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Sorbitol accumulation in rats kept on diabetic condition for short and prolonged periods[1]

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AIM: To study the influence of the course of diabetes, aging, and glycemia on the sorbitol accumulation in diabetic rats. **METHODS:** Streptozocin (Str) diabetic rats were obtained by Str iv (35 mg·kg^{-1}). Glycemia and sorbitol levels from sciatic nerve and lens were measured after 1 d, 2, 5, and 8 months of diabetes. Sorbitol concentrations in serum, heart, diaphragm, small intestine, and kidney after 8 months of diabetes were measured. **RESULTS:** Diabetic rats after Str injection showed hyperglycemia (>1.7 g·L^{-1}), hyperphagia, polyuria, polydipsia, and loss of body weight. Sorbitol levels in lens and sciatic nerve increased in normal and diabetic rats; the increase was higher in diabetic rats. No relationship was shown between glycemia and sorbitol levels. An increased sorbitol level after 8 months of diabetes was found in small intestine and kidney. **CONCLUSION:** The sorbitol levels increased in lens and sciatic nerve with aging and this process was accelerated by diabetes.

In insulin-independent tissues hyperglycemia increases the intracellular concentration of glucose and thus the net flux of glucose through the cell membrane. Because sorbitol does not easily diffuse across cell membranes, cell damage occurs where accumulated sorbitol levels are high. Clinically, sorbitol accumulation in cells contributes to pathological conditions in several tissues including lens, peripheral nerve, myocardium, and kidney. The streptozocin (Str) diabetic rat is the most commonly used animal model to demonstrate sorbitol accumulation[1-4]. A considerable body of animal data confirms a crucial role for the sorbitol pathway in the etiology of experimental neuropathy and cataract[5-8]. On the other hand, a survey of publications from 1959 dealing with sorbitol accumulation in sciatic nerve and lens from diabetic rats[1-3,7,9] showed a period of diabetes limited from 1 wk (the shortest period) to 24 wk (the most prolonged period).

In the present investigation this period of time was expanded in both directions by assessing the sorbitol accumulation in lens and sciatic nerve occurring both at later (32 wk) and a very early stage (d 1) of diabetes. In addition, the influence of glycemia and aging was investigated.

MATERIALS AND METHODS

Rats Albino ♂ Wistar rats weighing 287 ± *s* 4 g were used. Diabetes was induced with an iv injection of Str 35 mg·kg^{-1} dissolved in sodium citrate 0.05 mol·L^{-1}, pH 4.5, after an overnight fast. Control rats were injected with vehicle alone. Str rats were accompanied until 8 months and several parameters of diabetes were monitored until the day immediately before the experiments.

Experimental design On the day of the experiment, the rats were anesthetized with ip pentobarbital sodium 35 mg·kg^{-1}, and after laparotomy blood was collected from vena cava for determination of glycemia[10]. The tissues were transferred to liquid nitrogen. Sorbitol content[11] was estimated as NADH-induced fluorescence (λ = 340 nm) produced from sorbitol reacting with sorbitol dehydrogenase (SDH).

$$D\text{-sorbitol} + NAD^+ \xrightleftharpoons{SDH} D\text{-fructose} + NADH + H^+$$

Reagents NADH, NAD^+, SDH, and Str were purchased from Sigma, USA. All others reagents were of the highest purity obtainable.

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Statistical analysis Data were treated by t test or ANOVA using a computer program (Primer biostatistics: the program).

RESULTS

Str-treatment resulted in a diabetic syndrome. Hyperphagia, polydipsia, polyuria, and loss of body weight were observed (Tab 1).

Tab 1. Body weight (BW), daily food intake (DFI), daily water consumption (DWC), and daily urine elimination (DUE) in control (C) and diabetic (D) groups. $n = 9$ **rats,** $\bar{x} \pm s$. $^cP<0.01$ *vs* **control.**

	BW/g	DFI/ $g \cdot kg^{-1}$	DWC/ $mL \cdot kg^{-1}$	DUE/ $mL \cdot kg^{-1}$
2nd month				
C	414 ± 20	86 ± 13	96 ± 12	20 ± 9
D	290 ± 40^c	206 ± 28^c	593 ± 81^c	305 ± 76^c
5th month				
C	459 ± 19	74 ± 11	99 ± 18	17 ± 8
D	286 ± 43^c	217 ± 44^c	725 ± 127^c	363 ± 141^c
8th month				
C	456 ± 19	66 ± 9	95 ± 34	14 ± 7
D	282 ± 32^c	205 ± 24^c	742 ± 157^c	384 ± 132^c

Sorbitol levels in lens and sciatic nerve increased with age and this process was accelerated by diabetic condition (Tab 2).

Tab 2. Sorbitol concentration ($mg \cdot kg^{-1}$) in lens and sciatic nerve from diabetic (D) and control (C) groups. (n) = **number of rats,** $\bar{x} \pm s$. $^cP<0.01$ *vs* **control.**

		Lens	Sciatic nerve
d 1	C	11.0 ± 1.0(9)	3.0 ± 0.5(9)
	D	50 ± 16 (9)^c	26 ± 4 (9)^c
2nd month	C	23 ± 14 (3)	8 ± 4 (3)
	D	34 ± 22 (3)	28 ± 13 (3)
5th month	C	41 ± 15 (3)	18 ± 7 (4)
	D	228 ± 63 (3)^c	34 ± 12 (3)
8th month	C	81 ± 6 (5)	23 ± 7 (3)
	D	597 ± 244 (5)^c	127 ± 31 (6)^c

However, sorbitol levels showed no correlation with glycemia in both tissues, in different periods of diabetes (Fig 1).

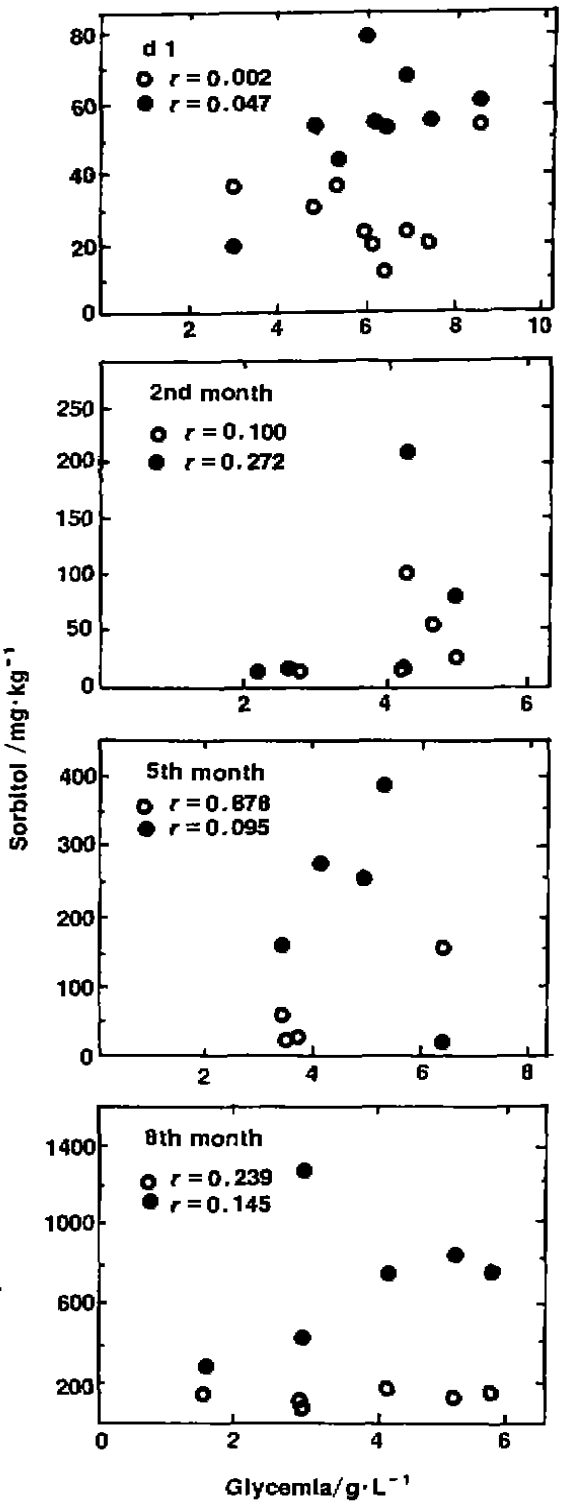

Fig 1. Relationship between sorbitol concentration and glycemia in streptozocin-diabetic rats with different periods of diabetes. (○) **Sciatic nerves;** (●) **Lens.**

The levels of sorbitol after 8 months of diabetes were increased in small intestine and kidney, but basically the same as in serum, heart, and diaphragm (Tab 3).

Tab 3. Sorbitol concentration (mg/kg fresh wt) in tissues from diabetic and control groups. (n) = number of rats, $\bar{x} \pm s$. $^bP<0.05$, $^cP<0.01$ *vs* control.

	Control	Diabetic
Serum	0.30±0.10 (4)	0.30±0.10 (3)
Heart	14.5±1.6 (5)	20.2±5.7 (5)
Diaphragm	5.1±2.5 (5)	3.6±1.6 (4)
Small intestine	4.2±2.7 (7)	10.5±6.9^b (7)
Kidney	40.9±2.4 (9)	56±10^c (8)

DISCUSSION

We demonstrate sorbitol accumulation in lens and sciatic nerve *in vivo* 24 h after Str injection (Tab 2). This time phase represents the earliest accumulation of sorbitol after the confirmation of diabetes. Our results supported the view that sorbitol accumulation was largely the consequence of aging. The acceleration of sorbitol accumulation in diabetic rats by aging (Tab 2) is understandable since aldose reductase (AR) activity increased with aging[12] and diabetes[13]. Although AR plays an important role in the sorbitol accumulation[4-6], cytosolic NADH/NAD$^+$ ratio was increased during diabetes[14]. Because SDH catalyses the oxidation of sorbitol to fructose, with the concomitant reduction of NAD$^+$, an increased cytosolic NADH/NAD$^+$ ratio is not favorable for this reaction. This biochemical mechanism could explain part of the acute sorbitol accumulation in lens and sciatic nerve described in this study (Tab 2). However, additional mechanisms must be involved. For example, although livers from diabetic rats showed increased NADH/NAD$^+$ ratio (unpublished results) and loss of regulation by sympathetic nerves[15], the liver did not accumulate sorbitol.

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309-311

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山梨醇在糖尿病大鼠体内的短期和长期积聚状态

关键词 链佐星; 糖尿病; 山梨醇; 晶体; 坐骨神经; 高血糖; 衰老

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