

Trehalase activity in genetically diabetic mice (serum, kidney, and liver)*

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SUMMARY Trehalase activity was determined in serum, liver, and kidney in alloxan treated Swiss mice and in homozygous (Ob/Ob, Db/Db) and heterozygous (Ob/+, Db/m+) diabetic mice.

Both alloxan and genetic diabetic mice exhibited a large increase in serum and liver trehalase activity with no change in kidney trehalase activity. The heterozygotes (Ob/+, Db/m+) showed only a slight increase of enzyme activity.

Further quantitative differences were noticed between the genetic and alloxan diabetic animals. The liver enzyme activity increased from 10- to more than 20-fold in the liver of the homozygous Ob/Ob and Db/Db strains and only 3-fold (not significant compared to controls) in the alloxan treated animals. The above results suggest a regulatory relationship between the genes coding for trehalase and the enzymes of glucose metabolism activity involved in the development of the metabolic anomalies of diabetes. The structural gene for trehalase may well have survived elimination of selective pressure during phylogenesis and remained part of a co-regulated group of glucose metabolising enzymes. This could explain its sensitivity to mutations affecting glucose metabolism and its sensitivity to insulin directed regulatory mechanisms.

Trehalase (glucoside 1,1 glucohydrolase, EC 3.2.1.28) is widespread in nature.¹ In mammals, it is present in kidney, liver, small intestine, urine, and bile. Its natural substrate, α, α -trehalose, has, however, never been characterised in mammals. All the enzymes necessary for its synthesis are nevertheless present in the renal cortex,² and some years ago Sacktor and Berger³ described the synthesis of ¹⁴C-trehalose by incubating rabbit renal cortex slices with ¹⁴C-glucose. In spite of these results, the biological role of trehalase in mammals remains unknown. Trehalase present in the brush border membrane of small intestine may be responsible for the hydrolysis of trehalose in the intestinal tract absorbed with food.⁴ Alternatively, trehalase may have no role to play in the organism whatsoever.

Serum trehalase activity has been shown to be fairly constant in the same subject, although large variations have been noticed from one person to another.⁵

An increased serum trehalase activity has been

reported in human diabetes mellitus,⁶ without any apparent relationship to serum glucose levels.⁷ It was tempting therefore to explore the possibility of a relationship between disturbed glucose metabolism and serum and tissue trehalase levels. As a first approach we used alloxan diabetic mice. The results obtained confirmed the existence of such a relationship and warranted further experiments using the genetic diabetes models available in the C57 Black mouse strain. Although quantitative differences were observed between the Swiss and C57 Black strains, both chemical and genetic models confirmed the existence of such a relationship.

Material and methods

α, α -trehalose was purchased from Merck. Swiss albino mice, EOPS, were used as controls. They were provided by Charles Rivers (Saint-Aubin les Elbeuf). Diabetes was induced in Swiss mice by a single intravenous injection of alloxan monohydrate (90 mg/kg body weight). The animals were killed 3 weeks after injection. Spontaneously diabetic animals

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were obtained from the Centre de Sélection et d'Elevage des Animaux de Laboratoire, CNRS (45045 Orléans Cedex). We used C57 B1/6 Orl/Ob mice,⁸ both homozygous Ob/Ob and heterozygous Ob/+, and C57 B1 Ks Orl-Db mice,⁹ both homozygous Db/Db and heterozygous Db/m+. As a control, we used C57 B1/6 mice. All the animals were exsanguinated through the orbital sinus.

Organs were excised, rinsed in cold saline, weighed, and homogenised in cold saline (10 ml final volume) for kidneys, and sodium bicarbonate/calcium chloride (1 mol, 0.5 mol v/v) 10 ml/1 g for liver, with Ultraturrax homogeniser-disintegrator (30 sX² at setting 20 000/min).

Trehalase activity was estimated in plasma as described previously on aliquots of 100 µl.¹⁰ One unit of enzyme (U Tr) was defined as the quantity of trehalase that hydrolyses 1 µmol substrate in one minute at 37°C.

In each determination of trehalase activity, the conditions of steady state kinetics were respected and the activity was expressed as U Tr/g organ.

The computations were done on the logarithm, to the base 10 of 10 or 100 times the enzyme activity (units-units/g for organs). The data are thus normalised. The factor 10 or 100 avoided the introduction of negative logarithms. It has no effect on the variances.

Results

Trehalase activity in control and alloxan diabetic Swiss mice serum and organs expressed as log

trehalase activity in units is shown in table 1. It can be seen that serum trehalase activity was significantly higher in the alloxan treated mouse serum than in the control mouse serum.

The liver trehalase activity appeared to be increased also, but the difference is not statistically significant because of the large variability of the results.

The kidney trehalase activity decreased slightly but significantly. The blood sugar was significantly increased in alloxan treated mice.

Table 1a shows the antilogarithms of the results calculated in table 1.

Serum trehalase activity was 2.19 times higher in alloxan diabetic mice than in the controls and hepatic trehalase activity was 2.95 times higher, but there was a 12% decrease in renal trehalase activity. Blood sugar was 3.6 times higher in the alloxan diabetic mice serum as compared to the control serum. Glycosuria was increased in diabetic mice (250 times, results not shown).

Table 2 shows the serum, kidney, and liver trehalase activities for the heterozygous and homozygous diabetic mice strains derived from the original C57 B1/6 strain. In these strains the hyperglycaemic syndromes are considered to be related to single gene mutations.¹¹ Serum trehalase activity was found to be increased in both heterozygous strains (Ob/+, Db/m+) compared to the original strain, although the difference on the logarithms is not significant for Ob/+ and only slightly significant for Db/m+. Both homozygous strains exhibited an important and significant

TABLE 1 Serum, liver, and kidney trehalase activity of normal and alloxan diabetic Swiss mice. Enzyme activity is expressed as log 10 for serum (10 × units/ml) and kidney (10 × units/g organ), and log 100 for liver (100 × units/g organ). The numbers indicate the average values (M), the standard deviation (SD), and the standard error of the mean (SEM). *t* is calculated for 95% confidence limits.

	Serum				Liver				Kidney				Blood sugar			
	M	SD	SEM	tSEM	M	SD	SEM	tSEM	M	SD	SEM	tSEM	M	SD	SEM	tSEM
Control mice	2.25	0.17	0.039	0.08	1.40	1.07	0.24	0.50	2.025	0.05	0.0128	0.02	1.99	0.039	0.008	0.017
Alloxan treated mice	2.59	0.15	0.033	0.06	1.87	0.84	0.19	0.40	1.92	0.08	0.018	0.04	2.548	0.05	0.011	0.023
	***				NS				***				***			

NS, difference between control and alloxan treated groups not significant.

***, difference between control and alloxan treated groups significant (p < 0.001).

TABLE 1a Geometric mean and factors corresponding to SD and SEM of serum, liver, and kidney trehalase activity in units, obtained by taking antilogarithms of results shown in table 1

	Serum			Liver			Kidney			Blood sugar		
	Geom mean (units)	SD factor	SEM factor	Geom mean (units)	SD factor	SEM factor	Geom mean (units)	SD factor	SEM factor	Geom mean (units)	SD factor	SEM factor
Control mice	17.7	1.48	1.09	0.251	1.18	1.74	10.6	1.12	1.03	9.815	0.845	0.189
Alloxan treated mice	38.9	1.41	1.08	0.741	6.91	1.55	8.31	1.2	1.04	35.075	3.541	0.223

TABLE 2 Serum, liver, and kidney trehalase activity of homozygous and heterozygous mice strains exhibiting spontaneous diabetes as compared to the original mouse strain (C57 Bl/Orl). Enzyme activity is expressed as log 10 for serum ($10 \times \text{units/ml}$) and kidney ($10 \times \text{units/g organ}$), and as log 100 for liver ($100 \times \text{units/g organ}$). The numbers indicate the average values (*M*), the standard deviation (*SD*), and the standard error of the mean (*SEM*). *t* is calculated for 95% confidence limits.

	No	Serum				Liver				Kidney			
		<i>M</i>	<i>SD</i>	<i>SEM</i>	<i>tSEM</i>	<i>M</i>	<i>SD</i>	<i>SEM</i>	<i>tSEM</i>	<i>M</i>	<i>SD</i>	<i>SEM</i>	<i>tSEM</i>
C57 Bl/orl	10	1.023	0.614	0.194	0.02	0.97	0.192	0.0607	0.12	1.69	0.187	0.059	0.12
ob/+	10	1.30	0.018	0.008	0.03	1.09	0.195	0.0617	0.18	1.62	0.0809	0.0255	0.04
		NS				NS				NS			
ob/ob	10	2.87	0.187	0.0593	0.14	2.02	0.280	0.0886	0.20	1.64	0.094	0.0297	0.07
		***				***				NS			
db/m+	16	1.41	0.225	0.062	0.13	1.22	0.186	0.0589	0.14	1.80	0.112	0.028	0.06
		*				**				NS			
db/db	16	1.99	0.168	0.042	0.08	2.35	0.313	0.099	0.23	1.87	0.064	0.016	0.04
		***				***				*			

Significance was calculated between the original strain and the heterozygotes and between the heterozygotes and homozygotes. Homozygous values differing significantly from heterozygous values also differ significantly from those of the original strain.

NS, difference between control and spontaneous diabetic group not significant.

*, difference between control and spontaneous diabetic group significant ($p < 0.05$).

**, difference between control and spontaneous diabetic group significant ($p < 0.01$).

***, difference between control and spontaneous diabetic group significant ($p < 0.001$).

TABLE 2a Geometric mean and factors corresponding to SD and SEM of serum, liver, and kidney trehalase activity in units, obtained by taking antilogarithms of results shown in table 2.

	Serum			Liver			Kidney			blood sugar		
	Geom mean (units)	SD factor	SEM factor	Geom mean (units)	SD factor	SEM factor	Geom mean (units)	SD factor	SEM factor	Geom mean (units)	SD factor	SEM factor
Control mice (C57/Bl/orl)	1.05	4.11	1.56	0.0933	1.56	1.15	4.90	1.54	1.15	3.719	0.624	0.197
ob/+	2.00	1.04	1.00	0.123	1.57	1.15	4.17	1.21	1.06	8.274	1.817	0.813
ob/ob	74.1	1.54	1.14	1.04	1.91	1.23	4.37	1.24	1.07	11.3	0.756	0.239
db/m+	2.57	1.68	1.15	0.166	1.53	1.15	6.31	1.29	1.07	10.756	2.205	0.697
db/db	9.78	1.47	1.10	2.24	2.06	1.25	7.42	1.16	1.04	21.010	3.763	0.316

increase of serum trehalase activity compared to the corresponding heterozygous strains or to the original strain.

Liver trehalase activity was also increased slightly in Ob/+ and significantly in Db/m+, as compared to the controls. A very big increase was noted again in both homozygous strains, compared to the corresponding heterozygous or the original strains.

The figure shows a histogram comparing the variations in blood serum and liver trehalase activity in the genetically and chemically diabetic mice. There is an apparent and good correlation between the increase in serum and liver trehalase activity in the homozygous strains.

No important variation of trehalase activity could be found in the kidneys of homozygous and heterozygous strains.

The blood sugar level was significantly increased in both heterozygous strains. Glycosuria was slightly positive for db/m+ mice but was negative for ob/+ mice.

Table 2a shows the antilogarithms of the results calculated in table 2. In this case, there was an increase of serum trehalase activity of 1.9 times for ob/+, 70 times for ob/ob, 2.4 times for db/m+, and 9.3 times for db/db.

For liver trehalase, the increase of activity was 1.3 times for ob/+, 11 times for ob/ob, 1.8 times for db/m+, and 24 times for db/db. The renal trehalase activity showed only slight variation: an increase of 1.7 times for ob/+, 1.12 for ob/ob, and 0.77 and 0.66 times for db/+ and db/db, respectively.

An analysis of variance of the above results was performed and the results are shown in table 3 comparing C57 B1, ob/+, ob/ob, db/m+, and db/db mice using Scheffe's test.¹² The results showed a globally significant difference between sera, liver, and glycaemia for the five mouse strains. In table 4 we list the comparison of the means according to Scheffe's test.

In order to explore the possible relationship between serum and liver trehalase activities and blood glucose levels, we compare in table 5 the

TABLE 3 Analysis of variance of log 10 (10 × serum, 100 × liver, 10 × kidney) trehalase activity comparing C57 Bl, ob/+, ob/ob, db/m+, db/db.

		Df	Sums of squares	Mean squares	Variance ratio
Serum	Between diabetic states	4	21.229	5.307	54.65***
	Residual	49	4.758	0.0971	
	Total	53	25.987		
Liver	Between diabetic states	4	15.248	3.812	66.41***
	Residual	45	2.584	0.0574	
	Total	49	17.832		
Kidney	Between diabetic states	4	0.6126	0.153	12.33***
	Residual	57	0.707	0.0124	
	Total	61	1.32		
Glycaemia	Between diabetic states	4	2.941	0.735	141.34***
	Residual	40	0.211	0.0052	
	Total	44	3.152		

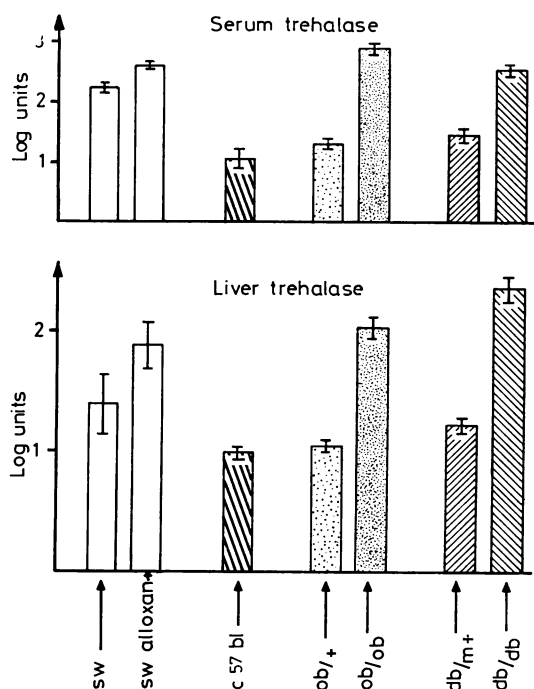


FIGURE Comparison of variations in blood serum and liver trehalase activities in the genetically and chemically diabetic mice.

percentage variation of these variables in the alloxan treated Swiss mice and in the heterozygous and the homozygous mutants of C57 Black.

It can be seen that there is no apparent relationship between the increase of serum and liver trehalase activities and the increase of blood glucose levels. The percentage increase of serum trehalase activity is of the same order in the alloxan treated animals as

TABLE 4 Scheffe's test for comparison of means.

	Trehalase activity			
	Serum	Liver	Kidney	Blood sugar
Bl/ob+	NS	NS	NS	*
Bl/obob	***	***	NS	**
Bl/dbm	**	NS	*	**
Bl/dbdb	***	***	**	***
Ob+/obob	***	***	NS	NS
Ob+/dbm	NS	NS	**	NS
Ob+/dbdb	**	***	***	*
dbm/dbdb	**	***	NS	NS
dbm/obob	***	***	**	NS
Obob/dbdb	***	*	**	*

TABLE 5 Relative increase of serum and liver trehalase and blood sugar levels expressed as percentage increase with respect to the control value.

	Δ% variation		
	Serum trehalase	Liver trehalase	Blood sugar
Alloxan treated			
Swiss mice	119	196	250
ob/+	100	32	122
ob/ob	740	1015	205
db/m+	144	78	189
db/db	765	2311	467

in the heterozygous mutants and significantly lower than the percentage increase in the homozygotes.

Similar results were obtained for the liver trehalase activities. The relative increase after alloxan is a little higher than the one observed in the heterozygotes and much lower than the increase observed in the homozygotes.

On the other hand, the relative blood sugar increase is of the same order with alloxan as in the homozygous strains. These results indicate a lower insulin sensitivity of trehalase levels than of those enzymes involved in blood sugar regulation.

Discussion

The comparison of trehalase activity in spontaneously diabetic mice and that in alloxan diabetic mice may be of value in the study of the mechanisms involved in the development of this metabolic disorder.

Since the work of Dunn *et al.*,¹³⁻¹⁵ alloxan is known to destroy the β cells of the islets of Langerhans thereby causing a lack of insulin. There is a morphological and functional involution of the pancreas and a progressive degranulation of the β cells.^{16 17}

The ob/ob mice show moderate spontaneous diabetes with moderate hyperglycaemia, hyperinsulinaemia, and a high gluconeogenic enzyme activity. They are markedly obese.

The db/db mice show severe diabetes with higher hyperglycaemia than ob/ob mice, transitory hyperinsulinaemia, high gluconeogenic enzyme activity, and moderate obesity.

The heterozygous strains are apparently normal.¹⁸ We nevertheless found significant hyperglycaemia compared with C57 B1/6 strain.

Both mouse strains carry autosomal mutations at either the diabetes (db) or the obesity (ob) loci. The db locus is on chromosome 4, linkage group VIII, and the ob mutation is on chromosome 6, linkage group XI.

Chick *et al.*¹⁹ showed abnormalities in glucose tolerance primarily restricted to male heterozygous mice (db/m+). In this context it should be mentioned that we noticed a significant difference between the fasting blood glucose levels and the serum trehalase activity of normal male and female C57 B1/Orl mice.

It is interesting to note that serum and liver trehalase activities increased very strongly in both homozygous diabetic strains. This increase was much more pronounced than the one produced by alloxan treatment. The heterozygous strains (ob/+, db/m+) showed only a moderate or no increase of serum or liver trehalase levels. No variation of kidney trehalase activity could be found either with alloxan or in the genetically diabetic strains. These results are in agreement with our former proposition concerning the possible hepatic origin of the serum enzyme trehalase.²⁰

No direct correlation could be found between serum and liver trehalase activities and blood sugar levels. The largest increase in blood sugar (approximately 3.5 times) was seen in the alloxan treated animals. Furthermore, a large increase in blood sugar was found in the heterozygous (ob/+, db/m+) strains with moderate or no increase of liver trehalase activity. These results are in agree-

ment with the highly improbable idea that trehalase is involved in the regulation of blood glucose levels.

However, trehalase in liver and serum may well be the result of the 'survival' of a gene, probably in close association with other genes, coding for enzymes involved in the regulation of glucose metabolism. In this case a correlation might be expected between the expression of these genes and the one coding for trehalase, both being sensitive to the same hormonal and genetic regulatory mechanisms.

The increase of the liver trehalase activity may thus be related to the previously noted increase of the liver enzymes involved in gluconeogenesis in the same homozygous strains.^{21 22} The lack of difference in Ob/+ mice and the slight increase in Db/m+ mice, as compared to the very large increase in the homozygous mice, suggests the possibility of a repression mediated mechanism. Such a repression mechanism could depend on the synthesis of a substance coded for by the modified genes. The presence of one set of normal genes out of two could be nearly enough to ensure the synthesis of an adequate amount of the repressor substance. In the absence of both sets of normal genes it could be synthesised, leading to the full expression of the structural genes coding for trehalase.

The gene(s) coding for trehalase may be part of a set of structural genes for glucose metabolising enzyme, which evolved together from bacteria and insects where trehalose is the major circulating carbohydrate. The trehalase gene could have remained in association with the other genes of glucose metabolising enzymes without playing any further role in intermediary metabolism of vertebrates. It would then react to the same stimuli as the other enzymes and its activity would co-vary with those of other glucose metabolising enzymes. The regulatory influence of insulin or insulin dependent mechanisms or both would then be expected to play a role in the regulation of the expression of the trehalase gene, which has been localised on chromosome 14. The fact that the homozygous Ob/Ob and Db/Db strains show a much higher increase of liver trehalase than the alloxan treated mice (compared to their respective controls) is in favour of a genetically determined regulatory mechanism.

Although this mechanism is compatible with our results, it remains entirely speculative and other explanations can also be offered. It should be remembered also that several different biosynthetic pathways appear to be disturbed in the genetic diabetic mouse strains, for instance, the regulation of the relative rates of synthesis of type I and type III collagens.²³ These considerations, as well as the

sex and strain differences in serum and liver trehalase activity (Baumann and Labat-Robert, unpublished work, and tables 1 and 2), suggest complex regulatory mechanisms for trehalase activity, one of which appears to be closely related to those involved in the metabolic determination of diabetes.

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