Sialic Acid and Lipid Profiles of Individuals with Diabetes Mellitus Type 2 and Cardiovascular Disease

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Authors' contributions

This work was carried out in collaboration between all authors. Author HA designed the study and wrote the first draft of the manuscript. Authors MS and SBS managed the sample collection and laboratory analyses. Author AAI managed the literature searches. Authors HA and AAI shared in data analysis and interpretation of results. Author HA was responsible for manuscript writing and final editing. All authors read and approved the final revised manuscript.

Original Research Article

ABSTRACT

Background: Diabetes Mellitus type 2 is among the top ranked non communicable diseases in Nigeria. There is an increasing incidence of this disease condition and its subsequent complications particularly cardiovascular complications. Sialic acid is a constituent of cell membrane and is present in most acute phase proteins. Several studies have indicated changes in sialic acid concentration in different pathological conditions and some studies in different populations of the world have suggested a link between sialic acid concentration, diabetes mellitus, lipid disorders and cardiovascular disease.
Aim: This study was carried out to determine sialic acid concentrations and lipid abnormalities in patients with diabetes mellitus type 2 with and without cardiovascular complications hypothesizing

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that sialic acid concentration will have an association with lipid parameters in diabetic groups.
Method of Study: Blood samples collected from 100 patients with diabetes mellitus type 2 and 50 apparently healthy individuals (controls) were subjected to sialic acid analysis by the Thiobarbituric acid assay and a lipid profile analysis where total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), triglyceride (TG) were evaluated. Test of significance was calculated by unpaired student's t test between study groups. Pearson's correlation was performed between the variables of serum sialic acid and serum lipid profile.
Results: Significance was set at $P=.05$. Significantly higher mean serum levels of sialic acid were found in diabetic groups ($P=.001$) as compared to the apparently healthy group with highest concentrations of sialic acid observed in the diabetic group with cardiovascular disease. However, no significant association was found between sialic acid levels with lipid parameters.
Conclusion: The findings in this study validate other studies linking elevated serum sialic acid levels with diabetes mellitus type 2 and diabetic cardiovascular disease. The study showed lipid abnormalities in all groups indicating rising incidence of diabetic dyslipidemia and dyslipidemia even within healthy population.

1. INTRODUCTION

Diabetes mellitus type 2 is one of the most prevalent non communicable diseases presently in Nigeria. There is an increasing incidence of this disease condition and its subsequent complications particularly cardiovascular and renal complications in our community. Studies have shown an association between serum sialic acid and cardiovascular mortality in general populations across the world. There is data suggesting a positive relationship between serum sialic acid, diabetes mellitus, stroke and cardiovascular mortality. Sialic acid is a terminal carbohydrate residue of the non-reducing end of oligosaccharide chains of glycoproteins and glycolipids in sera and tissues. More than 50% of sialic acid in serum is bound to the acute phase proteins. Serum sialic acid (N-acetyl neuraminic acid) concentration is a marker of the acute phase response, since many of the acute phase proteins (e.g. al-acid glycoprotein, fibrinogen and haptoglobin) are glycoproteins with sialic acid as the terminal sugar of the oligosaccharide chain, thus, sialic acid can be used as a measurement of the acute-phase response [1]. Serum sialic acid is also increased during inflammatory processes because of increased concentrations of richly sialylated acute phase glycoproteins [2]. Some prospective studies have investigated the relationship between sialic acid and risk of cardiovascular diseases and documented that circulating serum sialic acid, an inflammatory marker has been shown to be a strong predictor of cardiovascular mortality [3]. Nigam et al. [4] also reported an elevated serum concentration of sialic acid to be a strong predictor in the general population of cardiovascular death. In both men and women, serum concentration of sialic acid predicts coronary heart disease and stroke mortality both during short and long term (>15 years) follow-up, independently of serum total cholesterol level, diastolic blood pressure and body mass index [5]. Also several different mechanisms may lead to increased sialic acid concentrations in various pathological conditions, when combined with other markers, sialic acid concentrations could be helpful in disease screening and follow- up, as well as in monitoring of treatment. In the last few years, different workers all over the world have demonstrated that concentration of sialic acid in the human serum is abnormally high in a number of pathological states where the underlying pathology is either of tissue destruction, tissue proliferation, depolymerization or inflammation [6]. The clinical usefulness of serum sialic acid determination in inherited sialic acid storage diseases is well established. Thus, several different mechanisms may lead to increased sialic acid concentrations in various pathological conditions. However, several authors have reported that the non specificity of serum sialic acid limits its clinical usefulness. Nevertheless, when combined with other markers, sialic acid concentrations are helpful in disease screening and follow- up, as well as in monitoring of treatment [7]. Several population based studies conducted across the world have clearly demonstrated that an elevated serum sialic acid level is linked directly to various cardiovascular risk factors like increased body mass index (BMI) and increased lipid profile levels in diabetic patients [4]. Dyslipidemia is a well recognised and modifiable risk factor for cardiovascular diseases which is currently a leading cause of

morbidity and mortality world-wide [8]. The detection and treatment of dyslipidemia is now considered in most countries of the world as a means of reducing cardiovascular risks and hence the determination of serum lipid levels in people with diabetes is considered a standard of care. Oluyomi et al. [9] observed that lipid and lipoprotein levels vary widely depending on ethnic groups, genetic and environmental factors as well as severity and duration of the diabetic state in non-insulin-dependent patients and disturbances in lipoprotein transport and overt hyperlipidemia are frequently encountered in non insulin dependent diabetic mellitus and in persons at high risk of developing diabetes, such as those with impaired glucose tolerance. To the authors' knowledge, few studies have been carried out on sialic acid association with lipid parameters which are cardiovascular markers in this environment, and given the rising incidence of non communicable diseases in Nigeria such as diabetes, obesity, cardiovascular and renal diseases, there is need for additional studies to further validate other studies on the potential of sialic acid as a cardiovascular marker within the population group. The present study was carried out to determine sialic acid and lipid profile in individuals with diabetes mellitus type 2 with and without cardiovascular disease and attempt to evaluate the possible relationship between sialic acid levels and lipid parameters in diabetic patients.

2. MATERIALS AND METHODS

2.1 Study Area and Ethical Approval

The study was carried out at the Muhammadu Abdullahi Wase Specialist Hospital Kano, North Western Nigeria. Ethical approval was obtained from the ethical committee of the Ministry of Health Kano State Nigeria.

2.2 Study Subjects

A total of 150 individuals were enrolled for the study comprising 100 individuals diagnosed with Diabetes Mellitus (DM) type 2 attending endocrinology clinic and 50 apparently healthy individuals at the blood donor clinic of the Muhammadu Abdullahi Wase Specialist Hospital Kano, Nigeria. Diabetes was defined using standard World Health Organization criteria and diabetes mellitus type 2 was assigned if the subject is on oral hypoglycemics or hypoglycemic diet alone for more than 2 years. Apparently healthy individuals were recruited for the study

from the blood donor unit of the hospital. Informed consent was sought from the subjects as per declaration of Helsinki and baseline clinical details obtained from hospital records including basic demographic information.

2.3 Inclusion Criteria

Individuals with diabetes mellitus type 2 for ≥ 5 years without any diagnosis or symptoms of diabetes mellitus related complications specifically cardiovascular complications, also using clinical criteria such as present usage of oral hypoglycemic agents or a combination of insulin along with oral hypoglycemic agents who agreed to participate were included in the study. Also individuals with diabetes mellitus type 2 for ≥ 5 years with established cardiovascular complications obtained from hospital records and apparently healthy individuals with normal blood glucose levels who agreed to participate were also included in the study.

2.4 Exclusion Criteria

Individuals that were diabetic who declined to give consent as well as diabetic patients with renal complications and on lipid lowering medication were excluded from the study. For the control group, individuals that were diabetic, had cardiovascular complications and also on lipid lowering medication were excluded.

2.5 Sampling Techniques/ Data Collection

At the endocrinology clinic, demographic data and necessary medical history of subjects was obtained from hospital records following consent given by subjects. Basic demographic information was obtained from control group through personal interviews. Subjects were then divided into three groups. Group 1(n=50) –DM-CVD (diabetes mellitus type 2 with cardiovascular disease); Group 2 (n=50) –DM (diabetes mellitus type 2 without cardiovascular disease) and Group 3 (n=50) –Control (apparently healthy individuals).

2.6 Sample Collection and Processing

Exactly 4 ml of blood sample was collected from peripheral vein of each subject using a 5ml syringe and then dispensed into plain sample container without anticoagulant, allowed to clot and retract. Serum was extracted and then stored at -20℃ until needed for analyses.

2.7 Biochemical Analyses

2.7.1 Serum sialic acid determination

Free Serum Sialic Acid (FSSA) concentration was determined by the thiobarbituric acid (TBA) assay of Aminoff [10]. To 0.15 ml of serum, 0.25 ml periodic acid was added and incubated in a water bath at 37℃ for 30 mins. This was followed by addition of 0.1 ml of sodium arsenite solution to destroy excess periodate. One (1 ml) of TBA solution was then added and the mixture was heated in a boiling water bath for seven and half minutes after which it was cooled in an ice bath. Exactly 2.5 ml of acid-butanol mixture was added and the mixture was vigorously shaken to extract the chromophore. After which it was centrifuged at 1000 g for 5 minutes. The clear organic phase was then transferred to a cuvette and absorbance read at 549 nm against blank containing water instead of the sialic acid solution.

2.8 Lipid Profile Analyses

Each blood sample was analyzed for total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL), by enzymatic methods, using commercially available kits (Randox Laboratories Ltd., Crumlin England, UK).

2.9 Statistical Analysis

Data recording was done on Microsoft excel before being exported to Statistical Package for Social Sciences (SPSS) programme version 16 (Chicago, IL, USA). Analyses were carried out using both inferential and descriptive statistics with mean and standard deviations (SD) range and percentages. Microsoft Excel and Word in Windows 2007 were used for graphics and tables. The student t test and ANOVA were used to test for significant differences in means of various groups. Pearson's correlation was performed between the variables. All reported p-values =.05 were considered statistically significant.

3. RESULTS

3.1 Socio Demographic Characteristics

The mean age of study population was 48.07±13.81 years. The highest number of individuals was found in the 30-50 year age range (Fig. 1). Lowest number of individuals in the <30 years was found in the diabetic groups while the control group had the highest number of individuals in the <30 years group.

Gender distribution between groups showed a higher percentage of females in all the diabetic groups when compared to the control group (Fig. 2). Fifty two percent of the study population were females while forty eight percent were males.

3.2 Biochemical Analyses

The mean serum sialic acid concentration was 2.422±191 mg/ml for study population. There was significant difference (P=.001) between the three groups in sialic acid concentration with mean values of 4.833±1.076 mg/ml, 1.882±0.267 mg/ml and 0.551±0.230 mg/ml for the DM-CVD group, DM group and the control group respectively (Fig. 3).
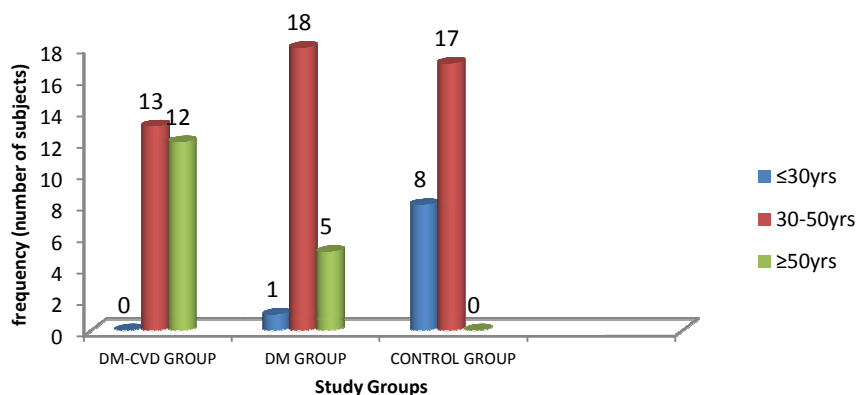

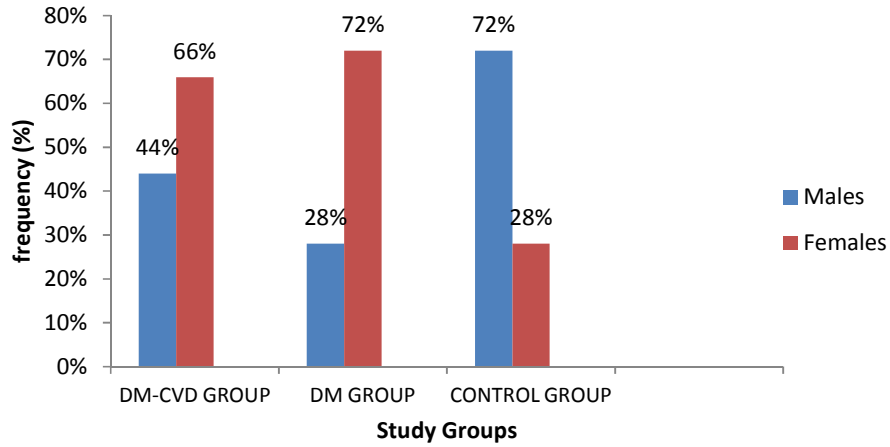**Fig. 1. Age distribution of study population based on decades across groups**

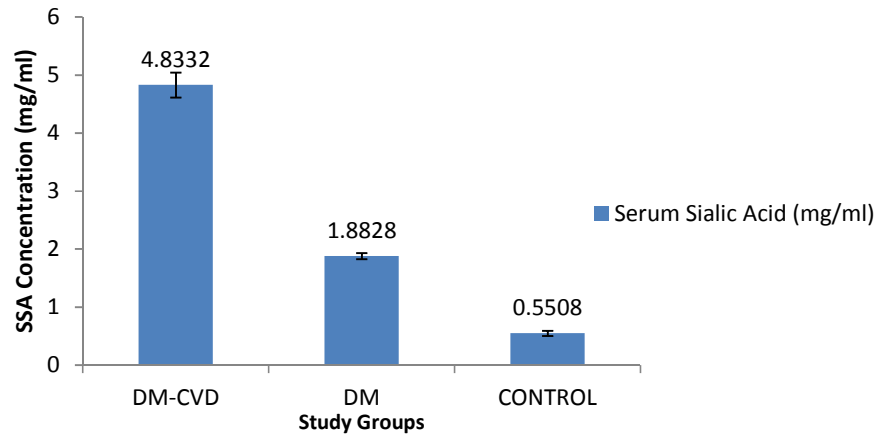**Fig. 2. Gender distribution across groups of study population**

Fig. 3. Serum sialic acid levels of study population
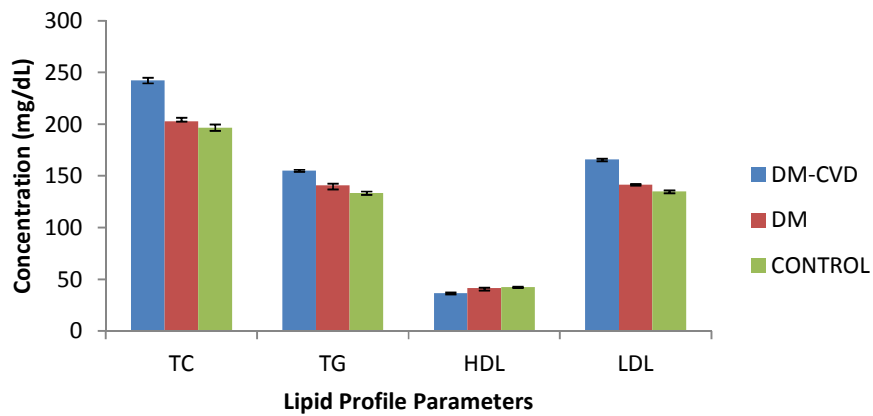*Data represent mean±SE. *p<0.05 and **p<0.01*

Fig. 4. Lipid profile parameters (TC, TG, HDL, and LDL) across study population

Table 1. Lipid profile parameters of diabetic and control subjects

Parameter	DM-CVD n=50	DM n=50	Control n=50
TC (mg/dL)	242.41±13.32[b]	202.79±18.21[a]	196.84±15.52[a]
TG (mg/dL)	155.19±5.07[b]	140.79±9.92[a]	133.59±7.90[a]
HDL (mg/dL)	36.64±5.05[b]	41.54±3.36[a]	42.56±3.05[a]
LDL (mg/dL)	165.75±56.31[b]	141.46±5.57[a]	134.88±6.66[c]

Mean ± SD values of lipid profile parameters in study population
a, b, c,=Data along the same row with different superscript alphabets are statistically significant (P=0.05)

The diabetic groups (DM-CVD and DM) had higher levels of TC, TG, LDL and lower levels of HDL compared to control group (Fig. 4). There was significant difference (*P*=.05) between the DM-CVD group and the other two groups (DM and control). However no significant difference (*P*=.05) in TC, TG and HDL was found between the control group and DM group (Table 1 above). No significant correlation (*P*=.05) was found between serum sialic acid level and all lipid profile parameters in all groups.

4. DISCUSSION

The higher number of individuals found in the 30-50 years age range in the diabetic groups compared to the control group with mean age of 48 years validates the fact that the onset of diabetes mellitus type 2 is usually from the third decade of life and that cardiovascular complications mostly occur above the fourth decade. This finding agrees with reports by several authors who recorded mean age of diabetic type 2 patients in the third decade range. Uttra et al. [11] found the mean age of subjects with diabetes mellitus type 2 was 53 years. The higher number of females found in the diabetic groups suggests that females in our environment may be more prone to diabetes mellitus type 2 than men or could be due to the fact that in some communities/societies females are more likely to seek treatment than males when encountered with medical issues. Ogbera et al. [12] reported more diabetic females (66%) than males (34%) in their study in Nigeria. In the control group, the higher number of males than females found could be attributed to the fact that the control group comprised a high number of blood donors from the donor clinic. The higher rate of male donors than females may be a result of screening criteria of the donors usually excluding females from blood donation. Higher levels of serum sialic acid (SSA) found in the diabetic groups (DM-CVD and DM) as compared to the non diabetic group (control) suggests a relationship between sialic acid concentration and diabetes mellitus type 2. Several authors

have documented that in diabetes mellitus type 2, the circulating sialic acid concentration is elevated in comparison with non diabetic subjects, especially in those with metabolic syndrome [13,14,15]. This has led to the hypothesis that a cytokine-induced acute-phase response is an integral part of the pathophysiology of this type of diabetes. Crook et al. [1] reported that though the mechanistic aspect of raised levels of serum sialic acid is not very clear, several possibilities have been suggested for elevated levels in diabetic patients. They postulated that there may be generalized endothelial cell dysfunction or macrovascular disease, either through loss of sialic acid containing glycoproteins from vascular cells into blood stream or through an acute phase response. Diabetes mellitus type 2 may be considered an acute phase disease, since in type 2 diabetes , even without tissue complications, the serum levels of acute phase proteins, C-reactive protein and haptoglobin are elevated [16]. Also since sialic acids are terminal sugars on oligosaccharide chains of glycoproteins which are components of membranes, elevated sialic acid levels may possibly indicate cell membrane damage of vascular tissues as seen in diabetes mellitus. The highest level of sialic acid concentration seen in the DM-CVD group validates studies that have indicated a positive association between sialic acid and cardiovascular disease and consequently mortality and has been proposed to be a long term predictor of coronary artery disease. Elevated total serum sialic acid concentration is a risk factor for cardiovascular mortality in humans. Englyst et al. [17] suggested that percentage of body fat , may also contribute to the higher levels of plasma sialic acid observed in people with diabetes mellitus type 2, although other factors may also be important and that circulating sialic acid is an independent risk factor for cardiovascular disease and is higher in people with diabetes mellitus type 2. Lindberg et al. [18] have shown that the predicting power of sialic acid for coronary heart disease is more or of the same magnitude to that of cholesterol. Some

studies have shown that serum sialic acid does play an important role in the pathogenesis of atherosclerosis and it could be useful in screening of population at cardiovascular risk. Further more, Lindberg et al. [19] showed that serum sialic acid may also reflect the existence or activity of an atherosclerotic process. Another explanation for the increased sialic acid level seen in the DM-CVD group could probably be as a result of inflammatory process or tissue destruction as seen in acute phase cardiovascular disease. Moreover, serum level of glycoproteins have been reported to increase in atherosclerosis which could lead to high sialic acid level since glycoproteins are richly sialylated. The presence of sialidase enzyme could also be a possible explanation for the increased sialic acid levels found in the DM-CVD group. Parkash et al. [20] documented an increase in the activity of sialidase enzyme in myocardial cell membrane surface. This enzyme causes hydrolytic release of α-glycosidically bound sialyl residue of sialoglycoconjugates and sialooligosaccharides and causes increase in sialic acid concentration in acute myocardial infarction. The authors demonstrated that increased plasma sialidase activity in these patients might be associated with clumps of desialylated erythrocytes that may alter blood flow in the capillaries. An association between serum total sialic acid and cardiovascular mortality in the general populations in different parts of the world has been found but the mechanisms underlying this are unknown [21]. Also, the higher sialic acid level found in the diabetic groups (DM and DM-CVD) could be as a result of aging since plasma sialic acid level increases with age. Many authors have found that plasma sialic acid levels increase as a function of age in humans. Mehdi et al. [22] noted that the increase in oxidative stress during aging causes increased sialic acid decomposition from erythrocyte membrane which may lead to altered biophysical property of the membrane affecting many enzymatic and transporter activities. In this study, though diabetic subjects (DM-CVD and DM) had higher mean levels of TC, TG, LDL and lower levels of HDL compared to controls, only the DM-CVD group had values of all lipid parameters above normal ranges further affirming the role of dyslipidemia in cardiovascular disease. Diabetic dyslipidemia which is characterized by elevated serum low-density lipoprotein (LDL), triglycerides (TG) and decreased high-density lipoprotein (HDL) is one of the major risk factors for cardiovascular disease. Ogbera et al. [12] reported that the prevalence of dyslipidemia in diabetes mellitus is 95% and that according to the CDC, 97% of adults with diabetes have one or more lipid abnormalities. The high triglyceride and high low density lipoprotein levels (above normal range) found in both the DM and control group, and the lack of statistically significant difference found in some of the lipid parameters between these two groups indicates the rising prevalence of lipid disorders in both diabetic individuals without complications and apparently healthy individuals in our community. This could probably be linked to diet and some lifestyle choices made by the larger population in the community probably as a result of socio economic status which predispose individuals to insulin resistance, dyslipidemia and cardiovascular disease. Maduka et al. [23] reported that diabetic individuals are more prone to dyslipidemia as compared to normal individuals therefore, the chance of mortality and morbidity is high in diabetic individuals and that geographic location, social and economic status of population affects the prevalence of dyslipidemia. The finding of the lack of association between sialic acid and lipid parameters in this study was unexpected as most reports have documented an association between elevated sialic acid levels, dyslipidemia and cardiovascular disease. Several workers have documented the correlation between serum TC, LDL-C and TG with serum sialic acid and revealed a significant positive correlation of sialic acid with TC, LDL-C and TG and a negative correlation with HDL-C [21,24,25]. The finding in this study however agrees with report from Crook et al. [1] who found no significant correlation between plasma sialic acid and coronary heart disease in both males and females with diabetes mellitus type 1. They reported no significant correlation between plasma sialic acid and coronary heart disease in either sex. Although reports of association between sialic acid and risk factors for cardiovascular diseases like lipid parameters have been reported, studies investigating the correlation between serum total sialic acid and severity of atherosclerosis are conflicting. Clinical and epidemiological studies indicate that serum total sialic acid is a marker of a sustained inflammatory response in cardiovascular disease, rather than causal in nature [21]. This study however has some limitations as it reflects only associations between blood lipid parameters and sialic acids in the study subjects, variables such as body mass index and waist circumference were not investigated, also the study groups could have been age and gender matched.

5. CONCLUSION

The findings in this study agree with reports of studies from other population cohorts in different parts of the world, linking high sialic acid levels with diabetes mellitus type 2 and cardiovascular disease as indicated by the higher levels seen in the diabetic groups particularly the DM-CVD group. The higher sialic acid levels in diabetic groups compared with the non diabetic group indicates a relationship between sialic acid concentration and diabetes mellitus type 2. While measurement of sialic acid levels in diabetics could possibly be used as a marker of cardiovascular disease as has been suggested by some workers , more studies using a larger population cohort need to be done as this may probably provide more insight into how these sugars could help in predicting diabetes and cardiovascular disease. There is also rising incidence of dyslipidemia in general healthy population.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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