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Fructose metabolizing enzymes from mouse liver: influence of age and caloric restriction

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Abstract

The influence of caloric restriction (CR) on the activities of liver fructose metabolizing enzymes and metabolite levels were studied in young (3 months) and old (30 months) mice. Fructokinase activity was increased ($P<0.05$) in both young and old CR mice when compared to controls while triokinase activity was increased ($P<0.05$) only in old CR versus control mice. Aldolase was not altered by CR in either old or young mice. No age-related differences in activities were observed in controls although a trend towards an increase was observed for triokinase, while significant age-related increases were observed for fructokinase and triokinase, but not aldolase, in CR mice. Both young and old mice on CR showed significant decreases in fructose and fructose-1-phosphate, however, no age-related changes in metabolite levels were observed for either control or CR mice. A fructose-1-phosphate kinase activity was also measured and found to be unchanged in both young and old mice on CR, but the activity was significantly lower in the old mice compared with young. We show here that the enzymes involved in fructose metabolism are influenced by CR and that this could contribute to alterations in gluconeogenesis and glycolysis observed with CR.

Keywords: Aging; Aldolase; Calorie restriction; Fructokinase; Fructose-1-phosphate; Triokinase

1. Introduction

Caloric restriction (CR), without malnutrition, is the only environmental intervention that delays age-associated pathophysiological changes in laboratory rodents and has been shown to extend maximum life span in a variety of species [1,2]. The association of increased longevity and lowered age-related diseases with CR has been known for almost 70 years [3], and subsequent studies in laboratory rodents have shown that a 30–60% decrease in food intake results in a similar percentage increase in average and maximum life span [2]. Reduction in the overall caloric intake and avoidance of malnutrition, rather than limiting any specific nutrient, is critical in achieving the life-extending benefits of CR [2].

Fructose is naturally present at high levels in fruits, honey and also in some vegetables, and is considered to be the sweetest of all simple sugars. The overall consumption of fructose by the human population has increased significantly because of its use as a sweetener in the food industry in the form of high-fructose corn syrup. Similarly, fructose is a major component of the semi-purified diets frequently used for CR and aging studies in rodents [4], thus fructose metabolism is a significant site of energy flux in many CR studies. Dietary fructose is present either as a monosaccharide or combined with other sugars (e.g., glucose to give sucrose) and its metabolism has been

Abbreviations: CR, caloric restriction; F-1-P, fructose-1-phosphate; F-1,6-BP, fructose-1,6-bisphosphate; DHAP, dihydroxyacetone phosphate; GAP, glyceraldehyde-3-phosphate; 1-PFK, 1-phosphofructokinase; FK, fructokinase; TK, triokinase; PFK-1, phosphofructokinase-1

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previously reviewed [5–8]. The liver contains an active enzyme system for fructose metabolism, therefore, displaying a great capacity for the sugar's metabolism [7]. Hepatic hexokinase phosphorylates most hexoses including fructose, but at physiological levels of glucose the fructose phosphorylation is inhibited, although in muscle and adipose tissue this is how fructose enters metabolic pathways [7]. In the liver, the more important pathway for fructose to enter metabolism is the one described by Hers [9], where the enzymes fructokinase, aldolase and triokinase metabolize fructose. The activities of the three enzymes have been reported to decrease dramatically after 48–72 h of fasting and were recovered within 24 h of fructose feeding [10]. The effects of feeding fructose and/or sucrose on the activities of fructose metabolizing enzymes have been described [7,11,12]; however, to our knowledge there are no studies concerning the effects of CR on these enzymes. Carbohydrates are the major energy component of common rodent diets used for CR and aging studies, and the carbohydrate metabolizing pathways are thus the major sites of the energy flux with these diets.

A reduction in energy metabolism has long been considered a potential mechanism for life span extension with CR. To better understand the effects of CR on metabolism, we determined the activities of enzymes involved in the major energy metabolism pathways in CR and control mice. Previously we have reported that CR decreased glycolysis [13], increased gluconeogenesis and transamination [14] and differentially regulated Krebs cycle components [15]; however, there are no other studies on complete pathways that show how CR influences metabolism and what role, if any, it plays in the utilization of available and alternative energy sources. In the current study, we have expanded our previous work characterizing changes in glycolysis and gluconeogenesis by investigating the influences of age and CR on the activities of the hepatic enzymes and metabolites involved in fructose metabolism.

2. Materials and methods

2.1. Chemicals

All chemicals were purchased from Sigma Chemical Company (St. Louis, MO) or Roche diagnostics corporation (Indianapolis, IN). Auxiliary enzymes and coenzymes used in the assays were purchased from Roche Diagnostic Corporation (Indianapolis, IN). Slide-A-Lyzer mini dialysis units (10,000 MWCO), for the removal of ammonium sulfate from the auxiliary enzymes, were from Pierce (Rockford, IL).

2.2. Animals

Male C57BL/6J mice were purchased from Charles River Laboratories (Wilmington, MA) at 1 month of age, housed singly in accordance with the Institutional and Federal Guidelines for Ethical Animal Experimentation and were kept on a 12-h light/12-h dark cycle. The mice were fed ad libitum a non-purified diet, PLI 5001 (Purina Laboratories, St. Louis, MO), for 1 month and at 2 months of age they were assigned either to the control or CR group and fed semi-purified diets, as described elsewhere [4]. At the time of sacrifice, young animals were 3 months of age (1 month on either the control or CR diet) and old animals were 30 months of age (28 months on either the control or CR diet). The daily caloric intake per animal was 12 kcal for the controls and 9 kcal for the CR mice. The macronutrient compositions were 20.9% protein, 50.6% carbohydrates, and 28.5% fat (expressed as a percentage of metabolizable energy) for the control diet and 26.6% protein, 44.6% carbohydrates, and 28.5% fat (expressed as metabolizable energy) for the CR diet.

2.3. Tissue harvesting and preparation

All mice were fasted overnight and their livers harvested between 9 and 10 AM the next day, as described previously [15]. The livers were rapidly freeze-clamped in situ, placed immediately in liquid nitrogen, and powdered under liquid nitrogen in a mortar and pestle and the powders stored in liquid nitrogen.

2.4. Measurement of enzyme activities

Frozen powders were removed from liquid nitrogen as rapidly as possible and placed in an ice-cold glass homogenizer, weighed and homogenized at a 1:10 ratio (w/v) and the supernatants kept for assays. Fructokinase (EC 2.7.1.3), also called ketohexokinase, aldolase (EC 4.1.2.13) and triokinase (EC 2.7.1.28) were assayed according to previously published methods [16–18, respectively]. Also assayed was fructose-1 kinase activity, using the PFK-1 method as described previously [13], in the presence of F-1-P as substrate. All assays were performed at 30 °C, except for triokinase which was at 25 °C, using a Perkin Elmer Lambda 25 UV/VIS spectrophotometer set at 340 nm and equipped with a Peltier heating control and nine-cell changer. Control assays were also performed in parallel for all enzymes and the resulting rates subtracted from those of assays. Enzyme activities were expressed as μmol/min/mg protein using an extinction coefficient of 6.22 mM^{-1} cm^{-1}.

2.5. Measurement of metabolite concentrations

Frozen powders were weighed and homogenized in ice-cold perchloric acid (6%, w/v) using motor-driven, glass-Teflon homogenizers kept on ice during the homogenization. The homogenates were centrifuged and supernatants removed and further treated for the measurement of fructose [19] and fructose-1-phosphate [20]. Rat liver aldolase, needed for the determination of fructose-1-phosphate, was