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RIBOFLAVIN INCREASES SERUM GLUTAMINE LEVELS IN RATS WITH STREPTOZOTOCIN-INDUCED DIABETES

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Abstract

Diabetes mellitus is one of the most common chronic diseases in the world. Metabolomic studies have demonstrated altered blood levels of glutamate and glutamine during type 1 diabetes and type 2 diabetes. Riboflavin is a precursor of flavin adenine dinucleotide and flavin mononucleotide, which are coenzymes necessary for the function of enzymes involved in various biochemical reactions, including those affecting amino acid metabolism. In this study, we investigated the effects of riboflavin on serum glutamate and glutamine levels in rats with streptozotocin-induced type 1 diabetes. Diabetic rats received riboflavin (25 mg, 50 mg, or 100 mg) dissolved in the drinking water daily for 2 weeks. Our results showed that riboflavin supplementation did not affect serum glutamate levels but increased serum glutamine levels in diabetic rats. We speculate that increased serum glutamine levels resulting from riboflavin supplementation may have beneficial effects during diabetes.

Key words: diabetes, riboflavin, glutamate, glutamine

Introduction. Diabetes mellitus (DM) is one of the most common chronic diseases in the world, characterized by hyperglycemia resulting from progressive loss of pancreatic β cell mass and/or function [1]. Previous studies have shown

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that type 1 diabetes (T1D) and type 2 diabetes (T2D) can affect the metabolism of glutamate and glutamine. For example, brain glutamate levels are elevated in people with T1D and correlate with glycemic control and age of disease diagnosis [2]. Streptozotocin-induced T1D reduces glutamate oxidation and glutamine synthesis in the retina of rats [3]. Metabolomic studies have shown various blood metabolites to be associated with disease pathogenesis in both T1D and T2D [1]. It has been found that high plasma glutamate and low glutamine-to-glutamate ratio are associated with T2D [4]. Also, low plasma levels of glutamine were reported in patients with T1D [5] and rats with streptozotocin-induced T1D [6]. It has been suggested that hyperglutamatemia, possibly generated by α-cell dysfunction, platelet activation, and/or abnormal glutamate metabolism in the liver and muscle, may play an important role in the progressive β-cell loss occurring in T1D and T2D [7]. High levels of glutamate may contribute to the development of DM by excessive activation of NMDA receptors in β-cells, acceleration of β-cell dysfunction, and apoptosis induced by hyperglycemia [8].

Riboflavin, also known as vitamin B2, plays an essential role in a wide range of enzyme functions, including: mitochondrial bioenergetics, redox status regulation, basic protein disulphide maturation, neurotransmitter catabolism, cellular methylation, amine catabolism, and DNA replication [9]. Riboflavin was previously demonstrated to inhibit glutamate release from rat cerebrocortical nerve terminals, thereby exerting a neuroprotective role [10]. In the present study, we investigated the effects of riboflavin on serum levels of glutamate and glutamine in rats with streptozotocin-induced T1D.

Material and methods. ***Experimental animals.*** Male Wistar rats, (2 months of age, body mass range 190 ± 15 g) were used in the experiment. They had ad libitum access to standard chow pellets and water. Animals were housed in a temperature-controlled room (20–22 °C) on a 12:12-h light-dark cycle (07:00 to 19:00 h). The experiment was conducted in accordance with the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Drugs. Riboflavin (#10438870) was purchased from Thermo Scientific Chemicals.

Experimental protocol. T1D was induced by a single intraperitoneal (i.p.) injection of streptozotocin (50 mg/kg, dissolved in citrate buffer, pH 4.5) in fasted rats. At the fifth week after the injection of streptozotocin, the rats were characterized by typical manifestations of T1D. They showed polydipsia, polyuria, polyphagia, loss of fur, and difficulty in gaining body mass. Their average body mass at week five was 220 ± 30 g. During the fifth week, diabetic rats received daily riboflavin (25, 50, or 100 mg) dissolved in 600 ml of drinking water for two weeks. The animals were divided into four groups ($n = 10$ animals per group): the diabetic group (Diabetic), the diabetic group receiving 25 mg riboflavin every day (Diabetic + Rf 25 mg), the diabetic group receiving 50 mg riboflavin every day

(Diabetic + Rf 50 mg), and the diabetic group receiving 100 mg riboflavin every day (Diabetic + Rf 100 mg). After these two weeks, animals were anesthetized with the combination of xylazine (10 mg/kg, i.p.) and ketamine (80 mg/kg, i.p.) and blood was collected by cardiac puncture.

Measurement of serum glutamine and glutamate. Blood serum was separated by centrifugation and samples stored at $-20\,^{\circ}\mathrm{C}$ until further use. Measurement of serum glutamate and glutamine levels was performed using the Glutamine and Glutamate Determination Kit (#GLN1, Sigma-Aldrich, Germany) as per the manufacturer's instructions. This kit is designed for the spectrophotometric measurement of L-glutamine and/or L-glutamate via enzymatic deamination of L-glutamine and dehydrogenation of L-glutamate with conversion of NAD^+ to NADH.

Statistical analysis. Statistical analysis was performed using SigmaPlot 12.5 (Systat Software GmbH, Erkrath, Germany) software. The Shapiro–Wilk test was used for normality testing. The parametric data were analyzed by a one-way ANOVA. The non-parametric data were analyzed by using a Kruskal–Wallis one-way ANOVA on Ranks, followed by a Dunnett's multiple comparison test. A p-value less than 0.05 was considered to be statistically significant.

Results. Riboflavin supplementation did not affect serum glutamate levels in diabetic rats. The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability. There was not a statistically significant difference (Fig. 1; one-way ANOVA: $F_{3,36} = 0.824$, $p = 0.489$). However, the power of the performed test with $\alpha = 0.050$ is 0.049, which is below the desired power of 0.800. Less than desired power indicates that a difference is less likely to be detected when one actually exists, and therefore negative results should be interpreted cautiously.

In contrast, riboflavin supplementation affected serum glutamine levels in diabetic rats. The differences in the median values among the treatment groups were greater than would be expected by chance. There was a statistically significant difference (Fig. 2; Kruskal–Wallis one-way ANOVA on Ranks: $H = 20.561$ with 3 degrees of freedom, $p < 0.001$). Multiple comparisons versus the control group (Dunnett's method) showed a significant difference only between the "Diabetic" vs. "Diabetic + Rf 50 mg" (Fig. 2; $q = 3.683$, $p < 0.05$) and "Diabetic" vs. "Diabetic + Rf 100 mg" (Fig. 2; $q = 3.429$, $p < 0.05$) groups. The post-hoc analysis revealed that diabetic rats receiving water supplemented with 25 mg riboflavin showed no statistically significant difference in serum glutamine levels compared to the diabetic group (Fig. 2; $q = 1.219$, $p > 0.05$).

Discussion. In this study, we demonstrated that riboflavin supplementation did not affect serum glutamate levels but increased serum glutamine levels in rats with streptozotocin-induced T1D. However, the mechanism by which riboflavin can increase serum glutamine levels in diabetic rats has not been elucidated. Riboflavin is a precursor of flavin adenine dinucleotide and flavin mononucleotide,

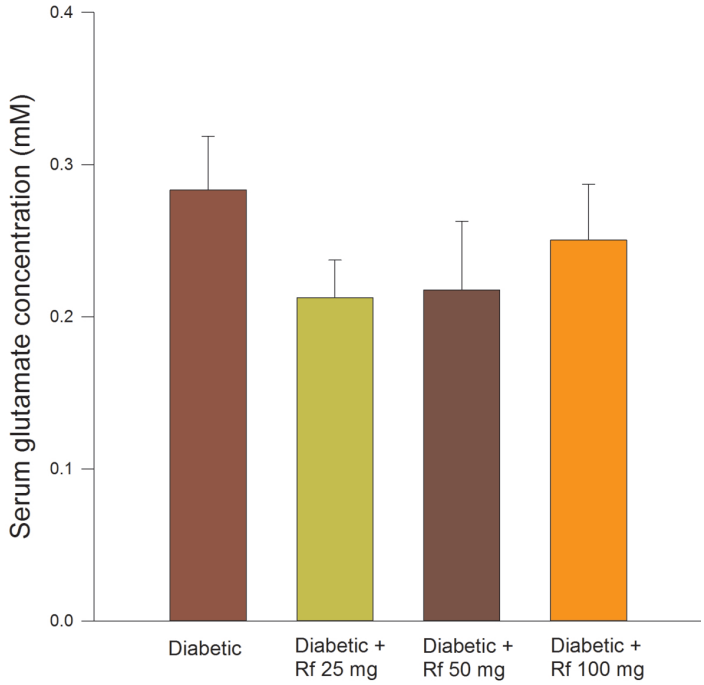


Fig. 1. Bar graph showing the effect of riboflavin supplementation on serum glutamate levels in diabetic rats. Values represent the mean ± standard error of the mean, $n = 10$

which are coenzymes necessary for the function of enzymes involved in various biochemical reactions, including those affecting amino acid metabolism [9]. Previous studies have shown that riboflavin intake can alter plasma levels of amino acids. For example, plasma homocysteine levels are inversely associated with riboflavin intake in humans [11, 12]. Interestingly, it has been demonstrated that vitamin B2 supplementation can affect the gut microbiome. In broiler chickens, riboflavin supplementation modulates gut microbiota, with the highest dosage being more effective in increasing the abundance of health-promoting bacterial groups, including *Bifidobacterium*, leading to boosted butyrate production [13]. It is well known that the gut microbiome can influence blood metabolites [14].

Riboflavin and glutamine have been suggested to increase survival in lipopolysaccharide-induced septic shock by facilitating tissue heat shock protein expression [15]. We speculate that the effect of riboflavin on serum glutamine levels during diabetes may be beneficial. It has been demonstrated that glutamine reduces postprandial glycemia, enhances postprandial insulinemia, and augments postprandial active glucagon-like peptide responses [16]. Also, glutamine stimulates protein-synthetic and inhibits protein-degradative signalling pathways in the skeletal muscle of rats with streptozotocin-induced T1D, suggesting that glutamine supplementation is potentially useful for slowing the progression of muscle

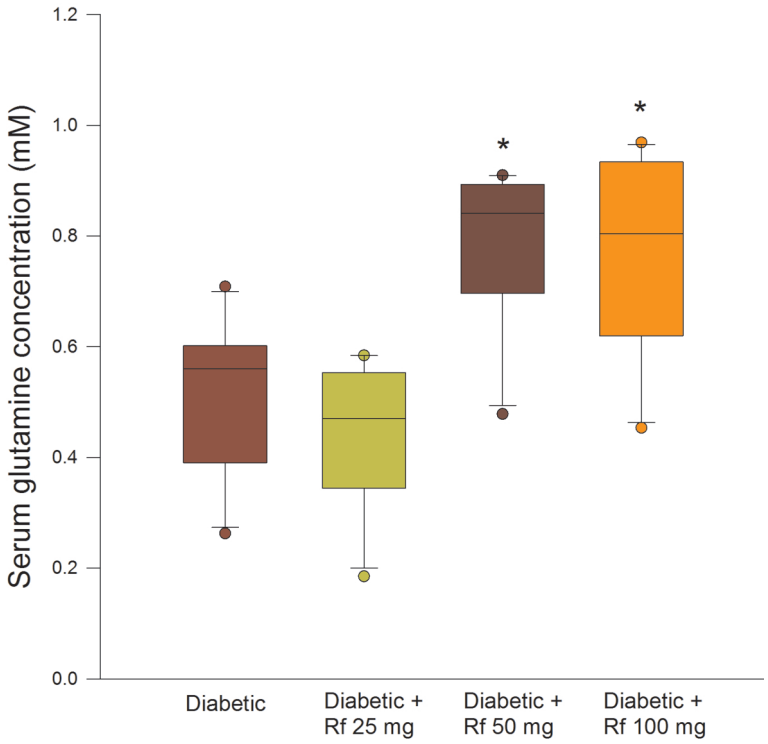


Fig. 2. Box plot showing the effect of riboflavin supplementation on serum glutamine levels in diabetic rats. The ends of the boxes define the 25th and 75th percentiles, with a line at the median and error bars defining the 10th and 90th percentiles. $^*p < 0.05$ vs. Diabetic group, $n = 10$

atrophy observed during diabetes [6]. In addition, glutamine prevents the development of experimental diabetic cardiomyopathy in streptozotocin-nicotinamide induced diabetic rats [17]. Furthermore, oral glutamine supplementation has been reported to decrease blood glucose in adolescents with T1D after exercise [18].

Conclusion. In the present study, we have demonstrated that riboflavin supplementation increases serum glutamine levels in rats made diabetic by streptozotocin administration. We speculate that riboflavin-induced elevation of serum glutamine levels may have a beneficial effect during diabetes.

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