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Note

Dietary restriction prevents diabetogenic effect of streptozotocin in mice

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The beneficial role of dietary restriction (DR) was studied in streptozotocin (STZ)-induced diabetes in mice. The DR mice exhibited the lower blood glucose (mg/dl) level as compared to *ad libitum* (AL) fed ones. After 3 months' DR, STZ treatment to both AL and DR mice showed significant ($p<0.001$) elevation of the blood glucose level in AL-fed mice, while a lower level of glucose was maintained in DR-fed mice. The ability of maintaining a low blood glucose level in STZ-treated DR mice indicated that STZ might have been ineffective from its action on beta cells of pancreas by long-term DR. Thus, these findings suggested that DR may be an important tool for preventing the diabetic conditions. However, further studies are required to know the mechanism(s) of DR protection against diabetogenic action of STZ in experimental animals.

Keywords: Dietary restriction, *Ad libitum*, Streptozotocin, Diabetes, Mice

Dietary restriction (DR) i.e. a reduction in calorie intake without malnutrition is known to influence physiological processes viz., immunological, protein and amino acid metabolism and also endocrinological system[1,2]. It is an efficacious mode to increase longevity and delay the incidence and severity of various age-associated pathologies, including cardiomyopathy, nephropathy and spontaneous and chemically-induced tumerogenesity[3-5]. DR is known to extend the mean and maximum life span in numerous organisms from yeast to rodents and possibly primates[6-8]. It also increases longevity by potentiating the immune response and lowering the oxidative stress/damage[4,5]. DR evokes anti-inflammatory and antineoplastic effects and also protects aging in rodents against diabetes, impaired tissue growth and reproductive senescence[9].

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Streptozotocin (STZ) is a naturally occurring glucosamine-nitrosourea class of compound which is toxic to the insulin-producing beta cells of pancreas in mammals. It is transported into beta cells by the glucose transporter protein (GLUT2) and damages the DNA by alkylation reaction. Such DNA damage induces ADP-ribosylation, leading to depletion of cellular NAD^+ and ATP. These events result in the formation of superoxide radicals, and consequently H_2O_2 and hydroxyl radicals are also generated. As a result, beta cells undergo the destruction by necrosis, leading to diabetogenecity[10]. Treatment of rats with STZ produces a diabetic state, characterized by weight loss, polydipsia, polyurea, glucosuria, polyphagia, hypoinsulinaemia and hyperglycaemia[11]. Heart rate and its variability, physical activity and body temperature are rapidly reduced following treatment with STZ[12]. The pathophysiology of STZ-induced diabetes includes cardiomyopathy, frequently associated with contractile dysfunction and heart rhythm disturbances. Contractile dysfunctions, including reduced amplitude of contraction and prolonged time course of contraction and relaxation have been frequently reported in myocytes from STZ-treated rats[13,14].

STZ is widely used to induce diabetes in animal model for studying disorders during diabetic state. Several studies have been reported during STZ-induced diabetes relating to oxidative energy metabolism in rat liver mitochondria[15], low density lipoprotein receptor expression in rat adipose tissue[16], downregulation of rat kidney AT2 receptors[17], and also on the activity of hypoglycemic agents[10]. As the DR is a powerful tool for preventing many disease-related metabolic disorders, in this study, we have investigated the beneficial role of DR in STZ-induced diabetes in mice.

Materials and Methods

Albino (BALB/c strain) male mice of 8 weeks, maintained under normal laboratory conditions (24 ± 2ºC; 12 h light/dark cycle) were used for the study. The animals were caged in polycarbonate cage and fed with a standard pellet diet (Amrut Laboratory, Pune) as per experimental schedule. The mice were divided into two groups. The *ad libitum* (AL) group

had continuous supply of food, whereas the animals subjected to DR were provided with food on every alternate day for a period of three months. However, water was supplied regularly to both the groups. The record of body weight of both AL and DR animals exhibited a significantly lower body weight (-38%) in DR animals as compared to AL-fed animals, ensuring their DR status[19]. Mice maintained on such regimen are known to consume 30% less food over a period of time and live upto 30% longer, compared to AL-fed animals[18,19]. Institutional Ethics Committee (IEC) guidelines on use of animals were followed during experimentation.

Trial experiments were carried out to determine the time and dose response of STZ. The doses of STZ ranged from 5 to 40 mg/100 g body weight of mice and the time of response monitored on every 4th day till 12 days. Maximum response was obtained with a single dose of 20 mg STZ/100 g body weight after 12 days of the treatment. Thus, both the AL and DR mice were administered intraperitonially (i.p.) with 20 mg of STZ/100 g body weight dissolved in 0.3 ml of 0.1 M citrate buffer (pH 4.5). The blood glucose level in all groups of animals was measured using ASCENSIA ETRUST Glucometer (Bayer). The data obtained from different sets were analyzed according to Student's *t*-test.

Results and Discussion

The blood glucose level (mg/dl) during 3 months was lower in DR than AL-fed mice. After 3 months of DR, STZ treatment elevated blood glucose level significantly ($p<0.001$) in AL mice in 12 days, but did not change glucose level in DR mice (Fig. 1), suggesting that DR prevents diabetes induced by STZ. During long-term DR, STZ might have been ineffective owing to an adaptive response of DR in maintaining lower levels of free radicals, such as superoxide and hydroxyl radicals which are known to cause STZ-induced damage of β-cell of pancreas[10]. Our finding corroborated with the previous report that DR improves health and extends life span by improving insulin sensitivity and lowering blood glucose levels[20,21]. Many organs and tissues of type II diabetic individuals tend to age faster than normal[20]. In humans, high calorie diets cause numerous pathological conditions including increased glucose and insulin levels, leading to diabetes, cardiovascular disease and non-alcoholic fatty liver diseases, a condition for which there is no effective treatment[22].

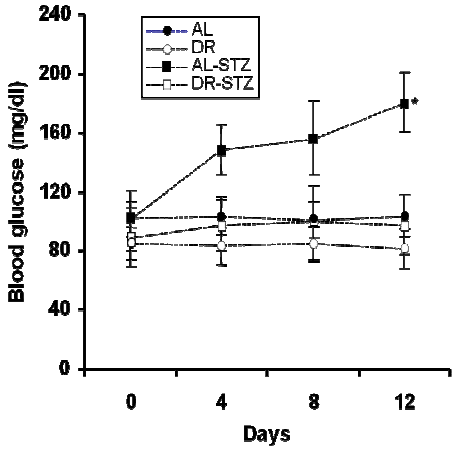

Fig. 1—Effect of streptozotocin (STZ) on induction of diabetes in *ad libitum* (AL) and dietary restricted (DR) mice [Values are mean from five mice in each group. Bars represent standard deviation. Asterisk (*) exhibits statistically significant ($p<0.001$) value of glucose level (mg/dl) in STZ-treated AL (AL-STZ) mice as compared to AL, DR and STZ-treated DR (DR-STZ) mice]

Diabetes has become one of the major health complications and growing at an alarming proportion worldover and in particular in India. Approximately 140 million people worldwide suffer from diabetes[23]. The disease is becoming a major problem of public health in developing countries, where its prevalence is increasing steadily and adequate treatment is often expensive or unavailable[24]. Management of diabetes without any side effects is still a challenge to the medical community. There is continuous search for alternative drugs. Therefore, there is a growing interest in herbal remedies due to their minimal side effects and relatively low cost. In fact, they are prescribed widely, even when their biological active compounds are unknown[25]. The use of such herbal products may alternatively exert many of the similar beneficial effects of DR, without cutting the food intake[5].

DR can play an important role in managing metabolic disorders, particularly diabetes[5]. DR even at a later stage in life is reported to have beneficial effects in reversing some of the biochemical changes during aging[26]. It also improves metabolic responses and restores age-related decline in such functions[19]. Although, few contradictory reports[27-29] are available on the benefits of DR in experimental animals, one can expect that DR will help to prevent or delay the onset of age-related diseases and increase functional longevity in terms of improved quality of life and health span in humans[5,30]. The ability of maintaining a low blood glucose level in STZ-treated

DR mice in the present study indicated that DR might provide metabolic protection in preventing STZ-induced diabetes. We suggest that DR might be an alternative measure in maintaining blood glucose level in diabetes.

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