Biorhythms of Activities of Liver and Blood Dehydrogenases and Changes in Body Weight of the Rats Feeding Normal Diet or Excess of Sugar Substitutes

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Biorhythms with higher levels of activity of sorbitol dehydrogenase and lactate dehydrogenase in blood plasma, specific activity of sorbitol dehydrogenase, lactate dehydrogenase, and malate dehydrogenase in the liver, and body weight of rats were more pronounced in the spring-summer period than in the autumn-winter period. These specific features were revealed in animals feeding a normal diet or food with 54 and 27% sugar substitute sorbitol. However, specific activity of glucose-6-phosphate dehydrogenase in the liver was higher in the autumn-winter period. Activity of sorbitol dehydrogenase in blood plasma increased by tens of times due to induction of sorbitol synthesis (substrate) in the liver. Sugar substitute xylitol is structurally similar to sorbitol, but is not the substrate for sorbitol dehydrogenase. However, the effect of xylitol on activities of lactate dehydrogenase, malate dehydrogenase, and glucose-6-phosphate dehydrogenase in the spring-summer period was similar to that of sorbitol.

Key Words: *dehydrogenases; sorbitol; xylitol; sucrose*

Little is known about regulation of carbohydrate metabolism under conditions of excessive consumption of sugar substitutes for prevention and therapy of obesity, diabetes mellitus, caries, and other diseases. It remains unclear which molecular mechanisms mediate the action of sugar substitutes in healthy people during excessive consumption to reduce body weight. There are no data on biorhythms of dehydrogenase activity in the liver, which plays a direct or indirect role in carbohydrate metabolism. Previous studies showed that circadian and seasonal variations in enzyme activity reflect adaptation to environmental conditions [3,5].

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Here we studied the effect of excessive dietary intake of sugar substitutes (sorbitol and xylitol) on activity in liver and plasma dehydrogenases directly or indirectly involved into carbohydrate metabolism. These data will elucidate the mechanism of the negative effect of seasonal fluctuations under conditions of excessive dietary intake of sugar substitutes in the absence or presence of excess sucrose.

MATERIALS AND METHODS

The rats (n=81) aging 1 month were divided into 6 groups. Group 1 animals (n=18, control) fed standard diet over 1 month. The rats of groups 2-6 (n=10-12) received standard diet (46%) and sugar substitutes (54%): group 2, sucrose; group 3, sorbitol; group 4, xylitol; group 5, sorbitol and sucrose (equal

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TABLE 1. Body Weight of Rats after Consumption of Various Diets in Different Seasons (g, *M*±*m*)

Group	Autumn	Winter	Spring	Summer
1	168.5±12.0	166.4±8.2	229.0±10.2	234.0±20.8
2	150.3±7.9	145.0±7.1*	158.3±6.4**	252.0±26.5
3	113.2±10.7**	103.6±5.5***	146.4±9.0***	162.0±10.4**
4	—	—	153.3±9.4***	147.8±3.9**
5	123.8±10.3	134.7±10.2*	185.5±9.4*	181.0±10.9
6	—	—	160.0±6.5***	177.0±8.5*

Note. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ compared to standard diet.

parts); and group 6, xylitol and sucrose (equal parts). The animals had free access to water.

The rats were weighted and killed under barbiturate anesthesia. The liver was isolated. Blood plasma was obtained. Activity of enzymes in blood plasma (per 1 liter) and specific activity of enzymes in the liver (per 1 mg protein) was measured spectrophotometrically (λ=366 nm) using the following substrates: pyruvate for lactate dehydrogenase (LDH, by oxidation of $NADH+H^+$ to NAD^+); glucose-6-phosphate for glucose-6-phosphate dehydrogenase (G-6-PDH, by reduction of $NADP^+$ to $NADPH+H^+$); fructose for sorbitol dehydrogenase (SODH, by reduction of $NADP^+$ to $NADPH+H^+$); and malate dehydrogenase by oxidation of $NADH+H^+$ to NAD^+ in coupled reactions catalyzed by aspartate transaminase and malate dehydrogenase. Protein concentration was measured by the method of Lowry.

The results were analyzed by Student's *t* test.

RESULTS

The appearance and behavior of animals significantly differed after consumption of various feeds. As differentiated from clean and well-fed animals of groups 1 and 2, group 3-6 rats were characterized by agitation and body weight loss. These rats had dirty, wet, and bound hair and watery stool.

Body weight loss was most pronounced in rats receiving 54% sugar substitutes, but less significant in animals feeding sucrose. Body weight tended to increase in summer (compared to the control). The rats of groups 5 and 6 were intermediate in body weight between group 2-4 animals. Body weight of rats receiving standard diet or feeding sorbitol and sucrose was much lower in autumn and winter. These data allowed us to combine 4 values of body weight in the spring-summer and autumn-winter period (Table 2).

Enzyme assays were also combined in two half-years. Feeding 54% sorbitol was followed by an increase in plasma SODH activity in the autumn-winter and spring-summer periods (by 33 and 18 times, respectively, compared to standard diet; Fig. 1). Enzyme activity in group 5 rats increased by 4 and 3 times, respectively. However, enzyme activity remained practically unchanged in animals of groups 2, 4, and 6. Specific activity of SODH in the liver of group 3 rats increased by 4 times during each half-year. This parameter in group 5 rats increased by 30 and 50%, respectively. The diet with 54% sucrose had little effect on specific activity of SODH. Plasma SODH activity in rats receiving various diets did not differ in half-year periods.

Plasma LDH activity in rats feeding 54% sorbitol increased by 3 and 1.3 times during the spring-summer and autumn-winter periods, respectively. These changes were less significant than the increase in SODH activity (Fig. 2). The dietary regimen of group 5 rats had little effect on LDH activity in the autumn-winter period. However, LDH activity in these animals increased by 2 times during the spring-summer period. Dietary intake of 54% sucrose was followed by an increase in plasma LDH activity during the autumn-winter and spring-summer periods (by 1.5 and 2.5 times, respectively). As differentiated from specific activity of SODH, specific activity of LDH in the liver of group

TABLE 2. Body Weight of Rats after Consumption of Various Diets in Different Half-Year Periods (g, *M*±*m*)

Group	Autumn-winter half-year	Spring-summer half-year
1	167.5±7.3	231.5±14.1^{++}
2	147.6±5.0	205.1±17.4^{++}
3	108.4±6.4	154.2±7.6^{+}
4	—	150.6±6.5
5	129.3±7.8	183.3±8.0^{+++}
6	—	168.5±7.0

Note. $^{+}p<0.05$, $^{++}p<0.01$, and $^{+++}p<0.001$ compared to the autumn-winter period.

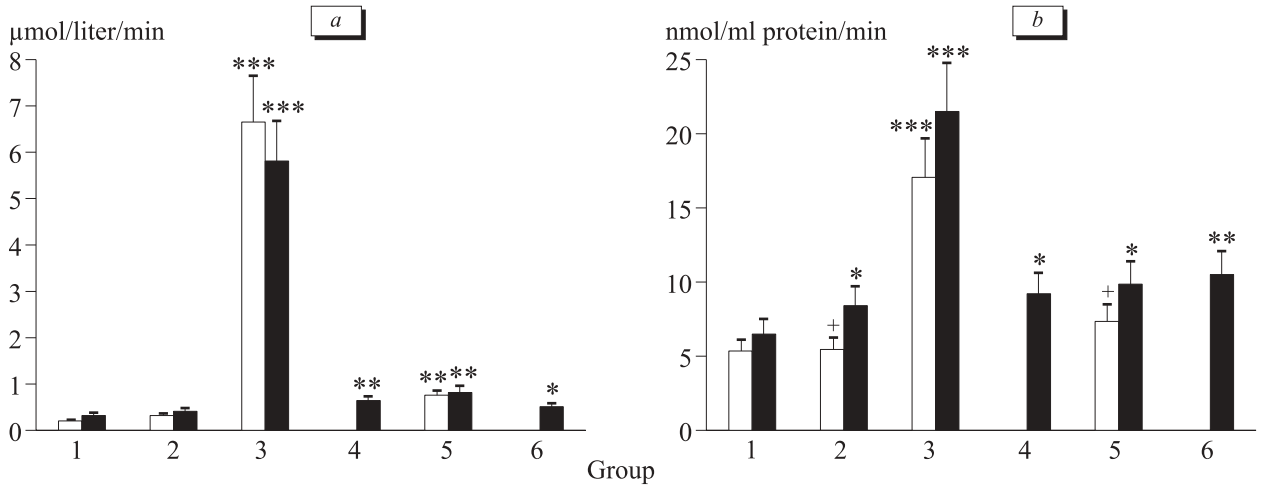

Fig. 1. Specific activity of SODH in blood plasma (*a*) and liver (*b*) of rats under various dietary regimens. Here and in Figs. 2 and 3: light bars, autumn-winter half-year; dark bars, spring-summer half-year. $^{*}p<0.05$, $^{**}p<0.01$, and $^{***}p<0.001$ compared to the control; $^{+}p<0.05$, $^{++}p<0.01$, and $^{+++}p<0.001$ compared to the spring-summer half-year.

2-4 rats did not significantly increase during the autumn-winter period. However, specific activity of LDH in rats of various dietary groups was 2-3 times higher in the spring-summer period than in the autumn-winter period.

Specific activity of malate dehydrogenase in the liver of group 2 and 5 rats during the autumn-winter period was much higher compared to animals feeding standard diet. However, enzyme activity after dietary intake of sucrose or xylitol during the spring-summer period did not differ from the control (Fig. 3).

During the autumn-winter period, specific activity of G-6-PDH in the liver of group 2, 3, and 5 rats increased by 2-3 times compared to animals feeding a standard diet (Fig. 3). During the spring-summer period, enzyme activity in control rats did not differ from that in animals of dietary groups 2 and 5. Specific activity of G-6-PDH in the liver significantly decreased in group 3 rats, but increased in group 4 animals (by 1.5-2 times).

Specific activity of SODH, LDH, and malate dehydrogenase in the liver of various dietary groups was higher during the spring-summer period. By contrast, specific activity of G-6-PDH was lower in this period.

The mechanism of significant increase in SODH activity was evaluated during excessive dietary intake of sorbitol. We studied the role of photoperiodism and temperature conditions in seasonal biorhythms of liver dehydrogenase activity and body weight of rats.

There are published data on seasonal variations in activity of liver enzymes catalyzing the key stages of metabolism in mammals: tyrosine hydroxylase and dopamine-β hydroxylase, synthesis of norepi-

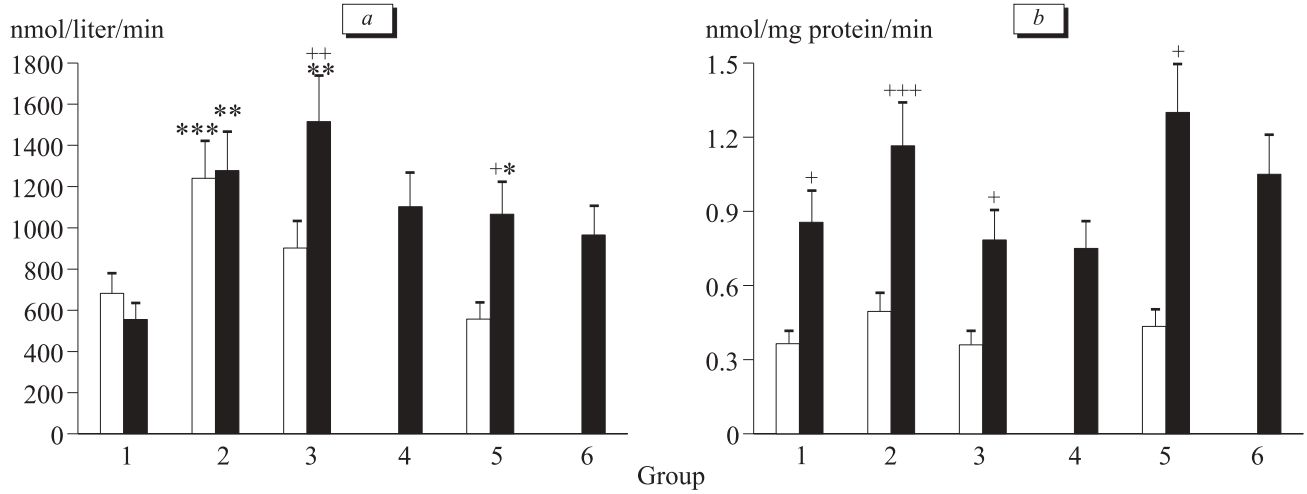

Fig. 2. Specific activity of LDH in blood plasma (*a*) and liver (*b*) of rats under various dietary regimens.

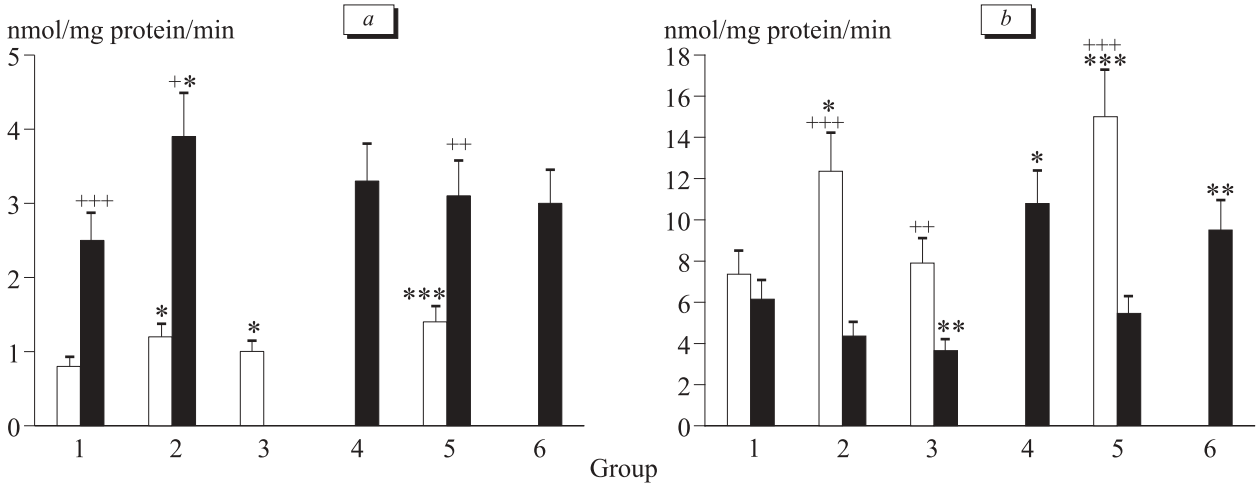

Fig. 3. Specific activity of malate dehydrogenase (*a*) and G-6-PDH (*b*) in the liver of rats under various dietary regimens.

nephrine and dopamine [11]; nNO synthase, formation of NO from arginine [12]; NADPH diaphorase, NO marker in neurons [13]; $25(OH)D_3$ hydroxylase, synthesis of active form of vitamin D_3 (calcitriol, $1,25(OH)_2D_3$) [7]; antioxidant enzymes [6]; and aminoacyl-tRNA synthase [14]. Similar results were obtained for plasma LDH [3] and other enzymes of the blood.

The hypothalamic suprachiasmatic nuclei are a central pacemaker of biorhythms in mammals. These structures receive the light and dark signals through retinal receptors and retinohypothalamic tract [1,2]. Pinealocytes synthesize neurohormone melatonin (N-acetyl-5-methoxytryptamine) from serotonin under dark conditions. Melatonin is not deposited, but secreted from these cells. Melatonin regulates circadian and seasonal biorhythms in organs and tissues due to seasonal differences in the length of darkness [2,4,10]. In addition to the hypothalamic suprachiasmatic nuclei, pinealocytes constitute another part of the general system for regulation of biorhythms. The sympathetic nerves are responsible for connection of these nuclei with pinealocytes. The neurotransmitter norepinephrine stimulates melatonin secretion through β_1-receptors on pinealocytes. Light exposure inhibits melatonin synthesis. There are 6 genes for the regulation of circadian rhythms. A pathogenetic relationship was revealed between variations in melatonin secretion and development of internal diseases [8].

Variations in environmental temperature also serve as a serious chronobiological factor [3]. Temperature in the vivarium during winter was lower than during summer.

Specific activity of SODH in the liver after dietary intake of 27% sorbitol was 2-fold higher compared to 10% sorbitol. Moreover, specific activity of SODH in the liver after dietary intake of 54% sorbitol was 4-fold higher compared to 27% sorbitol. Hence, enzyme induction with the substrate is a possible mechanism for the increase in SODH activity in rats after excessive dietary intake of sorbitol.

Among two similar sugar alcohols, only sorbitol served as a SODH-inducing agent. Specific activity of SODH increased to a lesser degree under the influence of xylitol (compared to sorbitol). The increase in enzyme activity at 10% xylitol was more significant than at 27% xylitol. Moreover, the increase in enzyme activity at 27% xylitol was more significant than at 54% xylitol. However, sorbitol and xylitol had the same effect on body weight and specific activity of LDH (Tables 1 and 2, Fig. 2).

Our results show that a sharp increase in SODH activity under the influence of sorbitol can be related to enzyme induction. These changes probably play an adaptive and protective role, since excess sorbitol causes urolithiasis, diarrhea, damage to the lens and retina [9], and other disorders. Half-year biorhythms in activities of SODH, glycolytic enzyme LDH, tricarboxylic acid cycle (malate dehydrogenase), and pentose phosphate pathway of liver G-6-PDH reflect adaptation to seasonal variations.

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