto the Tes-Tape^R. All diabetic animals were matched with a nondiabetic control of the same age and sex. If possible, the diabetics were also matched with a nondiabetic sibling of the same sex. All blood samples used for analysis reported in this study were obtained from the orbital sinus [43].

Control of diabetes. The majority of diabetics received no therapy and were allowed food (Purina Mouse Breeder Chow) and water *ad libitum*. In some early studies, ketonuria of diabetics was controlled by limiting food and/or administration of NPH insulin with no attempt to control glucosuria. These animals are referred to as controlled ketotic diabetics. Limitation of diet was accomplished by cup feeding 2 ml of liquid diet (MILLER and KRAKE [37]) in the morning and 3 ml in the afternoom. This feeding regime was based on maximum consumption of nondiabetic control hamsters. The liquid diet preparation contained 1.4 calories per ml. The dose of insulin required to control ketosis varied from 0.25 to 2.0 units b.i.d. In all cases, the last dose of insulin was given 24 hours before blood analysis or removal of tissues for studies *in vitro*. When liquid diet and/or insulin was given to the diabetic, appropriate nondiabetic control animals were treated similarly.

Blood and urine glucose determinations. Glucose concentrations were analyzed on 0.05 ml of blood by the AutoAnalyzer microglucose procedure, which uses a modification [19] of the method described by HOFFMAN [28]. Urinary glucose excretion was measured on urine collected while the animals were housed in stainless steel metabolism cages. Five consecutive 24-hour urine collections were made after at least a week of acclimatization to the metabolism cages. Urine glucose concentrations were determined by the AutoAnalyzer.

Glucose tolerance test. Those animals which were injected with insulin received their last dose 24 hours before the glucose tolerance was started. All hamsters were fasted overnight (16 hours) and a zero-hour blood sample was obtained. Immediately after the zero hour sampling, they were injected intraperitoneally with 250 mg/kg of glucose (0.1 ml/10 g body weight of 2.5% glucose in sterile physiological saline). The animals were bled again at 30, 60 and 120 minutes after glucose injection. Blood sugar was determined as described above.

Plasma free fatty acids (FFA) and blood ketone determinations. Plasma FFA were determined on 0.1 or 0.2 ml samples by the method of DOLE [11] as modified by TROUT [49]. Blood ketones were determined on 0.1 ml of whole blood by the procedure of

LYON and BLOOM [32] which measures acetoacetate and acetone. Acetoacetate is decomposed to acetone by acid and heat in a modified Thunberg tube and the total acetone is distilled into furfural. A reddish violet color is developed by addition of a strong acid to the difurfurylidene-acetone complex and the color intensity measured on a Beckman Model B. Spectrophotometer.

Liver glycogen. Liver glycogen was determined on samples of fresh tissue using the Anthrone method (SEIFTER et al. [44]) following digestion of tissue in 30% hot KOH.

Plasma and pancreatic insulin. Plasma insulin was determined on 0.1 or 0.2 ml of plasma. Blood was collected in a heparinized tube, and the plasma separated and kept frozen until assayed. The pancreatic tissue was removed following exsanguination and decapitation, and kept frozen until extraction. Extraction of pancreatic insulin was carried out by grinding individual pancreata in 1 ml of acid alcohol (750 ml of absolute ethyl alcohol, 15 ml concentrated HCl and 235 ml of distilled water) using the micro attachment on a Servall Omnimixer. The mixer was rinsed with two 1 ml portions of acid alcohol which were combined with the original material and the slurry left at room temperature overnight and centrifuged. The precipitate was resuspended in 2 ml of acid alcohol, allowed to stand for 2 hours and recentrifuged. This procedure was repeated twice and the supernatants were pooled. The acid-alcohol extract was adjusted to pH 8.0 with ammonium hydroxide, and assayed at various dilutions made with 1% BSA in borate buffer (pH 7.4). All insulin assays were done by a slight modification of a double antibody system described by MORGAN and LAZAROW [39, 40, 41]. The differences between the published procedure and the procedure used in these studies were that ^{131}I-insulin[1] with a specific activity greater than 100 microcuries/mg was used, and 4 μunits of ^{131}I-insulin was used or approximately 0.04 microcuries of ^{131}I per tube. The guinea pig anti-insulin serum was prepared as described by MORGAN and LAZAROW [39]. Bovine insulin (preparation PJ4609[2]) was used as a standard throughout these studies.

Several studies were done to determine the influence of insulin on plasma immunoreactive insulin levels. These were done after it was observed that the nondiabetics and diabetics which had received insulin had very high levels of plasma immunoreactive insulin 24 hours after the last insulin injection. In one study (Table 13), normal male hamsters were injected b.i.d. with 0.25 units of NPH insulin for 2 weeks. Plasma insulin was determined at various intervals after the last insulin injection. In a second experiment (Table 14), nondiabetic female hamsters were injected with 0.25 units of NPH insulin for one day (at 8.00 A.M. and 4.00 P.M.) and plasma insulin was determined on blood obtained 18 hours after the second injection.

Glucose oxidation and incorporation of glucose carbon into glycerol and fatty acids by epididymal fat pads in vitro. The procedure used for hamster fat pads was that described previously for studies using rat epididymal fat by GERRITSEN and DULIN [19, 21]. Insulin-treated animals received their last injection 24 hours prior to removal of tissues. Hamsters were sacrificed by exsanguination and decapitation, and epididymal fat pads removed. One pad was placed in Krebs-Ringer bicarbonate buffer, pH 7.4, and the other in buffer containing 250 μunits of insulin per ml. All flasks contained 3 ml of buffer with 0.5 microcuries of glucose-1-^{14}C. The experiment was designed so that one-half of the left pads and one-half of the right pads were treated. The CO_2 released was collected and counted by liquid scintillation counter (Packard Series 3000). The fat tissue was extracted by the procedure described by FOLCH [17]. Extracted lipids were saponified and dried, and the dried material was acidified and re-extracted by the Folch procedure. Aliquots of the lower chloroform phase containing fatty acids and the upper aqueous layer containing glycerol were counted by scintillation techniques. An external standard was used to determine the degree of quenching. Blood sugar was determined at the time tissues were taken.

Glucose uptake by diaphragm muscle in vitro. The procedure used was a modification of that described by VALLANCE-OWEN and HURLOCK [50]. Insulin-treated animals received their last insulin injection 24 hours before exsanguination and decapitation. The whole diaphragm was removed and placed in cool, balanced salt solution for 10—15 minutes. They were transferred to a salt solution containing 300 mg% of glucose and aerated for 5 minutes with 5% CO_2 and 95% oxygen. Incubation was carried out in a Warburg for 90 minutes and the glucose remaining in the medium was measured by an Auto-Analyzer procedure. This procedure measures glucose with sufficient accuracy to detect changes as small as 1 mg%. Blood sugar was determined at the time tissues were taken.

Results

Glucose tolerance test. Blood sugar of nondiabetic Chinese hamster rose from a fasting level of 110 mg% to a peak of 175 mg% thirty minutes after a glucose load of 250 mg/kg (Fig. 1). Thereafter blood sugar fell slowly to 130 mg% at 2 hours which was still significantly elevated above the initial fasting level.

Glucose tolerance curves for the two groups of control animals [nondiabetic nonrelated (NDNR) and nondiabetic siblings] were not different. Since these nondiabetic animals have identical glucose tolerance curves and were indistinguishable by all other measurements, the data from these were pooled and reported in this paper as nondiabetic controls.

[1] Abbott, List No. 7791 porcine glucagon-free insulin.
[2] Kindly supplied by Dr. MARY ROOT, ELI LILLY and Company. This insulin preparation contained less than 0.0003% glucagon and assayed 23.8 units/mg.

The blood sugar of the diabetics rose from a fasting level of 250 mg% to a maximum of 325 mg% at 30 minutes after the glucose load and then slowly declined to 280 mg% during the next 90 minutes. The variation

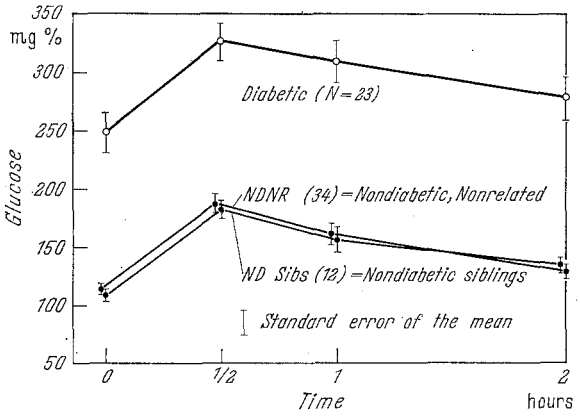

Fig. 1. Glucose tolerance curves of Chinese hamsters

of the blood sugar of these diabetics during the glucose tolerance was condiderably greater than that of the controls as evidenced by the large standard error. However, the shape of the mean glucose tolerance curves for the diabetics and controls were remarkably similar. The maximum increase in blood sugar after the glucose load was 65 mg% for nondiabetics and 75 mg% for diabetics. Blood sugar declined 45 mg% in both groups from 30 to 120 minutes after the glucose load.

Blood sugar. Fasting and nonfasting blood sugar levels of diabetics were significantly higher than those of the appropriate controls (Table 1). The nonfasting blood sugar levels of diabetics were significantly higher than the fasting levels. In contrast, there was no difference between fasting and nonfasting blood sugar of nondiabetic hamster. Fasting blood sugar of ketotic diabetics was significantly higher than the fasting blood sugar of the nonketotic diabetics. Blood sugar of diabetic and nondiabetic hamsters was not influenced by sex, age, duration of diabetes, age of onset of diabetes, diet (Table 3) or insulin therapy[3] (Table 1).

Urine glucose excretion. Glucosuria in the diabetic Chinese hamster fed *ad libitum* varies from 51 to 3000 mg of glucose per 24 hours whereas glucosuria in the nondiabetic is insignificant. The glucosuria of uncontrolled ketotic diabetics fed *ad libitum* increased significantly from 939 to 1342 mg/24 hours during a four-month period (Table 2). No change in glucosuria

Table 1. *Blood sugar levels of diabetic and nondiabetic Chinese hamsters*

Type	Diabetic		Nondiabetic	
	Blood sugar (mg%) ± S.E.[1]		Blood sugar (mg%) ± S.E.[1]	
	Fasted	Nonfasted	Fasted	Nonfasted
Ketotic[2] (uncontrolled)	300 ± 19[3,4] (7)	441 ± 45[3] (6)	112 ± 5 (11)	112 ± 4 (11)
Ketotic (controlled)	326 ± 24[4] (10)	—	136 ± 5 (14)	—
Nonketotic (uncontrolled)	219 ± 19[3,4] (16)	371 ± 33[4] (16)	108 ± 3 (24)	125 ± 5 (24)

[1] Standard error of the mean.
[2] Ketosis defined as consistent positive Ketostix® test on urine.
[3] FBS vs. NFBS, $P < 0.01$.
[4] Ketotics vs. mild, $P < 0.01$.
() = Number of animals.

Table 2. *Urinary glucose excretion of diabetic and nondiabetic Chinese hamsters*

Type	Diabetic		Nondiabetic
	Urine glucose (mg/24 hr) ± S.E.[1]		Urine glucose
	Initial	+ 4 months	(mg/24 hr) ± S.E.[1]
Ketotic[2] (uncontrolled)	939 ± 85[3] (6)	1342 ± 59[3,4] (7)	1.9 ± 0.1 (9)
Ketotic (controlled)	673 ± 111 (10)	—	2.4 ± 0.2 (14)
Nonketotic (uncontrolled)	657 ± 168 (5)	766 ± 214[4] (5)	1.7 ± 0.1 (10)

[1] Standard error of the mean.
[2] Ketosis defined as consistent positive Ketostix® test on urine.
[3] $P < 0.05$.
[4] $P < 0.05$.
() = Number of animals.

[3] Last injection of insulin was 24 hours prior to bleeding.

was observed in nonketotic diabetics during the same interval. In some ketotic diabetics, glucosuria was quantitated while ketonuria was controlled by insulin administration and/or limitation of diet (ketotic, controlled group, Table 2). A dose of insulin just sufficient to control ketonuria decreased glucosuria (uncontrolled 939 mg/24 hours, controlled 672 mg/24 hours, Table 2). When three diabetic hamsters that had been maintained on a limited diet (liquid diet, 2 ml in the A.M. and 3 ml in the P.M.) were switched to Purina Mouse Breeder Chow *ad libitum*, glucosuria increased significantly from 776 to 1118 mg/24 hours (Table 3).

There was a tendency toward a positive correlation between urine glucose excretion and nonfasting blood sugar levels in diabetic hamsters (linear correlation regression coefficient of +0.73).

Liver glycogen. Fasted, controlled, ketotic diabetics had six times as much liver glycogen as the nondiabetic controls (Table 4). Liver glycogen level of fasted nonketotic diabetics was ten times greater than that of the controls. The difference between the liver glycogen levels of the ketotic and mild diabetics is not significant. No correlation was found between fasting blood sugar and fasting liver glycogen levels (linear regression correlation coeficient of +0.5).

The effect of sex, age, duration of diabetes, age at onset of diabetes, insulin and/or diet on liver glycogen could not be established due to an insufficient number of observations.

Table 3. *Effect of restricted diet on blood sugar and glucosuria of Chinese hamsters*

		Restricted diet[1]			Ad libitum diet		
		Blood sugar (mg%) ± S.E.[2]		Urine glucose (mg/24 hr) ± S.E.[2]	Blood sugar (mg%) ± S.E.[2]		Urine glucose (mg/24 hr) ± S.E.[2]
Type	No.	Fasted	Nonfasted	(4/22/66)	Fasted	Nonfasted	(5/5/66
Diabetic	3	268 ± 76	434 ± 54	776 ± 38[3]	317 ± 28	492 ± 67	1118 ± 84[3]
Nondiabetic	7	120 ± 4	130 ± 6	2.0 ± 0.3	103 ± 3	114 ± 9[4]	1.8 ± 0.1

[1] Restricted for diabetics only, since quantity of diet given was amount maximally consumed by nondiabetics.
[2] Standard error of the mean.
[3] $P > 0.05$.
[4] Mean of 6 values.

Table 4. *Liver glycogen levels of fasting diabetic and nondiabetic Chinese hamsters*

	Diabetic	Nondiabetic
Type	Liver Glycogen (% fresh wt) ± S.E.[1]	Liver Glycogen (% fresh wt) ± S.E.[1]
Ketotic (controlled)	2.5 ± 0.9[2] (6)	0.4 ± 0.07[2] (10)
Nonketotic (uncontrolled)	4.2 ± 1.0[2] (6)	0.4 ± 0.06[2] (8)

[1] Standard error of the mean.
[2] $P < 0.001$, compared with nondiabetic controls.
() = Number of animals.

Plasma free fatty acids (FFA). The fasting FFA level of controlled ketotic hamsters 24 hours after last insulin injection was significantly greater than those of the nondiabetic controls (3300 vs. 1800, Table 5). The nonfasting FFA of the uncontrolled ketotics is significantly higher than the controls (3100 vs. 1900), but the fasting FFA levels of these animals were not different (2100 vs. 1500). This lack of difference may be due to the small number of observations and large variation. The FFA of mild diabetics were not significantly different from the FFA of their nondiabetic controls.

Table 5. *Plasma free fatty acids (FFA) of diabetic and nondiabetic Chinese hamsters*

	Diabetic		Nondiabetic	
Type	FFA (μE/1) ± S.E.[1]		FFA (μE/1) ± S.E.[1]	
	Fasted	Nonfasted	Fasted	Nonfasted
Ketotic (uncontrolled)	2100 ± 221 (4)	3100 ± 479[2] (4)	1500 ± 175 (9)	1900 ± 156[2] (9)
Ketotic (controlled)	3300 ± 456[3] (10)	—	1800 ± 100[3] (19)	—
Nonketotic (uncontrolled)	2100 ± 130 (14)	2700 ± 392 (5)	1800 ± 111 (21)	1900 ± 415 (8)

[1] Standard error of the mean.
[2] $P < 0.05$ compared with control.
[3] $P < 0.001$ compared with control.
() = Number of animals.

The average fasting FFA level was not higher than the nonfasting level in either diabetic or nondiabetic hamsters. FFA of diabetic and nondiabetic hamsters were not influenced by sex, age, duration of diabetes, age at onset of diabetes or insulin therapy (last injection of insulin was 24 hours prior to bleeding).

Blood ketones. Fasting levels of blood ketones were significantly higher in ketotic diabetic Chinese hamsters than in nondiabetic controls (Table 6). Blood

ketones of nonketotic diabetics were similar to those found in their nondiabetic controls. There was no significant difference between blood ketones of the uncontrolled ketotic diabetics compared with controlled ketotic diabetics 24 hours after the last insulin injection. The average fasting blood ketones did not increase in either diabetics or nondiabetics compared with nonfasting levels. No correlation was found between plasma free fatty acids and blood ketone levels.

Blood ketones were not influenced by sex, age, duration of diabetes, age at onset of diabetes or insulin treated controls. Glucose utilization by diaphragms from nonfasted diabetics (ketotics that had not received insulin or nonketotic diabetics) also did not differ from glucose uptake by diaphragms of nonfasted nondiabetic animals (Table 8). Although not shown, there was no difference due to sex, diet, duration of diabetes, age of onset of diabetes or age of animal at the time of this study.

Glucose oxidation and synthesis of fatty acids and glycerol by epididymal fat pad tissue in vitro. The epididymal fat pads from fasted or nonfasted nondiabetic

Table 6. *Blood ketone levels of diabetic and nondiabetic Chinese hamsters*

Type	Diabetic		Nondiabetic	
	Ketone (mg%) ± S.E.[1]		Ketone (mg%) ± S.E.[1]	
	Fasted	Nonfasted	Fasted	Nonfasted
Ketotic (uncontrolled)	14.3 ± 4.3[2] (6)	11.0 ± 4.6 (6)	6.2 ± 0.6[2] (14)	5.0 ± 0.5 (14)
Ketotic (controlled)	9.3 ± 3.5[2] (10)	—	4.2 ± 0.6[2] (19)	—
Nonketotic (uncontrolled)	4.0 ± 0.8 (16)	5.9 ± 1.2 (12)	3.7 ± 0.5 (23)	3.8 ± 0.3 (16)

[1] Standard error of the mean.
[2] $P < 0.01$, compared with nondiabetic controls.
() = Number of animals.

Table 7. *Glucose uptake by isolated diaphragm from fasted nondiabetic and diabetic Chinese hamsters*

Type animal	Insulin treatment	No.	Glucose uptake (mg%/10 mg dry weight) ± S.E.[3]	Fasted blood sugar (mg%) ± S.E.[3]
Nondiabetic	0	26	18.6 ± 0.5	136 ± 9
Nondiabetic	+[1]	4	18.5 ± 2.3	124 ± 7
Diabetic	0	13	19.0 ± 3.4	304 ± 18
Diabetic	+[2]	5	20.6 ± 1.5	312 ± 24

[1] NPH insulin-dose 0.25—0.5 u. b.i.d. Last dose 24 hours before sacrifice.
[2] NPH insulin-dose 0.25—1.75 u. b.i.d. Last dose 24 hours before sacrifice.
[3] Standard error of the mean.

Table 8. *Glucose uptake by diaphragm muscle from nonfasted normal and diabetic Chinese hamsters*

Type animal	No.	Nonfasting blood sugar (mg%) ± S.E.[1]	Glucose uptake (mg%/10 mg dry weight) ± S.E.[1]
		Ketotic	
Nondiabetic	4	109 ± 3.6	27.6 ± 2.6
Diabetic	6	437 ± 61	28.4 ± 5.0
		Nonketotic	
Nondiabetic	15	126 ± 4.7	23.2 ± 1.1
Diabetic	11	371 ± 21.7	19.7 ± 1.1

[1] Standard error of the mean.

Table 9. *Oxidation of glucose-1-^{14}C to $^{14}CO_2$ by epididymal fat pads from fasted nondiabetic and diabetic Chinese hamsters*

Type animal	No.	$^{14}CO_2$-CPM/mg wet weight of fat tissue ± S.E.[1]		Fasted blood sugar (mg%) ± S.E.[1]
		Without insulin	Insulin (250 μU/ml)	
Nondiabetic	21	11.8 ± 1.10	23.9 ± 2.12	133 ± 3
Diabetic	11	10.5 ± 0.83	21.1 ± 2.65	288 ± 28

[1] Standard error of the mean.

therapy (last injection of insulin was 24 hours prior to bleeding).

Glucose uptake by diaphragm muscle in vitro. Glucose uptake by diaphragms of fasting non-diabetic and diabetic hamsters was essentially identical (Table 7). Glucose uptake by the diaphragms of the insulin-treated diabetics (when last dose was 24 hours before removing tissues) did not differ from that of similarly and diabetic hamsters converted glucose-1-^{14}C to $^{14}CO_2$ at the same rate (Table 9 and 10). The response of these fat pads to insulin was also similar measured by $^{14}CO_2$ production from glucose-1-^{14}C. The epididymal fat tissue from fasted diabetics converted carbon 1 of glucose 1-^{14}C to ^{14}C- labelled fatty acids and glycerol at the same rate as tissues obtained from nondiabetic animals (Table 11).

Pancreatic insulin content. The insulin content of the pancreas of diabetics not treated with insulin was significantly lower (2.03 vs. 0.56 units/g) than in the pancreas of the non-diabetics (Table 12). Diabetics that had received insulin to control their ketosis also had lower pancreatic insulin than insulin-injected non-diabetics. The pancreatic insulin of non-diabetics injected with insulin was not significantly lower than that of the nontreated non-diabetics. In contrast, diabetics that had received insulin had lower pancreatic insulin than diabetics not injected with the hormone (0.56 vs. 0.16 units/g). There was no significant difference in the insulin content due to sex, age, onset of diabetes or duration of diabetes. Consequently, the data from animals of different sexes, ages, and onset and duration of diabetes were pooled.

Plasma insulin. There was no difference in fasting plasma insulin of ketotic diabetics, nonketotic diabetics and nondiabetic animals (Table 13). The plasma insulin levels of the nondiabetic and the mild diabetic were significantly increased with feeding (Table 13). In contrast, the plasma insulin of ketotic diabetics did not change after feeding. There was no difference in plasma insulin level due to sex, onset of diabetes, age, or duration of diabetes, consequently data were pooled when taken from animals of different ages, sex, and duration of diabetes.

Plasma immunoreactive insulin of animals that had been treated with insulin was considerably increased compared with that of animals which had never received insulin, and this increase was greater in the nondiabetic animals than in diabetic animals (Table 14). When nondiabetic hamsters were injected with 0.25 units of NPH insulin for 2 weeks and bled at various intervals following the last dosage, a significant increase in plasma immunoreactive insulin was still observed 26 days after the last insulin injection (Table 15). When nondiabetic animals were injected with 0.25 units of NPH insulin for one day and the plasma immunoreactive insulin levels determined 18 hours after the last injection, no significant increase in plasma insulin was observed (Table 16).

Table 10. *Glucose-1-^{14}C oxidation by epididymal fat pads of nonfasted nondiabetic and diabetic Chinese hamsters and response of fat pads to insulin*

Type animal	No.	Nonfasted Blood sugar (mg%) ± S.E.[1]	$^{14}CO_2$-CPM/mg tissue ± S.E.[1]	
			No insulin	With insulin
Diabetic	3	324 ± 12.6	9.1 ± 2.6	16.0 ± 5.2
Nondiabetic	4	132 ± 4.7	19.1 ± 5.4	37.5 ± 5.1

[1] Standard error of the mean.

Table 11. *Conversion of carbon 1 of glucose-1-^{14}C to ^{14}C glycerol and ^{14}C fatty acids by epididymal fat pads from fasted nondiabetic and diabetic Chinese hamsters*

Type animal	No.	Fasted blood sugar (mg%) ± S.E.[1]	CPM/mg tissue wet weight ± S.E.[1]	
			^{14}C Glycerol	^{14}C Fatty acids
Nondiabetic	19	134 ± 4.6	42.6 ± 5.2	4.5 ± 0.5
Diabetic	11	277 ± 28	33.8 ± 4.0[2]	5.0 ± 1.4[2]

[1] Standard error of the mean.
[2] $P < 0.2$ between diabetic and nondiabetic.

Table 12. *Pancreatic insulin of fasted diabetic and nondiabetic Chinese hamsters with and without insulin treatment*

Type animal	No.	Insulin[1]	Pancreatic insulin (units/g wet weight) ± S.E.[2]	Fasted blood sugar (mg%) ± S.E.[2]
Nondiabetic	18	0	2.03 ± 0.29	125 ± 3
Nondiabetic	5	+	1.52 ± 0.9	125 ± 2.4
Diabetic	4	0	0.56 ± 0.19[3]	238 ± 42
Diabetic	9	+	0.16 ± 0.06[4]	311 ± 18.5

[1] NPH insulin injected to control ketosis. Last dose given 24 hours before sacrifice.
[2] Standard error of the mean.
[3] $P < 0.01$ compared with nondiabetics not treated with insulin.
[4] $P < 0.01$ compared with diabetics not treated with insulin or nondiabetics treated with insulin.

Discussion

The objectives of these studies on the Chinese hamsters were twofold. The first objective was to obtain data on physiological and biochemical changes in diabetic animals and to relate these changes to probable causes of diabetes. Those causes that have received the most attention are: 1. inability of pancreas to synthesize and/or secrete insulin; 2. inability of muscle and fat tissue to utilize glucose; 3. alterations in liver metabolism resulting in increased gluconeogenesis. The second objective was to compare changes in diabetic hamsters with those reported for spontaneous diabetic man and animals as well as alloxan, anti-insulin serum or surgically-induced diabetes in animals.

Some of the data reported here support the concept that diabetes is related to decreased beta cell function in the Chinese hamster. These observations are: 1. pancreatic insulin is decreased in diabetics and there is a correlation between pancreatic insulin and plasma insulin; 2. plasma insulin of the severe ketotic diabetic does not increase with feeding; and 3. even though

Table 13. *Fasting and nonfasting plasma insulin and blood sugar of ketotic and nonketotic diabetic and nondiabetic Chinese hamsters*

Type animal	Blood sugar ± S.E.[1] (mg%)		Plasma insulin ± S.E. (μU/ml)	
	Fasted	Nonfasted	Fasted	Nonfasted
		Ketotic		
Nondiabetic	125 ± 6.1 (8)	118 ± 5.4 (8)	79 ± 26 (8)	220 ± 40 (8)[2]
Diabetic	299 ± 11.3 (5)	396 ± 43 (7)	72 ± 24 (5)	75 ± 16 (7)
		Nonketotic		
Nondiabetic	105 ± 5.0 (14)	127 ± 7.1 (14)	59 ± 5.3 (14)	216 ± 38.7 (14)[3]
Diabetic	200 ± 12.2 (11)	367 ± 22.0 (11)	59 ± 5.0 (11)	124 ± 18.0 (11)[4, 5]

[1] Standard error of the mean.
[2] $P < 0.01$ between fasted and nonfasted.
[3] $P < 0.01$ between fasted and nonfasted.
[4] $P < 0.01$ between fasted and nonfasted.
[5] P 0.1—0.05 nonfasted nondiabetics and diabetics.

Table 14. *Plasma insulin of nonfasting diabetic and nondiabetic Chinese hamsters and effect of insulin treatment on plasma insulin*

Type Animal	No.	Insulin[1]	Plasma Insulin (μU/ml) ± S.E.[5]
Nondiabetic	20	0	255 ± 56
Nondiabetic	4	+	2797 ± 216[2]
Diabetic	12	0	176 ± 28
Diabetic	6	+	850 ± 196[3, 4]

[1] Treated b.i.d. with 0.25—2.0 units of NPH insulin for several months. Last injection 24 hours before bleeding.
[2] $P < 0.01$ compared with noninsulin treated.
[3] $P < 0.01$ compared with noninsulin treated.
[4] $P < 0.01$ compared with insulin treated nondiabetic.
[5] Standard error of the mean.

Table 16. *Plasma insulin levels after one day of insulin treatment (0.25 unit b.i.d.) of nonfasted nondiabetic Chinese hamsters*

No.	Treatment	Plasma insulin[1] (μU/ml) ± S.E.[2]
3	Control	246 ± 59
3	Insulin	343 ± 30[3]

[1] Eighteen hours after last dose of insulin.
[2] Standard error of the mean.
[3] $P < 0.02$.

Table 15. *Plasma insulin levels at various intervals after withdrawal of insulin in nonfasting nondiabetic Chinese hamsters*

No.	Treatment	Plasma insulin (μU/ml) ± S.E.[1]						
Day after last dose		1	2	3	9	16	26	34
5	Control	245 ± 26	214 ± 33	248 ± 25				
5	Insulin[5]	1458 ± 432[2]	2408 ± 701[2]	1967 ± 540[2]				
6	Control		272 ± 57		338 ± 47	289 ± 55	233 ± 37	181 ± 30
7	Insulin[5]		1774 ± 532[3]		1165 ± 243[3]	590 ± 116[3]	427 ± 55[4]	249 ± 46

[1] Standard error of the mean.
[2] $P < 0.01$ (from control at same day).
[3] P 0.01—0.02 (from control at same day).
[4] P 0.02—0.05 (from control at same day).
[5] 0.25 unit b.i.d. for 14 days.

plasma insulin significantly increases with feeding in the nonketotic diabetic, this increase was consistently less than that in the nondiabetic. Contradictory to this concept is that, although fasting hyperglycemia is present in the diabetic, the fasting insulin levels were similar in diabetics and nondiabetics implying that tissues utilizing glucose do not respond to the available insulin and therefore there is an insufficient quantity of insulin. However, it must be emphasized that this observation could be interpreted that diabetes in this animal is still a pancreatic-related disease since the increased blood sugar would be expected to result in sufficient insulin to control blood glucose levels.

Comparison of pancreatic insulin of hamsters and man show that they are similar since diabetics of both species have less pancreatic insulin than nondiabetics [55]. Further, in both species the severe ketotic diabetic has less pancreatic insulin than the nonketotic diabetic [55]. Another similarity of diabetic man and hamster is that plasma insulin of severe diabetics does not increase with a glucose load [58, 13, 25, 24].

Fasting plasma insulin in the mild-nonketotic-diabetic man and hamster is normal [57, 59]. It is difficult to compare the relative increase in plasma insulin due to a glucose load in mild-diabetic man and Chinese hamster since measurements were made in the non-fasted hamster and following a glucose load in man. Nevertheless, in both species plasma insulin increases significantly with hyperglycemia. This comparison is further complicated by conflicting reports on the quantitative plasma insulin changes following a glucose load in the mild diabetic man [57, 13, 30, 25].

An unexpected finding was the high level of plasma immunoreactive insulin (IRI) in diabetic and nondiabetic hamsters treated with insulin for several months and bled 24 hours after the last injection. This increased IRI in the insulin-treated animals could be due to antibody-bound insulin. Supporting this explanation are the observations that hamsters injected with insulin for two weeks had elevated levels of IRI for 26 days; whereas insulin injection for only one day did not result in increased IRI 18 hours after the injection. It was also unexpected to find that the insulin-treated diabetics had significantly less plasma insulin than treated nondiabetics. This could be the result of increased metabolism of insulin in the diabetic, reduced synthesis and/or release of the endogenous insulin by the diabetic resulting in a smaller net increase in plasma IRI, a decreased production of insulin antibodies in the diabetic or a combination of these factors. There is no evidence to support the possibility that the decreased IRI of the diabetic was due to increased metabolism of insulin. Since it was shown that the plasma and pancreatic insulin of diabetics was decreased and since it had been reported that diabetics are less capable of producing antibodies [12] it seems likely that a combination of these two factors could explain the decreased IRI in plasma of insulin treated diabetic hamsters compared with insulin-treated nondiabetic hamsters.

A second possible explanation for diabetes is that the muscle and the fat tissue of diabetic animals and man are unable to utilize glucose at a normal rate either because of some inherent deficiency in the transport or enzyme systems of the tissue or due to relative or absolute deficiency of insulin. Since *in vitro*, diaphragm muscle of ketotic and nonketotic diabetic hamsters utilized glucose at the same rate as tissue of the nondiabetic animals, it suggests that the transport and enzyme systems of the muscle of the diabetic hamster are unimpaired. This observation is different from that for muscle of animals made diabetic by anti-insulin serum (Gregor et al. [22]), by alloxan (Krahl and Cori [31] and Hall [26] or pancreatectomy (Migliorine and Chaikoff [36] and Beatte [1], since the muscles of these animals utilized glucose at a decreased rate. The observation that glucose utilization by muscle from diabetic Chinese hamsters was normal is in agreement with the observation of Magyar [33] that muscle tissue from mild human diabetics took up normal amounts of glucose *in vitro*. A fundamental difference therefore exists in muscle tissue from diabetic hamsters and man compared with muscle from animals made diabetic through insulin deficiency.

It has been the general finding that adipose tissue of alloxan-diabetic animals (Hausberger et al. [27], Millstein and Hausberger [38], Winegrad and Shaw [53] and Winegrad and Renold [54]; depancreatized rats (Migliorine and Chaikoff [36] and Fain et al. [14]; and antiinsulin-induced diabetes (Tarrant and Ashmore [48] and Dixit et al. [10]) have a decreased ability to oxidize glucose to CO_2 or convert glucose to fatty acids and glycerol. Fat tissue from human diabetics has been reported to oxidize glucose to CO_2 and synthesize fatty acids at a normal rate (Bjorntorp [2] and Carlson and Ostman [8]). The adipose tissue from diabetic hamsters is similar to human fat tissue since diabetes in the hamster does not alter the ability of adipose tissue to oxidize glucose to CO_2 or convert glucose to fatty acids and glycerol *in vitro*. It can therefore be concluded that the transport and enzyme systems for glucose utilization in adipose tissue and diaphragm muscle of the hamster are functional. However, since these studies on fat and muscle tissue were done *in vitro*, it is possible that in the environment of the diabetic animal the tissues do not possess normal metabolism. Further support for the view that the utilization of glucose is similar in diabetic and nondiabetic hamsters is that the glucose tolerance curves of both types of animals were similar in shape. It is possible that plasma insulin of diabetics was sufficient to maintain the transport system and enzyme system of both muscle and fat tissue but not able to control blood glucose levels.

A third possible explanation for diabetes is abnormal metabolism of the liver. In the present studies, even though fasting plasma insulin levels were normal

in the diabetics and tissues of diabetic hamsters used glucose at normal rates, the diabetics still exhibit a fasting hyperglycemia and glucosuria. This would suggest that the liver must be producing glucose at an increased rate in the diabetic. It has been observed that glucose metabolism by the liver of diabetic animals is different from that of normals [23, 52, 5]. Additional support implicating the liver as a cause of hyperglycemia in the diabetic is that glucose output by the liver of diabetic man and animals is increased [3, 56, 16]. That the liver of the diabetic Chinese hamster is abnormal is suggested by data reported by RENOLD [42] that oxidation of glucose-1 and glucose-6-^{14}C to CO_2 by liver of the diabetic hamster is depressed. An additional observation suggesting that the liver of the diabetic hamster is not similar to that of the normal animal is that fasting liver glycogen of diabetics is markedly elevated. This elevation in liver glycogen has also been reported to occur in alloxan-diabetic rats [18] and possibly diabetic man [4].

In conclusion, data reported here for the Chinese hamster and available information on human diabetics suggest that diabetes in these two species may be a result of abnormal insulin secretion by the pancreas and abnormal glucose output by the liver rather than a decreased glucose metabolism by muscle and fat tissue.

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