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Impact of Age on Biochemical Parameters Associated with Cardiovascular Risk Factors in Growing Male Albino Rats

Bamidele Salau[1*], Charles Adeosun[1], Paulina Adeniji[2] and Adeleke Atunnise[3]

[1]Department of Chemical Sciences, College of Natural Sciences, Redeeemer's University, Ede, Osun State, Nigeria.
[2]Department of Hospitality and Tourism, College of Management Sciences, Redeeemer's University, Ede, Osun State, Nigeria.
[3]Department of Biochemistry, Obafemi Awolowo College of Health Sciences, Ikenne Campus, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author BS designed the work, data analysis, critically revised the manuscript and final approval for publication. Authors CA and PA did data acquisition and revised the manuscript. Author AA did data analysis and drafting of the manuscript. All authors read and approved the final manuscript

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ABSTRACT

Aims: This study investigated the cardiovascular risk factors associated with ageing in growing rats.
Place and Duration of Study: Department of Biochemistry, Olabisi Onabisi University, Ogun State, Nigeria. The study was carried out for twelve weeks.
Methodology: Twenty weanling albino rats strain were used to investigate the relationship between normal ageing process for twelve weeks and cardiovascular risk factors by assessment of some selected parameters such as antioxidant status, haematology and hemorheology, calcium content as well as lipid profile.

**Corresponding author: E-mail: delesalau@gmail.com, dele62@yahoo.com;*

Results: Significant increase ($p<0.05$) was seen in plasma uric acid, Total serum cholesterol, White Blood Cells, Packed Cell Volume, Plasma Viscosity, Fibrinogen, Serum calcium, Bone calcium, Teeth calcium and Fecal calcium; while significant reduction ($p<0.05$) was observed in High Density Lipoprotein (HDL cholesterol). No significant difference was observed in other parameters investigated.

Conclusion: This study revealed that ageing process may induce cardiovascular risk factors in normal rats by elevating some parameters in plasma lipids, oxidative stress as well as calcium content in the plasma indicating that the effect of ageing may be more profound on some parameters than others.

Keywords: Ageing; cardiovascular risk factors; lipid profiles; calcium and antioxidant.

1. INTRODUCTION

Ageing which usually starts after maturity, is an unpreventable physiological stage that every organism undergoes to reach terminal point (death). In higher animals it is characterized by physiological changes that are influenced either by endogenous agent like hormonal [1] or exogenous variables like environmental stress and illness, which may include smoking, alcohol abuse, infectious disease, malnutrition, poverty, lack of access to education, dangerous work conditions, violence, poor health care, injuries experienced early in life or throughout the life [2]; are the key players that facilitate the manifestation of ageing [3,4]. Though ageing may be referred to as inevitable natural occurrence that succeed maturity phase [4] however, it conveys the deterioration of major physiological processes and results in increased susceptibility to morbidity [4], which may contributes to the risk of many diseases, in turn advances to the development of other metabolic ailments such as diabetes, kidney disease and cardiovascular disease [5]. Some of the common chronic disorders experienced by the aged are hypertension, obesity, hypeglycemia and hyper-calcemia [6]. Though, these diseases overtly appeared in the aged, however, their gradual manifestation could be monitored through some biochemical parameters thus giving the opportunity of controlling these diseases through moderation of these parameters.

Cardiovascular disease, being the leading cause of sudden death around the World maintains anterior spot of health challenges [7]. In the past, it used to be rampant among the aged however, in last few decades its prevalence among young people especially children is dramatically increasing [8]. Although, there is general belief that ageing is not a disease, however, physiological changes caused by ageing are associated with increased blood cholesterol, increased body fat, decreased lean body mass and loss of bone density [9]. It increases the chances of dying by dramatically reducing the rate of survival or recovery from diseases [10]. In addition, a potentiating relationship may exist between ageing and disease because ageing amplifies the impact of diseases by weakening the immune system while disease promotes ageing process by causing injury to body tissue [11]. There are indications that ageing and diseases such as cardiovascular disease may take place spontaneously and cause damages to body systems [12].

Reports have shown that cardiovascular disease could potentiate ageing and vice versa; recently more reports have proved that ageing seem to be more and responsible for the exponential increase of cardiovascular disease [13]. Though, death from cardiovascular diseases may be sudden [14]. However, some parameters can be monitored before the full manifestation of the disease which includes lipid profiles [15], heamorrheological and haematological factors [16], antioxidant status [17] and calcium status [18]. However, these parameters associated with ageing vary considerably in time and magnitude. Therefore, this study investigated changes in these parameters as signs to normal ageing process in albino rats.

2. MATERIALS AND METHODS

Twenty, weanlings (4-6weeks old), males albino rats of the Wister strain ,weighing 48g -65g were used in this study; acclimatized for 2 weeks, fed water and rat chow *ad-libtum* for twelve weeks. The care and use of the animals were in accordance with the U.S. Public Health Service Guidelines [19].

2.1 Grouping of Animals

The experimental animals for this study were grouped and managed as shown below:

G1 rats made up of 10 male rats (Base line group) which were sacrificed at the onset of the investigation.

G2 rats made up of 10 male rats (Ageing group) were placed on commercial diet only and sacrificed at the completion of the investigation.

The baseline group, 10 rats, were taken, fasted overnight, sacrificed and used for various analyses to obtain baseline data. Ageing group (G2) rats were fed with commercial and the experimental diets, *ad libtum* for twelve weeks, anaesthetized at the end of week twelve after overnight fasting and the required tissues were removed for biochemical analyses. Bones were removed by scraping off the flesh from the bones and heads using clean razor blade. The carcasses were washed, oven dried and used for calcium analysis. Blood was collected for whole blood use while part of it was centrifuged for plasma collection. Fecal droppings and urine were collected for urea and fecal calcium determination.

2.2 Bioassay

2.2.1 Antioxidant and lipid peroxidation determination

Catalase activity was determined by method of Sinha [20] while, glutathione concentration and lipid peroxidation as Malondialdehyde (MDA) concentration were determined using methods of Beutler et al. [21] and Varshney and Kale [22] respectively. Uric acid and albumin concentrations were determined according Randox Laboratory kit purchased from Randox Laboratory Ltd, United Kingdom.

2.2.2 Lipid profile

Triglyceride, total cholesterol, HDL cholesterol were assayed according Randox laboratory kit. While LDL cholesterol and VLDL cholesterol were calculated using Friedwald [23] formulae.

2.2.3 Heamatology and hemorheology assessments

Red blood cells were counted by method of Kasper and Wallerstein [24]; white blood cells were determined using Booth and Thompson [25] method, while packed cell volume by method of Dacie and Lewis [26]. Plasma and blood viscosities by modified method of Reid and Ugwu [27], plasma fibrinogen by direct clot weight procedure as described by Ingram [28] and Erythrocyte sedimentation rate was determined by using Western green method of Westergreen [29].

Serum calcium was determined by using Randox kit purchased from UK laboratory while fecal, tooth and urine calcium were determined by method of Belcher and Nutler [30].

2.3 Statistical Analysis

Data were analysed by using T-test statistical method, while values are expressed as means and standard errors of mean for ten replicates.

3. RESULTS AND DISCUSSION

Physiological modulations span through the entire life of an organism. Relatively, major physiological transitional phase of an organism occurs at the early stage and it engrosses almost all metabolic machineries especially hormones, which modulates energy absorption from diet. Alteration in metabolic status with attendant risk factors such as in cardiovascular disease may propagate onset of ageing.

Results in Table 1 showed the antioxidant status of rats fed with standard diet for twelve weeks. Since ageing is gradual progressive phenomenon which spans over a period of time. The gradual accumulation of free radicals may be responsible for the significant ($p<0.05$) increase in plasma uric acid concentration observed in G2. Uric acid may be one of the first line defence components against age induced oxidative stress in mitochondrial [31,32]. Nieto et al. [33] suggested that hyperuricemia may be a compensatory mechanism to counteract oxidative damage related to atherosclerosis and aging in humans. Briviba, et al. [34] also suggested that there is a particular relationship between the site of uric acid formation and its need for biologically potent radical scavenger and antioxidant. Unlike uric acid, Catalase activities, reduced glutathione, malondialdehyde and albumin levels in ageing group (G2) did not change significantly ($p>0.05$), although, a further elongation of experimental period could suggest otherwise. Perhaps, body defence mechanism has the capability to mop-up cellular reactive oxygen species generated to an extent until the endogenous antioxidant machineries is over stressed [14].

Table 1. Plasma antioxidants and lipid peroxidation in experimental and control male albino rats

Group	Catalase activity (µmol/minute)	Glutathione (mg/dl)	MDA (mg/dl)	Uric Acid (mg/dl)	Albumin (g/dl)
G1	52.60 ±0.60	22.22±0.55	6.94±0.63	103.04±2.88[a]	4.09±1.02
G2	51.21±0.59	24.41±0.64	7.54±0.57	131.71±1.28[b]	3.50±0.94

Values are expressed as mean ± SEM for ten rats per group. Values with different superscripts are significantly different (p<0.05)

Table 2. Plasma lipid profiles in experimental and control male albino rats

Group	Triglyceride (mg/dl)	Total cholesterol (mg/dl)	HDL-Cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	VLDL cholesterol (mg/dl)
G1	134.25±0.57	78.60±0.70[a]	65.35±1.22[b]	21.51±1.27	26.98±0.09
G2	132.36±0.57	91.09±3.30[b]	60.56±1.27[a]	15.27±1.53	26.46±0.11

Values are expressed as mean ± SEM for ten rats per group. Values with different superscripts are significantly different (p<0.05)

Table 3. Haematology and hemorheology values in experimental and control male albino rats

Group	RBC (10^6/mm^3)	WBC (10^3/mm^3)	PCV (%)	Plasma viscosity	Blood viscosity	Fibrinogen (mg/dl)	ESR (mm/hr)
G1	5.40±0.83	3.30±0.00[a]	48.12±1.32[a]	1.46±0.01[a]	11.77±0.56	226.01±4.03[a]	3.41±0.07
G2	6.41±1.90	3.48± 0.01[b]	58.83±2.73[b]	1.76±0.01[b]	11.62±0.43	270.18±3.32[b]	3.75±0.06

Values are expressed as mean ± SEM for ten rats per group. Values with different superscripts are significantly different (p<0.05)

Table 4. Calcium status in experimental and control male albino rats

Group	Serum Ca^{2+} mg/dl	Bone Ca^{2+}mg/dl	Teeth Ca^{2+} mg/dl	Urinary Ca^{2+}mg/dl	Fecal Ca^{2+}mg/dl
G1	6.465±0.30[a]	61.50±1.20[a]	55.75±0.52[a]	2.61±0.57	16.09±0.61[a]
G2	8.302±0.43[b]	90.00±0.28[b]	89.39±0.97[b]	2.94±0.49	22.02±0.61[b]

Values are expressed as mean ± SEM for ten rats per group. Values with different superscripts are significantly different (p<0.05)

As shown in above Table 2, there were significant (p<0.05) increase in total cholesterol and significant (p<0.05) decrease in HDL cholesterol in G2 when compared to G1. This hyperlipidaemia observed in G2 could be the concomitant effect of cellular reactive oxygen species which force cholesterol to serve as endogenous antioxidant and ensued in the accumulation of cholesterol as well as the reduction in HDL cholesterol level [11,35,36]. Though, plasma triglycerides, LDL and VLDL cholesterol in G2 were not significantly different (p>0.05) from the G1.

A significant (p<0.05) increase in packed cell volume (PCV) and plasma viscosity was observed in ageing group (G2) when compared with baseline (G1) in above Table 3. Reduced thirst reduces the blood fluidity which may in turn explain the increased PCV [37]. Ageing has been reported to alter hormone secretion especially renin, an enzyme that regulate kidney functions in the process of water reabsorption as well as thirst, hence resulted in hypovolemia - a disorder characterised by decreased blood volume which is influenced by the amount of water intake as well as arterial size. Though, no significant difference (p>0.05) was observed in red blood cell (RBC), white blood cell (WBC), blood viscosity and erythrocyte sedimentation rate (ESR), however, fibrinogen level in G2 was significantly higher (p<0.05) than G1. The notable increase in fibrinogen level could be initiated by apoptotic tissue stimulated by plaque formation - a contributing factor to the narrowing of blood vessels. This may as well induce inflammation and consequently provoke fibrinogen formation [38]. No significant (p>0.05) difference was observed in erythrocyte sedimentation rate, this may be due to indifference in the component responsible for ESR such as blood viscosity and increase in plasma viscosity.

Plasma calcium level in G2 significantly ($p<0.05$) increased when compared with G1. This could be as a result of the deteriorating effect of ageing on body tissues including muscle mass. According to Houpt et al. packed cell volume reduced significantly when both food and water were withheld. Food deprivation or malabsorptions in GIT determine the proportions of thirst stimulation that can be attributed to plasma hypertonicity and to hypovolemia [37]. Also, less energy expenditure due to life style may impair hormonal secretion or/and gastrointestinal tract (GIT) resistance to GIT hormones, thus results to malabsorption essential minerals like calcium, in turn initiate hypercalcemia, a significant component of cardiovascular risk [1,39]. Furthermore, hypercalcemia may result to changes in cardiovascular hemodynamics such as reduced cardiac output and systemic hypertension. It may also play a role in functional reduction in some vital organs and evoke endothelial cell dysfunction which may enhance changes in vasoactive mediators resulting in increased atherosclerosis, hypertension and glomerulosclerosis [40].

Bone and teeth calcium contents in ageing group (G2) were also significantly ($p<0.05$) higher than weanling rats group (G1). Calcium plays a key role in a wide range of biologic functions, either in the form of its free ion or bound complexes. One of the most important functions as bound calcium is in skeletal mineralization. Non-bone calcium in the body are in constant and rapid exchange within the various calcium pools and is responsible for a wide range of essential functions, including extra- and intracellular signalling, nerve impulse transmission, and muscle contraction [41]. However, serum calcium may induce toxicity if the concentration of serum ionized calcium is not tightly maintained within a physiologic range [41]. Change in calcium status may adversely affect the effects of calcium toxicity include increased clothing time, osteoporosis, chronic kidney disease (CKD), Alzheimer disease as a result of poor signal transmission among others [42].

Weakened skeletal structure is one of physiological defects associated with ageing and accounts for impaired skeletal support and mobility. Contrarily, a significant increase ($p<0.05$) in bone calcium was recorded in G2 compared to G1. Though, decalcification and demineralisation caused by ageing may have set in but in an insignificant manner, while weanling rats (G1) may have not attained their maximum calcification and mineralization state hence, peak bone mass seemed less appearing. Hypercalceuria noted in G2 may either be the consequence of hypercalcemia-induced homeostasis imbalance or impaired kidney function induced by reactive oxygen species (ROS) release during mitochondria metabolism. Epithelial layer of the gastric tracts are also subject to ageing which may lead to reduced signal transmission [43] as well as resistance to hormones such as vasopressin [44]. While fecal calcium content significant elevation ($p<0.05$) in G2 may be due to depleted absorption of calcium in the gastric tract (GIT) in turn, altering endogenous cholecalciferol synthesis that promotes exogenous calcium absorption across the epithelial layer of the digestive tracts which may be impaired as a result of degenerative event of dermal tissue. Bar and Hurwitz, (1981) reported that severe dietary calcium restriction resulted in a lower 1-hydroxylase activity as well as a duodenal Calcium-binding protein [45]. Also, it has been reported that deprivation of food intake may reduced thirst as a tool in plasma osmoregulation [46]. Meal size (satiation) is affected by various factors including olfactory stimuli [47], stretch receptor signals originating in the stomach and proximal small intestine [48-50], nutrients (glucose, amino acids), metabolites (lactate, pyruvate and ketones) and alterations in gut hormones including cholecystokinin (CCK), glucagon like peptide -1 (GLP-1) and ghrelin in response to nutrient ingestion.

4. CONCLUSION

Ageing remains a progressive deterioration that occurs in all living system. Though, variation in its manifestation is subjected to ranging factors such as environmental stress, rate of cellular metabolism, and genetics among others. The present study indicates that ageing is associated with some biochemical parameters that are indices of cardiovascular risks. Among the parameters are uric acid, fibrinogen, white blood cells count, packed cells volumes, plasma cholesterol, calcium and HDL-cholesterol that occur earlier than other parameters. Thus, they may be good tools of assessing ageing and cardiovascular disease.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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