



Effect of Melatonin in High Sugar Diet fed Male Wistar rats on the levels of Plasma Glucose, Magnesium, and interleukin-6

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Abstract

Type 2 Diabetes Mellitus (T2DM) is a metabolic disorder in which there is insulin resistance and it is experienced by insulin sensitive tissues like skeletal muscle, adipose tissue, liver, and small part of cardiac tissue. The present study was conducted for 12 weeks in the male Wistar rats to investigate the effect of High Sugar Diet (HSD) and melatonin in relation to plasma glucose, serum levels of interleukin-6 (IL-6), and levels of Magnesium (Mg) in serum, liver, pancreas and kidney tissues. The study also investigate that melatonin administration increases the Mg levels in serum and above mentioned tissues. We observed increased levels of plasma glucose, serum IL-6, and lower levels of Mg in serum and kidney tissues in HSD rats when compared with control rats. After administration of melatonin to HSD rats, significant decrease was observed in the values of plasma glucose and serum IL-6. On the contrary, we observed significant increase in the levels of Mg in the serum and kidney tissues when melatonin was administered to HSD rats. Thus, we conclude that the response to melatonin administration for glucose, Mg, and IL-6 parameters is related to the degree of insulin resistance and the duration of the study. The response to melatonin is also related to physical stress, to the types of diet consumed.

Keywords: *Diabetes Mellitus, Insulin Resistance, Hypomagnesemia, Interleukins.*

Introduction

Worldwide Type 2 Diabetes Mellitus (T2DM) is a major medical, social, and economic problem and is the leading cause of hospitalization for patients. T2DM is a metabolic disorder in which there is insulin resistance (IR) and it is experienced by insulin sensitive tissues like skeletal muscle, adipose tissue, liver, and small part of cardiac tissue^[1]. Such tissues eventually experience a dysbalance between the catabolism and anabolism of bio-molecules including carbohydrates, proteins,

and lipids^[1-3]. In due course of time after IR, the glucose increases in blood (hyperglycemia) primarily, which later in the presence of reactive species (also due to hyperglycemia), conjugates with building blocks of organism like proteins, lipids, and deoxyribonucleic acid (DNA) respectively^[2-4]. This irreversible conjugation leads to a conformity change in bio-molecules. Certain changes in structure induce a pro-inflammatory response and therefore hyper activating immune cells lead to a low grade

inflammation which persists in T2DM^[5,6]. Apparently, the pro-inflammatory response triggers heterogeneous group of secondary disorder like atherosclerosis, cardiovascular disease, nephropathy, retinopathy, and peripheral neuritis in T2DM^[1-4]. In short, hyperglycemia has a principle factor in the development and progression of diabetes and eventually its complications.

Interleukin-6 (IL-6) is an important biomarkers involved in IR^[7] as well as in inflammation^[8]. IL-6 is reported to involve in cellular responses to stimuli such as free radicals and oxidative stress^[9]. Awazawa et al^[10] stated that IL-6 down-regulates proteins such as IRS-1 involved in IR. However, Yu et al^[11], found that improved glycaemic control is associated with increased PPAR-g and decreased IL-6 protein expression in pancreas.

Magnesium (Mg) is an essential cofactor for a number of kinases that are important in carbohydrate and lipid metabolism, including hexokinase, phosphofructokinase, and adenylylate cyclase^[12]. The mechanism by which Mg exerts anti-diabetic effects has not been studied in detail. However, Mg stimulates the activity of glucokinase in the liver and improves pancreatic beta islet cell function to enhance insulin regulation and secretion^[13]. Studies have shown lower levels of Mg in patients suffering with T2DM^[14-17]. In addition, a study has shown that lower level of Mg is associated with IR^[16,17]. The mechanism behind hypomagnesemia in T2DM is not clearly known. Garland et al., reported that glycosuria which is present in the diabetic state may be the cause for lower levels of Mg in diabetes^[18].

Melatonin is a hormone secreted in vertebrates in large amounts by pineal gland and small quantities by extrapineal sites including retina, Harderian glands, and gastrointestinal tract^[19]. A study had suggested that melatonin greatly enhances glucose uptake in a murine skeletal muscle cells^[20]. It was reported that melatonin is capable of enhancing glucose disposal in obese rats^[21,22]. Melatonin is

profoundly known for alleviating oxidative stress in obese rats^[23]. Elevated level of IL-6 is associated with inflammatory disease when there is lower concentration of melatonin is present in the blood^[24]. In another study it has demonstrated that melatonin enhances the synthesis of IL-6^[25]. However, Clapp-Lilly et al, reported that administration of melatonin reduced the IL-6 production amyloid- β brain slices^[26].

Therefore, the aim of the present study was to investigate the effect of HSD and melatonin in relation to plasma glucose, serum levels of IL-6, and levels of Mg in serum and liver, pancreas and kidney tissues. The study also investigate that melatonin administration increases the Mg levels in serum and above mentioned tissues.

Materials and Methods

High Sugar Diet (HSD) was used to induce IR to derange the homeostasis of glucose and produce hyperglycemia, and eventually T2DM as previously described^[27,28]. Acclimatization period for rats was given for 10 days. The animals were kept at room temperature at 25°C with 12 hr dark and light cycles. Both control and experimental rats were kept fasting for 12 hr prior to HSD induction. Rats developed IR, showed hyperglycemia (blood sugar > 200 mg/dl or > 11.1 mmol/L), weight increased, increased water intake and became less active. The total duration of experiment was 12 weeks excluding the acclimatization period. HSD consists of white bread and containing 1 mg of sugar per one ml of water.

The control rats were given limited amount of food and water per day. On the other hand, to determine the effect of HSD in the development of IR, hyperglycemia, IL-6, and Mg status of the animals, thus, these animals received unlimited HSD until the end of the study. To the third group to determine the effect of melatonin in the development of IR, T2DM, and magnesium status of the animals, thus, these animals received unlimited HSD and melatonin of 200 μ g/animal/day until the end of the study. Oral

gavage was the administration route of melatonin for experimental rats. At the end of 12 weeks, all the rats used in the study were fasted overnight. The next day morning, the study rats were anesthetized with chloroform and blood samples were collected by cardiac puncture. Collected blood of rats was centrifuged at 2000 g for 15 minutes and serum was isolated and was stored at 80°C for analyses of parameters. The tissue samples including liver, pancreas, and kidney were removed using laprotomy procedure. Tissues were cleaned with normal saline and were stored at 80°C. The tissues were homogenized in 50mM phosphate buffer using a Remi high speed homogenizer. The homogenate was then centrifuged at 2000 g for 30 minutes and the resultant supernatant was used for measurement of interleukin (IL-6).

Plasma glucose was estimated for control as well as experimental rats at the end of the study period after a 12 hr fast using a commercial Accurex glucometer. Mg and IL-6 in tissues were estimated according to the instructions provided in the Abcam Mg colorimetric kit and IL-6 single analyte elisa kit respectively.

Statistical Analysis

Biochemical parameters mentioned in the study were measured in the blood, liver, kidney, and pancreas tissues obtained from control and melatonin treated animals after the termination of the study. All the values obtained were expressed as mean \pm SD. The statistical analysis of results was carried out by one way analysis of variance (ANOVA) followed by Bonferroni test.

Results

In the Fig. 1, HSD-fed rats have high plasma glucose levels compared to control rats ($P < 0.05$). There was also significant increase in weight (230 %) of the animals in the HSD-fed group as reported earlier [27,28]. When HSD group rats' plasma glucose was compared with the HSD group rats treated with melatonin group, we observed a significant change in the level of

plasma glucose concentration ($P < 0.05$). The data (Fig. 1) suggest that HSD induces hyperglycemia and when the experimental conditions are provided melatonin enhances the disposal of glucose. The data in Fig. 1 also shows that the level of pro-inflammatory cytokine IL-6 was increased in HSD fed rats when compared with control group rats ($P < 0.05$). Melatonin significantly reduced the levels of IL-6 even after consuming experimental unlimited sugar diet (HSD) ($P < 0.05$). On the contrary, the level of serum Mg was significantly increased after treatment with melatonin (Fig. 1) ($P < 0.05$).

It is evident from the results shown in Fig. 1 that rats develop hyperglycemia due to HSD have lower levels of Mg and high levels of IL-6 both in plasma and tissues. The changes in the levels of serum Mg and IL-6 are more obvious when the ratio between Mg and IL-6 (Mg/IL-6) is compared between the groups as shown in Table 1 ($P < 0.05$). It is evident from these results that the balance between Mg and IL-6 is altered to a significant degree in HSD fed rats compared with the control group rats ($P < 0.05$). This alteration in the Mg/IL-6 ratio was restored to near normal levels in HSD group rats treated with melatonin ($P < 0.05$). These results suggest that melatonin has the ability to normalize the level of Mg and suppress the synthesis of IL-6 (Table 1, Fig. 1).

In Fig. 2, we observed significant difference in rat tissues of pancreas and kidney in the levels of Mg when compared between control group versus HSD group and HSD group versus HSD+melatonin group ($P < 0.05$). On the contrary, no significant difference was observed in the liver tissue of rats in the levels of Mg when compared between control versus HSD, control versus HSD+melatonin, and HSD versus HSD+melatonin ($P < 0.05$).

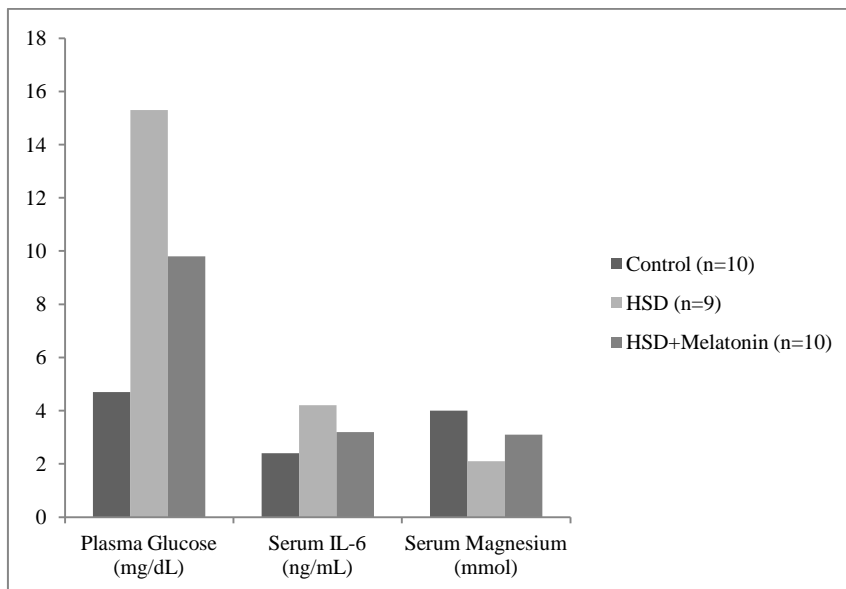


Figure 1 Concentration of plasma glucose, serum IL-6, and serum magnesium in groups of control, High Sugar Diet (HSD), and HSD treated with melatonin (HSD+Melatonin).

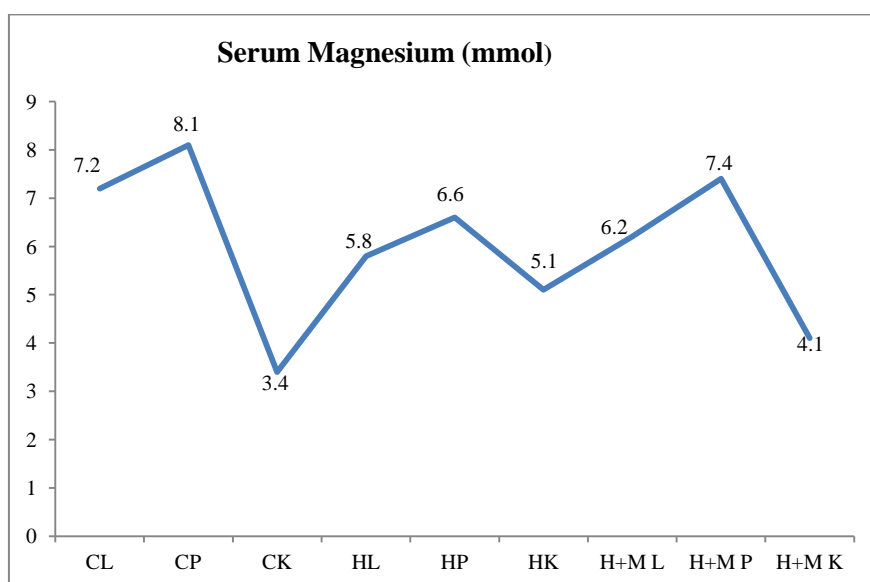


Figure 2 Concentration of serum magnesium in rats of control liver (CL), control pancreas (CP), control kidney (CK), High Sugar Diet fed rats liver (HL), HP, HK, and melatonin treated HSD liver (H+M L), H+M pancreas (H+M K), and H+M kidney (H+M K).

Table 1 Effect of High Sugar Diet (HSD) and melatonin treatment on the Magnesium/IL-6 ratio in the serum of male Wistar rats

Group	Serum magnesium/IL-6 ratio
Control	3.70 ± 1.95
HSD	0.35 ± 0.09
HSD+Melatonin	0.61 ± 0.10

Discussion

The present study observed hyperglycemia in the rats consuming HSD (2nd group) compared to control group rats (Fig. 1). Similarly, the HSD

group rats also observed increased level of pro-inflammatory cytokine IL-6 compared to control rats. It is clearly evident from the previous studies that consumption of high caloric diet along with

less amount of physical activity leads to obesity [29-32]. Since, the utilization of consumed calories if not broken down to generate energy in the form of ATP to perform physical activity, will lead to accumulation of excess consumed calories in the form of lipid molecules in adipose tissue [29,30]. There are reports suggesting that intake of excess calories lead to obesity [31,32]. Thus, the present report infers that due to unlimited intake of HSD progressed towards the increased weight in animals (data not shown). Moreover, weight increase of the animals needs more oxygen consumption to metabolize the bio-molecules to sustain the needs of the newly accumulated molecules around the body surface of the animals. Evidences suggest that oxygen reduces to water by inducing free radical production, which eventually cause oxidative stress [33,34]. Oxidative stress affects the nuclear transcription factors to generate pro-inflammatory cytokines.

Experimental and clinical studies reported that IL-6 is known to induce IR in experimental animals and humans as well [6-8]. In the present study it is clear that increase in IL-6 levels in the HSD group rats is the reason for hyperglycemia which is the result of IR. After treatment with melatonin to HSD rats (3rd group), we observed lower concentrations of glucose and IL-6. Melatonin is known to reduce oxidative stress by combating the free radicals, thus reducing oxidative stress, in turn reducing the levels of IL-6 and improving insulin sensitivity [22,23]. Since, the reduction of free radicals leads to better disposal of glucose from the blood.

We report lower levels of Mg in serum and kidney tissue in HSD rats (2nd group) compared with control rats serum and kidney tissue. However, significant difference was observed in melatonin treated HSD rats (3rd group) in the levels of serum Mg. Similarly, the present study also report significant increase in the levels of Mg in kidney tissue of melatonin treated HSD rats (3rd group) when compared with HSD rats (2nd group). IL-6 is the reason for lower levels of Mg in HSD serum and kidney tissues (2nd group). It is demonstrated

that insulin sensitivity is required for the reabsorption Mg in the renal tubules [35]. Studies have shown that concentration of Mg inside the cell is purely dependant on the insulin as it regulates the stimulation of ATPase pumps and increasing free Mg entry into the cells [35-37]. This also causes the influx of calcium ions which in turn closes calcium channels, which leads to improvement of insulin sensitivity [38-40]. The lower Mg level in the serum and kidney tissues is attributed to the consequence of IR [35,36,40]. Therefore, the reduction in insulin resistance by melatonin administration and consequent improvement in insulin function would increase the reabsorption and also prevent the loss of Mg from the kidney (in 3rd group).

Another important aspect of the present study is the observation that even serum levels of Mg and IL-6 were reversed to relatively near normal level following melatonin administration (in 3rd group). Thus, the present study also shown that it is possible to restore the level of Mg and IL-6 to near normalcy only when glucose levels are restricted to below the cut-off level to diabetes mellitus. The present study differs from Navarro-Alarcon et al [41], in many aspects. For example, Navarro-Alarcon et al [41], used ZDF and ZL rats in their study and reported only Mg status in various tissues. On the other hand, we used HSD to induce hyperglycemia and measured the levels of plasma glucose, change in weight, and Mg levels in serum and also in tissues including liver, pancreas, and kidney. There are no reports as per to our knowledge as to the effect of melatonin on Mg and IL-6 levels in HSD induced diabetes mellitus. Our study also differs from that of Navarro-Alarcon et al [41], in that we used oral gavage technique to administer melatonin to experimental animals and showed that even oral melatonin is equally effective in restoring the Mg and IL-6 levels to normalcy in HSD induced diabetes.

Conclusion

The present conclude that the response to melatonin administration for glucose, Mg, and IL-6 parameters is related to the degree of insulin resistance and the duration of the study. The response to melatonin is also related to physical stress, to the types of diet consumed. The beneficial effects could be attained when the experimental conditions are provided. In view of the present study results, it will be interesting the possible use of melatonin to prevent hyperglycemia and to restore the dys-balance levels of Mg and IL-6.

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