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Refractive Power and Blood Glucose Changes: Studies in Alloxan-Induced and Insulin-Treated Diabetic Rabbits

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ABSTRACT

This study was carried out to determine the refractive power and blood glucose changes in alloxan-induced and insulin-treated diabetic rabbits. Eighteen healthy Dutch rabbits of both sexes weighing between 1.1kg and 1.9kg were used for this study. A 5% solution of alloxan monohydrate was administered intravenously to create a diabetic state. Refraction and blood glucose measurement was measured at approximately 5 days interval for 15 days before and after induction of diabetes by Streak retinoscopy. Results show a significant increase in hyperopia from + 1.49 D (+/- 0.59) to + 2.00 D (+/- 0.52) with increased blood glucose level after induction of diabetes. There was a significant positive correlation (P = 0.01) between hyperglycemic and hyperopic changes in the diabetic rabbits (r = +0.913, P<0.05). On the other hand, there was no significant correlation between insulin-induced blood glucose and refractive changes in insulin treated diabetic rabbits (r = +0.31, P > 0.05).

Keywords:
Refractive power; blood glucose; diabetes; insulin; hyperopia

INTRODUCTION

The refractive function of the eye is an important determinant of ocular health and it is known to be dependent on both local and systemic biochemical variables. Among the local variables, the axial length and refractive power of the eye (as contributed by the curvature, thickness, and refractive index of the cornea and lens) are known to be the primary factors in which abnormal changes may lead to refractive errors, including myopia, hyperopia, astigmatism, and presbyopia. The influence of biological molecules in the systemic circulation on refractive error and refractive development is an important area in current scientific research that may provide information necessary for developing strategies for prevention of refractive errors or their associated visual morbidity. Several studies[1-6] have shown the influence of hormones, neurotransmitters,cigarette smoking, or medications on ocular refraction. In this regard, the nature of the refractive complications of metabolic disorders could give a clue to the underlying biochemical mechanisms.

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Diabetes mellitus is a metabolic disorder due to insulin deficiency. It is characterized by glycemic, protein, lipid, fluid and electrolyte abnormalities[7]. The ocular refractive complications of diabetes mellitus was brought to limelight by Duke- Elder[8] who claimed that increased blood glucose leads to a myopic shift or increase in refractive power while a decrease in blood glucose leads to a hyperopic shift or decreased refractive power . While this hypothesis was supported by some clinical and in vitro studies[9]-[12], others have found hyperopia, not myopia, in association with hyperglycemia and anti-diabetic drug therapy[12-15]. Thus, the nature of the relationship between refractive error and blood glucose changes in diabetes is yet to be clarified[16].This study was designed to re-examine the nature of the relationship between refractionand blood glucose changes using diabetic rabbits.

MATERIALS AND METHODS

The materials used for this study include: Glucometer (Roche Diagnostics, Germany), Streak Retinoscope and Direct Ophthalmoscope (Heine, Germany), Trial lens set, electronic analytical balance (Ohaus, USA), weighing scale, wooden animal holders, test tubes, syringes and needles. Human Isophane Insulin (Novo Nordisk Germany), Aloxan Monohydrate (Qualikems, India), tropicamide 1% solution (Alon, Belgium), lignocaine hydrochloride (Maxima, Germany), sterile water, and Isopropyl Alcohol (New Healthway, Lagos).

Ethical approval was obtained from the Animal and Ethics Committee of the Abia State University Department of Pharmacology and Therapeutics, Uturu, Nigeria where the study was carried out.

Eighteen healthy Dutch rabbits (Oryctolaguscuniculus) of both sexes, weighing between 1.1kg and 1.9kg were obtained from Michael Okpara University of AgricultureUmudike Animal Farm and certified healthy by a veterinarian. They were kept in clean cages under a 12-hourly light-dark cycle with free access to food and water for seven days to enable them acclimatize to the new environment. The animals were weighed, sex-matched, and divided into two groups:

Group A (n=6) served as normal control while group B (n=12) served as experimental diabetes group.

Induction of Experimental Diabetes

A 5% solution ofalloxan monohydrate (a pancreatic B cell selective cytotoxic agent) was made and single doses of 100mg/kg body weight were administered intravenously via the marginal ear vein of the rabbits in the experimental group after fasting them overnight. A diabetic state (fasting blood glulose≥200mg/dl) was confirmed 5 days after alloxan injection via measurement of blood glucose levels.

Effect of diabetes on refractive state in rabbit eyes

Refraction was measured at approximately 5 days interval for 15 days before and after induction of diabetes by Streak retinoscopy.

Effect of insulin therapy on blood glucose and refraction in diabetic rabbits

The experimental diabetic rabbits were sub-divided into two groups of six animals each with

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one group which served as the diabetic control group and received sterile water (B1), while the other sub-group received daily doses of 2.5-4.5 units of Isophane insulin for 15days. Refraction and blood glucose measurements were done subsequently at 5 days intervals in the two groups.

Statistical analysis

Data were expressed as mean +/- standard deviation (SD) and analyzed statistically using Student's t-test, z-test and Pearson's correlation. P values of less than 0.05 were considered significant.

RESULTS

Table 1 shows refractive error (REF, in diopters) and blood glucose (B/G, mg/dl) changes in control (non-diabetic) and diabetic rabbits after two weeks period. A significant increase in hyperopia from + 1.49 D (+/- 0.59) to + 2.00 D (+/- 0.52) was observed with increased blood glucose level after induction of diabetes.Table 2 shows the refractive error (REF) and blood glucose(BG) changes in alloxan-induced diabetic rabbits treated with twice daily doses of insulin for two weeks, showing a significant increase in hyperopia with decrease in blood glucose. Figure 1 is a graph showing correlation between refractive and blood glucose changes after a single 100mg/kg i.v dose alloxan exposure in rabbits. There was a significant positive correlation ($P = 0.01$) between hyperglycemicand hyperopic changes in the diabetic rabbits ($r = +0.913$, $P<0.05$). Figure 2 is a graph showing correlation between decreasing blood glucose and refractive error changes in insulin treated diabetic rabbits. There was no significant correlation between insulin-induced blood glucose and refractive changes in insulin treated diabetic rabbits ($r = +0.31$, $P > 0.05$)

DISCUSSION

The normal adult rabbits were found to be hyperopic at baseline (T0) with mean refractive error of $+1.49D(SD, \pm 0.52)$ and mean fasting blood glucose of 93.8 mg/dl(SD, ± 3.17) (Table 1). Alloxan treatment produced a significant and sustained hyperglycemia(mean FBG=293.8 mg/dl) and increased hyperopia to a mean of +2.00D(SD, +/-0.52) as seen over the two week post-induction period (T15,table1). The hyperopic refractive change correlated positively with the glycemic change (Figure 1) suggesting a possibility of the two conditions being driven by the same pathologic mechanisms. Previous investigations have reported hyperopic shift during hyperglycemia in diabetic patients[14]. However, studies by Duke-Elder[8], Gwinup and Villarreal[9] and Furushimaet et al[12]revealed a myopic shift in refraction in diabetic patients as well as in glucose-induced hyperglycemia in healthy subjects. Diabetes-induced acute myopia could be attributed to other factors such as dehydration and acid-base abnormalities. This link is evident by considering the pharmacologic properties of myopigenic drugs such as topiramate, thiazide diuretics, retinoic acid derivatives, etc.[5] Insulin treatment of alloxan-induced diabetic rabbits produced a significant reduction in blood glucose from 291.7 ± 42.9mg/dl to 136.0 ± 51.03mg/dl over a two week period ($P<0.05$, Table 2). This demonstrated the efficacy of human insulin in treating alloxan –induced diabetes in animals. There was also an increase in the hyperopic state (+2.00 +/-0.52D to +2.91+/-1.13D, $P<0.05$) with a maximum change of+1.50D (fig 2). However, there was no significant correlation between the hyperopic change and blood glucose reduction in

insulin treated diabetic animals (fig2). This could imply a difference in mechanisms for both the hypoglycaemic and refractive effect of insulin. A hyperopic shift during insulin therapy has also been reported by Okamoto et al.[17] and Lin et al[18]

Saito et al[19] have suggested that intracellular accumulation of glucose and its derivatives in the lens could lead to transient hyperopia as a result of osmotic-induced changes in refraction. Huntgens and O'Donnell[20] explained that hyperopic and myopic shifts during hyperglycemia could arise from different causes; the former as a result of decrease in the refractive index of the crystalline lens, while the latter is as a result of changes in corneal geometry such as alteration in corneal lens curvature, position and size. This explanation is further corroborated by Okamoto et al[17] who suggested that changes in the intraocular refractive components could cause a reduction in refractive index of the eye leading to hyperopia. However, other possible mechanisms are suggested.

Firstly, glucose entry into the lens is facilitated by transport proteins on the lens epithelium, such as GLUT1, which may be inhibited in diabetes due to insulin deficiency or glucose toxicity. The rate of glucose transport into the lens is not increased with increased extracellular glucose. Thus, hyperopia in diabetes cannot be explained based on intracellular accumulation of glucose or it's metabolite (sorbitol) as suggested by Saito et al.[19] The refractive power of the lens is dependent on the difference between refractive indices of aqueous humour and anterior lens. Increased glucose in aqueous humour increases its refractive index but reduces the differential refractive index and the lens refractive power. Secondly, diabetes and insulin have been associated with increased blood-retinal barrier breakdown leading to retinal oedema. This has been attributed to sodium retention or increased activity of vascular endothelial growth factors (VEGF).[21]Sodium retention and peripheral oedema is a known adverse effect of insulin and oral insulin secretagogues.[22] Accumulation of fluid underneath the central sensory retina could move the central retinal plane anteriorly with consequent axial shortening and hyperopia. Hernandez et al[23] had observed increased macular volume and thickness with blurred vision in diabetic patients under insulin therapy.Thirdly, alloxan-induced diabetes is also associated with decreased Na^+-K^+AtPase activity and increased corneal hydration.[24,25.] Sodium is an effective osmol and its retention in the cornea or lens could lead to osmotic changes and consequently hyperopic shift. Another possible mechanism of the hyperopic complication of diabetes and insulin therapy may be due activation of dopamine via the hypothalamic-pituitary-adrenocortical(HPA) axis[26]. The finding by Schartau et al[27] that dopamine (D_1) decreased the refractive power of Cichild fish lens could further support this hypothesis.

In conclusion, these findings indicate that a decrease in refractive power occurred early in the course of diabetes and during insulin therapy in rabbits. There was however no significant correlation between the hyperopic change in refraction and the hypoglycemic effect of insulin. This may suggest that the hyperopic shift may be due to metabolic changes other than blood glucose. For example, insulin therapy may lead to sodium retention, increased cornea and lens hydration with subsequent reduction in refractive index.[28]In long-lasting diabetes with marked hyperglycemia, the opposite may be the rule, whereby metabolic acidosis

and dehydration leads to myopia as has been reported with certain medications.

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TABLES

Table 1: Refractive error and blood glucose changes in diabetic and non- diabetic rabbits

Animal	Time	T_0	T_{15}	P-value
Control (n=6)	REF	+1.41(+/-0.22)	+1.31+/-0.24	>0.05
	B/G	92.3+/-2.66	95.0+/-6.39	>0.5
Experimental Group (n=12)	REF	+1.49+/-0.52	+2.00+/-0.52	<0.05
	B/G	93.8+/-3.17	293.8+/-31.5	<0.05

Table 2:Refractive error and blood glucose changes in alloxan-induced diabetic rabbits treated with insulin

Animal	Time	T_{15}	T_{30}	P-value
Diabetic control (n=6)	REF	+2.13+/-0.56	+1.90+/-0.50	>0.50
	BG	296.0+/-18.1	247.0+/-47.3	10.50
Insulin treated (n=6)	REF	+2.00+/-0.52	+2.91+/-1.13	<0.05
	BG	291.7+/-42.9	136.0+/-51.03	<0.05

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FIGURES

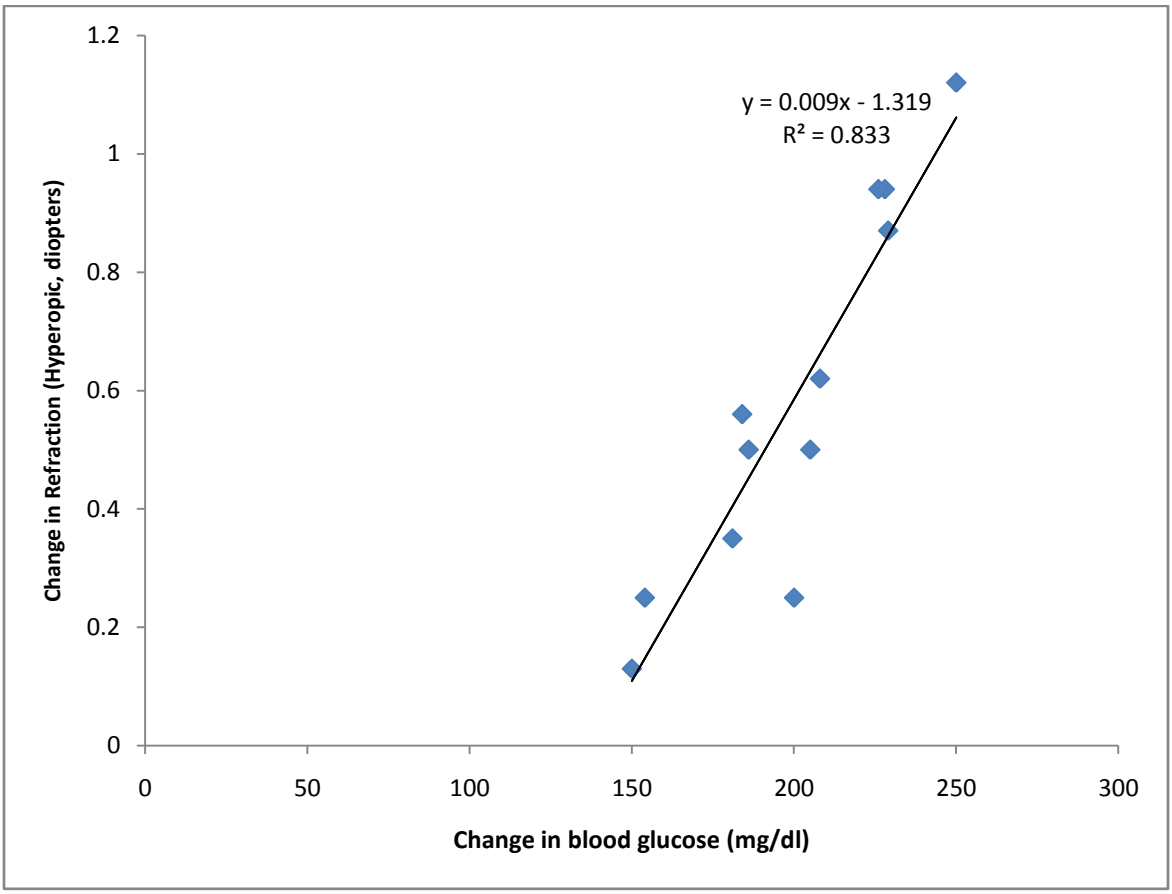

Figure 1: Correlation between refractive and blood glucose changes after a single 100mg/kg i.v dose alloxan exposure in rabbits.

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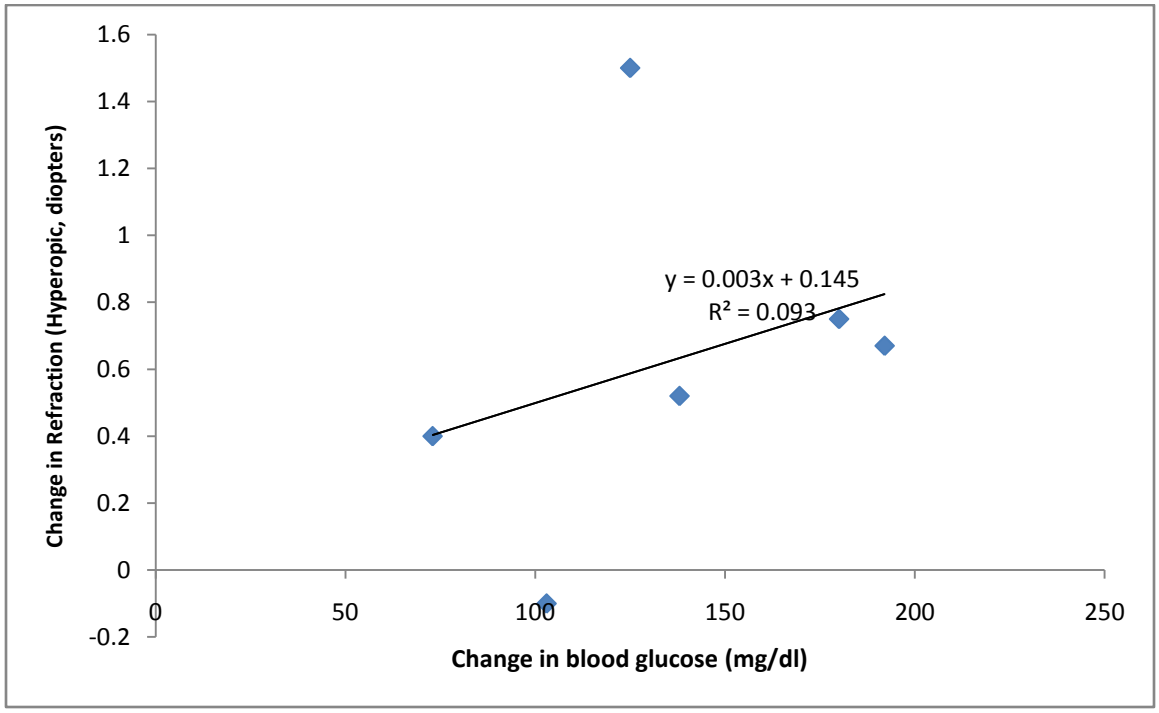

Figure 2: Correlation between decreasing blood glucose and refractive error changes in insulin treated diabetic rabbits