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Vitamin C improves basal metabolic rate and lipid profile in alloxan-induced diabetes mellitus in rats

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Diabetes mellitus (DM) is a multi-factorial disease which is characterized by hyperglycaemia, lipoprotein abnormalities and oxidative stress. This study evaluated effect of oral vitamin C administration on basal metabolic rate and lipid profile of alloxan-induced diabetic rats. Vitamin C was administered at 200 mg/kg body wt. by gavage for four weeks to diabetic rats after which the resting metabolic rate and plasma lipid profile was determined. The results showed that vitamin C administration significantly ($P<0.01$) reduced the resting metabolic rate in diabetic rats; and also lowered plasma triglyceride, total cholesterol and low-density lipoprotein cholesterol. These results suggest that the administration of vitamin C in this model of established diabetes mellitus might be beneficial for the restoration of basal metabolic rate and improvement of lipid profile. This may at least in part reduce the risk of cardiovascular events seen in diabetes mellitus.

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1. Introduction

Diabetes mellitus (DM) is a multifactorial disease which is characterized by hyperglycaemia, lipoprotein abnormalities (Scoppola *et al* 2001) and altered intermediary metabolism of major food substrates (Unwin *et al* 2001). In addition, generation of free radicals often worsen the complications of DM such as hypertension, atherosclerosis and micro-circulatory disorders (Caballero *et al* 1999; Sower and Melvin 1999). Changes in lipid levels and consequent disorders of lipid metabolism and stress have been observed in DM (Betteridge 1994).

Due to altered intermediary metabolism in DM, a feature present in both type 1 and type 2 DM, persons with type 2 DM, even in the absence of clinical cardiovascular disease, have a reduced maximal oxygen consumption compared with non-diabetic persons (Regensteiner *et al* 1995). In other studies, a reduced cardiac output during exercise in persons with DM and reduced maximal O_2 consumption during exercise have been reported (Roy *et al* 1989; Regensteiner *et al* 1998).

On the other hand, several studies have reported that the basal metabolic rate is increased in diabetic condition (Fransilla-Kallunki and Groop 1992; Avesani *et al* 2001). This increase is a reflection of the increased metabolic demands in DM.

Many minor components of food, such as minerals and antioxidant vitamins, have been shown to alter biological processes which may reduce the risk of chronic diseases in humans. For instance ascorbic acid, an aqueous phase antioxidant has been reported to improve whole body glucose disposal in healthy subjects and in diabetic patients (Paolisso *et al* 1994). Since vitamin C has been described to be beneficial in DM, the present study investigates contribution of antioxidant vitamin C in resting metabolic rate and lipid profile of alloxan-induced diabetes mellitus in rats, as there have been relatively few studies on this model.

Keywords. Ascorbic acid; diabetes mellitus; lipid profile; metabolic rate; vitamin C

Abbreviations used: BMR, Basal metabolic rate; DM, diabetes mellitus; HDL, high density lipoprotein; LDL, low density lipoprotein.

2. Materials and methods

2.1 *Animals*

Male Wistar rats, weighing approximately 120 g, were used for the study. The animals were obtained from animal house of the Department of Physiology, University of Calabar, Calabar, Nigeria. Approval for the study was obtained from the Animal Ethical Committee of the College of Medical Sciences. The rats were maintained under standard animal room conditions of temperature (29°C - 30°C) and a 12 h light/dark cycle. They were provided with feed and tap water *ad libitum*. The animals were divided into three groups of six rats each.

2.2 *Induction of DM*

Diabetes mellitus was induced according to the method of Sheweita *et al* (2002), in two groups of rats by intraperitoneal injection of 120 mg/kg body weight of alloxan (Sigma Chemical Co., Poole, UK) dissolved in normal saline. This dose was administered in the morning and repeated in the evening of the same day. Control rats received only normal saline. Diabetes was confirmed 48 h after injection in rats showing glycosuria using clinistix (Bayer Diagnostics, Mannheim, Germany) and fasting blood glucose of 11.0 mmol/l. Vitamin C (Sigma Chemicals Co. Poole, UK) was administered to one group of diabetic rats by gavage at a dose of 200 mg/kg body wt. daily for 8 weeks.

2.3 *Measurement of basal metabolic rate*

After eight weeks of vitamin C administration, basal metabolic rate was determined in all groups of rats using the method previously described (Osim *et al* 1994). This method involves adding water into the chamber to replace the amount of oxygen extracted from the system, thus, equilibrating the pressure within the system, using manometer to note pressure difference. Each rat was allowed an overnight fast. This we did by withdrawing food for 12 h prior to determination of basal metabolic rate. The measurement was done in the day between 10.00 and 14.00 h at room temperature (30°C) where animals were housed. Each rat was placed inside a metabolic chamber containing soda lime (Fischer Sci. Co., USA) to absorb carbon dioxide. The rat was allowed to settle down for about 5 min in the chamber before closing the lid. The chamber was closed and made airtight using silicon grease.

Normal breathing was allowed for 5 min from the time the system was made airtight. Thereafter, water was added into the system to restore pressure to the original level. The volume of oxygen extracted by the rat was determined based on the amount of water added into the system. Each measurement was repeated five times at 10 min intervals. The average value was determined and used to calculate the basal metabolic rate per hour. Records of the weight of each animal were taken after the experiment. Basal metabolic rate was calculated by dividing the volume of oxygen consumed per hour by the weight of the rat (Hoar and Hickman 1975).

2.4 *Biochemical assay*

After basal metabolic rate determination, the rats were euthanized by anaesthesia using chloroform vapour and dissected. Blood was collected from the heart by cardiac puncture and transferred into EDTA treated tubes immediately. Blood was then centrifuged at 4,000 *g* for 10 min to remove red blood cells and recover plasma. The plasma thus obtained was used for biochemical analysis.

Triglycerides and total cholesterol concentrations as well as high-density lipoprotein cholesterol were analysed using assay kits (purchased from Sclavo Division Diagnostici, Sienna, Italy). Blood glucose level was analysed by using automatic glucose analyser (Accu-Chek Advantage, Roche Diagnostics GmbH, Maannheim, Germany).

2.5 *Statistics*

The results obtained were analysed by one-way analysis of variance (ANOVA) using SPSS statistics software package (version 10) followed by post-hoc LSD test where F value was significant. All data are expressed as mean $\pm$ SEM. Differences between groups were considered significant at $P<0.05$ and 0.01.

3. Results

3.1 *Effect of vitamin C administration on basal metabolic rate*

The basal metabolic rate values in diabetic rats and control are presented in figure 1. The basal metabolic rate (BMR) in diabetic rats was 1.19 ± 0.15 ml/h/g, while the BMR in control rats was 0.76 ± 0.89 ml/h/g. The BMR value in diabetic rats treated with vitamin C was 0.77 ± 0.08 ml/h/g. The BMR value was significantly higher ($P<0.05$) in diabetic rats compared with control. Vitamin C supplementation significantly ($P<0.05$) reduced BMR in diabetic rats to a value comparable with the non-diabetic control rats.

3.2 *Effect of vitamin C on lipid profile*

The mean values of lipid profile, body wt. and blood glucose level in normal, diabetic and vitamin C treated diabetic rats

is presented in table 1. There was a significant increase (P<0.05) in fasting blood glucose and decrease (P<0.05) in body wt. in alloxan-diabetic rats when compared with normal control. Vitamin C administration significantly (P<0.05) reduced blood glucose level and improved the body wt.

Plasma triglyceride, total cholesterol and low density lipoprotein (LDL)-cholesterol concentrations were significantly (P<0.01) higher in untreated diabetic rats compared with the diabetic rats administered with vitamin C. The plasma lipid profile of diabetic rats administered with vitamin C were significantly (P<0.05) higher than the values of the normal control rats. However, the atherogenic index (ratio of LDL-cholesterol to high density lipoprotein (HDL)-cholesterol) was not significantly different in the three groups of rats.

4. Discussion

Diabetes mellitus is a metabolic disease associated with impaired glucose metabolism which in effect adversely alters intermediary metabolism of lipids and proteins. Formation of protein glycation products releases free radicals; subsequently causing oxidative stress (Ceriello and Motz 2004). Most of the complications of the diabetic state are initiated by the generation of free radicals: for instance LDH oxidative modification, leading to atherosclerosis (Felmenden *et al* 2003; Bhadki *et al* 2004) occurs only in the presence of free radicals. Basal metabolic rate, which represents energy expenditure at rest, is elevated (Avesani *et al* 2001; Nawata *et al* 2004). The rise in BMR is attributed to increased breakdown of lipids and proteins – as lipids have a higher concentrate of energy than do carbohydrates hence in their breakdown BMR is raised.

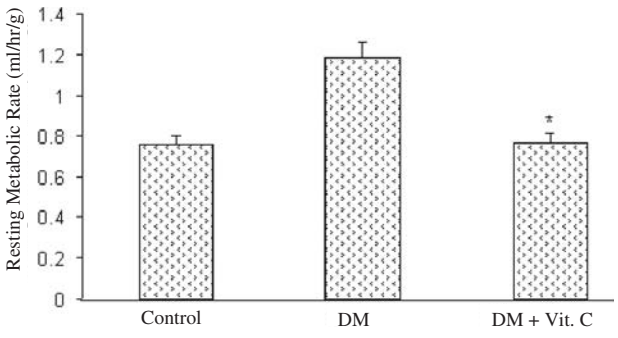

Figure 1. Mean resting metabolic rate in control, diabetic and diabetic treated (with vitamin C) rats).

Antioxidants have been described to ameliorate metabolic dysfunction observed in DM whether type 1 or type 2. We have in our study used alloxan to induce hyperglycaemic condition which is an initial signal in DM leading to altered metabolism, free radical generation and subsequent complications. In this generalized model which resemble type 1, we have administered antioxidant vitamin C. The results of this study indicate that the administration of vitamin C produced an improvement in the glycaemic status (decreased in glycose level), and affected lipidaemic control as observed in the reduction of lipid fraction in the diabetic group relative to control. Body energy expenditure was equally reduced.

BMR reflects energy expenditure at rest and it is the metabolic rate determined at rest 12 to 14 h after the last meal (Guyton and Hall 2006). In diabetic condition, BMR is raised (Fontvielli *et al* 1992; Avesani *et al* 2001). The present study shows that vitamin C supplementation reduced BMR in diabetic condition. Vitamin C supplementation decreasing metabolic rate in type 1 diabetic rats observed in our study agree in part with the previous studies. For instance, there is evidence that oxygen consumption is decreased in DM (Regensteiner *et al* 1995, 1998), and there is increased rate of glycolysis (Simoneau and Kelly 1997). The possible explanation for the decrease in BMR may be linked to the sparing of lipid degradation and oxidation as evidence in low level of this lipid fragment in the diabetic group supplemented with vitamin C. Since lipids have a higher concentrate of energy than do other energy substrates, the BMR tended to decrease.

Table 1. Lipid profile and glucose level in diabetic and control rats.

Parameters	Control	Diabetic group	Diabetic group + vitamin C
Triglyceride (mmol/l)	1.48 ± 0.35	4.26 ±0.16^{c}	2.2 ± 0.21
Total cholesterol (mmol/l)	1.9 ±0.29	9.2 ± 0.8^{c}	3.2 ± 0.9^{a}
HDL-cholesterol (mmol/l)	0.91 ± 0.14	3.4 ± 0.25^{c}	1.62 ± 0.17†
LDL-cholesterol (mmol/l)	1.64 ± 0.32	7.72 ±0.91^{c,†}	2.55 ± 0.86^{a}
HDL-cholesterol/LDL-cholesterol ratio	1.97 ± 0.45	2.38 ± 0.37	1.61 ± 0.46
Plasma glucose (mmol/l)	3.74 ± 1.0	12.6 ± 1.8^{b}	8.4 ± 0.5^{a,b}
Body weight (g)	157.0 ± 7.6	132.0 ±13.2^{b}	149.0± 3.0^{a}

^{a}P<0.05 compared with diabetic control, ^{b}P<0.05 compared with control and ^{c}P<0.001 vs control, $^{\dagger}P$<0.05 vs control.

Vitamin C is established biochemically as an antioxidant which mops up free radicals produced in the body. The decrease in free radical load consequently produces a decrease in complications of the diabetic state. Although our study did not measure qualitative lipoprotein modification or apolipoprotein interaction, there are reports however that show the ability of vitamin C to scavenge superoxide, hydrogen peroxide, hydroxyl radicals etc. in DM (Young and Woodside 2001).

Lipid profile, which is altered in diabetes state (Betteridge 1994), is one of the significant factors in development of cardiovascular diseases. Studies have shown that increased plasma triglyceride and cholesterol levels may be a risk factor for vascular disease (Kamata and Yamashita 1999; Kamata *et al* 2001; Shahar *et al* 2003). Also oxidative modification of LDL is an important step in the development of atherosclerosis (Felmeden *et al* 2003; Bhadki *et al* 2004). This oxidation is initiated and propagated by free radicals where antioxidants become depleted (Young and Woodside 2001; Kaviarasan *et al* 2005). In this study, vitamin C supplementation significantly reduced lipid profile in diabetic rats when compared to untreated diabetic rats. There was also a reduction in blood glucose level. This improvement in lipid profile in the present study is supported by previous studies (Anderson *et al* 1999; Kurowska *et al* 2000): that vitamin C prevents oxidation of LDL-cholesterol; decreases total and LDL-cholesterol and triglyceride; and also raises HDL-cholesterol level. The possible explanation for the hypocholesterolaemic effect of vitamin C is that vitamin C prevents LDL-cholesterol from oxidative damage and aids in degradation of cholesterol. Secondly, it has been suggested that this vitamin is needed by the enzyme in the first step of bile acid synthesis (cholesterol 7α-hydroxylase) by directing cholesterol towards bile acid synthesis it reduces its level in serum (White *et al* 1994). Kaviarasan *et al* (2005) reported that level of total cholesterol, triglyceride, lipid peroxidation and glucose increased in hyperlipidemic patients with or without DM whereas there was decreased plasma concentration of vitamin C and other antioxidants. Taking the above evidence together suggest that vitamin C supplementation improves the lipid profile in alloxan-induced DM in rats by acting through cholesterol 7α-hydroxylase to direct cholesterol into bile synthesis. Furthermore, by scavenging free radicals it decreases oxidative damage to oxidized LDL-cholesterol.

In conclusion, the results of the present study indicate that, in alloxan-induced DM in rats, the basal metabolic rate is raised and that lipid profile is altered adversely. The administration of vitamin C in this model of established DM might be beneficial for the restoration of BMR and improvement of lipid profile. This may at least in part reduce the risk of cardiovascular events seen in diabetes mellitus.

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