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Short Communication

POLYOL AND WATER ACCUMULATION IN MUSCLE OF GALACTOSE-FED RATS

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Abstract—Skeletal muscle contains high levels of aldose reductase that catalyzes the reduction of galactose to the polyol galactitol. Galactitol and water were measured in muscle of rats fed a high galactose diet with or without addition of the aldose reductase inhibitor sorbinil. Galactitol, measured in isolated samples of muscle by HPLC, reached steady-state levels (5.9 ± 1.0 mg/g tissue) within 3 days. Muscle water, determined *in vivo* by magnetic resonance imaging, increased (51 ± 5%, $P < 0.02$) to steady-state levels within 7 days. Both the increased galactitol and water remained constant for the 4-month duration of this study. Aldose reductase activity also remained constant. Sorbinil prevented both the increase in galactitol and the increase in water. These results suggest that the increase in water is due to the osmotic effects of galactitol accumulation and demonstrate that galactitol and water accumulation neither up-regulate nor down-regulate aldose reductase expression in skeletal muscle.

Key words: aldose reductase; galactitol; water; MRI

The polyol pathway, a two-enzyme pathway that utilizes aldose reductase and sorbitol dehydrogenase to convert glucose to fructose by way of the intermediate sugar alcohol sorbitol, has been implicated in the development of a wide range of diabetic complications [1, 2]. Galactose is also a substrate for aldose reductase in the first reaction of the polyol pathway. However, the product galactitol is a poor substrate for sorbitol dehydrogenase. Galactitol can accumulate because it does not readily diffuse through cellular membranes. The galactose-fed rat has been widely used as a model for studies of the role of the polyol pathway in the development of diabetic complications, especially with respect to complications of the eye [3–7]. It has been observed that galactose feeding results in up-regulation of aldose reductase mRNA levels in lens epithelial cells [8, 9].

In rat kidney, sorbitol production appears to be a normal part of the osmoregulatory mechanisms of the medulla [10, 11]. The galactose-fed rat has been used to study the effects of high polyol levels on regulation of expression of aldose reductase in rat kidney. In this case, galactitol accumulation results in down-regulation of mRNA for aldose reductase [12].

Most aldose reductase in humans and animals is present in skeletal muscle due both to the high intrinsic activity in this tissue and to the large size of this tissue compartment. In the present study, we have addressed the question of the rates of accumulation of galactitol and water in skeletal muscle and the effects of this accumulation on expression of aldose reductase.

Materials and Methods

Animal diets and treatment. Male Sprague–Dawley rats, 150–200 g, were fed one of three diets: (1) rat chow (controls), N = 10; (2) rat chow supplemented with 30% galactose, N = 10; or (3) rat chow supplemented with 30% galactose and 0.7% sorbinil (Pfizer), N = 5. Prior to imaging, the animals were anesthetized with an i.p. injection of Inactin (BYK), 10 mg/100 g body weight. For determinations of galactitol and aldose reductase, animals were killed, and samples of skeletal muscle were taken, weighed and frozen at −70° until used.

Magnetic resonance imaging (MRI). MRI experiments were conducted on a General Electric 1.5 Tesla Signa system. The method of Dixon was used to quantify proton signal from water or fat by subtraction or addition of the fat in-phase or fat out-of-phase images, using an internal standard of copper sulfate [13, 14]. Partial saturation pulse sequences were employed with TE = 25 msec and TR = 2000 msec, respectively. The image resolution was set to 0.4 mm × 0.4 mm × 3 mm with a 12-cm field of view and a 256 × 256 matrix. A general Electric 17-cm diameter Helmholtz-type extremity coil was employed in all studies. Anesthetized rats from each diet group were imaged side-by-side to provide internal reproducibility on the same axial slice. If one of the rats was slightly out of the plane of the axial slice, a comparable inferior or superior axial slice was selected to provide an equivalent anatomical area. Quantification of image intensity was determined digitally using the software provided with the Signa system and was related to a standardized water phantom containing 0.01 N copper sulfate. Eight volumes of interest composed of 9 pixels each were selected in skeletal muscle from each animal, intensity measurements were taken, and the eight values were averaged. Hydration levels of muscle were expressed as a fraction of the proton intensity of the copper sulfate standard. Statistical analysis was performed with a paired Student's *t*-test. Three sets of three rats (control, galactose-fed and galactose/sorbinil-fed) were studied repeatedly over the 120-day experiment.

Galactitol determinations. Skeletal muscle samples (50 mg) were homogenized in 2 mL cold water containing

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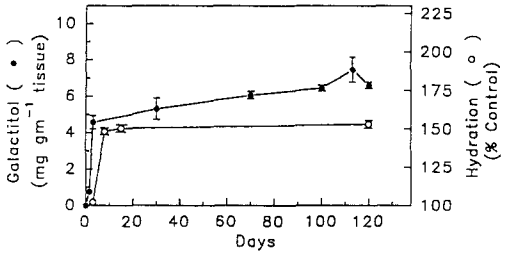

Fig. 1. Rates of galactitol and water accumulation in skeletal muscle of galactose-fed rats. Error bars indicate standard deviations from triplicate determinations.

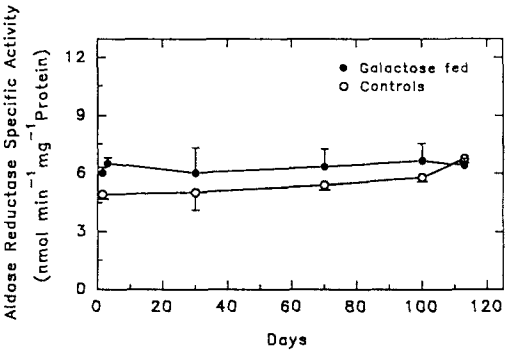

Fig. 2. Aldose reductase activity in skeletal muscle of galactose-fed rats. Error bars indicate standard deviations from triplicate determinations.

sorbitol as an internal standard. Proteins were precipitated with 70% ethanol, and the supernatant was dried under vacuum. Polyols were derivatized by addition of 0.2 mL phenylisocyanate (Kodak) and 0.1 mL pyridine [15]. Derivatized polyols were quantified by HPLC using a Spectra Physics SP 8700 system with a 25-cm Nucleosil 5, C_{18} MN reversed phase column (Macherey–Nagel). Standard curves for sorbitol and galactitol were colinear.

Aldose reductase activity. Samples of skeletal muscle (50 mg) were homogenized in 0.25 mL sodium phosphate buffer, 0.1 M, pH 7.0, and were centrifuged for 15 min at 12,000 *g*. The supernatant fraction was analyzed for aldose reductase activity using the same buffer containing 10 mM glyceraldehyde and 0.1 mM NADPH by following the decrease in absorbance at 340 nm [16]. Proteins were determined by the dye binding method of Bradford. Specific activities are expressed in nanomoles per minute per milligram protein from triplicate assays. Specific activities were determined at pH 7 rather than at lower pH values close to the pH optimum due to increased background rates of oxidation of NADPH at lower pH. Individual points were reproducible ±5% at pH 7.

Results

Rates of galactitol accumulation. In animals fed the galactose-supplemented diet, galactitol levels in skeletal muscle increased rapidly to an apparent steady state by day 3, as shown in Fig. 1. There was no significant change after day 3. Galactitol concentrations remained at 5.9 ± 1.0 mg/g tissue between day 3 and day 120. No galactitol was detected in animals fed the control diet.

Effects of sorbinil on galactitol accumulation. In animals fed the high galactose diet supplemented with sorbinil, the presence of sorbinil completely inhibited the accumulation of galactitol at day 1.5 and markedly inhibited galactitol accumulation during prolonged feeding. Animals on this diet for 120 days showed a galactitol level of 0.62 ± 0.07 mg/g tissue, representing a 90% reduction in the steady-state level of galactitol. Animals fed the high galactose diet developed cataracts after 6 weeks, while those on the sorbinil-supplemented diet did not develop cataracts.

Rates of water accumulation. Proton intensity measurements were taken in 4 areas each of paraspinus and quadraceps muscle and averaged. The hydration levels in control rats were constant at 0.44 of the internal standard during the 120-day study. Animals fed the high galactose diet exhibited increased muscle water that reached apparent steady state by day 7 (Fig. 1) and remained at 150–168% of control ($P < 0.02$) between day 7 and day 120. Thus, the increase in water parallels the increase in galactitol but reaches steady state 2–3 days later.

Effects of sorbinil on water accumulation. Animals fed the high galactose diet supplemented with sorbinil exhibited water levels that were 105–110% of control after day 7, an increase that was not statistically significant ($P > 0.05$). Thus, accumulation of both galactitol and water is inhibited markedly by sorbinil.

Effects of galactose feeding on aldose reductase levels. Aldose reductase activities in muscle from animals on the high galactose diet were not significantly different from those of control animals during the entire 120-day period of this study, as shown in Fig. 2. Specific activities remained at 6.0 ± 0.7 nmol/min/mg protein with no indication that aldose reductase was up-regulated or down-regulated in response to elevated water or elevated galactitol.

Discussion

The rapid accumulation of galactitol in skeletal muscle of galactose-fed rats to steady-state levels within 3 days demonstrates the usefulness of this animal model for studies of the role of the polyol pathway in normal and abnormal physiology. Steady-state levels of 5.9 ± 1.0 mg galactitol/g tissue represent greater than 50 mM concentrations of this polyol in skeletal muscle, assuming 50% muscle water content. This concentration of galactitol would be expected to produce an osmotic effect if there were no compensatory mechanisms to counteract this osmolyte. The fact that water accumulated in skeletal muscle, reaching steady state later than galactitol, and the fact that both galactitol and water remained at elevated levels during fairly long-term feeding (4 months) are consistent with the conclusion that water accumulates as a result of the osmotic effects of galactitol and with the conclusion that skeletal muscle does not appear to compensate for this osmotic effect.

The lack of up-regulation or down-regulation of aldose reductase at the enzyme level is also consistent with the conclusion that skeletal muscle does not compensate for the osmotic effects of galactitol nor for the presence of high levels of galactose by altering the expression of aldose reductase. This is in contrast to other tissues. Increased levels of aldose reductase have been detected immunohistochemically in the lens of diabetic and galactose-fed rats, where enzyme activity also appears to increase [17, 18]. Up-regulation of the gene for aldose reductase in the lens of galactose-fed rats has been easier to demonstrate at the mRNA level where significant increases were observed within 12–15 days in rats on galactose diets [8, 9]. Likewise, down-regulation of aldose reductase in rat medulla of galactose-fed rats after 10 days of feeding was demonstrated at the mRNA level [12]. Clearly, aldose reductase levels respond to galactose feeding and/or galactitol and water accumulation differently in muscle compared with lens epithelium [8, 9] and kidney [12], suggesting that regulation

of the gene for aldose reductase is complex and tissue specific.

Monitoring the accumulation of water or galactitol in muscle of galactose-fed rats may also provide a convenient and rapid method to assess the bioavailability of potential aldose reductase inhibitors.

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