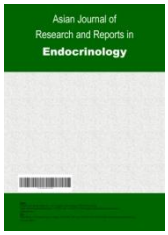

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Tetrahydrobiopterin Administration Restores Sex Hormones Imbalance in Streptozotocin-induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Diabetes mellitus results in the state of imbalance in oestrogen and testosterone; more so, females exhibit increased circulating testosterone along with decreased in oestrogen level in diabetes mellitus. It is suggested that, sex hormones are important regulators of glucose and lipid metabolism. Tetrahydrobiopterin bioavailability which is an important modulator of these metabolites may be involved in sex hormones metabolism. In current work we investigated the effect of tetrahydrobiopterin administration on sex-hormones levels in diabetes mellitus.

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Materials and Methods: Thirty (30) albino Westar rats (15 males and 15 females) weighing 100-120 g was divided into three groups: Controls (5 males and 5 female), diabetics (5 males and 5 females) and diabetics supplemented with tetrahydrobiopterin (5 males and 5 female). Diabetic groups received a single intra venous injection of Streptozotocin (STZ, 60 mg/ kg) while the control group were injected with a similar volume of citrate buffer. Tetrahydrobiopterin 20 mg/kgbw/day was supplemented for two weeks. Fasting Plasma Glucose, oestrogen and testosterone were estimated in all the groups.
Results: The mean Fasting plasma glucose concentration was significantly lower ($p<0.05$) in controls males (5.24±0.15 mmol/l) and females (5.43±0.15 mmol/l) and treated males (7.66±0.22 mmol/l) and females (7.20±0.36 mmol/l) compared to untreated males (15.68±2.84 mmol/l) and females (19.40±4.13 mmol/l). The mean plasma oestrogen concentration in control males (12.75±4.52 g/ml) and females (111.72±6.42 pg/ml) and treated males (15.16±2.08 pg/ml) and females (103.74±2.41 pg/ml) were significantly higher ($p<0.05$) than untreated males (5.33±1.36 g/ml) and females (66.84±20.88 pg/ml). The mean plasma testosterone concentration for females in control and treated groups were significantly lower ($p<0.05$) than untreated while there is no significant difference in mean values for males across the groups ($p>0.05$).
Conclusion: Diabetes resulted in oestrogen and testosterone imbalance and tetrahydrobiopterin administration restores level to near normal.

Keywords: Diabetes mellitus; oestrogen; testosterone; tetrahydrobiopterin.

1. INTRODUCTION

Diabetes mellitus is defined based on laboratory findings as the fasting blood glucose of ≥ 7.1 mmol/L and 2 hour postprandial of ≥ 11.1 mmol/L on more than one occasion [1]. Cardiovascular mortality and morbidity differs greatly between men and women. Women seem to be more protected and are affected by these diseases more rarely and later in life compared to men [2]. However, women who have diabetes mellitus have an increased risk of heart disease because diabetes mellitus cancels out the protective effects of oestrogen [3]. It is suggested that, there may be distinct genetic and hormonal factors related to gender in the development of heart disease in diabetes mellitus [4].

Hormonal differences between men and women may speculativel play a role in these discrepancies regarding cardiovascular morbidity and mortality [5]. In light of such hormonal differences, it is expected that testosterone have an adverse effect or estrogen to have a protective effect or both. Increasing evidence suggests that diabetes mellitus is a state of imbalance in sex hormone levels [6]. In essence, this imbalance in hormone levels in women results in a hormonal profile that resembles that of a man, with a higher testosterone/estrogen ratio [2]. Based on this, it would be reasonable to think that hormone supplementation to restore the normal testosterone/estrogen ratio would be beneficial in women with diabetes mellitus and co-inflicted with CVD complications. Indeed, a few studies have shown that hormone therapy in women with diabetes mellitus is associated with a reduction in visceral adiposity and improvement in lipid and glucose metabolism, all of which are risk factors of CVD [7].

A major weapon of endothelial cells to fight vascular disease is endothelial nitric oxide synthase (eNOS), an enzyme that generates the vasoprotective molecule nitric oxide (NO) [8]. eNOS uncoupling plays a major role in endothelial dysfunction seen in diabetes mellitus [9] and its cofactor, tetrahydrobiopterin (BH_4) is highly sensitive to oxidation by $ONOO^-$. Diminished levels of BH_4 promote $O_2^{\cdot -}$ production by eNOS (referred to as eNOS uncoupling) [9]. This transformation of eNOS from a protective enzyme to a contributor to oxidative stress has been observed in several in vitro models, in animal models of cardiovascular diseases, and in patients with cardiovascular risk factors [9]. In many cases, supplementation with BH_4 has been shown to correct eNOS dysfunction in animal models and patients [10].

In current study, attempt was made to supplement STZ-induced diabetic rats with BH_4 and evaluate the effect of this supplementation on sex hormones of both male and female diabetic rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Thirty (30) Albino Westar rats (15 males and 15 females) 100-120 g were purchased from The Animal House, National Veterinary Research Institute (NVRI), Vom. The animals were housed in standard cages at room temperature, in the month of July/August in the animal house, Department of Pharmacology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto.

2.2 Research Design

Thirty (30) male and female rats were divided in to three (3) groups as follows:

Group 1: 5 male and 5 female non diabetic rats as controls.

Group 2: 5 male and 5 female diabetic rats untreated.

Group 3: 5 male and 5 female diabetic rats treated with BH_4.

2.3 Induction of Diabetes Mellitus

Experimental diabetes mellitus was induced by a single intraperitoneal injection of freshly dissolved Streptozotocin (60 mg/kg) [11], in normal saline maintained at 37°C, to group 2 and 3 rats after overnight fasting. Control rats (group 1) received a similar injection of normal saline alone. Glucose solution (5%) was used as their drinking water for 24 hours. Streptozotocin causes degeneration of β-cells of the islet of Langerhans by generating free radicals in these cells [11].

2.4 Inclusion and Exclusion Criteria

Seventy two (72) hours after Streptozotocin injection, the rats were fasted overnight and their fasting blood glucose obtained through tail artery was estimated. Only rats from groups 2 and 3 that have fasting blood glucose level of ≥ 7.1 mmol/L (≥126 mg/dl) were included in the study.

2.5 Treatments

The following treatment was administered after diabetic induction:

Tetrahydrobiopterin 20 mg/kg b.w./day given to group 3 rats orally for two weeks [12].

2.6 Sample Collection

At the last day of the treatment, the rats were fasted overnight and anaesthetized by dropping each in a transparent plastic jar saturated with chloroform vapour. About 5 mls of blood sample was obtained through cardiac puncture and divided into fluoride oxalate and lithium heparin anti-coagulated containers. Humane procedure was used as adopted [13]. Blood samples were centrifuged at 3000 rpm for five (5) minutes and plasma obtained was used for analysis.

2.7 Analytical Methods

All reagents and chemicals used are of analytical grade or higher.

Plasma glucose was estimated by glucose oxidase-peroxidase method [14], plasma testosterone and oestrogen was estimated by ELISA [15].

2.8 Statistical Analysis

The data generated from the laboratory experiments were analyzed using 'SPSS version 20' statistical software and presented as mean ± Standard Error (SE) of the concentration. Differences between means of the variables were compared using one way ANOVA and *P*-value <0.05 was considered significant. The differences were analysed using Turkey post hoc test.

3. RESULTS

Table 1 shows the diabetogenic effect of Streptozotocin and effect of tetrahydrobiopterin on fasting blood glucose. There was significant increase *(P*<0.05) in mean fasting blood glucose concentration in groups injected with Streptozotocin when compared with controls. Administration of tetrahydrobiopterin caused a significant decrease (*P*<0.05) in fasting blood glucose of the treated group, when compared with untreated. There is no gender disparity among the groups.

Table 2 shows plasma level of oestrogen and testosterone of all the experimental groups.

Table 1. Fasting blood glucose (FBG) concentrations of males and females rats used in the study

Group	Gender (n)	Pre FBG (mmol/l)	Post FBG (mmol/l)
1 (Control)	Males (5)	5.30±0.16	5.24±.015
	Females (5)	5.68±0.18	5.43±0.15
2 (Untreated)	Males (5)	17.50±1.91	15.68±2.84
	Females (5)	20.50±4.76	19.40±4.13
3 (Treated)	Males (5)	17.52±2.35	7.66±0.22
	Females (5)	17.22±0.93	7.20±0.36
		Post hoc	
1 V 2	Males	<0.05	<0.05
	Female	<0.05	<0.05
1 v 3	Males	<0.05	>0.05
	Females	<0.05	>0.05
2 v 3	Males	>0.05	<0.05
	Females	>0.05	<0.05

Values are mean ± standard error of the mean of groups used in the study, analysed using one way ANOVA and P< 0.05 was considered significant

Table 2. Plasma oestrogen and testosterone concentrations of males and females rats used in the study

Group	Gender (n)	Oestrogen (pg/ml)	Testosterone (ng/ml)
1 (Control)	Males (5)	12.75±4.52	3.45±0.22
	Females (5)	111.72±6.42	0.20±0.04
2 (Untreated)	Males (5)	5.33±1.36	3.90±0.52
	Females (5)	66.84±20.88	0.88±0.16
3 (Treated)	Males (5)	15.16±2.08	2.94±0.54
	Females (5)	103.74±2.41	0.19±0.05
		Post hoc	
1 v 2	Males	<0.05	>0.05
	Female	<0.05	<0.05
1 v 3	Males	>0.05	<0.05
	Female	>0.05	<0.05
2 v 3	Males	<0.05	<0.05
	Female	<0.05	<0.05

Values are mean ± standard error of the mean of groups used in the study, analysed using one way ANOVA and P< 0.05 was considered significant

The mean plasma level of oestrogen in control and treated group was significantly higher (P<0.05) than in untreated group. Gender disparity exists across the groups with females having significantly higher (P<0.05) values than males.

The mean plasma level of testosterone in the control and treated groups were significantly lower (P<0.05) than untreated group for females while the mean values for males in control and untreated were similar (P>0.05).

4. DISCUSSION

In the current study, treatment with tetrahydrobiopterin significantly lowers blood glucose level in diabetic rats. The mean fasting blood glucose level in diabetic rats not treated with tetrahydrobiopterin was significantly higher (P<0.05) than the values in the treated group and controls while the difference between mean fasting blood glucose of diabetic rats treated with tetrahydrobiopterin and that of controls were not significant (P>0.05). In the current study however, there is no gender disparity on glucose lowering effect of tetrahydrobiopterin administration.

These findings are similar to those reported by Abudukadier et al. [16] which showed that tetrahydrobiopterin has glucose lowering effect by suppressing hepatic gluconeogenesis in an endothelial Nitric Oxide Synthase (eNOS)-

dependent manner in diabetic rats. Tetrahydrobiopterin availability is a critical determinant of Nitric Oxide production by NOS. Liver has a critical role in glucose lowering effect of tetrahydrobiopterin which activate AMP kinase and the suppression effect on gluconeogenesis was AMP kinase dependent. Furthermore, it is reported [16] that glucose lowering effect observed was similar to that of metformin.

In the current study also, BH_4 administration, 20 mg/kg body weight, in diabetic rats was enough to have caused significant decrease in blood glucose level through afore mentioned mechanisms.

In the current study also, we investigated the influence of diabetes mellitus and tetra-hydrobiopterin administration on plasma oestrogen and testosterone in males and females diabetic rats. The mean oestrogen level in diabetic untreated rats were significantly lower (P<0.05) than in both treated and non-diabetic control. Conversely, testosterone level in diabetic female rats was significantly higher (P<0.05) than in both control and treated group while the mean values for males in control and untreated were similar (P>0.05).

Diabetes mellitus resulted in the state of imbalance in oestrogen and testosterone; more so, females exhibit increased circulating testosterone along with decreased in oestrogen level in diabetes. This is in agreement with the work of Ding [17], who in particular, reported that, women with diabetes commonly exhibit increase in circulating testosterone alongside decreases in estradiol levels [17]. This is one of the important risks components for the development of cardiovascular disease in diabetes mellitus and likely explains why in diabetes mellitus, females protective factor against cardiovascular disease appeared to diminish.

Body of evidence [18,6,2] suggested strong associations between sex hormones imbalance and cardiovascular risk in both men and women; it is proposed that testosterone was protective in men, and estradiol levels seemed protective in women. However, diabetes mellitus modified the effect of sex hormones in both men and women [18]. These differences in the effects of sex hormones observed in women with diabetes can, at least partially, explain the elemination of the protection against acute myocardial infarction in these women [6].

It is reported [19] that, while the exact mechanism by which sex hormones imbalances are brought about in diabetes mellitus and how it contribute to the development of cardiovascular disease are still being investigated, both experimental and clinical studies suggested that, sex hormones are important regulators of glucose and lipid metabolism, dysfunction of which is a great risk factor for cardiovascular disease and tetrahydrobiopterin which is a critical determinant of nitric oxide bioavailability greatly influences glucose and lipid metabolism [20].

In the current study, treatment with tetrahydrobiopterin restores normal testosterone/ oestrogen ratio with values of the treated group being similar (P>0.05) to control. This may be explained by the work of Ulrich and William, (2012), who reported that, oestrogen and vascular endothelial growth factor phosphorylate endothelial Nitric Oxide Synthase mainly via Serine/Threonine kinase at the active site, which is an important regulator of its function. Increased endothelial Nitric Oxide Synthase activity via BH_4 supplementation, may in turns facilitate oestrogen metabolism and restoration of the imbalance observed in this study.

5. CONCLUSION

Present study therefore, demonstrated beneficial effect of tetrahydrobiopterin administration on correcting sex hormones imbalances observed in diabetes mellitus and the effect is presumably via Nitric Oxide Synthase dependent pathway. Diabetes resulted in oestrogen and testosterone imbalance and tetrahydrobiopterin administration restores level to near normal.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval was obtained from Faculty of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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