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AGEING IN AN ELDERLY GHANAIAN POPULATION; A CROSS SECTIONAL STUDY OF PHYSIOLOGICAL PARAMETERS, FOXO3A GENETIC VARIABILITY AND OXIDATIVE STRESS

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DECLARATION

I , Naa Adorkor Addy Sodzi-Tettey, declare that except for other people's investigations which have been duly acknowledged, this work is the result of my own original research, and that this dissertation, either in whole or in part has not been presented elsewhere for another degree.

……..

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DEDICATION

Dedicated to Team Sodzi

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ABSTRACT

Brief background

Worldwide, the population of the elderly has tripled in the last century. Projections indicate that the global population of persons aged 60 years and over, which was 11% in 2000 will reach 22% by 2050. Ageing is a gradual process with various determinants many of which are modifiable. Functional systemic changes, together with the effect of the environment, nutrition, lifestyle and genome of a person may insidiously lead to the development of chronic age-associated diseases. Biomarkers of ageing have been found to give good indication of the extent of ageing of the human body.

Aim

To compare selected physiological parameters in elderly Ghanaians with chronic illness to others without chronic illness and ascertain the presence of FOXO3a genetic variations which have been associated with longevity.

Methodology

Elderly Ghanaians aged 50 years and over were selected by purposive sampling based on specific inclusion and exclusion criteria. Selected physiological and anthropometric measurements were done. Blood samples were collected from participants for haematological examination and genetic studies. A questionnaire was administered to each participant to assess socioeconomic status, self-reported health status, general habits and cognitive function. The data collected was summarized in descriptive and analytical terms from which conclusions were drawn.

FINDINGS AND CONCLUSIONS

An average of 76% of the population studied showed abnormalities in the following parameters: Systolic and Diastolic Blood Pressure, Body mass index, Lower-limb weakness, Eye-sight or low Haemoglobin level. Fifty percent of respondents had anemia, 30.5% had blood pressure greater than 140/90 mmHg and BMI showed a significant decline with age. Worsening of eyesight, (85.9%), weakening of lower limbs, (50.8%), and hearing impairment (39.1%) were the most prevalent self-reported health parameters. The percentage of females who were found to have some deteriorated cognitive function was significantly higher than males (19.6% vs. 2.8%, $p=0.016$, Chi-square test). The results showed a positive correlation between the Mean Arterial Pressure and SOD activity. The three FOXO3a variants studied were found to exist in the Ghanaian population.

The Comet Assay demonstrated mechanical deterioration in DNA in young adults and in the elderly.

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LIST OF ABBREVIATIONS

APOE	Apolipoprotein E
BMI	Body Mass Index
BP	Blood Pressure
DNA	Deoxyribonucleic Acid
EDTA	Ethylene di-amine tetraacetic acid
FEV_1	Forced expiratory volume in one second
FOXO3A	Forkhead box O3A gene
Hb	Haemoglobin
IIS-pathway	Insulin-Insulin-like Growth Factor1 - pathway
IGF	Insulin-like growth factor
LMIC	Low and middle income countries
NGO	Non-Governmental Organization
NHIS	National Health Insurance Scheme
PEF	Peak expiratory flow
ROS	Reactive Oxygen Species
SOD	Superoxide dismutase
SPMSQ	Short portable mental status questionnaire
SRH	Self-Reported Health

SSNIT	Social Security and National Insurance Trust
WBC	White Blood Cells
WHO	World Health Organization
NHLBI	National Heart, Lung and Blood Institute
USA	United States of America

CHAPTER 1

1.0 BACKGROUND

Human longevity is a complex phenomenon. Genome, environmental factors such as exposure to high and low frequency radiation, poisons and toxins, diet and nutrition, psychological state as well as acute and chronic illness with the accompanying oxidative stress, inflammation and neurodegenerative changes all play roles in how long a person lives, the quality of life obtained and sustained and the extent to which the body changes with ageing (Kenyon 2010; Caballero and Coto-Montes, 2012).

Chronological age (in days, months, years) is determined by counting from the time of birth to the point of reference (Merriam Webster Medical Definition, 2013).

Chronological age, however, does not automatically reflect ageing of physiological systems and is not an accurate determinant of the state of health of an individual or an organ. There are variations in physiological deterioration of different organs and systems with age, in the presence or absence of disease (Fries, 2002). Many questions arise on the scientific explanation for the inconsistencies in the ageing process. Answers may be found in the role of the genetic composition of the individual, variations in effectiveness of the oxidative stress mechanisms and metabolic processes or differences in situation and lifestyle.

Distinguishing between normal attrition of function with ageing and the loss of function which accompanies pathological changes from diseases associated with longevity is often a challenge. Failing to recognize a normal change with ageing results in subjecting the elderly to barrage of tests and medications which they do not need and attributing

manageable complaints to ageing prevents proper diagnosis and health care management of the elderly (Minto and Biccard, 2013).

In Ghana, the elderly are receiving more attention now than ever before. The National Ageing Policy, revised in 2010, mentions "Old age and health challenges" as one of the important issues regarding the elderly and states "Improving Health, Nutrition and Well-Being of Older Persons" as part of its policies and strategies. The National Health Insurance Scheme covers persons aged 70 years and above without premium payment (Ministry of Employment and Social Welfare Ghana, 2010).

HelpAge Ghana is an NGO whose goal is to champion the well-being of all old people in the country. Established in 1988 it is a full member of HelpAge International, London. Help Age Centers were used for data collection because they are numerous, easy to find and have resting areas and washrooms for participants. Management of Help Age Ghana was willing to have the project sited at their facilities without limiting participation to HelpAge members.

1.2 Problem Statement

Currently, there are 810 million persons (one out of every nine persons) aged 60 years and above in the world (United Nations, 2012). Projections show that this number will rise to 2 billion (one out of every five) by 2050 (United Nations, 2012) with the population of the elderly in less developed regions is growing faster than in the developed countries (United Nations, 2012).

With the increasing aged population will come the increased incidence of chronic age-associated diseases such as coronary heart disease, diabetes, cerebrovascular accidents, Alzheimer's disease etc. Age-related diseases decrease quality of life while increasing the socio-economic burden of caring for the aged.

There is inadequate data on the lifestyle and challenges of the aged in Ghana. There is an acute paucity of genetic data on possible inheritance of longevity in the Ghanaian. There is no data on how genetic variations in the FOXO3a gene, which has been found to be linked to longevity in other populations (Flachsbart et al, 2008) correlates with ageing in Ghana. Such data is needed for the building of models of ageing for the development of targeted strategies to prevent or manage age-related diseases.

1.3 Justification

Justification of this study lies in the quest to understand the ageing phenomenon so as to be able to intervene as needed for successful ageing. A person should have the benefit of looking forward to a future, age notwithstanding. In order to give to the community benefits of experience in an active manner the older person must be in reasonable health. The prudent way of intervening and helping the older person medically for example, lies in knowing the mechanism of ageing in both specific and general terms. Gathering and studying information on the elderly will contribute to comprehension of the ageing process and help in the identification of individuals who are likely to develop certain age-associated diseases in order to put in early preventive mechanisms, facilitate development of interventions which can delay onset of age-associated diseases and enable adequate preparation for old age. Effective interventions for the ailing Ghanaian elderly call for investigations on ageing mechanisms conducted in local populations. The approach used here integrates clinical physiological parameters with biochemical analysis and molecular studies.

1.4 Study hypotheses

a. There are no significant differences in physiological parameters in the elderly Ghanaian with and without chronic illness.

b. FOXO3a genetic variations which have been associated with longevity in other populations are absent in Ghanaians.

1.5 Aim

To compare selected physiological parameters in elderly male and female Ghanaians with chronic illness to elderly Ghanaians without chronic illness and ascertain the presence of some FOXO3a genetic variations.

1.6 Specific Objectives

1. To assess changes in physiological parameters with ageing in an elderly Ghanaian population.
2. To compare selected physiological and biochemical parameters in the elderly Ghanaian population with and without chronic illness
3. To determine FOXO3a genetic variation in the elderly with and without chronic illness.
4. To demonstrate mechanical ageing of cells with the Comet Assay.

CHAPTER 2

LITERATURE REVIEW

2.1 Ageing

Ageing is a natural and inevitable process in living biological systems. In the case of the human, changes occur from the cellular entity at the time of conception till the time of death. In the developing embryo these changes are termed embryogenesis, in the newborn through infancy and puberty to maturation in early adulthood it is termed growth. It is believed that most systems function at their very best during early adulthood, as demonstrated by the peak period in the life of energetic sports persons after which a gradual decline in function of systems begins. Embryogenesis, puberty maturation, adulthood and beyond are all stages in ageing. Even in the face of multiple mechanisms of maintenance and repair the body undergoes definite deteriorative changes as it lives (Rattan, 2013).

In adulthood there is a plateau phase during which the body is able to maintain homeostatic stability with great functional reserve capacity. That notwithstanding, gradual asymptomatic deterioration of organs and systems occur (Dodds, 2012). This deterioration is contained so well by homeostasis and functional reserve that there are no known milestones of ageing during the adult period. As such, severe distortions such as impaired glucose control can go on for years without notice until the development of frank diabetes (Fries, 2002). The difficulty in the recognition of signs and symptoms of ageing brings us to the multidimensional notion of successful ageing.

The 1987 model of successful ageing by Rowe and Kahn (Rowe and Kahn, 1987) described three components of ageing

a. Freedom from disease and disability
b. High cognitive and physical functioning
c. Social and productive engagement.

Ann Bowling and Paul Dieppe define successful ageing in terms of both biomedical and psychosocial factors (Bowling and Dieppe, 2005). The biomedical approach defines successful ageing in terms of absence of disease with good physical and mental functioning whiles the socio-psychological is more about life satisfaction, social functions and participation. Naturally, such a definition will have some variations in different cultures. A Ghanaian model defining successful ageing is yet to be established.

The concept of successful ageing can be traced to as far back as the 1950's through to the 1980's when ideas on positive ageing were popularized, removing the stigmatization of the aged and stopping the characterization of the aged as burdens and repositories of disease (Tyas *et al*., 2007, Depp and Jeste, 2006). In Ghana, we still have some amount of stigmatization of the aged with existing witches' camps and occasional lynching (Adinkrah, 2004)

Crowther and associates in 2002, proposed the concept of positive spirituality as an enhancement of the Rowe and Kahn model (Crowther *et al*, 2002). This concept, in combination with the Rowe and Kahn model can be used in the determination of successful ageing in Ghana.

Successful ageing has become a subject of major concern in the world today. This is the age of development and marketing of all sorts of dietary regimes, dietary supplements, lifestyle techniques and claims touted to augment successful ageing. The anti-ageing industry has grown into a multi-million dollar industry with a growth of anti-ageing clinics in developed countries (Cardona, 2008).

2.2 Longevity

Longevity is the duration of life.

The average length of life has increased all over the world. Advances in medical services which have improved the diagnosis and management of both acute and chronic illnesses and general improvements in lifestyle and nutrition account for this (Fries, 2002). Therefore, although the maximum lifespan does not seem to have increased there are more old people in the world than 100 years ago (Fries, 2002).

There are major differences in life expectancy between males and females and between developed and developing countries. Generally, black people and men have shorter lives and people living in developing countries have lower life expectancies than those in developing countries (United Nations, 2010).

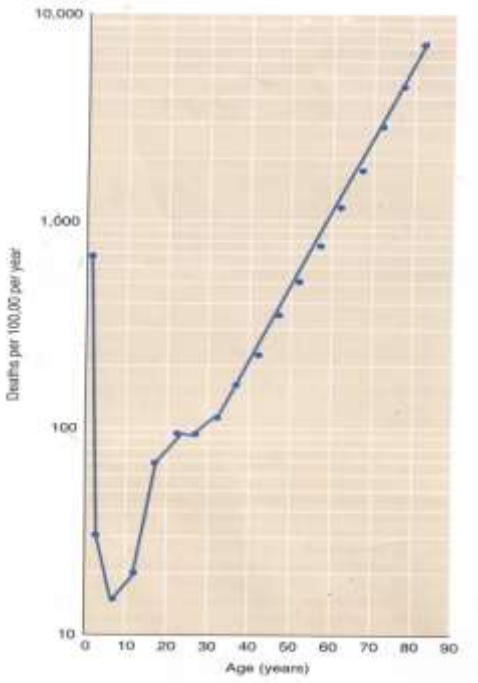
Life expectancy has risen to an unprecedented high in developed countries and continues to rise with Japan at the top of the United Nations Life expectancy list at 82.6 years (United Nations, 2007). It is noteworthy that African countries dominate the lower half of the list. Life expectancy in Ghana has moved from 53.1 years in 1980 to 64.2 years in 2011 (Ghana Statistical Service, 2011). Life expectancy at birth is a measure of overall quality of life in a country and summarizes the mortality at all ages (Centers for disease control USA, 2011). It is influenced by mortality figures of all age groups. Developing countries, which have the worst neonatal and infant mortality figures, are the ones with the lowest life expectancy (Salomon et al, 2013).

A parameter which measures length of life once adulthood is attained will give a better picture of the pattern of ageing in a group of people. In 1825, Benjamin Gompertz (Boron and Boulpaep, 2002) defined what he called the human age-specific death rate – the fraction of the population entering an age interval that dies during the age interval. He found that after early adulthood, the age-specific death rate increases exponentially with increasing adult age – the mortality curve. This implies that the older one grows, the greater the chances of dying. Based on this, it has generally been accepted that the Gompertzian slope reflects the rate of population ageing.

The rate of ageing of a given population can be quantified by calculating the slope of the mortality curve of the population. This approach has major challenges, one being its failure to take the cause of death due to a natural disaster or war into consideration.

Fig 2.1. The Gompertzian Curve

Age Specific mortality for the US Population for 2002. Data are projections from the US Population census 2002 (Boron and Boulpaep, Medical Physiology, 2nd edition, page 1283).0244089749



2.3 Demography

Globally, age 60 years is regarded as the lower chronological age threshold for the elderly. However, age 50 years is increasingly being used as the lower threshold by African gerontologists (WHO, 2000) who argue that life expectancy at birth in Sub Saharan Africa is typically ten or more years lower than in developed regions, and that the social construction of old age are set at a younger age (Kinsella and Phillips, 2005).

In Ghana, persons aged 60 years and above made up only 4% of the population in 1950. Currently they make up about 7.4% calculated from the 2010 National Population Census and the figure is projected to rise to 12 % by 2050 (United Nations Population Division, 2011). As such within 100 years the population of the elderly in Ghana is expected to triple.

Thus, the classic population pyramid as in Fig 2.2 below, Ghana 1990, is changing as seen in Fig 2.3 Ghana 2010, and is projected to look like Fig 2.4, Ghana 2050.

Fig 2.2. Ghana, 1990.

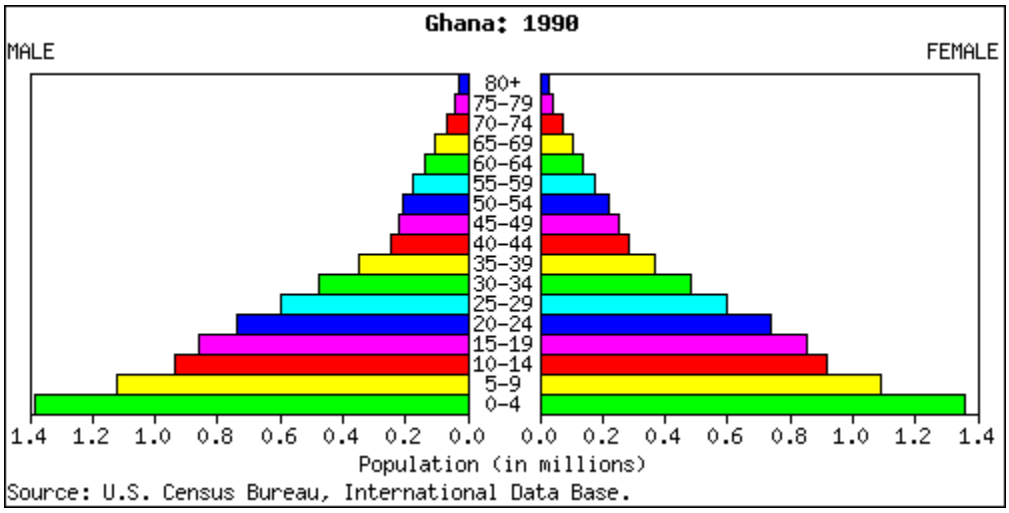


Fig 2.3. Ghana, 2010.

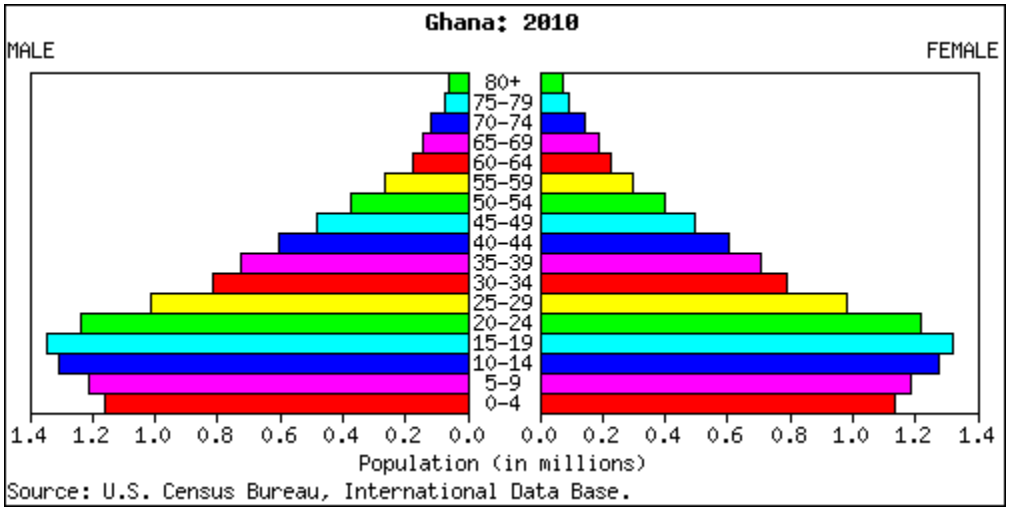
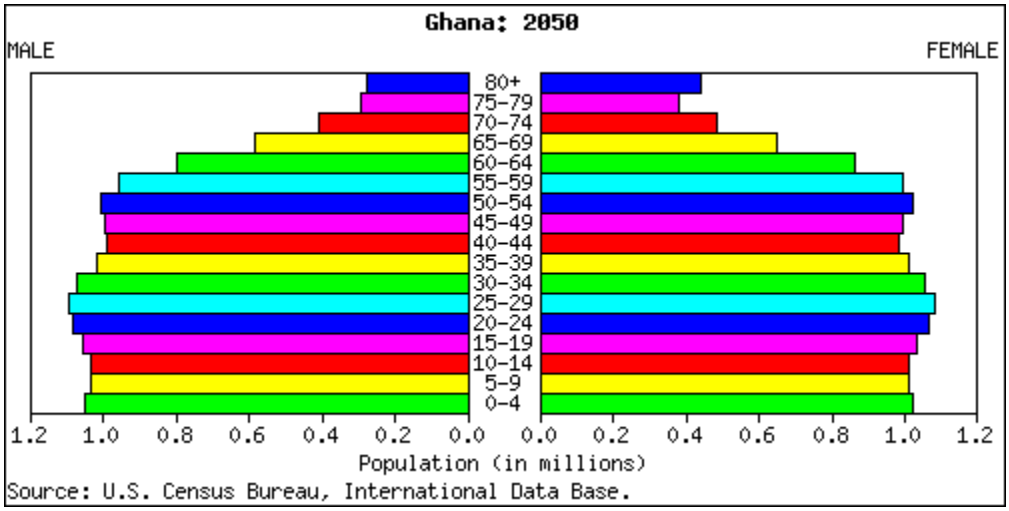


Fig 2.4. Ghana, 2050 (projected).



http://www.nationmaster.com/country/gh/Age_distribution

Healthcare

Healthcare is a major issue for old people as they have a greater incidence of chronic illness. Currently in Ghana there are no public healthcare facilities with geriatric departments. Geriatric health professionals are scarce and difficulties in assessing healthcare by the aged are yet to be addressed.

The National Health Insurance Scheme (NHIS) is of great help to the aged (source: Help Age Ghana, NGO). As of December 2010, 70% of the general population had registered with NHIS of which 34% were active card bearers (National Health Insurance Authority, 2010); Blanchet et al, 2012). In SAGE Ghana (2007/08), findings, only 26.8% had access to health insurance (Biritwum *et al*, 2013).

Income

The Social Security and National Insurance Trust (SSNIT) is the largest pension scheme in Ghana. It caters for only 17,229 people and they must have worked in the formal sector (public or private) to qualify. SSNIT did not have a scheme for the informal sector until 2005 when it started a flexible pilot project for informal workers. According to SSNIT's 2010 annual report, beneficiaries get between 26 and 1,250-plus dollars a month. Over 50% of beneficiaries receive less than 62 United States dollars a month. Therefore, majority of old people in Ghana are not on this scheme and the majority of those who are on it receive pensions which are too low to cater for their needs. The social support system made up of the extended family and religious institutions is largely responsible for sustaining old people in this country (Ofei-Kwapong, 2013).

Self-rated health (SRH)

Self-rated health is asking an individual to evaluate his/her own health on a 2, 3, 4 or 5 pointed scale or to compare his/her health with that of others, usually peers. (Jylha, 2009). It is recommended as a standard part of health surveys and also as a tool for disease risk screening and clinical trials (Robine, et al, 2003; WHO, 1996).

It is not known whether cognition is able to pick up sensation from the physiological and immune state of the body or that the answer given on self-rated health is just based on what one knows of or has been told of his or her own health. It has been found that people rate their health in a particular way based on different things (Obare, 2007). In the face of these challenges association of self-rated health with mortality has been seen in people from different ethnic backgrounds, in young people (Larsen et al, 2002) and in 90 year olds (Nybo et al., 2005) so it continues to be a useful tool.

Forty-two point five percent of rural elderly Ghanaians in the Kassena-Nankana district rated their health as good, 43.3% rated it as moderate and 14.2% rated their health as poor (Amon et al, 2011).

Household characteristics

Household characteristics are described by Biritwum and associates, 2013, as fundamental determinants of the health and wellbeing of the aged population. Results from SAGE Ghana Wave 1 study showed that 11 percent of households had only one member. Large households made up of 6 – 10 members made up 34.8 percent and households with two or five members made up 47.2 percent. Biritwum et al also reported that only 14.9 percent of households had good sanitation (Biritwum et al, 2013).

2.4 Theories of aging

Historically, theories on ageing have been numerous and diverse. In AD 129, Galen in his book *De sanitate tuenda* thought that ageing was due to change in body humours that begin in early life and cause a slow increase in dryness and coldness of the body. Roger Bacon was one of the first to suggest a wear and tear theory in ageing (Schroots and Birren, 1988). Charles Darwin attributed ageing to the loss of irritability in the nervous and muscular tissue (Schroots and Birren. 1988).

In modern times, there are three main biological theories of ageing.

1. Genetic cellular theories.
2. Non-genetic cellular theories.
3. Physiological system theories.

2.4.1 Genetic cellular theories.

These indicate that there are components in the ageing process which are passed through DNA from generation to generation. The syntheses of proteins which are required to maintain life are under the control of DNA and therefore depend on DNA integrity.

Induction of apoptosis, cell-cycle arrest and stress resistance are all under genetic influence (Tothova et al, 2007). Damage to DNA by radiation, free radicals etc may affect the ageing process (Sahin and DePinho, 2010). Mutations in DNA have also been found to be the underlying cause of ageing syndromes such as progeria (caused by mutations in the lamin A protein gene on chromosome 1), (Dreesen and Stewart, 2011).

Substantial evidence (Flachsbart et al, 2008) suggests that the lifespan of organisms is under genetic control. The relatively fixed life spans observed in mammals is well noted. Maximum lifespan of man remains essentially the same at about 120 years. The longest documented human lifespan is that of Jean Calment (1875-1997) of France, who died at the age of 122 years, 164 days; (Allaro *et al*., 1998).

The diversity which exists in longevity of different species lends credence to the notion of genetic control of lifespan. Further, the mechanisms influencing lifespan have been studied extensively in lower organisms with shorter lifespan - *Caenorhabditis elegans, Saccharomyces cerevisae, Drosophila melanogaster.* Hundreds of genetic variants that lead to life extension in these lower animals have been identified (Guarente *et al*., 2000; Patridge *et al*., 2002). These encourage human longevity genetic investigations.

Additionally, research suggests that longevity runs in families. Both parents and siblings of centenarians have been shown to have significantly longer life expectancies than the average for their birth cohorts (Perls *et al.,* 2003). In other words there are inheritable/genetic factors in longevity. Of the many plausible genes for human longevity, only the well-researched APOE gene has been replicated in multiple populations (Christensen *et al.,* 2006). This gene has widespread effects on ageing phenotypes, particularly cardiovascular disease and dementia, and as such influences the ability to achieve a long and healthy life.

2.4.2 Non-genetic cellular theories.

These theories attribute ageing to progressive cell damage caused by accumulation of waste products, the effect of glucose and damage by free radicals in the internal and external environment of cells. The ensuing effect is a gradual irreversible decline in function.

i. The Accumulation Theory.

This theory proposes that the gradual accumulation of body wastes in cells interferes with the usual metabolism procedures thus initiating ageing (Powell *et al*., 2005). Evidence supporting this theory is the presence of the waste product lipofuscin. Lipofuscin is often considered in a broader context as one of several forms of undesirable protein aggregations, others sometimes being called ceroids, inclusion bodies, plaques, or aggresomes depending on their source and composition (Grune *et al*., 2004). Increasing evidence suggests that lipofuscin is not benign but can impair the functioning of seemingly unrelated cellular systems (Gray and Woulfe, 2005). They may affect proteasomal activity and protein turnover and are common features of neurodegenerative diseases as well as ageing (Grune *et al*., 2004).

ii. The Cross Linkage Theory/Glycation Theory

Glycation (also called the Maillard reaction or non-enzymatic glycosylation) is a reaction by which reducing sugars become attached to proteins without the assistance of an enzyme. The imine formed is rearranged *in vivo* in a reversible reaction into an Amadori product which is then oxidized into Advanced Glycation End-Products (AGES), a substance which has been demonstrated to be linked to ageing by estimation of chronological age from histochemical analysis of the hippocampus of human cadavers (Sato *et al*, 2001).

Diabetes is often viewed as a form of accelerated ageing due to effects of glycation. Mehta and Ross, 2009 said that while other stressors promote ageing dietary restriction, slows ageing and preserves the characteristics of youth (Mehta and Ross, 2009). Although studies show that calorie restriction can improve longevity and health in model organisms and studies in humans demonstrate reduced risk factors for major diseases, the long-term effects on humans are still unknown (Spindler, 2010).

iii. The Free Radical Theory.

According to the free radical theory of ageing, oxidative stress increases with increasing age.

This theory postulates that ageing results from the accumulation of oxidative damage. (Shigenaga *et al.*, 1994). It attributes ageing to the operation of free radicals. Reactive oxygen species (ROS) generated during normal metabolic processes can cause DNA mutation, protein oxidation, and lipid peroxidation (Beckman and Ames, 1998).

Free radicals attack the structure of cell membranes, and the damage caused eventually results in physiological decline (Finkel and Holbrook, 2000).

2.4.3 Physiological system theories.

These attribute ageing to the gradual failure of physiological systems and to the decline of the ability of regulatory systems to integrate and coordinate bodily functions, ultimately leading to death (Weinert and Timiras, 2003). In humans, the nervous, endocrine, and immune systems play a key role by their actions in coordinating all other systems (Weinert and Timiras, 2003).

An important component of this theory is the perception of the hypothalamo-pituitary-adrenal (HPA) axis as the master regulator, which signals the onset and termination of each life stage. One of the major functions of the HPA axis is to coordinate the physiological adjustments necessary for preservation and maintenance of internal homeostasis despite the continuing changes in the environment. Chronic exposure to

severe stress from a multitude of physical, biological, or emotional stimuli may exhaust or weaken the capacity to adapt and lead to the so-called "diseases of adaptation" and death (McEwen, 2002). Ageing would then result from a progressive decrease in the ability to survive stress, suggesting a close relationship between stress and longevity (Weinert and Timiras, 2003).

The Neuroendocrine Theory has been supported by data showing that an ancestral insulin pathway controls stress responses and longevity in the nematode *C. elegans* (Kawano *et al,* 2000).

2.5 The FOXO3A gene

The FOXO gene is the human homologue of DAF-16 in lower organisms. The DAF-16 protein is a transcription factor and an evolutionary conserved key regulator of the Insulin-Insulin-like Growth Factor1 signaling (IIS) pathway (Murphy et al., 2003; White et al., 2003). It has been found to have an effect on metabolism and lifespan in model organisms (Murphy et al., 2003; White et al., 2003; Kenyon et al., 2005). When activated in *C. elegans* the lifespan of the worm significantly increases (Murphy et al., 2003).

The FOXO3 gene is located on chromosome 6, q21, in the human genome. It is 124952 bases in size. It has 4 exons. Exons 1 and 4 are non-coding but may function as gene regulators (http://www.genecards.org/cgi-bin/carddisp.pl?gene=FOXO3).

In man, members of the FOXO gene, of which there are four (FOXO1, FOXO3, FOXO4 and FOXO6) regulate metabolism, cellular proliferation, stress tolerance and lifespan (Flaschbart et al., 2008). The activity of FOXO is controlled by post-translational modifications, including phosphorylation, acetylation and ubiquitination (van der Horst and Burgering, 2007). Furthermore, oxidative stress which is believed to have major effects on ageing in man (Suzuki *et al*, 2010) has been linked to FOXO function via the P13K/Akt signaling cascade which is a downstream target of insulin signaling that inhibits

FOXO function. Antioxidant enzyme expression depends on P13K/Akt/FOXO3 activity (Kops *et al.*, 2002; Brunet et al, 1999).

Many variants of the FOXO3a gene have been published and several have been linked to longevity in different populations. Variations in FOXO3a are receiving a lot of attention because theoretically, one of them may be the variant for healthy ageing and longevity (Van der Horst and Burgering, 2007). It has been found, that variations of the gene in lower animals can result in significant differences in lifespan (Lin *et al*., 1997; Van der Horst and Burgering, 2007). Genetic variants have been identified in the study of lower organisms *(C. elegans, S. cerevisiae and D. melanogaster*) which lead to lifespan extension (Kenyon *et al*., 2000; Partridge *et al*., 2002.) The short maximum lifespan of these organisms make them particularly useful for this kind of study.

Initial FOXO3A studies were done on Japanese men when Wilcox and associates, (2008) described three single nucleotide polymorphisms which were statistically significantly associated with longevity and different ageing phenotypes [FOXO3A3 Genotype (rs2802292) TT, TG, GG]. Further studies were done on both male and female Germans in 2008, where 16 polymorphisms in FOXO3A were analyzed to confirm the association of FOXO3A with longevity (Flachsbart et al., 2008).

The Genome Reference Consortium (GHRCh37, NCBI dbSNP Build 134) released in 2011, lists 39 (August 2011) polymorphic variants of the FOXO3a gene, revised down to 38 (November, 2011). However, Donlon and associates, (2012) were unable to confirm these variants in a multi-ethnic population and concluded that some of them could be very rare while others were from misalignment with the FOXO3a pseudogene on chromosome 17 or the result of sequencing and/assembly errors (Donlon et al, 2012).

Donlon and associates concluded in 2012 that the variant of FOXO3a responsible for longevity is likely to be a non-coding sequence on intron 2. While they may be right this

is yet to be proven. Of interest is their recommendation on finding the potential relation between age-related diseases and variations in FOXO3 as a means of eventually isolating the longevity and healthy ageing variant (Donlon et al, 2012).

Insight into the effect of FOXO function and variation in humans is being obtained from work with model organisms. Mutant mice lacking FOXO3 (FOXO3-null mice) have been produced and are viable (The Jackson Laboratory, 2012). The female FOXO3-null mice exhibit an age dependent infertility (Carter and Brunet, 2007). FOXO1-null mice die at embryo stage, foxo4-null mice have no apparent phenotype, and foxo-6 null mice are being generated (Carter and Brunet, 2007). It is believed that foxo proteins may have overlapping functions, so to enable further investigation more complex, multiple deletion of foxo gene mouse models will have to be produced and studied (Carter and Brunet, 2007).

2.6 Antioxidants and ageing

Antioxidants are enzymes, proteins or small molecules which delay or inhibit oxidation of substrates. Some antioxidants suppress the formation of reactive oxygen species while others scavenge free radicals by removing active species rapidly before they can cause damage to cells and tissues [eg. superoxide dismutase (SOD)] (Niki, 2010). A third group of antioxidants repair damage caused by free radicals and the fourth group act as cellular signaling messengers to ensure that the appropriate antioxidants are produced at the right time (Gutteridge, 2000; Niki, 2010).

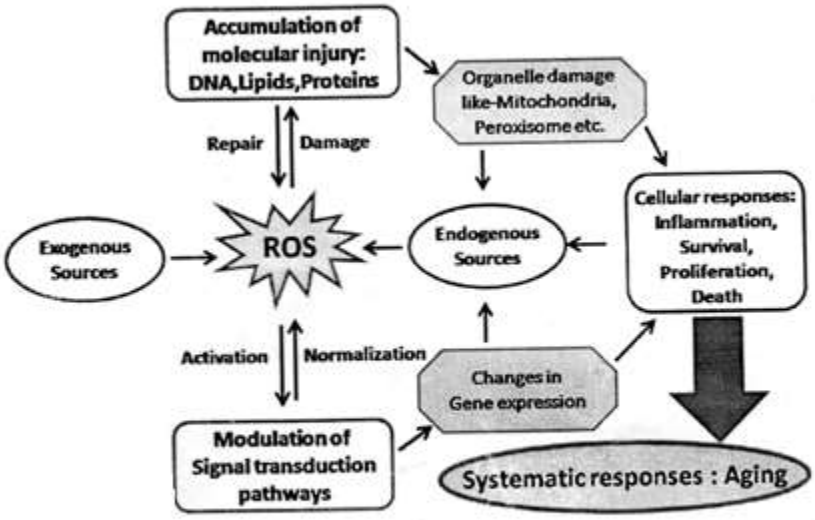
Oxidative metabolism produces highly reactive free radicals (Reactive Oxygen Species, ROS) which subsequently damage lipids, protein and DNA. This was first proposed by Harman Denhan in 1956 and continues to be updated (Cabiscol *et al*, 2010).

The commonest free radicals include the hydroxyl radical (HO^-), hydrogen peroxide (H_2O_2), ozone (O_3), superoxide anion (O_2^-) and peroxyl radicals. The free radicals exist

in the cell in dynamic balance with antioxidant molecules (eg superoxide dismutase, catalase).

This balance may be disrupted due to depletion of antioxidants or excess accumulation of ROS or both. When this happens, oxidative stress which can result in severe cellular damage leading to physiological dysfunction and cell death can occur (Pandey, 2010), Fig 2.5. Cells counteract oxidative stress by up regulating genes coding enzymes which mop up free radicals. By doing this, homeostatic limitations are maintained (Niki, 2010).

Fig 2.5. (Pandey et al, 2010) Reactive oxygen species (ROS) and ageing.



Reactive oxygen species (ROS) generated by endogenous as well as exogenous sources, cause damage and accumulation of proteins, lipids and [illegible], when defensive (repair) mechanisms of body become weak. These ROS also modulate the signal transduction pathways. These disturbances cause organelle damage, changes in gene expression followed by altered cellular responses which ultimately results into aging.

Oxidative stress is said to occur when free radicals build up in the body causing a disturbance in the redox balance. Radical scavenging enzymes and molecules are inducted to mop up the free radicals, hopefully before any damage is caused. Efficiency of this mechanism falls in circumstances where free radicals are produced in excess of mopping up capacity, hence oxidative stress. Oxidative damage is believed to eventually

lead to diseases and accelerated ageing (Niki, 2010). The presence of oxidative stress causes signaling messengers to increase gene expression to produce more scavenging antioxidants. Older obese men have been found to have more severe oxidative stress and a stronger relation between body mass index (BMI) and oxidant/antioxidant markers as compared to younger men (Karaouzene et al, 2011).

Low antioxidant activity is believed to accelerate ageing/deterioration of species. Superoxide dismutase 2 (SOD2) mutant *Drosophilia* which had very low SOD developed accelerated olfactory and neurological senescence with a progressive shortening of lifespan (Paul et al, 2007).

SOD catalyzes the destruction of the O^{2-} free radical. The SOD catalysed dismutation of superoxide may be written as

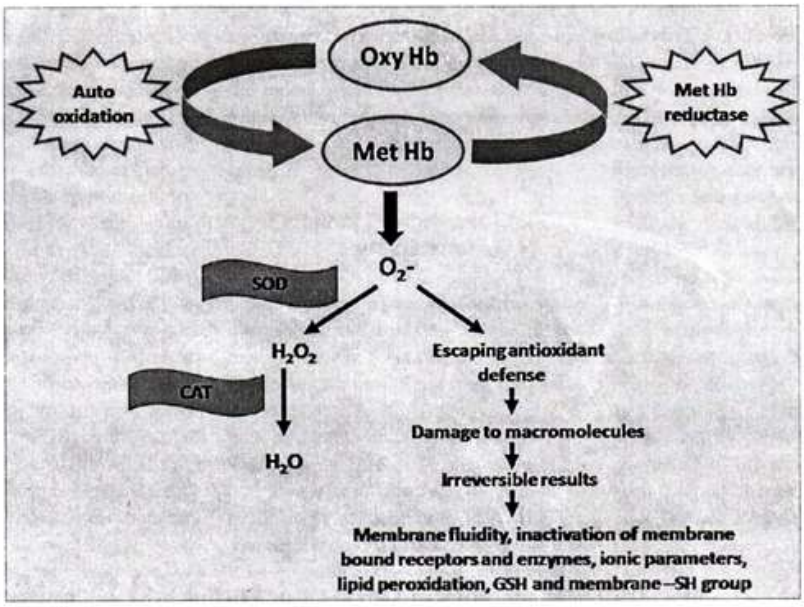
- $M^{(n+1)+}\text{-SOD} + O_2^- \rightarrow M^{n+}\text{-SOD} + O_2$
- $M^{n+}\text{-SOD} + O_2^- + 2H^+ \rightarrow M^{(n+1)+}\text{-SOD} + H_2O_2$.

where M = Cu (n=1) ; Mn (n=2) ; Fe (n=2) ; Ni (n=2).

Three forms of SOD are present in humans. SOD1 is cytoplasmic, SOD2 is mitochondrial and SOD3 is extracellular. SOD 1 and 3 contain Cu and Zn unlike SOD2 which contains Mn in its reactive center.

Fig 2.6 below summarizes the effect of reduced antioxidant capacity in erythrocytes with ageing.

Fig 2.6 (Pandey et al, 2010). The effect of reduced antioxidant capacity in erythrocytes with ageing.



Development of oxidative stress in erythrocytes. Hb; hemoglobin, SOD; superoxide dismutase, CAT; catalase. Under normal conditions, reactive oxygen radicals are buffered by endogenous defensive enzymes i.e., superoxide dismutase and catalase but due to reduced reducing/anti-oxidant capacity during aging or in other pathological conditions, reactive oxygen radicals escape and destroy the macromolecules, which ultimately results in altered erythrocytic behavior.

2.7 Non Subjective Prediction of Ageing

The first evidence that physiological age can be predicted non-subjectively is claimed by Melov, 2008. In 2008 he was able to predict the ages of nematode worms (*Caenorhabditis elegans)* with 70% precision by correlating whole-genome expression profiles of 104 individual wild-type worms with their age-related behaviour and survival (Melov, 2008). Melov's studies showed a set of genes that are actively involved in the ageing process.

The success in the study of the mechanisms influencing lifespan in lower organisms such as *Caenorhabditis elegans*, *Saccharomyces cerevisae,* and *Drosophila melanogaster* has motivated the search for corresponding genes in humans. The APOE gene, which codes for apolipoprotein E is the only one that has been found to be consistently associated with survival in various populations (Christensen *et al*., 2006) to date, although a lot of genetic studies on ageing have been conducted.

Genetic polymorphism within the human *Forkhead box O3A gene* (FOXO3A) has been strongly associated with human longevity (Wilcox *et al.,* 2008). Studies done in Japanese Americans (Wilcox *et al*., 2008) Italians and Chinese, (Yang *et al*, 2009) have consistently shown that certain polymorphisms of the FOXO3a gene are associated with longevity. It is also possible that genetic mutations which have occurred in some parts of the world, but not in others, influence ageing, justifying the need to study populations of different origins.

Twenty to thirty percent of variations in lifespan have been attributed to genetic influences (Hjelmborg *et al.,* 2006). Also, greater incidence of centenarians and supercentenarians has been found to occur in some families but not others (Kerber et al. 2001; Perls and Dellara 2003).

With demographic detail showing that the number of elderly in the whole world continues to increase in both absolute and relative terms, the need to know and preserve the health of the elderly has never been greater (United Nations World Population Prospects, 2012). Such knowledge will help to boost the probability that the increased lifespan will be made up of years of activity, free from disability.

2.8 Effect of Ageing on Physiological Parameters

Old age challenges the functional reserve of physiological functions which nature allows in youth. Functional reserve is well illustrated in biological systems especially in systems which have double organs when it is known that man is able to do well with just one. The deterioration of physiological processes with ageing is thought to be attributable to molecular/cellular changes over time as well as a decrease in cell numbers which lead to less effective homeostatic mechanisms (Huang et al, 2004). A decrease in the ability to step up activity when challenged or stressed as well as decreased ability to repair damage to cells occurs (Huang et al, 2004).

Chronic diseases generally progress slowly and may remain asymptomatic for many years. Manifestation of the atherosclerotic deposit as a vascular impedance or a thrombotic incident occurs many years after the atherosclerotic plaque begins to form. Frank diabetes may be diagnosed after years of glucose intolerance. In as much as one can live for many years with a chronic disease, especially when well controlled, chronic conditions are a major cause of morbidity and mortality in the world today. Thirty-six million people died from chronic disease in 2008, twenty seven million of whom were over 60 years of age (WHO, 2012). People who live long are generally found to have a lower prevalence of cancer and cardiovascular disease and high physical and cognitive function (Wilcox *et al.,* 2008).

Low and middle income countries now have more deaths due to chronic diseases than in the past when morbidity and mortality were due to infectious diseases; 80% of all cardiovascular disease-related deaths now occur in low and middle income countries (Marshall, 2004) Bulletin of the World Health Organization | July 2004, 82 (7).

2.9 Age related changes

Cellular senescence is the phenomenon by which normal diploid cells lose the ability to divide normally after about 50 cell divisions *in vitro* (Hayflick and Moorhead, 1961). Cellular senescence is closely interconnected with ageing, longevity and age-related diseases, either by sharing common genes and regulators or by protein-protein interactions and eventually by common signaling pathways (Tacutu *et al*., 2011). The most enriched pathways across age-related diseases and ageing-associated conditions (oxidative stress and chronic inflammation) are growth-promoting pathways and the pathways responsible for cell-extracellular matrix interactions and stress response (Tacutu *et al*., 2011).

Cellular senescence has been postulated as an important cause or consequence of type 2 diabetes and its complications. Cellular senescence becomes evident through phenotypic

changes in morphology, gene expression, and function (Goldstein, 1990). It has long been known that genomic instability, a feature of premature ageing disorders such as in the Werner syndrome (an autosomal recessive disorder characterized by rapid ageing and early onset of age-related disease), is associated with type 2 diabetes (Kipling *et al*.,2004).

Middle-aged offspring of long-lived families exhibit lower plasma levels of glucose and higher insulin sensitivity (Bartke, 2008). This is in agreement with findings from animal studies which revealed that the insulin/IGF1 signal transduction pathway is involved in lifespan (Bartke, 2008).

Prevalence of adult hearing loss increases with age. Adult hearing impairment is most commonly caused by presbycusis (Ciorba *et al*, 2012). Adult hearing loss is known to affect 40% of the population aged 75 and above (Ciorba *et al*, 2012).

Thirty-one percent high frequency hearing loss was found in a US population in a cross sectional nationwide study showed significant differences between men and women and between ethnicities with lower hearing loss in women and blacks (Agrawal *et al*, 2008).

2.9.1 Cardiovascular System

With changing lifestyle and nutrition in sub-Saharan Africa, prevalence of cardiovascular disease (CVD), e.g., atherosclerosis and hypertension that lead to heart failure and stroke has risen dramatically and has become a leading cause of morbidity and mortality (Addo *et al*., 2007; BeLue *et al*., 2009). According to The World Health Report, in 2001 CVD accounted for 9.2% of all deaths in the African region (WHO, 2002). In Accra, Ghana, CVD was the leading cause of death in 1991 and 2001 (de-Graft, 2007). Human longevity is characterized at middle age by lower prevalence of myocardial infarction, hypertension and type 2 diabetes (Westendorp *et al*., 2009).

In the United States, CVD is the leading cause of mortality, accounting for over 40 percent of deaths in those aged 65 years and above (Lakatta, 2002). Over 80 percent of all cardiovascular deaths occur in the same age group, making age the major risk factor for cardiovascular disease (Lakatta, 2002).

Clinical manifestations and prognosis of these cardiovascular diseases become altered in persons with advanced age because interactions occur between age-associated cardiovascular changes in health and specific pathophysiologic mechanisms that underlie disease (Lakatta, 2002) hence the need to study the elderly with and without disease.

Notable measurable changes occur in the cardiovascular system of the ageing healthy adult. Left ventricular hypertrophy, alteration in the diastolic filling pattern, impaired left ventricular ejection, alterations in heart rate reserve capacity and altered heart rhythm (Lakatta and Levy, 2003). These changes vary the substrate on which cardiovascular disease is superimposed (when it occurs,) with the likelihood of adverse effects on prognosis and progression of disease.

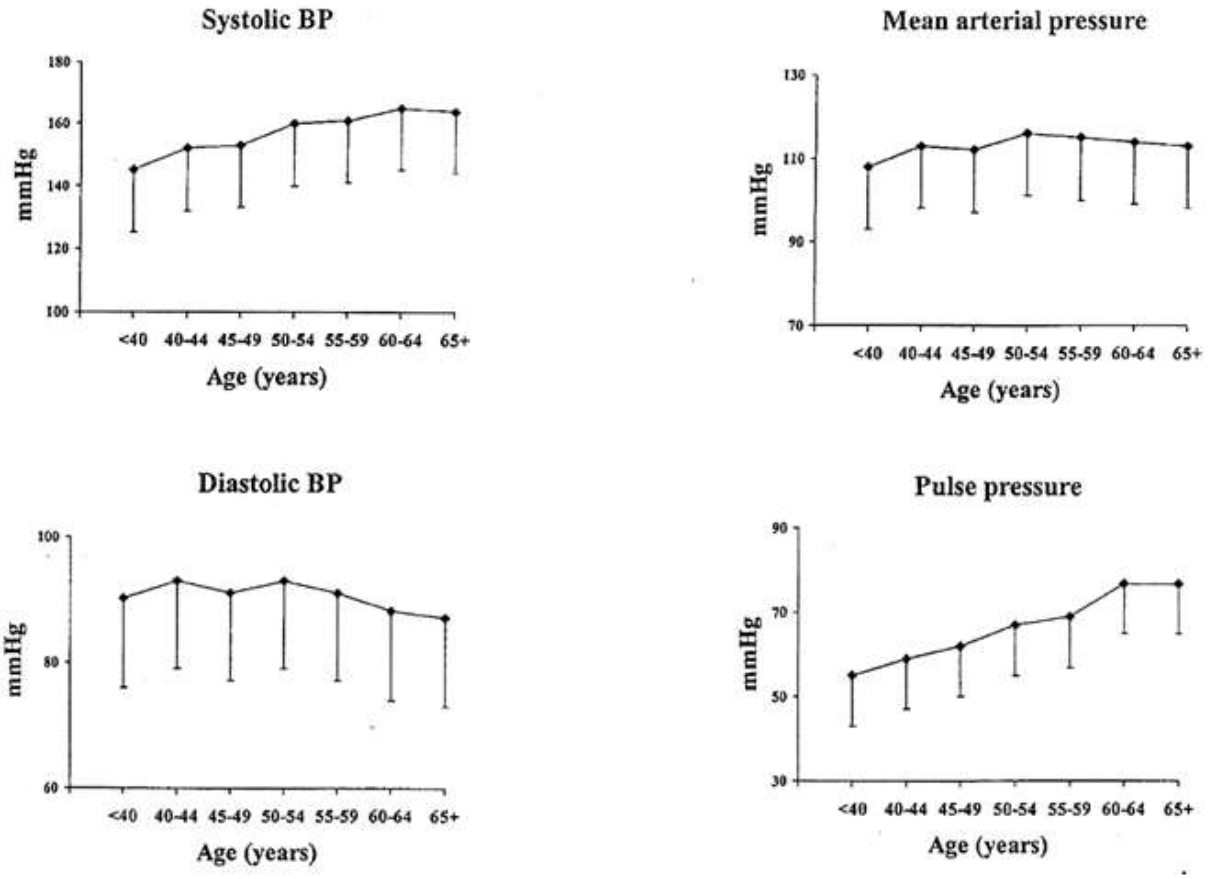
Lakatta and Levy in 2003 described left ventricular hypertrophy, heart failure and atrial fibrillation as the most dramatic cardiac changes with ageing. Rising blood pressure is also of particular importance especially since the prevalence of left ventricular hypertrophy increases with it. Furthermore, heightened pulse pressure (the difference between the systolic and diastolic blood pressure) is a risk factor for the development of atrial fibrillation *(http://ww.medscape.com/viewarticle/552468).*

Hypertension has been described as a major public health problem in Ghana with relatively low levels of awareness, drug treatment and blood pressure control (Amoah, 2003)

Ageing decreases the distensibility/compliance of arteries which causes an elevation of systolic blood pressure and a slight decrease in diastolic pressure, thus a widening of the pulse pressure (Mitchell et al, 2011)

It causes an increase in afterload, the resistance to ejection of blood from the left ventricle primarily because of the reduction in arterial compliance (Bolton and Rajkumar, 2011). The increased afterload causes a thickening of the left ventricular wall with increase in the size but not number of myocytes (Bolton and Rajkumar, 2011)

Fig 2.7 Changes in blood pressure, mean arterial pressure and pulse pressure with ageing (Khattar et al, 2001)



2.9.2 Respiratory system.

Anatomical, physiological and immunological changes affect pulmonary function with ageing (Delerme and Ray, 2008). Chest wall compliance decreases progressively with age and there's loss of diaphragmatic mass and strength which results in reduction of mechanical action of the muscles of respiration (Lalley, 2013). The work of breathing is increased when structural changes which occur in the chest wall and thoracic spine impair the compliance of the respiratory system (Deleme and Ray, 2006). Further, loss of the supporting structure of lung parenchyma causes airway closure, termed senile emphysema (Delerme and Ray, 2008) Ageing of the lungs results in decreased sensation of dyspnoea and increased susceptibility to lung infections due to decreased immunity (Gulshan and Goodwin, 2006). Ironically, the airway receptors become less responsive to pharmaceuticals with ageing, and together with all the other changes the respiratory system gets compromised greatly in the aged (Delerme and Ray, 2008).

Age related changes in forced expiratory volume in one second (FEV_1) were believed to be linear until recent studies suggested that the decline accelerates with ageing (Janssens, 2005).

Kawalinder and associates, 2012 reported FEV_1 averages based on measurements taken in groups as follows, 15 to 30 years, 2.73+/-0.53; 31 to 45 years 2.35+/-0.57; 46 to 60 years 2.05+/-0.58; 61 years and above 1.60 +/-0.49 (Kawalinder *et al*, 2012).

2.9.3 Anaemia in the elderly

Anaemia is defined as hemoglobin level which is less than a certain predetermined point. Haemoglobin level of less than 15g/dl in males and less than 11g/dl in females are used in the diagnosis of anaemia in the general population at the Research Laboratory, Medical Biochemistry, Korle-Bu where the hematological analysis for this study were done.

The prevalence of anaemia in the elderly in Ghana is not known. In other populations it has been found to be as high as forty percent (Rohrig et al, 2012), global anaemia prevalence in 2010 was 32.9%, (Kassebaum et al, 2013). Anaemia is not an age related disease, but it has been linked to disease and death in the elderly and is considered a major prognostic marker in the elderly (Artz, 2011)

The WHO criteria for anaemia in the elderly is a level less than the normal mean minus two standard deviations (Price and Schrier, 2008). The 1968 World Health Organization (WHO) criteria of a hemoglobin (Hgb) <13 g/dL (<130 g/L) in men and <12 g/dL (<120 g/L) in women have been used to define anemia since 1968 (WHO, 1968)

There is debate as to whether modifications in the definition of anaemia should take into consideration racial and ethnic peculiarities. Populations with thalassamia and sickle cell disease tend to have low-normal haemoglobin (Beutler and West, 2005). As much as these are important considerations optimal levels must be defined in terms of morbidity and mortality. It is generally well accepted that values for hemoglobin in apparently healthy older adults are lower than for younger adults and the differences in hemoglobin levels between the genders shrink with ageing (Patel, 2008)

Culleton and associates, 2006 suggest that the lower limits of hemoglobin for elderly men and women are 14.0g/dl and 13.0g/dl in males and females respectively (Culleton *et al*, 2006).

2.9.4 Other hematological parameters in the elderly

Reference ranges for other hematological parameters have not been specifically defined for the elderly in Ghana. The reference ranges of the Research Laboratory, Medical Biochemistry will be used in the analysis of the results of the hematological tests done (Appendix 4) and comparisons will be made with the results obtained.

The mean values obtained on studies done in anaemic (using WHO criteria) elderly persons above 65 years gave the following means and standard deviations (Price et al, 2011).

- Absolute neutrophil count: 5.6 x 10^3/microL (range: 1.4 to 18.6)
- Platelets: 219 x 10^3/microL (43 to 608)
- Hemoglobin: 11.2 g/dL (6.4 to 12.9

2.9.5 Fasting blood sugar in the elderly

Statistics on fasting blood sugar in elderly Ghanaians are not readily available. Arthur and associates, 2006 reported fasting blood glucose (mean and standard deviation) of 4.91 ± 1.08 mmol/L in non-diabetics from their study based in the Ashanti Region with reference range 0.076; $p< 0.01$). In the diabetics, the fasting blood sugar ranged between 3.9 to 22.5 mmol/L (Arthur *et al*, 2006). The ages of participants in that study are not reported in the publication.

Reference ranges of fasting blood sugar - In persons with no history of diabetes, fasting blood sugar less than or equal to 5.6mmol/l is considered normal, 5.7 to 6.9 is considered mild hyperglycemia and values greater than 7mmol/l are considered severe-hyperglycemia/diabetes mellitus.

2.9.6 Body Mass Index in the elderly

Forty-one percent of women were found to be underweight and 16.9 percent were overweight or obese in a study of elderly women in rural Ghana (Blankson and Hall, 2012). Tobacco and alcohol use, low visual acuity and difficulty in walking were found to be associated with low BMI of <18.5kg/m^2 (Blankson and Hall, 2012). In the United Stated the prevalence of obesity in men and women above age 60 is believed to be on the increase, with 30% of elderly Americans found to be obese (Zamboni and Mazzali, 2012).

Categorization of BMI (using the National Heart Lung and Blood Institute (NHLBI, USA) guidelines (Janssen et al, 2002)

Underweight (< 18.5 kg/m^2),

Normal (18.5 − 24.9 kg/m^2),

Overweight (25.0 − 29.9 kg/m^2)

Obese (≥ 30.0 kg/m^2)

The predictive value for this categorization in the elderly has been challenged (Heiat et al, 2001; Dolan et al, 2007).

Higher mortality risk has been seen in lowest quartile BMI as compared to second quartile BMI and after adjustment for age, gender, education, ethnic group, smoking, cancer and dementia the highest quartiles for BMI were also related to higher mortality (Jose et al, 2008)

2.10 Biomarkers of ageing

Biomarkers of ageing are physical properties in the human body which indicate that the body is ageing. In order to be called a biomarker, a factor has to satisfy a number of criteria. The best markers are the ones which are little affected by the outside environment.

By the High tech Bio-Medical Technologies for disease treatment and life extension experimental and clinical data (http://www.anti-aging-guide.com/62biomarkers_PFV.htm) a true biomarker will satisfy the following criteria:

A. The marker must predict the rate of ageing and be a better predictor of life span than chronological age.

B. It must be able to be tested on a regular basis.

C. It must work both for humans and other species, such as laboratory animals.

D. There is support from human clinical assessment and complementary research studies.

E. The studies on the biomarker are based on a significant representative sample.

F. The result is a clear association with ageing.

G. A relatively narrow standard deviation is present.

So far, about 24 factors have met the criteria and can be considered biomarkers. They may be indicated especially for males or for females, and figures may vary between the sexes. Here is their list of biomarkers:

1. 17-ketosteroid/17-hydroxycortiosteroid ratio (male)
2. Ascorbic acid
3. Basal Metabolic Rate
4. Blood pressure- pulse
5. Blood pressure- systolic
6. Body Mass Index (female)
7. Caries index
8. Creatinine clearance
9. DHEA-S
10. Fibrinogen
11. Hair baldness (male)
12. Hair grayness
13. Handgrip strength
14. Hemoglobin A1C
15. Lung capacity- FEV1
16. Lung capacity- FVC
17. Maximum oxygen update (male)
18. Near vision
19. Noradrenaline- plasma (male)
20. Periodontal index
21. PSA total (male)
22. Skin elasticity
23. Testosterone free (male)
24. Zinc- serum

All of the biomarker tests can be classified either as laboratory tests (e.g. blood and urine tests) or as physical tests.

2.11. The Comet Assay (Single Cell Gel Electrophoresis)

The Comet Assay is a technique for the detection of DNA damage in individual eukaryotic cells. It was developed by Ostling and Johansson in 1984 and modified by Singh and associates in 1988 (Shukla et al, 2011). It involves lysing cells embedded in agarose on a microscopic slide to expose supercoiled loops of DNA which is then subjected to electrophoresis. On observation by fluorescent microscopy Comet formations are seen. Interpretation is made by looking at the intensity of the tail relative to the head of the comet as the tail reflects the number of DNA breaks (Hartman et al, 2003)

Applications of the Comet Assay include the determination of the extent of DNA fragmentation in sperm cells which can be used to predict the outcome of *in vitro* fertilization and checking extent of DNA damage in toxicology studies.

In this study it is used to demonstrate DNA deterioration that occurs with ageing, expecting that longer Comet tails will be seen in the elderly as compared to the young, and thinking that with further work, the Comet Assay could be used together with other biomarkers of ageing in the prediction of physiological age.

CHAPTER 3

METHODS

3.1 Study design

The study is a cross-sectional quantitative assessment of selected physiological and genetic parameters in elderly Ghanaians aged 50 years and above with and without existing chronic diseases with comparison to a younger population in respect of Oxidative Stress Analysis.

Quantitative assessment

Subjects were required to fill a questionnaire, undergo some measurements and have their blood samples taken for laboratory investigation.

The elderly were divided into age groups by decades starting from age 50 through to 90 and above. Data was collected in two groups, namely;

1. Elderly with chronic illness – Systemic, metabolic or neoplastic, congenital or acquired.
2. Healthy elderly (without the above).

Three biomarkers of ageing were assessed – namely body mass index, lung capacity and blood pressure.

Oxidative stress analysis (superoxide dismutase), a comet assay to demonstrate DNA damage with ageing and studies on the FOXO3a gene, as described below were done in addition.

The biomarkers and tests were chosen carefully with the aim of the study, cost, time limits and available equipment in mind.

3.2 Study site

The study was conducted in the HelpAge Ghana Day Centers at Apenkwa, Achimota and Osu-Kaadzaano areas, all in the Greater Accra Region of Ghana. The HelpAge Day Centers serve as places for social interaction of the elderly in the communities where they are located. A resident nurse at the Osu-Kaadzaano Center helps with minor medical needs and facilitates hospital and home care for members as and when required. (Help Age Centers/ facilities have been caring for the aged in Ghana since 1988)

3.3 Study Population:

This study had two main populations; elderly and young adult populations. For the elderly group, all 18 communities consisting of 20 zones of Help Age Canters in the Greater Accra Region constituted the study population. From this number, three high functioning communities were purposively selected. This was done in order to get adequate numbers of the aged (54-90 years).

The functional definition of the healthy elderly in this study is men and women above 50 years of age who have not been diagnosed with any chronic physical, congenital or psychological illness for which they are not on regular medication.

Elderly men and women who fit the inclusion criteria and who consented to participate in the study were recruited until the period of data collection was over.

Inclusion criteria

i. Ghanaians aged 50 and above who have proof of age – birth certificate, voter's identification, national identification card, passport or any other nationally recognized document of identification.

ii. The young adult population was purposively selected from a Nursing Training College to undergo the oxidative stress analysis under the following inclusion criteria;

1. 20 – 25 year age group
2. No reported chronic illness e.g. diabetes mellitus, hypertension, cancer
3. Readiness to volunteer for study

This young adult population served as a control for the elderly population for the oxidative stress analysis. This was done because oxidative stress analysis results are known to have a lot of variations depending on test kits and reagents and circumstances of test. Using the same test kit for both elderly and young adult populations enabled the establishment of average baseline values in oxidative stress tests for the young adult population in order to compare with the averages from the elderly group.

Exclusion criteria

i. Persons less that age 50 years old.

3.4 Variables

The following constituted the independent and dependent variables that were studied:

Independent Variables: Age, Health Status – healthy or with presence of chronic illness – Diabetes, Hypertension, Cancer, congenital illness

Dependent Variables: Body Mass Index (BMI), Hematological parameters (Hemoglobin, Neutrophils, Lymphocytes, Platelets) and Superoxide Dismutase Activity (SOD), Mean Arterial Pressure (MAP), Forced Expiratory Volume$_1$ (FEV_1).

3.5 Sample size determination

The minimum sample size required for the study of each group was obtained using the formula

$$N \geq \{(Z_{1-\alpha/2})/m\}^2$$

Where Z is the coefficient of significance which is 1.96 for level of significance of $\alpha = 0.05$

N is the minimum sample size required for the study

m is the margin of allowable error which determines the power of the study (1-β)

The minimum sample size required such that there is 95% chance of absolute error estimate of the mean within ½ standard deviation of the mean would be

$N = \{1.96/0.5\}^2 = 16$

Therefore minimum sample size for each group would be 16, making a minimum total of 32.

A total of 128 elderly persons were studied.

3.6 Data Collection Techniques

The following data collection techniques were used;

i. Questionnaire - This covered socio-demographic parameters, self-reported health, nutrition activity and the Short portable mental state Questionnaire (SPMSQ) for assessment of cognitive function (Pfeiffer, 1975).

ii. Clinical and anthropometric measurements – Blood pressure, weight, height and lung function.

iii. Blood Analysis: Molecular, Hematological and Physiological blood analysis – FOXO3a variants, full blood count, superoxide dismutase activity and the Comet Assay.

3.6.1 Clinical and anthropometric measurement

Blood pressure was measured using an OMRON digital sphygmomanometer from the left or right upper arm in a sitting position after a minimum of ten minutes rest. The mean arterial pressure (MAP) was calculated from the blood pressure.

MAP = Diastolic pressure + 1/3(systolic pressure – diastolic pressure)

Body mass index (BMI) will be calculated by dividing body weight in kg by height in meters^2.

BMI = Weight (kg)/Height (m^2)

Subjects were weighed in kilograms wearing light clothing, standing on a digital bathroom scale.

Height (m) was measured using a wall mounted rule.

Lung function

FEV_1 and PEF (Peak expiratory flow) were measured with the Microlife® Digital Peak Flow Meter for Spirometry. (Microlife, Switzerland)

Measurements were taken with the subject comfortably sitting. The procedure was explained to all subjects. A nose clip was applied and tested. Each subject was then positioned with chin slightly elevated and neck stretched. Subject was allowed to practice breathing through the mouth piece. At the end of a normal expiration, subject was asked to take in a deep breath. At full inspiration, without a pause subject was instructed to breathe out as hard and as fast as possible. The FEV_1 and PEF readings were automatically recorded. The nose clip was left in place while subjects took 15 to 30 second breaks in between maneuvers. Three satisfactory maneuvers meeting criteria for the reproducibility of tests were obtained. A maximum of six maneuvers were done per person.

- FEV_1 – Forced Expiratory Volume in One Second - The amount of air which can be forcibly exhaled from the lungs in the first second of a forced exhalation.

- PEF – Peak expiratory flow (PEF) is the maximum flow generated during expiration performed with maximal force and started after a full inspiration.

3.7 Blood Analysis

Six mls of venous blood was taken from the cubital fossa of each participant following a twelve hour fast (7:00 pm to 7:00am).

Blood was collected into EDTA (ethylenediaminetetraacetic acid) tubes kept at room temperature for a maximum of four hours while being transported to the laboratory. On getting to the laboratory, haematological measurements were done using the Cell Dyn 1800 analyzer (Abbott Laboratories, Philippines). Blood was then centrifuged at 4000 rpm for ten minutes to separate plasma from the buffy coat and cells. All components were stored separately at -70 degrees Celsius.

Superoxide dismutase was determined from plasma using an SOD kit (refer to Appendix for details on SOD test kit.)

3.8 Genotyping

3.8.1 Genomic DNA extraction

The QIAGEN DNA kit (QIAGEN Co., Germany) was used to extract genomic DNA from the buffy coat of EDTA-preserved whole blood samples. The extracted DNA was stored in labeled Eppendorf tubes at -20°C until needed.

3.8.2 Genotyping of SNPs

Three tagging SNPs with primer sequences shown below from the FOXO3a gene were selected.

A. rs2253310

5’-GAGCTTGCTTTGGAGATGCA-3’/5’-CCCAGTCACTCACATAGTCCT-3’

B. rs4946936

5’-GGGTCCTGAGAACTTCTGAGT-3’/5’-GACATTCTGTAAGACATTCTGCCT-3’

C. rs2802292

5’-CTGAGGCTAACAGCTGGGTCT-3’/5’-CACTGGCTGCCTGACACCTAT-3’

(The three SNPs used in this study are identical to those studied by Yang *et al,* 2009. They were found to be associated with longevity in Chinese subjects).

Amplification was by PCR in 25 µl reaction mix containing 10µl template DNA, 0.125µl of the *Taq* polymerase enzyme (5U/ µl) (Sigma Missouri, USA), 1.0µl of each of the oligonucleotide primers at 10µM, 0.5µl of each of the four deoxyribonucleotide phosphates (dNTPs) at 10mM and 3µl of 10× PCR buffer (with $MgCl_2$). Three PCR were performed on each sample.

The amplification conditions were an initial denaturation at 94°C for 15 minutes, thirty five (35) cycles of denaturation at 94°C for 40 seconds, primer annealing at 52°C-rs2253310, 50°C -rs4946936 and 54°C - rs2802292 for one min each and strand extension by the *Taq* polymerase at 72°C for 2 minutes. A final strand extension at 72°C for 5 minutes was then performed to complete the reaction. The amplification reaction was performed using Techgene PCR machine, (Techne, United Kingdom).

After the reaction, 10µl of the PCR (polymerase chain reaction) product was run by electrophoresis at 120 volt (Labnet International, Power station 300) on 2% agarose gel (Biopioneer Co, USA) stained with 0.5µg/ml ethidium bromide (Life Technologies Co, USA) in 1X Tris acetate EDTA (TAE) running buffer (Biopioneer Co, USA) using 2µl of blue/orange DNA loading dye (6X) (Promega Co, USA). Hundred base pair nucleotide sequence molecular size marker (Sigma Mo, USA) was run alongside the PCR products on the gel. The gel was photographed using UV-illumination (UVIsave gel documentation system, model GAS9200/1/2/3, Version 12, UVITEC,United Kingdom) and analyzed.

Product sizes of 300, 250 and 350 base pairs were obtained for the SNPs rs2253310, rs4946936 and rs2802292 respectively.

3.9 Oxidative DNA damage analysis in white blood cells By Comet assay

The Comet Assay was done in two groups to demonstrate DNA damage with ageing.

1. A young adult (control) group
2. The aged (above 50 years)

3.9.1 Sample preparation

Glacial acetic acid was introduced into the buffy coat to get rid of all traces of red blood cells that were present as a result of the separation process. The buffy coat samples containing the white blood cells were then diluted with phosphate buffered saline (PBS) and counted to obtain a working concentration of 1×10^5 cells /ml.

3.9.2 Comet assay ™

DNA Comet Assay ™ (Trevigen Inc., Gaithersburg, MD, USA) was carried out as described by the manufacturer. In this assay, white blood cells were immobilized in a bed of low melting agarose on a Trevigen Comet slide. After a gentle cell lyses, samples were treated with alkali to unwind and denature the DNA and hydrolyze sites of damage. The samples were then subjected to TBE electrophoresis. The comet tails were scored according to DNA content (intensity). (Appendix 6)

3.9.2.1 Staining of Processed cells

The Comet Assay ™ SYBR® Green I nucleic acid gel staining kit (Trevigen Inc., Gaithersburg, MD, USA) was used to stain the processed cells from the comet assay as instructed by the manufacturer.

3.9.2.2 Photomicrography and Evaluation of DNA damage

For effective visualization and observation of the cells on the slides for DNA damage after SYBR® Green I nucleic acid gel staining, slides were viewed by an epifluorescence microscope using the fluorescein filter. SYBR® Green I maximum excitation and emission are respectively at 494nm and 521nm. Photomicrographs obtained from the various slides were taken immediately using a Konica Minolta 100VX film (Konica Minolta Co. Ltd., Japan) since the stain fades away with time under white light.

3.10 Superoxide dismutase activity detection

The SOD activity levels were determined by a colorimetric method using SOD Assay kit (*Cayman Chemicals, Michigan- USA*). Whole blood (2.5mls) obtained by venipuncture was centrifuged (4000rpm, 10mins at 4^{o}C), and plasma carefully separated. Two hundred and fifty microliters of the erythrocyte fraction was placed in one thousand microliters of ice-cold deionized distilled water (4^{o}C) and centrifuged at 6,000rpm for 20 minutes at 4^{o}C to lyse the erythrocytes. One thousand microliters of the supernatant (erythrocyte lysate) was collected for assay and stored on ice. The supernatant fluid was then diluted by a factor of 100 with sample buffer, and 10 µl of the diluted solution used to assay Cu/ZnSOD (Copper/Zinc Superoxide dismutase) activities as described by the manufacturer (*Cayman Chemicals, Michigan, USA*).

3.11 Quality Control

The following were done to ensure the quality of the data collected;

All blood samples were taken, tested and stored as per the test kit manufacturer's instructions. Samples were appropriately labeled to ensure a perfect match between study subject and the results of test.

Questionnaires were pretested to ensure good quality of raw data. All data was doubly entered with appropriate checks by researcher.

3.12 Analysis

3.12.1 Normal Ranges

The following physiological parameters were operationalized as follows

BP normal range

Systolic 90 to 140 mmHg

Diastolic 60 to 90 mmHg

BMI

Underweight <18.5

Normal .18.5 to 24.5

Overweight – 24.5 to 29.5

Obese - >29.5

Haematological

Haemoglobin	male 15 to 18 g/dl, female 11 to 16g/dl
White Blood Cells	2.6 - 8.5 x 10^3 per micro liter
Neutrophils	25 - 75% of WBC
Lymphocytes)	25 - 60% of WBC
Platelets 150	400 x10^3 per microliter

3.12.2 Statistical Analysis

Data Processing and Analysis

Questionnaires were coded and entered in Excel and later exported into SPSS. The resulting data was then analyzed and presented using bar charts, frequency distributions

and cross tables. The results were tested for statistical significance using Chi-square, independent-sample t-test and one-way ANOVA where appropriate, and reported at three levels of significance - 90%, 95% and 99%. A Multivariate OLS Linear Regression was also used to identify the determinants of ageing between males and females and across various age-groups.

3.12.3 Cognitive function.

THE SHORT PORTABLE MENTAL STATUS QUESTIONNAIRE (SPMSQ) was used to assess cognitive function. The SPMSQ is a widely used brief screening tool for dementia (Malhotra, 2013).

Scoring was done as follows

0-2 errors: normal mental functioning

3-4 errors: mild cognitive impairment

5-7 errors: moderate cognitive impairment

8 or more errors: severe cognitive impairment

One more error is allowed in the scoring if a subject has had a grade school education or less.

One less error is allowed if the subject has had education beyond the high school level.

See Appendix 3 for details on source.

3.13 Ethical Considerations

Ethical considerations included securing ethical clearance for study, informed consenting processes and adequate data handling.

Approval: This was sought from the Ethical and Protocol Review Committee of the University of Ghana Medical School to which a full research proposal was submitted. Protocol Identification Number: MS-Et/M.6 – P 5.1/2011-12.

Permission: Permission to base data collection at the HelpAge Centers was also sought from the Director of HelpAge Ghana. Permission to collect data from the young adult group was obtained from the Principal of the School of Nursing, Korle-Bu Nursing Training College.

Consenting Process: A letter of consent explaining the rationale for the study, its benefits and the confidential handling of information was drafted with relevant portions for respondents to sign to indicate their consent to assist with gathering the relevant data voluntarily. Participation was strictly voluntary. All participants either read the information material on the study or had it explained to them in an appropriate language. Participants were required to give consent to be part of the study by appending their signatures or thumbprints to the consent forms provided. Each participant was given a copy of the information sheet to take away (Appendix 1).

Risks/Benefits: The study involved invasive procedures. Participants were specifically informed of the invasive procedure (venepuncture) included in this study and instructed to report any adverse incidences associated with it to the investigator. In addition, adequate infection prevention control methods were implemented. The benefits of greater insight into the health condition of the aged in general and the positive implications of findings for Ghana's new policy on ageing were explained to participants.

Privacy/Confidentiality: Information gathered was kept in strict confidentiality and the identities of respondents protected. Interviews were also conducted in an atmosphere that guaranteed the privacy of the respondent with due regard for their comfort and the sensitive nature of the information gathered.

Data Storage & Usage: Data gathered was stored confidentially and used for the purpose for which it was gathered only. Approval will be sought from Ethical and Protocol Review

Committee of the University of Ghana Medical School if data is found useful for any other purpose.

CHAPTER FOUR

RESULTS

4.1 BACKGROUND CHARACTERISTICS OF RESPONDENTS

Out of the 128 elderly Ghanaians surveyed, 92 (71.9%) were females and 36 (28.1%) males. The majority (59, 46.1%) were aged between 70 and 79 years, with the youngest being 54 and the oldest 90 years. About half (65, 50.8%) were educated up to the primary or middle school level. A total of 63 (49.2%) had pension pay, and 105 (82.0%) were on a mutual health insurance scheme. Thirty-three (25.8%) were married, 67 (52.3%) widowed, and 28 (21.9%) divorced. A total of 105 (82%) were living with their families. Some 79 (61.7%) reported having chronic Illness, while the remaining 49 (38.3%) reported no case of chronic illness.

Females and the chronically ill tend to dominate the lower age groups, while respondents receiving regular pension and widows dominate the older age groups.

Table 4.1: Background Characteristics of Respondents (with Age Breaks)

BACKGROUND CHARACTERISTICS		**AGE GROUP**				**ALL**
		50-59	**60-69**	**70-79**	**80+**	
	Base	7	40	59	22	128
Sex	Male	0.0	20.8	28.8	50.0	28.1
	Female	100.0	80.0	71.2	50.0	71.9
Education	None	14.3	22.5	30.5	45.5	29.7
	Primary	28.6	5.0	25.4	9.1	16.4
	Middle School	42.9	50.0	25.4	27.3	34.4
	Secondary	14.3	20.0	15.3	9.1	15.6
	Tertiary	0.0	2.5	3.4	9.1	3.9
Income	Pension	28.6	37.5	47.5	72.7	47.7
Access to Healthcare	Health insurance	100.0	90.0	91.5	95.5	92.2
Marital Status	Married	57.1	27.5	16.9	36.4	25.8
	Widowed	14.3	42.5	59.3	63.6	52.3
	Divorced	28.6	30.0	23.7	0.0	21.9
Living Arrangement	Lives with family	100.0	87.5	76.3	81.8	82.0
	Lives alone	0.0	12.5	23.7	18.2	18.0
Health Status	Chronic Illness	85.7	62.5	61.0	54.5	61.7
	No Chronic Illness	14.3	37.5	39.0	45.5	38.3

Specific Objective One: Assessment of changes in physiological parameters with ageing in an elderly Ghanaian population

4.2 PHYSIOLOGICAL PARAMETERS WITH AGEING

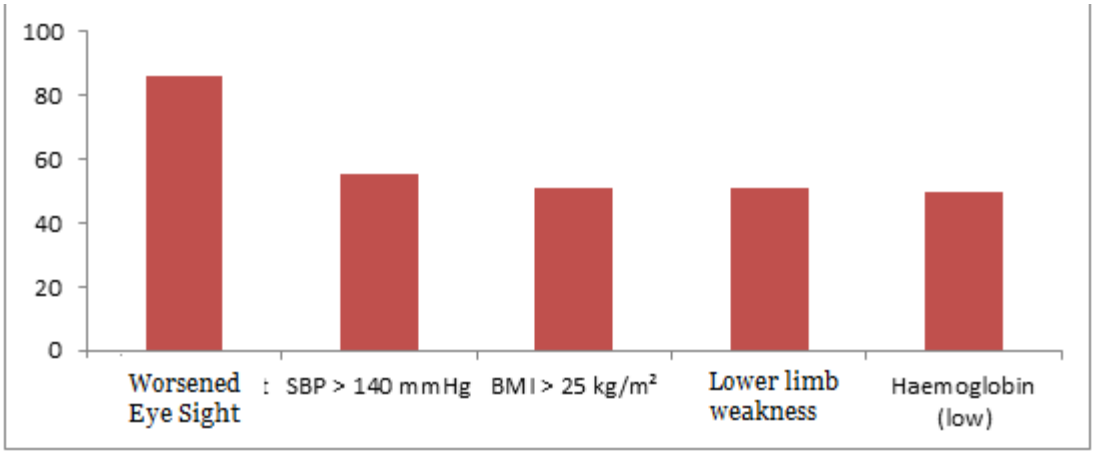
Nearly all respondents (99.2%) showed a poor result on at least one of the 13 factors studied.

1. Physiological parameters – Systolic and Diastolic Blood Pressure, Body Mass Index, Haemoglobin, White Cell Count, Neutrophils, Lymphocytes, Platelets,
2. Self-Reported Health parameters – Sight, Hearing, lower limb weakness, upper limb weakness and
3. Cognitive function

With all 13 factors listed above put together 95.3% of respondents had either one poor self-rating or one measured parameter which was not in the normal range. Seventy-five percent had values outside normal ranges of the measured physiological parameters with 58.8% out of normal range for haematological parameters. Over sixty percent (61.7%) had poor results on four to six parameters each and 9.4% in seven to nine parameters. The number of physiological parameters outside normal range results tended to increase more with age for males than for females ($r=0.288$, $p=0.088$ for males; and $r=0.124$, $p=0.240$ for females). Whereas only 50.0% of males aged between 60 and 69 years showed out of range results in one to three of the physiological parameters, as much as 76.5% of those aged between 70 and 79 years showed poor or out of range results in four to six parameters while 90.9% of those between 80 and 90 years showed poor or out of range results in four to six parameters.

The majority of poor or out of range results were recorded in the following physiological parameters: Eye sight, Systolic Blood Pressure, BMI, Lower-limb weakness, and low Haemoglobin level (see Fig. 4.1 below).

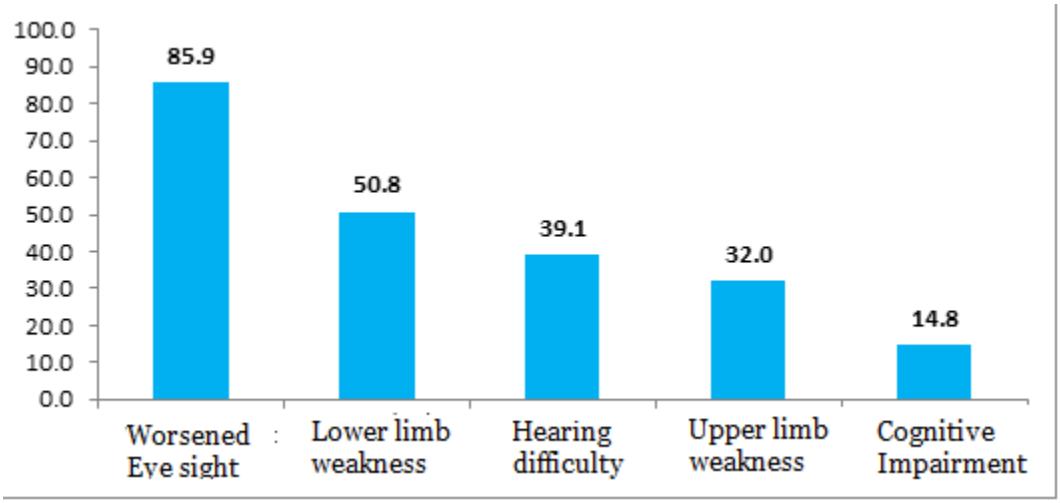
Figure 4.1: Percentage Distribution of Respondents on worsened eye sight, systolic blood pressure>140mmHg, BMI>25kg/m^2, lower limb weakness, and low hemoglobin.



4.3 SELF-REPORTED HEALTH (SRH) INDICATORS ON RESPONDENTS' HEALTH STATUS

Over eighty percent (85.9%) of the respondents reported worsening eyesight, 50.8% reported a weakening lower limb, 39.1% an increasing difficulty in hearing, 32.0% a weakening upper limb, and 14.8% a mild or moderate cognitive impairment.

Figure 4.2: Percentage Distribution of Respondents on Self-Reported Health



Self-Reported health status factors on Sight and Hearing Impairments did not show any significant difference by age or sex ($p>0.05$ in each case). Among males however, the older age-groups are more likely to report a deteriorating sight ($p=0.024$, Chi-square test) or hearing impairment ($p=0.083$, Chi-square test) than those in lower age-groups.

Lower and upper limb weakness tend to increase significantly with age ($p=0.006$ & $p=0.031$, respectively, Chi-square tests). However, these SRH factors did not show any marked difference by sex ($p>0.05$, Chi-square test).

While just a few respondents indicated a mild or moderate deterioration in cognitive ability, the percentage of females who reported a deteriorated cognitive function was significantly higher than males (19.6% vs. 2.8%, *p=0.016*, Chi-square test).

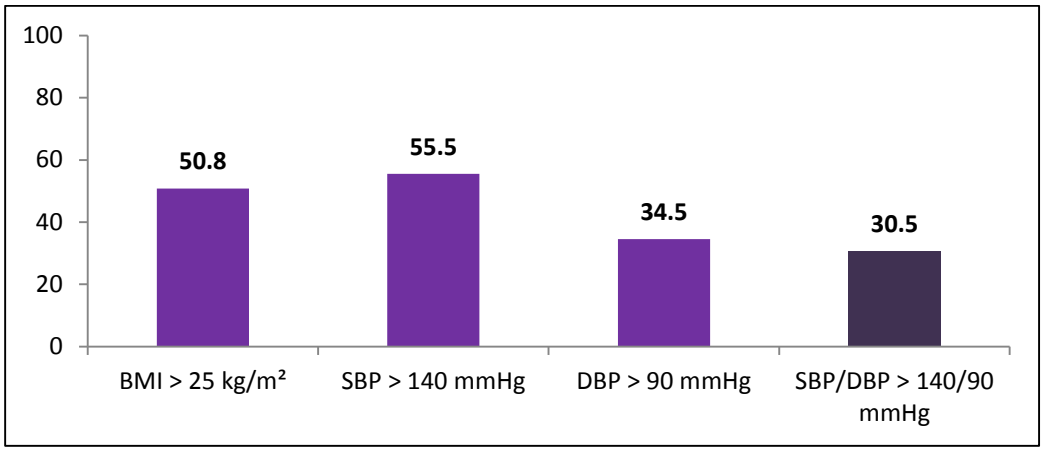
Table 4.2: Significant SRH Parameters by Age

SRH	**AGE GROUP**				
	50-59	**60-69**	**70-79**	**80+**	**ALL**
Base (Both sexes)	7	40	59	22	128
Lower limb	28.6	32.5	66.1	50.0	50.8
Upper limb	14.3	17.5	37.3	50.0	32.0
Base (Males)	0	8	17	11	36
Sight	-	62.5	94.1	100.0	88.9
Hearing	-	12.5	41.2	63.6	41.7

4.4 PHYSIOLOGIC AND ANTHROPOMETRIC MEASUREMENTS

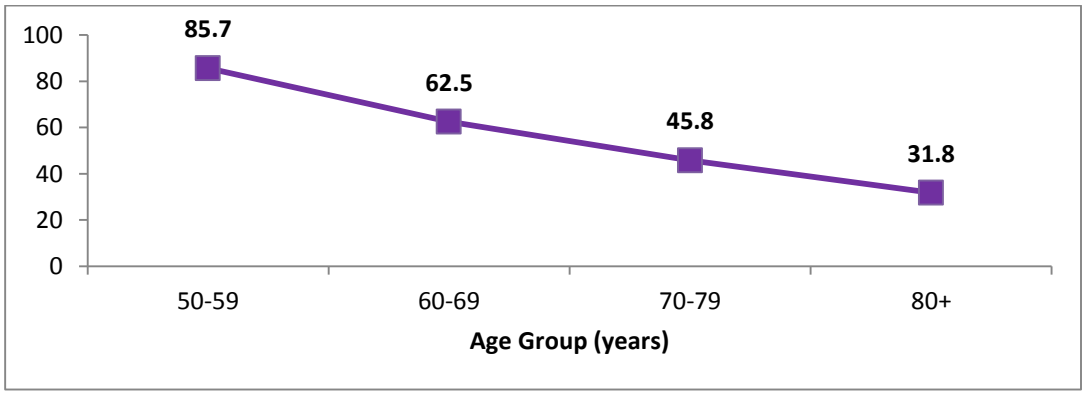
Over half of the respondents (55.5%) had a Systolic Blood Pressure above 140 mmHg, and about a third (34.5%) a Diastolic Blood Pressure above 90 mmHg. Some 30.5% had both their Systolic and Diastolic Blood Pressures falling within these ranges. About half of the respondents (50.8%) also had a BMI of 25 kg/m^2 or more, Fig 4.3.

Figure 4.3: Percentage Distribution of Physiologic and Anthropometric measures



A significantly higher percentage of females, compared to males, had a BMI that was greater than 25 kg/m^2 (60.9% vs. 25.0%, p<0.001, Chi-square test). Besides, BMI showed a significant decline by age (p=0.025, Chi-square test), Fig 4.4.

Figure 4.4: Percentage Distribution of Respondents with BMI > 25 kg/m2 by Age



Since only BMI showed significant results by age and sex, it was the only parameter used for further analysis.

Table 4.3 Age, chronic illness, hemoglobin cross tabulation.

AGE * CHRONIC ILLNESS * HB Cross tabulation

Count

HB			CHRONIC ILLNESS		Total
			YES	NO	
8-10	AGE	60-69 YEARS	4	2	6
		70-79YEARS	2	2	4
		80-89 YEARS	1	1	2
		90 and above	0	1	1
	Total		7	6	13
10.1-12	AGE	50 -59 YEARS	5	0	5
		60-69 YEARS	14	8	22
		70-79YEARS	24	12	36
		80-89 YEARS	8	5	13
	Total		51	25	76
12.1-14	AGE	50 -59 YEARS	1	1	2
		60-69 YEARS	7	5	12
		70-79YEARS	7	7	14
		80-89 YEARS	3	3	6
	Total		18	16	34
14.1-16	AGE	70-79YEARS	1	2	3
	Total		1	2	3
16.1-18	AGE	70-79YEARS	2		2
	Total		2		2

Table 4.4 Age Haemoglobin cross tabulation

AGE * HB Cross tabulation

Count

		HB					
		8-10	10.1-12	12.1-14	14.1-16	16.1-18	
AGE	50 -59 YEARS	0	5	2	0	0	7
	60-69 YEARS	6	22	12	0	0	40
	70-79YEARS	4	36	14	3	2	59
	80-89 YEARS	2	13	6	0	0	21
	90 and above	1	0	0	0	0	1
Total		13	76	34	3	2	128

From table 4.4 above, only two persons had Hb between 16.1 and 18g/dl, both of them within ages 70 and 79. Thirteen persons had Hb below 10g/dl.

Fig 4.5 Histogram showing numbers of persons in different age groups and Hbg/dl

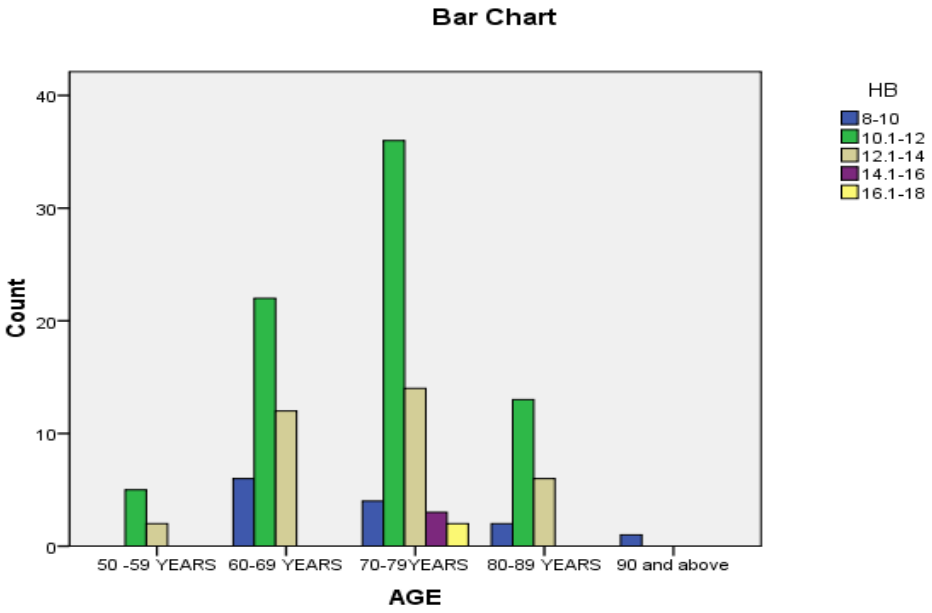


Table 4.5 Table showing age, chronic illness and FBS cross tabulation

AGE * CHRONIC ILLNESS * FBS Cross tabulation

Count

FBS			CHRONIC ILLNESS		Total
			YES	NO	
BELOW 4	AGE	60-69 YEARS	2	0	2
		70-79YEARS	0	1	1
	Total		2	1	3
4-5	AGE	50 -59 YEARS	1	0	1
		60-69 YEARS	12	6	18
		70-79YEARS	17	8	25
		80-89 YEARS	7	5	12
	Total		37	19	56
5.1-6	AGE	50 -59 YEARS	4	1	5
		60-69 YEARS	10	4	14
		70-79YEARS	14	11	25
		80-89 YEARS	2	3	5
	Total		30	19	49
6.1-7	AGE	50 -59 YEARS	1	0	1
		60-69 YEARS	1	2	3
		70-79YEARS	2	2	4
		80-89 YEARS	1	0	1
	Total		5	4	9
7.1-8	AGE	60-69 YEARS	0	3	3
		70-79YEARS	2	1	3
		80-89 YEARS	1	0	1
	Total		3	4	7
ABOVE 8	AGE	70-79YEARS	1	0	1
		80-89 YEARS	1	1	2
		90 and above	0	1	1
	Total		2	2	4

Table 4.6 Table showing age, FBS cross tabulation.

AGE * FBS Cross tabulation

Count		FBS						
		BELOW 4	4-5	5.1-6	6.1-7	7.1-8	ABOVE 8	Total
AGE	50 -59 YEARS	0	1	5	1	0	0	7
	60-69 YEARS	2	18	14	3	3	0	40
	70-79YEARS	1	25	25	4	3	1	59
	80-89 YEARS	0	12	5	1	1	2	21
	90 and above	0	0	0	0	0	1	1
Total		3	56	49	9	7	4	128

Fig 4.6 Histogram showing numbers of persons in different age groups against fasting blood sugar (FBS).

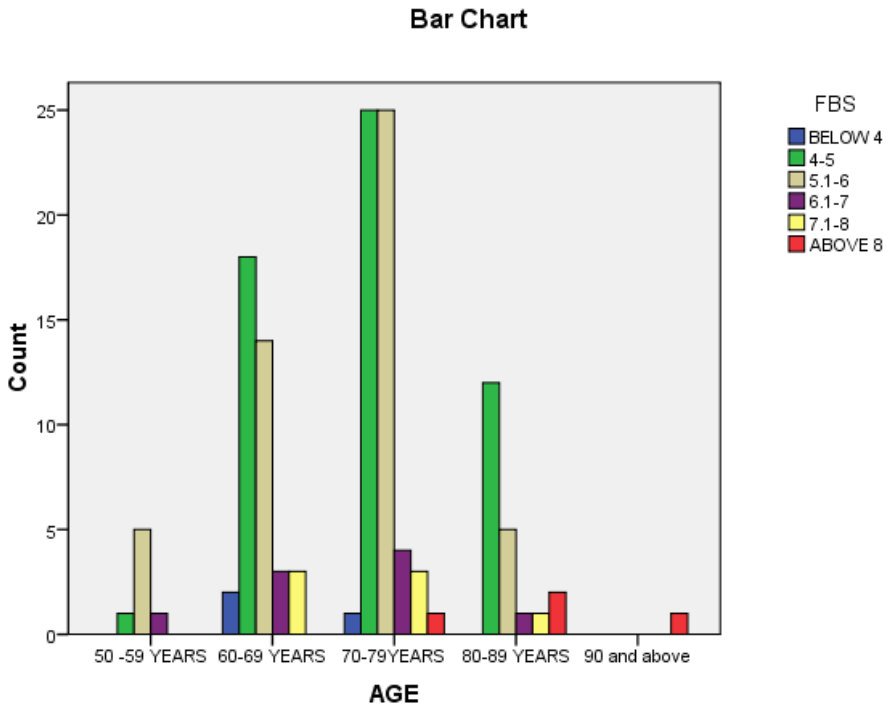
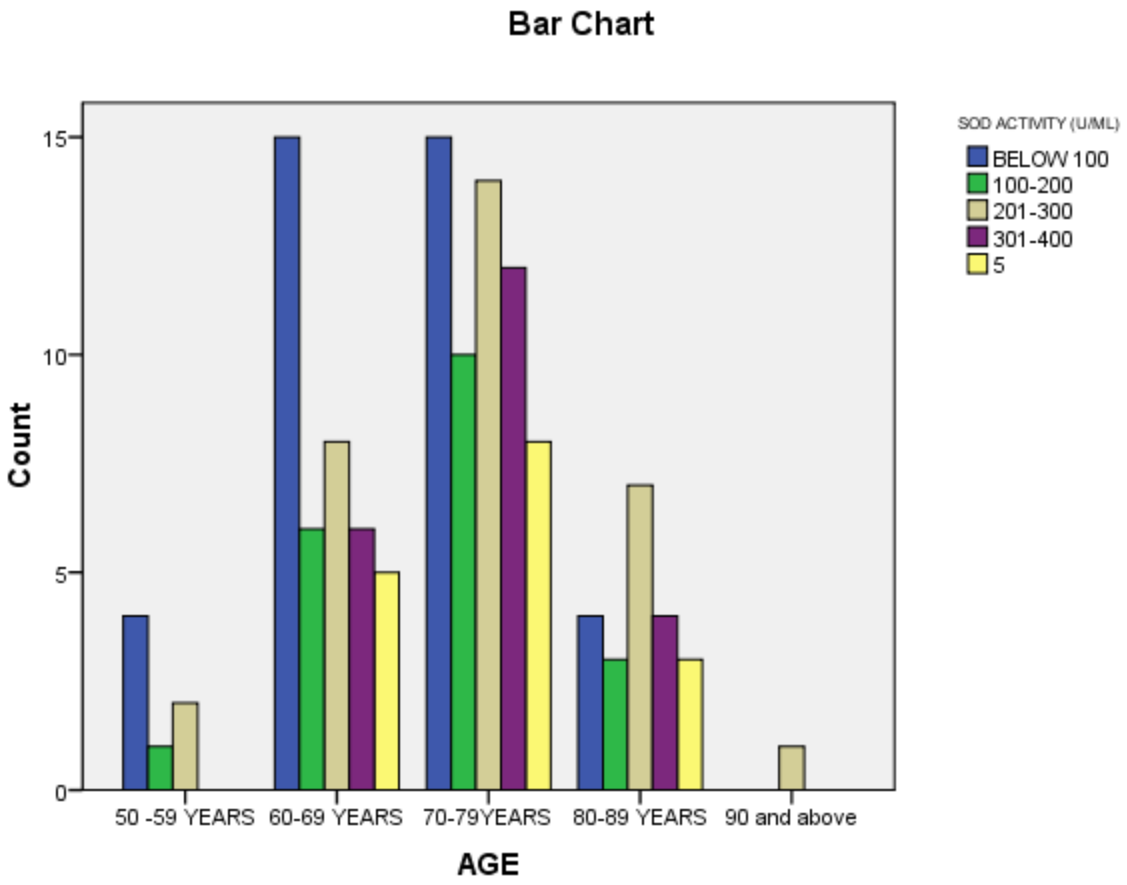


Fig 4.7 Histogram showing SOD activity in different age groups

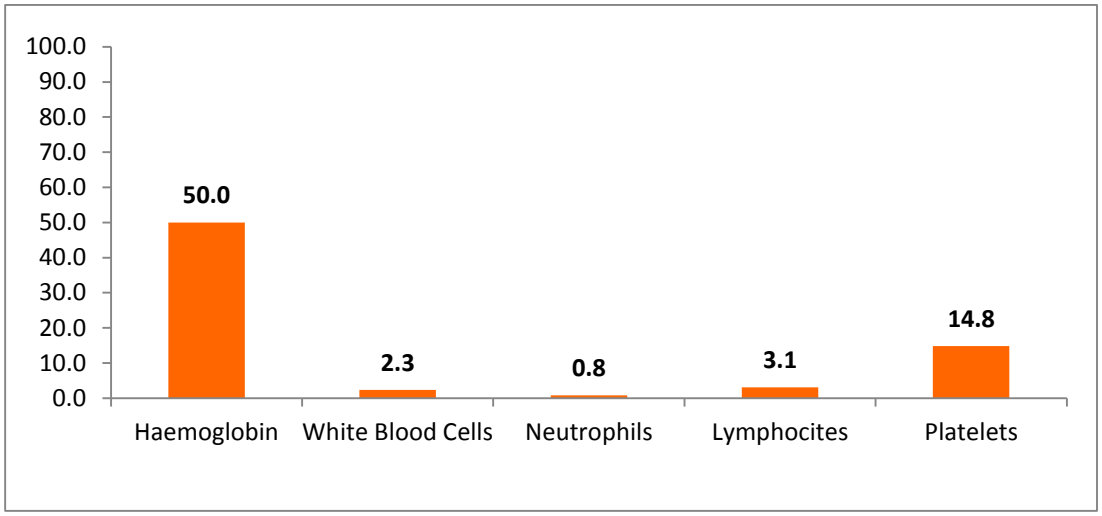


4.5 Haematological Parameters

A majority of the respondents (97.7%) had white blood cells within normal range (between 2.6 and 8.5 K/µL), with almost everyone (99.2%) having a neutrophil count of between 25 and 75% of total white blood cells. A high proportion (96.9%) also had Lymphocyte counts within normal range (25 to 69% of total White Blood Cells).

Exactly half of the respondents (50.0%) had Haemoglobin levels below the clinically normal range (15 to 18 g/dl, males and 11 to 16g/dl, females). There were also a substantial minority (14.8%) whose Platelet counts were below the normal range (150-140 K/ µL).

Figure 4.8: Percentage of Respondents with Haematological Parameters below lower limit.



Low levels of Haemoglobin and Platelets appear to occur more often with increasing age but this was not statistically significant ($p>0.05$, Chi-square test). However in terms of sex, a low level of Haemoglobin was prevalent among Males (97.2%) than Females (31.5%), ($p<0.001$, Chi-square test). Likewise, a relatively higher proportion of Males (25.0%) than Females (10.9%) had their Platelets below the normal range ($p<0.043$, Chi-square test).

Because males and females showed different results on Haemoglobin and Platelets, these were used for further analysis.

4.6 FOXO3a PCR Results

Fig 4.9 Foxo3a PCR gel electrophoresis

showing PCR products of approximately 300 Base Pairs

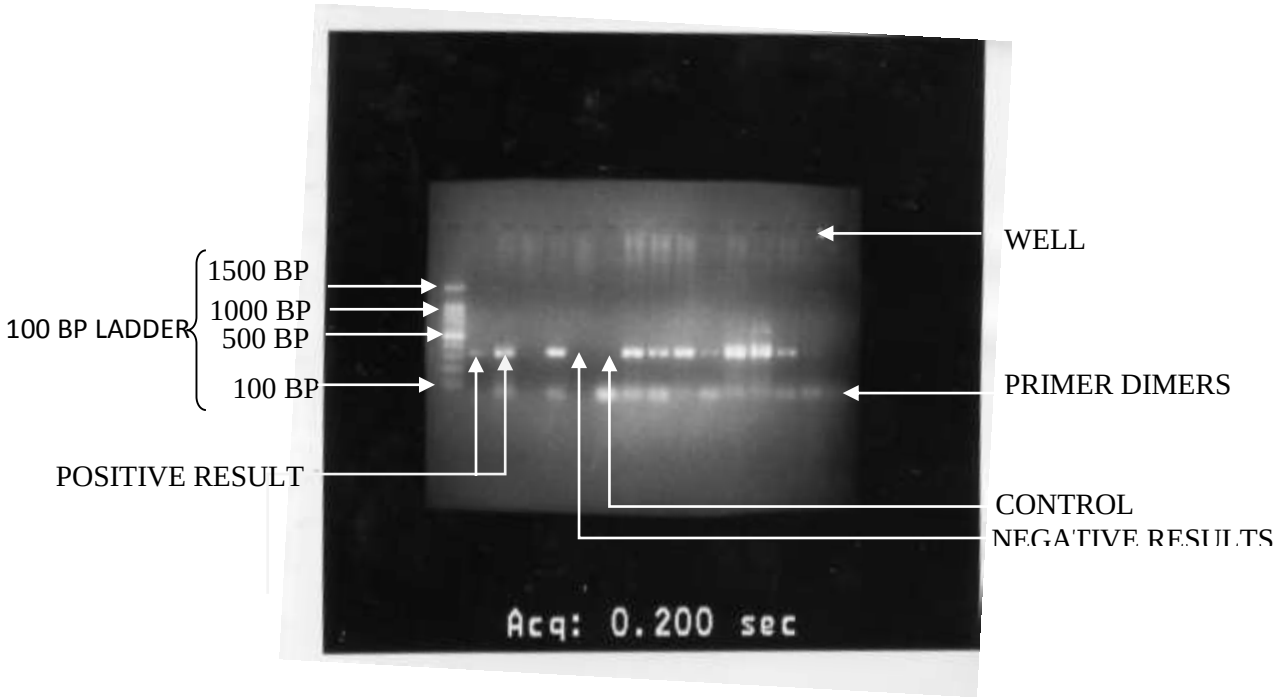


Table 4.7 FOXO3a PCR results.

	No Chronic Illness	Chronic Illness
Number of samples	49	79
Number of PCR	147	237
Number of positive results	100	163
% positive results	68.03	68.78

Overall, out of a total of 147 (49 x 3) PCRs done on samples of participants who did not have chronic disease 100 positive results were obtained (68.03 %). In the case of those who had chronic disease 237 (79 x 3) PCRs were done with 163 positive results (68.78). Ref. Table 4.7

4.7 Hierarchical Regression model for BMI

A summary of the Hierarchical Regression model for BMI is presented in Table 4.4 below. BMI is presented in the column while the independent variables are presented in the rows. For this model, socio-economic and lifestyle variables were further dropped from the model since they were weakly related to the level of BMI and caused adjusted R^2 to reduce.

The β for each independent variable shows the significance and relative importance of that variable in explaining variations in the dependent variable (in this case BMI). The R^2 shows the total variation in BMI explained by the regression model at each step. At each

step that a set of independent variables are introduced into the model, R^2 changes, to reflect the contribution of that set of variables in explaining the variance in work attitude. Table 4.4 shows that, overall, the regression model is statistically significant in explaining BMI score ($p < 0.01$). The model reveals that Sex, Age, FOXO3a gene and Chronic Illness jointly explained 5.6% of the total variance in BMI score. The results also indicate that FOXO3a gene strongly explains why the BMIs of Males are lower than Females. Moreover, Chronic Illness strongly explains why BMI declines by Age, and moderately explains differences.

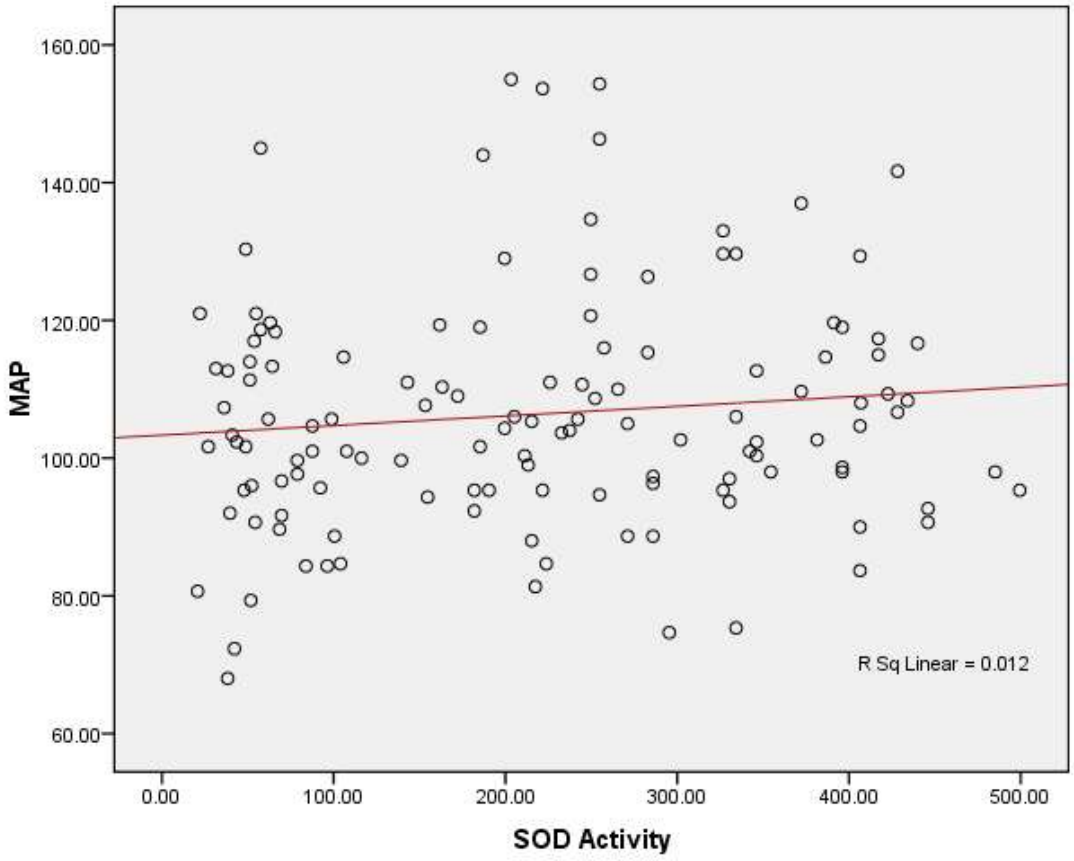
Table 4.8: A Hierarchical Regression Model on the BMI

Independent variables	**BMI (kg/m$^{2)}$**			
	Model 1	Model 2	Model 3	Model 4
S1: *Demographic*	β	β	β	β
Female	0.268**	0.262**	0.233*	
Age	-0.182*	-0.182*	-0.178*	
R^2 Adj.	**0.119*** **			
S2: *Genotype*				
FOXO3a gene		-0.172*	-0.168*	
R^2 Adj.		**0.136*****		
S3: *Health status*				
Chronic Illness			0.106	
R^2 Adj.			**0.139*****	
**P<0.05, **P<0.01, ***P<0.001* *All the βs are standardized coefficients*				

4.8 Correlation between MAP and SOD Activity

The results below show a positive linear relationship between MAP and SOD activity with SOD activity increasing with increasing MAP.

Figure 4.10 Showing Correlation between SOD Activity and MAP



Specific Objectives 2 and 3: To compare selected physiological, and biochemical parameters and FOXO3a genetic polymorphism in the elderly Ghanaian population with and without chronic illness.

A significant difference in systolic and diastolic blood pressure *(p value < 0.05)* between those who have chronic illness and those who are not chronically ill. FEV_1, anemia in men and women, overweight, superoxide dismutase activity all show no significant differences between respondents with and without chronic illness. (Table 4.9).

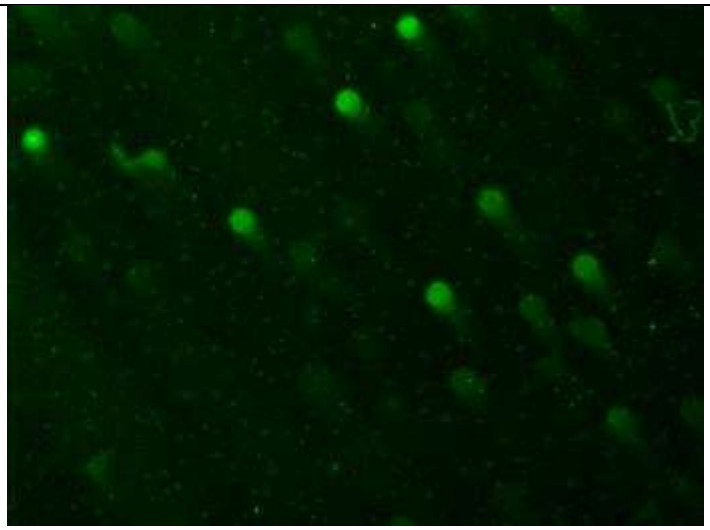
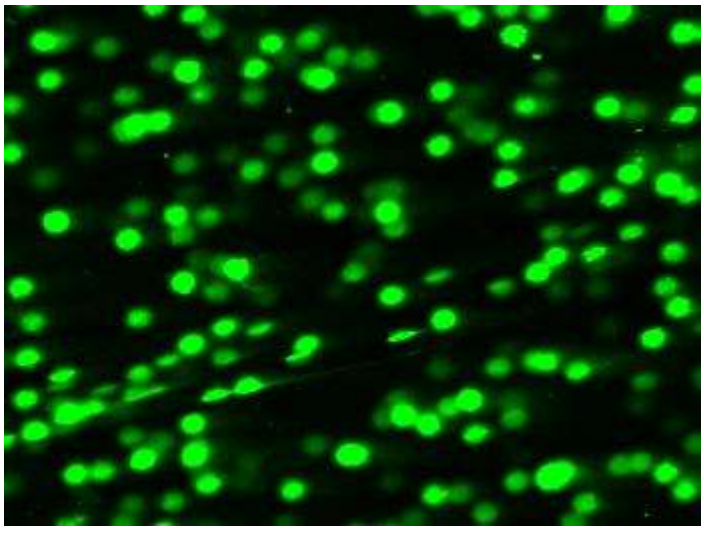
Table 4.9 Distribution of Selected Physiological and Hematological Parameters among Elderly Ghanaian Population with and without chronic illnesses

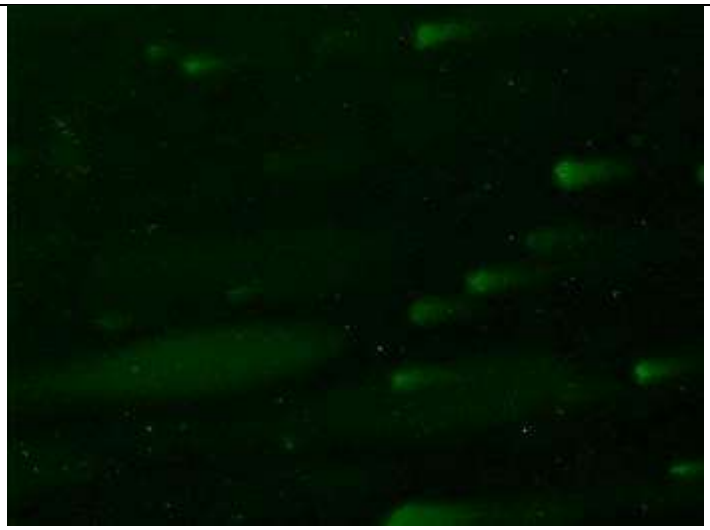
PARAMETER	CHRONICALLY ILL (N = 79)	NON-CHRONICALLY ILL (N = 49)	P VALUE
SYSTOLIC BLOOD PRESSURE (SBP> 140 mmHg)	67.1%	36.7%	0.001 Chi-S
FEV_1 (L per min)	1.18	1.35	0.061 t-test
Anaemia in Men (HB< 15G/DL)	93.3% (n=15)	100% (n=21)	0.230 Chi-S
Anaemia in Women (HB<11G/DL)	28.1% (n=64)	39.3% (n=28)	0.289 Chi-S
Overweight (BMI>25.5 KG/M SQ.)	53.2%	36.7%	0.070 Chi-S
Superoxide dismutase activity (SOD)	201.44	243.76	0.084 t-test
Systolic Blood Pressure	152.72	139.61	0.004 t-test
Diastolic Blood Pressure	88.52	81.16	0.004 t-test

4.9 Comet Assay Results

Specific Objective four: To demonstrate mechanical ageing of cells with the Comet Assay

Table 4.10 Comet Assay Results

AGE GROUP	RESULT OF COMET ASSAY	Percentage of DNA in tail	SCORE OF COMET ASSAY 0-4 IN INCREASING ORDER OF CELL DAMAGE
20 -25 A		25 %	2
20 – 25 B		0 %	0

60 YEAR OLD C		55%	3

Slide A and B show results from the Comet Assay in the young control group. Slide A showed 25% of DNA in the comet tail while Slide B had 0% DNA in the comet tail scoring 2 and 0 respectively. Slide C shows the result from a 60 year old who had 55% DNA in t

CHAPTER FIVE

DISCUSSION

The study is the first reported known one in Ghana to have assessed physiological, biochemical and genetic variations with ageing and chronic diseases.

Four point one percent of the population of Ghana are over 65 years old; male – 47% and female- 53% (Index Mundi, 2013). However, in this study gender was skewed towards the females, 71.9% as compared to males 28.1%. This might have occurred because social interactions among elderly males is known to be lower than what is seen in females, (Holwerda et al, 2012) and the study found that the HelpAge centers where the data was collected serve as places of socialization for the elderly. Secondly, there are known gender gaps in morbidity and mortality with females outliving males though these gaps are getting filled as lifestyles of women and men become more similar (Lui et al, 2013).

Only 3.9% of respondents had had tertiary education. In Ghana, the Secondary Net Enrolment ratio in 2007 was 45% while tertiary Gross Enrolment ratio was 6% (Atuahene and Owusu-Ansah, 2013) showing that the percentage of people who receive tertiary education is low. Socio-economic status, income, health and standard of living are directly related to education (Braveman et al, 2010) so this low level of persons with tertiary education is of concern.

The finding that 74% of respondents were either widowed or divorced at the time of the study with less than half of the respondents receiving regular pensions raise concerns about care for elderly and loneliness. The culture of elderly persons staying with family in Ghana results in a strong social support system for the elderly. Eighty-two percent of the elderly in this study were living with family, eighteen percent lived alone.

Although 92% of respondents had registered with the National Health insurance scheme there is no way of telling from this study whether or not they have access to it in the way that they should. This figure is in sharp contrast to the findings of SAGE Ghana of 26.8% access to health insurance in Ghana (Biritwum et al, 2013).

Thirty one point nine percent of the subjects said that they had increasing difficulty in hearing. Comparing this with 40% value obtained by audiometry by Cioba and associates in 2012 gives the impression that hearing loss in Ghana may be quite high since self-reported hearing loss is less sensitive than audiometric testing. Audiometric screening will detect the true prevalence of hearing loss and distinguish between high and low frequency hearing loss as well as unilateral and bilateral hearing loss. Unilateral hearing loss is very common in developing countries like Ghana due to the high incidence of chronic suppurative otitis media (Akinpelu et al, 2008) while bilateral hearing loss due to presbyopia is age related. Hearing has a large bearing on social interaction and morbidity especially in the elderly, with hearing loss associated with increased morbidity as social interaction decreases (Cioba *et al.*, 2012).

Sight was generally reported as poor in this study with 85.9% of respondents saying that their sight had worsened at present as compared to their middle age. A progressive loss in the ability to accommodate occurs in the eyes with ageing (Kam *et al*, 2010). Cataracts, glaucoma and macular degeneration are age associated conditions that result in poor sight in the elderly (Kam *et al*, 2010).

Self-reported lower and upper limb weakness increased significantly with increasing age, lower limb weakness reported by 50.8% of subjects and upper limb weakness reported by 32.0%. There was no marked difference by sex. Slow, progressive muscle weakening is known to occur with ageing and is believed to be due to muscle wasting (Degens, 2010).

Subjects who reported chronic illness made up 61 percent of the subjects studied. Chronic illnesses reported included hypertension, diabetes mellitus and cancer.

Females and the chronically ill dominated the lower age groups (50 - 69 years) while respondents receiving regular pension and widows dominated the older age groups (70 – 90 years). Overall, 75.0%, 58.8% and 95.3% respectively had physiological, hematological or self-reported health status parameters outside normal ranges. Results of physiological parameters outside normal ranges tended to increase more with age for males than for females.

Exactly half of the respondents (50.0%) had haemoglobin levels below the lower limit for their respective sexes. Low haemoglobin was more prevalent among males (97.2%) than Females (31.5%). Fourteen point eight percent had platelet counts below the lower normal range with a relatively higher proportion of males (25.0%) compared to females.

Blood pressure increased with age, both in the chronically ill and the non-chronically ill with 30.5% having blood pressure above 140/90 mmHg. There's a significant difference in systolic and diastolic blood pressure between those who have chronic illness and those who are not chronically ill.

FEV_1, anemia and superoxide dismutase activity all show no significant differences between respondents with and without chronic illness. For BMI however, a significantly higher percentage of females were overweight compared to males.

The percentage of females who reported a deteriorated cognitive function was significantly higher than males.

Older respondents had higher SOD activity, an indication of greater oxidative stress. In addition, the results showed a positive correlation between the Mean Arterial Pressure and SOD activity. These findings compare with those made by Yi and associates in 2003 when

they found that injection of extracellular superoxide dismutase caused a reduction in mean arterial pressure in spontaneously hypertensive rats (Chu *et al*, 2003).

The findings on BMI in this study speak to the issue of the rising incidence of non-communicable diseases linked to obesity in sub Saharan Africa. Zamboni and Mazzali (2012) showed that 30% of elderly Americans are obese while Blankson and Paul showed in 2012 that 16.9% of elderly women in rural Ghana were obese (de-Graft Aikens, 2007) also found that cardiovascular-related deaths were the leading cause of deaths in 2001. This study shows that 24.2% of respondents were obese with a significantly higher proportion of women were found to be overweight compared to men. This is comparable to the rates recorded by Zamboni and Mazzali (2012) about prevalence rates in obesity in the United States and reinforces the non-communicable disease burden facing low and middle income countries (LMIC). This finding is also consistent with projections by the WHO that up to 80% of all cardio vascular- related deaths now occur in LMIC.

The study shows that with age, men have a poorer health status compared to women. All over the world women have higher life expectancy than men (Lee et al, 2012).

Anaemia and low platelets counts in men are both reflective of a poor nutritional status. Since anaemia in the elderly is often indicative of other pathologies and is associated with high mortality this high rate deserves thorough studies.

The lack of significant differences in FEV1, BMI, haemoglobin and superoxide dismutase activity between respondents with and without chronic illness can be explained by the fact that there is still adequate physiological functional reserve to compensate for their chronic conditions together with the benefits of medication and lifestyle choices.

The study revealed that sex, age, and chronic illness jointly explained 5.6% of the total variance in BMI score. The results also suggest that the FOXO3a gene has an effect on the BMI, with that of males being lower than that of females. Moreover, chronic illness

strongly explains why BMI declines by age, and moderately explains differences in BMI by sex.

The positive correlation between MAP and oxidative stress is explained by the fact that as the body deals with the pathophysiologic mechanisms underlying the high blood pressure state, its redox balance is disturbed with the generation of more free radicals which by signal transduction cause an increase in antioxidant activity.

Age related changes in synovial joints leading to the development of osteoarthritis together with the sarcopenia explains difficulty in walking that many elderly people suffer. Ghana's health insurance scheme exempts persons aged 70 years and above from payment of premiums. The study's finding that 92% of the elderly had active health insurance coverage is significant as it indicates that they have some confidence in the health services offered and are likely to patronize geriatric services when made available.

The Comet Assay results show that deterioration in the DNA occurs in both the young (20 years) and the elderly (60 years). Factors that are known to cause destruction of DNA structures include exposure to radiation, poor nutritional status, ageing, intrinsic impaired ability to repair DNA, toxic materials (poisons) and sunlight ((Sahin and DePinho, 2010).

POSSIBLE LIMITATIONS

The study had the following possible limitations;

- Categorization of chronic ill health status could have been determined through more objective means, not dependent on self-reporting methods. In a health system where people may be unaware of their health status, it is possible that some of these elderly respondents might have been misclassified.

- The method of sampling used significantly limits the extent to which findings from this study can be generalized to the general population.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

This study has been conducted to address two hypotheses that:

a. There is no significant difference in physiological parameters in the elderly Ghanaian population with and without chronic illness.
b. Foxo3a genetic variations which have been associated with longevity in other populations are absent in Ghanaians.

The following conclusions can be drawn;

BMI was found to decrease with age while blood pressure, worsening eyesight, impaired hearing, limb weakness all increased with age with significant differences in both systolic and diastolic blood pressures between those with chronic and those without chronic illness.

FEV1, anemia in men and women, overweight, superoxide dismutase activity all show no significant differences between respondents with and without chronic illness. Significant levels of anemia however exist in the aged population.

Recommendations

- The exemption policy under the national health insurance scheme for the aged is having a positive impact on access to health care and it is recommended that it be maintained and strengthened *subscription level does not indicate impact on access*

- Given the high prevalence of anemia, poor vision, high blood pressure and overweight in the aged, it is recommended that the Ministry of Health and its service agencies develop deliberate programs and strategies to tackle these conditions. Determination of reference ranges of hematological parameters for the Ghanaian elderly must be developed. .
- Further genetic studies are recommended to identify which specific FOXO 3a variants may be available in the Ghanaian population and that are specifically associated with longevity. Knowledge in this will enable better understanding of the genetic basis of disease.

REFERENCES

Addo J, Smeeth L, Leon DA. (2007). Hypertension in sub-saharan Africa: a systematic review. Hypertension 2007; 50: 1012-8

Adinkrah, Mensah. "Witchcraft accusations and female homicide victimization in contemporary Ghana." Violence against women 10, no. 4 (2004): 325-356.

Agrawal Yuri, MD; Elizabeth A. Platz, ScD, MPH; John K. Niparko, MD (2008)

Arch Intern Med. 2008;168(14):1522-1530. doi:10.1001/archinte.168.14.1522.

Akinpelu, O. V., Y. B. Amusa, E. O. Komolafe, A. A. Adeolu, A. O. Oladele, and S. A. Ameye. "Challenges in management of chronic suppurative otitis media in a developing country." The Journal of Laryngology & Otology 122, no. 01 (2008): 16-20.

Allaro, M., V. Lebre, and J.-M. Robine. (1998). Jean Calment, From Van Gogh's Time to Ours, 122 Extraordinary Years. W. H. Freeman and Company, New York

Agrawal Yuri, MD; Elizabeth A. Platz, ScD, MPH; John K. Niparko, MD (2008)

Amoah AG, Hypertension in Ghana: a cross sectional community prevalence study in Greater Accra

Amon Exavery, Kerstin Klipstein-Grobusch, Cornelius Y. Debpuur, “Self-rated health (SRH) and healthcare utilization among rural elderly Ghanaians in Kassena-Nankana district” http://uaps2011.princeton.edu/abstracts/110332

Arthur, F. K. N., F. A. Yeboah, K. Nsiah, P. K. N. Nkrumah, K. A. Afreh, and K. Agyenim-Boateng. "Fasting blood glucose and glycosylated haemoglobin levels in

randomly selected ghanaian diabetic patients–the clinical implications." Journal of Science and Technology (Ghana) 25, no. 2 (2006): 13-17.

Artz, Andrew S MS; Chief Editor: Emmanuel C Besa, MD Updated: Jun 8, 20 http://emedicine.medscape.com/article/1339998-overview

Bartke, Andrzej. "Insulin and aging." Cell Cycle 7, no. 21 (2008): 3338-3343.

Beckman, Kenneth B., and Bruce N. Ames. "The free radical theory of aging matures." Physiological reviews 78, no. 2 (1998): 547-581.

BeLue R, Okoror TA, Iwelunmor J, Taylor KD, Degboe AN, Agyemang C. (2009). An overview of cardiovascular risk factor burden in sub-Saharan African countries: a socio-cultural perspective. Global Health 2009; 5: 10- doi: 10.1186/1744-8603-5-10 pmid: 19772644.

Beutler, Ernest, and Carol West. "Hematologic differences between African-Americans and whites: the roles of iron deficiency and α-thalassemia on hemoglobin levels and mean corpuscular volume." Blood 106, no. 2 (2005): 740-745.

Biritwum, Richard B., George Mensah, Nadia Minicuci, Alfred E. Yawson, Nirmala Naidoo, Somnath Chatterji, and Paul Kowal. "Household characteristics for older adults and study background from SAGE Ghana Wave 1." Global health action 6 (2013).

Blanchet N J, Fink G, and Osei-Akoto (2012) The Effect of Ghana's National Health Insurance Scheme on Health Care Utilisation. Ghana Med Journal 2012 June; 46(2): 76–84. PMCID: PMC3426378

Blankson, B., Hall, A. (2012). The anthropometric status of elderly women in rural Ghana and factors associated with low body mass index. Centre for Public Health Nutrition, School of Life Sciences, University of Westminster, London, UK.

Bolton Emily, and Chakravarthi Rajkumar. "The ageing cardiovascular system." Reviews in Clinical Gerontology 21.02 (2011): 99-109.

Boron and Boulpaep (2002), Medical Physiology, 2nd edition, page 1283. Saunders, Elsevier. , 2002

Bowling, Ann, and Steve Iliffe. "Which model of successful ageing should be used? Baseline findings from a British longitudinal survey of ageing." Age and Ageing 35, no. 6 (2006): 607-614.

Braveman, Paula A., Catherine Cubbin, Susan Egerter, David R. Williams, and Elsie Pamuk. "Socioeconomic disparities in health in the United States: what the patterns tell us." American Journal of Public Health 100, no. S1 (2010): S186-S196.

Brunet, A., Bonni, A., Zigmond, M. J., (1999). Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell. 96, 857 – 868.

Caballero, B., and A. Coto-Montes. "An insight into the role of autophagy in cell responses in the aging and neurodegenerative brain." Histology and histopathology 27, no. 3 (2012): 263-275.

Cabiscol, Elisa, Jordi Tamarit, and Joaquim Ros. "Oxidative stress in bacteria and protein damage by reactive oxygen species." International Microbiology 3, no. 1 (2010): 3-8.

Cardona, Beatriz. "'Healthy Ageing'policies and anti-ageing ideologies and practices: on the exercise of responsibility." Medicine, Health Care and Philosophy 11, no. 4 (2008): 475-483.

Carter, Matthew E., and Anne Brunet. "FOXO transcription factors." Current Biology 17, no. 4 (2007): R113-R114.

Centers for Disease Control and prevention, USA. http://www.cdc.gov/

Christensen K, Johnson TE, Vaupel JW (2006). The quest for genetic determinants of human longevity: Challenges and insights. Nat Rev Genet, 7:436-448.

Chu, Yi, Shinichiro Iida, Donald D. Lund, Robert M. Weiss, Gerald F. DiBona, Yoshimasa Watanabe, Frank M. Faraci, and Donald D. Heistad. "Gene transfer of extracellular superoxide dismutase reduces arterial pressure in spontaneously hypertensive rats role of heparin-binding domain." Circulation research 92, no. 4 (2003): 461-468.

Ciorba, Andrea, Chiara Bianchini, Stefano Pelucchi, Antonio Pastore, A. Ciorba, C. Bianchini, S. Pelucchi, and A. Pastore. "The impact of hearing loss on the quality of life of elderly adults." Clinical interventions in aging 7 (2012): 159

Crowther, M. R. , Parker, M. W. , Achenbaum, W. A. , Larimore, W. L. , and Koenig, H. G. (2002). Rowe and Kahn's Model of Successful Aging Revisited Positive Spirituality—The Forgotten Factor. The Gerontologist, 42 (5): 613-620. doi: 10.1093/geront/42.5.613

Culleton, B. F., Manns, B. J., Zhang, J. (2006). Impact of anemia on hospitalization and mortality in older adults. Blood Cells Mol Dis, 107:3841

Degens, H. "The role of systemic inflammation in age-related muscle weakness and wasting." Scandinavian journal of medicine & science in sports 20, no. 1 (2010): 28-38.

de-Graft Aikins, Ama. "Ghana's neglected chronic disease epidemic: a developmental challenge." Ghana medical journal 41, no. 4 (2007): 154.

Depp, Colin A., and Dilip V. Jeste. "Definitions and predictors of successful aging: a comprehensive review of larger quantitative studies." The American Journal of Geriatric Psychiatry 14, no. 1 (2006): 6-20.

Delerme Samuel, Patrick Ray. Acute respiratory failure in the elderly: diagnosis and prognosis Age Ageing (2008) 37 (3): 251-257. doi: 10.1093/ageing/afn060

Dodds, Chris. "Anaesthetic considerations in the elderly." Ophthalmic Anaesthesia (2012): 185.

Dolan, Chantal Matkin, Helena Kraemer, Warren Browner, Kristine Ensrud, and Jennifer L. Kelsey. "Associations between body composition, anthropometry, and mortality in women aged 65 years and older." American journal of public health 97, no. 5 (2007): 913-918.

Donlon, Timothy A., J. David Curb, Qimei He, John S. Grove, Kamal H. Masaki, Beatriz Rodriguez, Ayako Elliott, D. Craig Willcox, and Bradley J. Willcox. "FOXO3 gene variants and human aging: coding variants may not be key players." The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 67, no. 11 (2012): 1132-1139.

Dreesen, Oliver, and Colin L. Stewart. "Accelerated aging syndromes, are they relevant to normal human aging?." Aging (Albany NY) 3, no. 9 (2011): 889.

Finkel, T. and Holbrook N. J. (2000). Oxidants, oxidative stress and the biology of ageing. Nature, 408:239–247.

Flachsbart, F., Make, C., Rabea K., Helene, B., Eller-Eberstein, H. V., Nikolaus, S., Schreiber, S. and Nebel, A. (2008). Association of FOXO3A variation with human longevity confirmed in German centenarians.

Francis Atuahene, Anthony Owusu-Ansah. A Descriptive Assessment of Higher Education Access, Participation, Equity, and Disparity in Ghana. DOI: 10.1177/2158244013497725 Published 23 July 2013

Fries, J. F. (2002) Aging, natural death, and the compression of morbidity. Bull World Health Organ, 80(3): 245-250.

Ghana Statistical Service (2011). www.ghana.gov.gh

Goldstein, Samuel. "Replicative senescence: the human fibroblast comes of age." Science 249, no. 4973 (1990): 1129-1133.

Gray, D.A, Woulfe, J. (2005). Lipofuscin and Aging: A Matter of Toxic Waste. Sci. Aging Knowl. Environ. (5).

Grune, T., Jung, T., Merker, K., Davies K. J. (2004). Decreased proteolysis caused by protein aggregates, inclusion bodies, plaques, lipofuscin, ceroid, and 'aggresomes' during oxidative stress, aging, and disease. Int J Biochem Cell Biiol. 36 (12): 2519-30.

Guarente, Leonard. "Sir2 links chromatin silencing, metabolism, and aging." Genes & development 14, no. 9 (2000): 1021-1026.

Gulshan Sharma, and Goodwin James. "Effect of aging on respiratory system physiology and immunology." Clinical interventions in aging 1, no. 3 (2006): 253.

Gutteridge, John, and Barry Halliwell. "Free radicals and antioxidants in the year 2000: a historical look to the future." Annals of the New York Academy of Sciences 899, no. 1 (2000): 136-147.

Hartmann, A., E. Agurell, C. Beevers, S. Brendler-Schwaab, B. Burlinson, P. Clay, A. Collins et al. "Recommendations for conducting the in vivo alkaline Comet assay." Mutagenesis 18, no. 1 (2003): 45-51.

Hayflick L. & Moorhead P.S.; Exp. CELL res. 25; 585-621,1961

Heiat, A., Vaccarino, V., Krumholz, H. M. (2001). An Evidence-Based Assessment of Federal Guidelines for Overweight and Obesity as They Apply to Elderly Persons. Arch Intern Med., 161, 1194–1203.

Hjelmborg, Jacob vB, Ivan Iachine, Axel Skytthe, James W. Vaupel, Matt McGue, Markku Koskenvuo, Jaakko Kaprio, Nancy L. Pedersen, and Kaare Christensen. "Genetic influence on human lifespan and longevity." Human genetics 119, no. 3 (2006): 312-321.

Holwerda, T. J., A. T. Beekman, D. J. Deeg, M. L. Stek, T. G. Van Tilburg, P. J. Visser, B. Schmand, C. Jonker, and R. A. Schoevers. "Increased risk of mortality associated with social isolation in older men: only when feeling lonely? results from the Amsterdam Study of the Elderly (AMSTEL)." Psychol Med 42, no. 4 (2012): 843-853.

Huang Cheng, Chengjie Xiong, Kerry Kornfeld, 8084–8089, doi: 10.1073/pnas.0400848101

Index Mundi, 2013, http://www.indexmundi.com/ghana/age_structure.html

Janssens, Jean-Paul. "Aging of the respiratory system: impact on pulmonary function tests and adaptation to exertion." Clinics in chest medicine 26, no. 3 (2005): 469-484.

Janssen, Ian, Peter T. Katzmarzyk, and Robert Ross. "Body mass index, waist circumference, and health risk: evidence in support of current National Institutes of Health guidelines." Archives of internal medicine 162, no. 18 (2002): 2074-2079.

Jylha, Marja. "What is self-rated health and why does it predict mortality? Towards a unified conceptual model." Social science & medicine 69, no. 3 (2009): 307-316.

Kam, Jaimie Hoh, Eva Lenassi, and Glen Jeffery. "Viewing ageing eyes: diverse sites of amyloid Beta accumulation in the ageing mouse retina and the up-regulation of macrophages." PLoS One 5, no. 10 (2010): e13127.

Karaouzene, N., H. Merzouk, M. Aribi, S. A. Merzouk, A. Yahia Berrouiguet, C. Tessier, and M. Narce. "Effects of the association of aging and obesity on lipids, lipoproteins and oxidative stress biomarkers: a comparison of older with young men." Nutrition, Metabolism and Cardiovascular Diseases 21, no. 10 (2011): 792-799.

Kassebaum, Nicholas J., Rashmi Jasrasaria, Nicole Johns, Sarah Wulf, David Chou, Rafael Lozano, Mohsen Naghavi, and Christopher JL Murray. "A systematic analysis of global anaemia burden between 1990 and 2010." The Lancet 381 (2013): S72.

Kawalinder K. Girgla, Deepinder Kaur and Kiran. Variation in lung functions with age – a local study. International Journal of Basic and Applied Medical Sciences ISSN: 2277-2103 (Online) http://www.cibtech.org/jms.htm (2012) Vol. 2 (2) May-August, pp.148-153/Girgla et al

Kawano, T., Ito, Y., Ishiguro, M., Takuwa, K., Nakajima, T., and Kimura, Y. (2000). Molecular cloning and characterization of a new insulin/IGF-like peptide of the nematode Caenorhabditis elegans. Biochem Biophys Res Commun, 273: 431–436.

Kenyon, Cynthia J. "The genetics of ageing." Nature 464, no. 7288 (2010): 504-512.

Kerber, R., O'Brien, E., Smith, K., Cawthon, R. (2001). Familial excess longevity in Utah genealogies. J Gerontol A Biol Sci Med Sci, 56(3), 130–139.

Kinsella, Kevin G., and David R. Phillips. Global aging: The challenge of success. Vol. 60, no. 1. Population Reference Bureau, 2005.

Kipling, David, Terence Davis, Elizabeth L. Ostler, and Richard GA Faragher. "What can progeroid syndromes tell us about human aging?." Science 305, no. 5689 (2004): 1426-1431.

Khattar, Rajdeep S., John D. Swales, Caroline Dore, Roxy Senior, and Avijit Lahiri. "Effect of aging on the prognostic significance of ambulatory systolic, diastolic, and pulse pressure in essential hypertension." Circulation 104, no. 7 (2001): 783-789.

Kops, Geert JPL, Tobias B. Dansen, Paulien E. Polderman, Ingrid Saarloos, Karel WA Wirtz, Paul J. Coffer, Ting-T. Huang, Johannes L. Bos, René H. Medema, and Boudewijn MT Burgering. "Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress." Nature 419, no. 6904 (2002): 316-321.

Lakatta, Edward G., and Daniel Levy. "Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises part I: aging arteries: a “set up” for vascular disease." Circulation 107, no. 1 (2003): 139-146.

Lakatta, E. G. (2002). Age-associated cardiovascular changes in health: impact on cardiovascular disease in older persons. Heart Fail Rev., 7(1):29-49

Lalley, Peter M. "The aging respiratory system—Pulmonary structure, function and neural control." Respiratory physiology & neurobiology 187, no. 3 (2013): 199-210.

Larsen et al. (2002) Self-rated health, ethnicity and social position in a deprived neighborhood in Denmark. International Journal for Equity 2011, 10:5 http://www.equityhealthj.com/content/10/1/5

Lee, I-Min, Eric J. Shiroma, Felipe Lobelo, Pekka Puska, Steven N. Blair, and Peter T. Katzmarzyk. "Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy." The Lancet 380, no. 9838 (2012): 219-229.

Liu, Yan, Asuna Arai, Yoshihide Obayashi, Koji Kanda, Eugene Boostrom, Romeo B. Lee, and Hiko Tamashiro. "Trends of gender gaps in life expectancy in Japan, 1947–2010:

associations with gender mortality ratio and a social development index." Geriatrics & gerontology international 13, no. 3 (2013): 792-797.

Marshall S.J, (2004) Bulletin of the World Health Organization | July 2004, 82 (7).

Marja Jylhä (2009) What is self-rated health and why does it predict mortality? Towards a unified conceptual model Social Science & Medicine, Volume 69, Issue 3, Pages 307-316

McEwen B. S. (2002). The End of Stress as We Know It. Washington, DC: Joseph Henry Press.

Merriam Webster Medical Definition and more. (2013) http://www.merriam-webster.com/medical/chronological%20age

Mehta, Linda Hotchkiss, and George S. Roth. "Caloric restriction and longevity." Annals of the New York Academy of Sciences 1172, no. 1 (2009): 28-33.

Melov, S., (2008). Biomarkers of Ageing identified. Buck Institute of Ageing research

Merriam Webster Medical Definition and more. (2013) http://www.merriam-webster.com/medical/chronological%20age.

Ministry of Employment and Social Welfare Ghana, (2010). http://www.ghana.gov.gh/index.php/governance/ministries/334--ministry-of-employment-and-social-welfare-

Minto Gary, Biccard Bruce. (2013) Assessment of the high risk perioperative patient. Contin Educ Anaesth Crit Care Pain doi: 10.1093/bjaceaccp/mkt020

Mitchell, Gary F., Mark A. van Buchem, Sigurdur Sigurdsson, John D. Gotal, Maria K. Jonsdottir, Ólafur Kjartansson, Melissa Garcia et al. "Arterial stiffness, pressure and flow

pulsatility and brain structure and function: the Age, Gene/Environment Susceptibility–Reykjavik study." Brain 134, no. 11 (2011): 3398-3407.

Murphy, Coleen T., Steven A. McCarroll, Cornelia I. Bargmann, Andrew Fraser, Ravi S. Kamath, Julie Ahringer, Hao Li, and Cynthia Kenyon. "Genes that act downstream of DAF-16 to influence the lifespan of Caenorhabditis elegans." Nature 424, no. 6946 (2003): 277-283.

National Health Insurance Authority. Annual report on the National Health Insurance Scheme; Accra, (2010) http://www.nhis.gov.gh/files/8(1).pdf

Niki, Etsuo. "Assessment of Antioxidant Capacity< i> in vitro</i> and< i> in vivo</i>." Free Radical Biology and Medicine 49, no. 4 (2010): 503-515.

Nybo, Lars, Mads K. Dalsgaard, Adam Steensberg, Kirsten Møller, and Niels H. Secher. "Cerebral ammonia uptake and accumulation during prolonged exercise in humans." The Journal of physiology 563, no. 1 (2005): 285-290.

Obare Francis. (2007) SELF-ASSESSED HEALTH STATUS AND MORBIDITY EXPERIENCES OF TEENAGERS IN NAIROBI'S LOW INCOME SETTINGS African Population Studies/Etude de la Population Africaine, Vol. 22, No. 1, 2007, pp. 3-20

Ofei-Kwapong, Nana Oye. "ASHESI UNIVERSITY COLLEGE." PhD diss., University College, 2013.

Partridge, Linda, and David Gems. "Mechanisms of aging: public or private?." Nature Reviews Genetics 3, no. 3 (2002): 165-175.

Pandey, Kanti Bhooshan, and Syed Ibrahim Rizvi. "Markers of oxidative stress in erythrocytes and plasma during aging in humans." Oxidative medicine and cellular longevity 3, no. 1 (2010): 2-12.

Patel, K. V. (2008). Epidemiology of anemia in older adults. Semin Hematol, 45:210.

Paul, Anirban, Amy Belton, Sanjay Nag, Ian Martin, Michael S. Grotewiel, and Atanu Duttaroy. "Reduced mitochondrial SOD displays mortality characteristics reminiscent of natural aging." Mechanisms of ageing and development 128, no. 11 (2007): 706-716.

Perls, Thomas, and Dellara Terry. "Understanding the determinants of exceptional longevity." Annals of internal medicine 139, no. 5_Part_2 (2003): 445-449.

Powell, S.R, Ping W., David A., Teichberg S., Haridas V., McCloskey T. W, Davies K. J. A., Katzeff H. (2005). Aggregates of oxidized proteins (lipofuscin) induce apoptosis through proteasome inhibition and dysregulation of proapoptotic proteins. Free radical biology and medicine, 38, 1093 – 1101.

Price, E. A., Mehra, R., Holmes, T. H., Schrier, S. L. (2011). Anemia in older persons: etiology and evaluation. Blood Cells Mol Dis, 46:159.

Rattan, Suresh IS. "Healthy ageing, but what is health?." Biogerontology 14, no. 6 (2013): 673-677.

Robine J.M, Yasuhiko Saito, Carol Jagger. (2003). The relationship between longevity and healthy life expectancy. Quality in Ageing Volume 10 Issue 2 June 2009 © Pavilion Journals (Brighton) Ltd 2009

Rowe, J. and Kahn, R. (1987). Human aging: Usual and successful. Science, 237 (4811), 143–9. doi:10.1126/science.3299702. PMID 3299702

Rohrig, G., W. Doehner, R. M. Schaefer, and R. J. Schulz. "[Anemia and iron deficiency in the elderly. Prevalence, diagnostics and new therapeutic options]." Zeitschrift fur Gerontologie und Geriatrie 45, no. 3 (2012): 191-196.

Sahin E., DePinho, R.A., (2010). Linking functional decline of telomeres, mitochondria and stem cells during ageing. Nature, 464 (7288), 520–52.

Salomon, Joshua A., Haidong Wang, Michael K. Freeman, Theo Vos, Abraham D. Flaxman, Alan D. Lopez, and Christopher JL Murray. "Healthy life expectancy for 187 countries, 1990–2010: a systematic analysis for the Global Burden Disease Study 2010." The Lancet 380, no. 9859 (2013): 2144-2162.

Sato, Y., T. Kondo, and T. Ohshima. "Estimation of age of human cadavers by immunohistochemical assessment of advanced glycation end products in the hippocampus." Histopathology 38, no. 3 (2001): 217-220.

Schroots, J. J., and J. E. Birren. "The nature of time: implications for research on aging." Comprehensive gerontology. Section C, Interdisciplinary topics 2, no. 1 (1988): 1-29.

Shukla, Ritesh K., Vyom Sharma, Alok K. Pandey, Shashi Singh, Sarwat Sultana, and Alok Dhawan. "ROS-mediated genotoxicity induced by titanium dioxide nanoparticles in human epidermal cells." Toxicology in Vitro 25, no. 1 (2011): 231-241.

Shigenaga, M. K., Hagen, T. M., Ames B. N. (1994). Oxidative damage and mitochondrial decay in aging. Proc Natl Acad Sci USA, 91, 10771.

Spindler, S. R. (2010). Biological Effects of Calorie Restriction: Implications for Modification of Human Aging. The Future of Aging, 367–438

SSNIT'S 2010 Annual Report. www.ssnit.com/downloads/?item=1326906674

Suzuki, M., Willcox D. C., Rosenbaum M. W., Willcox B. J. (2010). Oxidative stress and longevity in Okinawa: an investigation of blood lipid peroxidation and tocopherol in Okinawan centenarians. Curr Gerontol Geriatr Res., 2010 : 380460

Tacutu R., Budovsky A., Yanai H., and Vadim E. F. (2011). Molecular links between cellular senescence, longevity and age-related diseases - a systems biology perspective. Impact Journals: Open Access Impact Journal on Aging. Online ISSN: 1945-4589

Tothova, Zuzana, and D. Gary Gilliland. "FoxO transcription factors and stem cell homeostasis: insights from the hematopoietic system." Cell stem cell 1, no. 2 (2007): 140-152.

Tyas, S. L., David A. S., Mark F. D., Kathryn P. R., William R. M. (2007). Healthy ageing in the Nun Study: definition and neuropathologic correlates. Age and Ageing. 36, 650–655.

United Nations (2012) Population Ageing and Development. Population Division www.unpopulation.org

United Nations Population Division, UNPD. New York: United Nations (2011). World population prospects: the 2010 revision. www.unpopulation.org

United Nations, Department of Economic and Social Affairs, Population Division (2007). World Population Prospects: The 2006 Revision, Highlights. Working Paper No. ESA/P/WP.202.

United Nations, World Assembly on Ageing (2001). The Ageing of the World's Population. United Nations.

Van der Horst A., Burgering B. M. (2007). Stressing the role of FOXO proteins in lifespan and disease. Nat Rev Mil Cell Biol, 8, 440 – 450.

Weinert B. T. and Timiras P. S. (2003). Invited Review: Theories of aging. J Appl Physiol, 95, 1706-1716.

Westendorp R. G., van Heemst D., Rozing M. P., Frolich, M., Mooijaart, S. P., Blauw, G. J., Beekman, M., Heijmans, B. T., de Craen A. J., Slagboom, P. E. (2009). Nonagenarian siblings and their offspring display lower risk of mortality and morbidity than sporadic nonagenarians: The Leiden Longevity Study. J Am Geriatr Soc., 57, 1634-1637.

WHO (1968). Nutritional anaemias. Report of a WHO scientific group. World Health Organ Tech Rep Ser, 405:5.

WHO (2002). The world health report: reducing risks, promoting healthy life. Geneva: World Health Organization; Available from: http://www.who.int/whr/2002/en/whr02_en.pdf

Wilcox BJ et al. (2008). FOXO3A genotype is strongly associated with human longevity. Proc Natl Acad Sci USA, 105, 13987-13922

Yang, L.,Wen-Jing W., Huiqing, C., Jiehua L., Chong, W., Fang-Yuan H., Jian G., Ling Z., Fan Y., Yi-Xin Z., Wei L., Gu-Yan Z., Hanbin C., Xiaomin, C., Zhiming Z., Hongbo, H., Birong, D., Xianming M., Yi Z., and Xiao-Li T. (2009). Genetic association of FOXO1A and FOXO3A with longevity trait in Han Chinese populations. Human Molecular Genetics, 18 (24), 4897–4904. doi:10.1093/hmg/ddp459. Advance Access published on September 29, 2009

Zamboni M and Mazzali G (2012). Obesity in the elderly: an emerging health issue. International Journal of Obesity 36, 1151-1152 (September 2012) | doi:10.1038/ijo.2012.120

APPENDICES

Appendix 1

Consent Form

Written informed consent will be obtained from all study participants or from family representatives if participants are not in the position to provide informed consent.

Consent form

Title: Relationship between FOXO3a genetic polymorphism and ageing in Ghanaians.

Principal investigator: Dr Naa Adorkor Addy Sodzi-Tettey

Address: Physiology Department, UGMS, Korle-Bu

Information (To be read or translated to participants)

Dear Volunteer,

This consent form contains information about the research entitled Relationship between FOXO3a genetic polymorphism and ageing in Ghanaians.

As a participant in this research you are required to read and understand the information and sign it or make your mark on it before a witness. Please ask for explanation and/or further information as required.

You will be given a copy of the form to take away with you.

Purpose of study

This study is being done to investigate ageing in Ghana. All over the world people are living longer. Recently in Ghana, we have been informed that our life expectancy has

risen from 58 to 64 which is still much lower than that seen in developed countries where average life expectancy is above 70 years. The trend now is to find ways and means to live a long and healthy life.

With the population of the aged all over the world increasing at an accelerated rate, there is an urgent need to understand the ageing process better so as to be better positioned to make interventions to improve/maintain quality of life in old age. Longevity research serves to reveal truths about human ageing from which avenues are created for intervention.

General information and your part in the study

To qualify to be a part of this study you must be a Ghanaian and your age should be verifiable by nationally recognized identification papers. (Birth certificate, national voter's identification card, passport, national health insurance card, driver's license)

You will be required to answer a questionnaire and undergo some physical examinations/measurements. A sample of your blood will be taken for laboratory analysis. Sterile techniques and disposable single use material will be used for all blood sample collection. Your privacy will be respected at all times.

Possible benefits

You will not receive any direct benefits from this study. However your participation will help us acquire more knowledge about ageing in Ghana to help in the development of needed interventions.

Possible risks

Little pain from the bruising at the bleeding site is expected. The total volume of blood which will be collected from you will not cause you any harm. You are not expected to

suffer any adverse effects from the examination and blood sample taking, but if you do, you will receive appropriate treatment.

Withdrawal from study Participation in this study is strictly voluntary. If you should decide not to participate there shall be no untoward consequences. In as much the same way you are free to withdraw at any stage and your decision will be respected.

Confidentiality

All information gathered will be treated as confidential material. You will not be named in any report.

Contacts

You may contact the principal investigator Dr Naa Adorkor Addy Sodzi-Tettey of the Department of Physiology, University of Ghana Medical School, on phone number 0206301110 with any questions, complaints or study related problems.

You may also contact the Chairman of the Ethical Review Committee, of the University of Ghana Medical School with any questions about the ethical aspects of the study or your rights as a volunteer.

Your rights as a participant

This research has been reviewed and approved by the University of Ghana Ethical Review Committee. The Ethical Review committee reviews research studies in order to ensure that participants' rights are protected even before the research is started. If you have any questions about your rights as a research participant, you may contact the Chairman, Ethical Review Committee, University of Ghana Medical School.

VOLUNTEER AGREEMENT

The above document describing the benefits, risks and procedures for the research title Relationship between FOXO3a genetic polymorphism and ageing in Ghanaians has been read and explained to me. I have been given the opportunity to ask questions which have all been answered to my satisfaction. I agree to participate as a volunteer.

---------- ----------

Date Signature/Thumbprint of Volunteer

If a volunteer cannot read himself/herself a witness must sign below:

I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered and the volunteer has agreed to participate in the research.

---------- ----------

Date Signature/Thumbprint of Witness

I certify that the nature, purpose, potential benefits and possible risks associated with participating in this research have been explained to the above individual.

APPENDIX 2

QUESTIONNAIRE

Name:

Demographic characteristics

1. Age/Date of birth……..
2. Sex M F

Social and Economic Status

3. Marital Status

   Married Not married Divorced Widowed

4. Level of formal education attained

   None Primary Middle School/JSS Secondary School/SSS

   Vocational Tertiary

5. Do you live alone? Yes / No

   If not who are the members of your household?

……

6. Assessment of housing. (circle where true)

Adequate ventilation	Leaky roof
Tap running water	Indoor plumbing
Electricity	Toilet If yes what type
Garbage pick-ups	

Compound house/Shared baths with other households

7. Do you have social security? Yes / No
8. Do you have health insurance? Yes /No
9. What is/are your sources of income?

   …….

10. Did you vote in the last election? Yes /No

Health Status and Risk Factors

11. "In general, how would you rate your health at present?

    Very good Good

    Moderate Poor Very poor

12. Sight

How would you rate your vision now as compared to your middle years? (40 years)

The same Worse Better

13. Hearing

How will you rate your hearing now as compared to you middle years? (40 yrs)

The same Worse Better

14. Have you been diagnosed with any non-communicable diseases/chronic illnesses/disorders? Yes / No

15. If yes which condition Duration Medication(s)

Diabetes

HPTN

SCD

Cardiovascular Disease

Cancer

Other regular medication/s including herbals …….

16. Do you currently use tobacco? Yes / No

If yes, how often and how much?

Daily Occasionally

17. If you smoked in the past when did you stop smoking?

18. Do you drink alcohol? Yes / No

Lifetime abstainer;

Non-heavy drinker;

Infrequent heavy drinker (2-3 days, 5+ standard drinks per week, last 7 days);

Frequent heavy drinker (4+days, 5+ standard drinks per week, last 7 days).

Health care

19. Have you assessed health care from any facility or person in last 30 days? Yes / No

If yes from hospital/clinic pharmacy traditional healer

Other, Specify……

20. Do you need daily care? Yes / No

If yes what for

Physical activity indicator

21. Do you have lower body weakness?

Able to walk normally Walks with difficulty Unable to walk.

22. Do you have weakness in your upper limbs? (grip strength) Yes / No

23. Are you able to perform activities of daily living without assistance? Yes / No

Nutrition

24. How many meals do you have each day? One/Two/Three/Four

25. Do you eat fruits and vegetables? If yes how often and how much?

26. Can you or your household give a 24 hour dietary recall?

27. Assessment of level of physical activity

Mode	Frequency	Duration	Intensity
Walking			Brisk/Slow
Aerobics			Brisk/Slow
Domestic work			Heavy/Light
Cycling/Swimming/Tennis/Football			Heavy/light
Other			Heavy/light

28. Do you have any exercise/sports equipment for personal use? Yes/No

If yes,

specify……

29. Cognitive function

*THE SHORT PORTABLE MENTAL STATUS QUESTIONNAIRE (SPMSQ)	Response	Incorrect Responses
i. What are the date, month, and year?		
ii. What is the day of the week?		
iii. What is the name of this place?		
iv. Memorize this address – One Mensah Street.		
v. What is your phone number?		
vi. How old are you?		
vii. When were you born?		
viii. Who is the current president?		
ix. Who was the president before him?		
x. What was your mother's maiden name?		

xi.	Can you count backward from 20 by 3's?		
xii.	Recall the address I gave you earlier.		

Anthropometric measurements

Measurement name	Measurement	Comments.
Weight (kg)		
Standing height (m)		
Arm span (cm)		

Appendix 3

SCORING:* THE SHORT PORTABLE MENTAL STATUS QUESTIONNAIRE (SPMSQ)

0-2 errors: normal mental functioning 3-4 errors: mild cognitive impairment 5-7 errors: moderate cognitive impairment 8 or more errors: severe cognitive impairment *One more error is allowed in the scoring if a patient has had a grade school education or less.

*One less error is allowed if the patient has had education beyond the high school level.

Source: Pfeiffer, E. (1975). A short portable mental status questionnaire for the assessment of organic brain deficit in elderly patients. Journal of American Geriatrics Society. 23, 433-41.

Genomic DNA extraction from buffy coat (Qiagen Co. Ltd., UL)

- **Principle**

DNeasy kits are advanced silica gel membrane technology for the rapid and efficient purification of total cellular DNA. The buffer system is optimized to allow direct cell lysis followed by selective binding of DNA to the DNeasy membrane. Simple purification processes completely removes contaminants and enzyme inhibitors such as proteins and divalent cations.

- **Materials and Reagents**

  DNeasy Kit

  Proteinase K

  Microcentrifuge

  Heating block

  Microcentrifuge tube

  Phosphate buffered saline

  Vortex

  Pipette and tips

  Eppendorf tubes

  Absolute ethanol

  Collection tubes

- **Methodology**

1. 20µl of proteinase K was pipetted into 1.5 ml microcentrifuge tube

2. 100µl of buffy coat of the sample was added onto the proteinase K
3. 100µl of phosphate buffered saline (PBS) was added to adjust the volume to 220µl
4. 200ml buffer AL was added and mixed thoroughly by vortexing
5. The mixture was incubated for 10min at 56°C
6. 200ml of absolute ethanol was added to the sample and mixed thoroughly by vortexing
7. The mixture from step 6was pipette into DNeasy mini spin column and centrifuged at 8000rpm for 1min. The flow-through and collection tube were discarded.
8. The DNeasy mini spin column was placed into a new 2ml collection tube. 500ml buffer AW1 was added and centrifuged at 8000rpm for 1min. The flow-through and collection tube were discarded.
9. The DNeasy mini spin column was placed into a new 2ml collection tube. 500ml buffer AW2 was added and centrifuged at 14000rpm for 3min to dry the DNeasy membrane. The flow-through and collection tube were discarded.
10. The DNeasy mini spin column was placed into a new 1.5ml collection tube. 50µl buffer AE was added and incubated at room temperature for 1min and centrifuged at 8000rpm for 1min. The resulting DNA sample was divided into 2 aliqouts of 25µl each and stored at -20°C.

Appendix 5

Polymerase chain reaction and gel electrophoresis

- **Principle**

The Polymerase Chain Reaction (PCR) is a molecular biology technique that provides an extremely sensitive means for amplifying small quantities of DNA across several orders of magnitude to generate exponential copies of that particular DNA sequence. It consists of cycles of repeated heating and cooling (thermal cycling) of the reaction for DNA denaturation, primer annealing and enzymatic replication of the target DNA sequence. Key components to enable selective amplifications include the target DNA whose portion is to be amplified, primers with sequences complementary to the target DNA, deoxyribonucleotides (dNTPs) and a heat-stable DNA polymerase. PCR is self propagating in the sense that as the reaction progresses, the DNA generated is itself used as a template for replication, setting in motion a chain reaction in which the DNA template is exponentially amplified.

Gel electrophoresis is a method adopted in molecular biology to separate DNA by length, size and charge. Nucleic acid molecules are separated by applying an electric field to move the negatively charged molecules through an agarose matrix with the smaller molecules migrating faster and farther than the larger molecules as a result of the ease with which they migrate through the pores of the gel. This phenomenon is referred to as sieving. Loading dyes such as Cresol Red and Orange Green are used to enable viewing of the post electrophoresis in natural light. The gel is treated with ethidium bromide which intercalates into the major groove of the DNA and fluoresces reddish-orange under UV light. Hence, by running a sample through an Ethidium bromide treated gel, any band containing more than 20ng DNA become distinctly visible.

- **Materials and reagents**

  10x PCR buffer

  1M HCl

70% ethanol

Aerosol Filter Pipette Tips

Agarose

Electrophoresis set up (Labnet International, Power station 300)

Ethidium Bromide

Gel photography system (UVIsave gel documentation system, model GAS9200/1/2/3, Version 12)

$MgCl_2$

Micro-centrifuge

Micro-centrifuge tube

Nuclease free water

PCR Machine (Techgene)

Pipette

Taq polymerase

Hundred base pair of DNA molecular size markers (Sigma Mo, USA) The DNA mole.

- **Reagents preparation**

Working solution of FOXO3a primers

Concentration of stock solution (C1) -100 µM

Volume of stock solution (V1) ?

Concentration of working solution (C2) -10 µM

Volume of working stock needed (V2) - 200µl

Number of Moles of primer ÷ Volume of primer = Concentration

Thus; Concentration x volume = Number of moles

Initial number of moles of the primer = final number of moles of the primer

$C1 \times V1 = C2 \times V2$

$V1 = \frac{C2 \times V2}{C1}$

$V1 = \frac{10\ \mu M \times 200 \mu l}{100\ \mu M}$

V1= 20µl

20µl of the primer stock solution was pipetted and added to 180µl of nuclease free water to make a 10µM working concentration, the resulting solutions was vortexed to mix and stored at -20 ºC for future use.

- **Working solution of Deoxynucleotide triphosphates (dNTPs).**

Concentration of stock solution (C1) - 100 mM

Volume of the stock concentration used in making the working concentration (V1) - ?

Concentration of working solution (C2) -10 mM

Volume of working solution needed (V2) - 100µl

$C1 \times V1 = C2 \times V2$

$V1 = \frac{C2 \times V2}{C1}$

$V1 = \frac{10\ mM \times 100 \mu l}{100\ mM}$

V1= 10µl

10µl stock solution was added to 90µl of nuclease free water to make 10 mM working concentration, the resulting solutions was vortexed to mix and stored at -20 ^{o}C for future use.

- **1X Tris acetate (TAE) Buffer**

Stock concentrations of TAE buffer (C1) - 50X

Volume of the stock concentration used in making the working concentration (V1) - ?

Concentration of working solution needed (C2) - 1X

Volume of working solution needed (V2) = 500ml

C1 X V1 = C2 X V2

$$V1 = \frac{C2 \times V2}{C1}$$

$$V1 = \frac{1 \times 500ml}{50}$$

V1= 10ml

10ml of the 50x stock solution at room temperature was taken into a 500ml volumetric flask and then topped up to the 500ml mark with distilled water, the resultant solution was mixed thoroughly and stored at room temperature for future use.

- **Two percent (2%) agarose gel preparation and casting**

Two grams (2g) of agarose was weighed into a heat resistant bottle and 100ml of 1x TAE added. The solution was heated to dissolve the agarose, cooled down to just above room temperature and mixed thoroughly with 5µl of ethidium bromide. The resultant solution was poured into a gel casting stray with combs to create the sample wells and allowed to set.

Human foxo3a PCR reaction condition

Reagents	**X 1**
DNA Template	10µl
10 X Buffer + 15mM $MgCl_2$	3µl
dATP (10mM)	0.5µl
dGTP (10mM)	0.5µl
dTTP (10mM)	0.5µl
dCTP (10mM)	0.5µl
Forward Primer	1 µl
Reverse primers	1 µl
Taq Polymerase	0.125µl

Nuclease free water	7.875µl
Total	25µl

Appendix 6

COMET ASSAY

- **Principle**

Comet Assay or single cell gel electrophoresis assay provides a simple and effective method for evaluating DNA damage in cells. The principle of the assay is based upon the ability of denatured, cleaved DNA fragments to migrate out of the cell under the influence of electric field. Undamaged DNA migrates slower and remains within the confines of the nucleoid when current is applied. Increase in DNA damage results in smaller fragments which migrate faster in the electric field. Evaluation of the DNA "comet" tail shape and migration patterns allows for assessment of DNA damage.

- **Materials and Equipment**

Lysis Solution

Comet low melting agarose (LMA)

Trevigen CometSlide™

200 mM EDTA, pH 10

10X PBS, Ca++ and Mg++ free

NaOH Pellets

Dimethylsulfoxide (DMSO)

10X TBE Buffer

Silver staining kit

Glacial acetic acid

Methanol

Deionised water

Pipette and tips

Boiling water bath and 37° C water bath

Horizontal electrophoresis apparatus

Light transmission microscope

1 L graduated cylinder

Eppendorf tubes

Improved Neubauer counting chamber

- **REAGENT PREPARATION**

1. **TBE (1X)**

100ml of TBE (10X) was added to 900mls of distilled water to obtain TBE (1X).

To prepare 10X TBE:

Tris Base = 108g

Boric acid = 55g

EDTA = 9.3g

Tris base was dissolved in 900ml of distilled water and the volume adjusted to 1litre. The solution was stored at room temperature.

2. 5% Acetic acid v/v

25ml of acetic acid added to 475ml distilled water to obtain the needed total volume of 500ml.

3. **70% ethanol**

280ml of ethanol was added to120ml of distilled water to obtain the needed total volume of400ml.

4. 1X PBS (Ca ++ and Mg ++ free)

Used concentration w/v: weighed 9.55g of the PBS and dissolve in 1litre of the distilled water, Homogenized and autoclaved.

5. Lyses solution

Add 40mls of lysis solution (from manufacturer) to 4mls of dimethylsulfoxide (DMSO). Chill at 4°C or in ice for at least 20mins before used. (Addition of DMSO is optional and is required only for samples containing heme, such as blood cells or tissue samples). Buffer formulation is proprietary.

- **Methodology**

1. Melt low melting agarose in a beaker or boiling water (100°C) for 5min. (loosened cap)

2. Transfer to 37°C water bath for at least 20mins to cool.

3. Add low melting agarose (37°C) 500µl + 50µl PBS + cells

4. 500µl + 50µl cells 1:10

5. 1:10 cells (PBS) + 50µl agarose

6. Pipette 75µl immediately unto comet slides and spread evenly. (When working with many samples place aliquots of molten agarose in a pre-warmed microcentrifuge tubes placed at 37°C to prevent hardening. If cells (sample) are not spreading evenly on the slide; warm the slide at 37°C before application).

7. Place slide flat at 4°C in the dark (refrigerator) for 10mins. A 0.5mm clear ring appears at the edge of CometSlide area. Increasing gelling to 30mins improves adherence of samples in the high humidity environments.

8. Prepare lysis solution 20mins after chilled on ice before use. For 10 slides prepare 40ml lysis + 4ml DMSO chilled at 20mins before use.

9. Immerse slide in prechilled lysis solution and leave on ice or at 4°C for 30min to 60mins.

10. Tap excess buffer from slide and immerse in freshly prepared Alkaline solution, pH>13.

    (Alkaline solution is prepared by dissolving 0.6g of NaOH pellets in a mixture of EDTA (200mM, 250µl) and distilled water (49.75ml). The solution warms during preparation, so should be allowed to cool to room temperature.

11. Leave CometSlide in alkaline solution for 20mins to 60mins at room temperature, in the dark.

- **TBE ELECTROPHORESIS**

12. Remove slide from alkaline solution; gently tap excess buffer from slide and wash by immersing in 1X TBE buffer for 5mins, twice.

13. Transfer slide from 1X TBE buffer to a horizontal electrophoresis apparatus. Place slides flat onto a gel tray and align equidistant from the electrodes. Pour 1X TBE buffer until level just covers samples. Set power supply to 1 volt per cm (measured electrode to electrode). Apply voltage for 10mins.

14. Gently tap off excess TBE, and dip slide in 70% ethanol for 5mins.

15. Air-dry samples. Drying brings all the cells in a single plane to facilitate observation. At this stage, samples may be stored at room temperature, with dessicant. Sample must be well dried before staining.

- **EPIFLUORESENCE STAINING PROCESS**

➢ **Preparation of SYBR Green Staining Solution**

Composition

1) SYBR Green I 1µl

2) 1×TE buffer, pH 7.5, (TE: 10mM Tric-Cl pH 7.5, 1mM EDTA) 10µl

Add 5ml of 1M Tris-HCl to 1ml 0.5M EDTA and add distilled H_2O to 500 ml

Prepare stain when ready to stain immediately.

➢ **Staining Reaction**

Cover sample area with 50µl of diluted staining solution (SYBR Green 1).

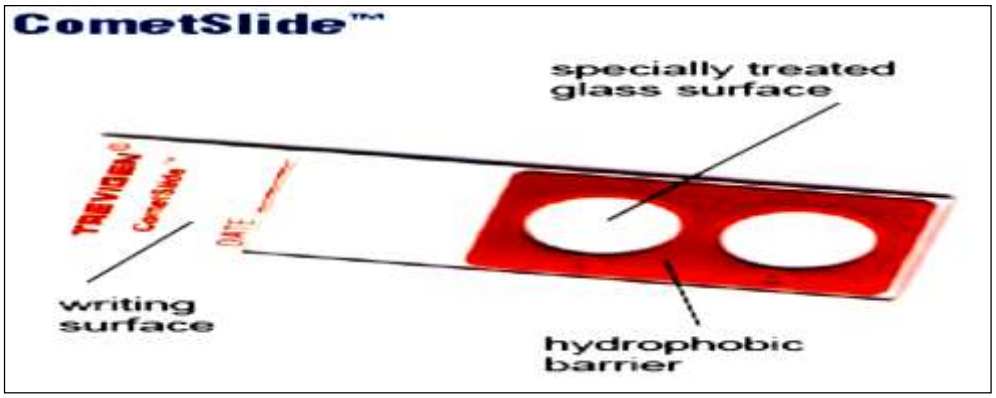


Fig. 6 CometSlide

Analysis and evaluation table showing visual classification of DNA damage, according to the relative proportion of DNA in the tail (score 0-4), obtained by single-cell electrophoresis. Score 0 represents undamaged cells and score 4 represents the most heavily damaged cell.

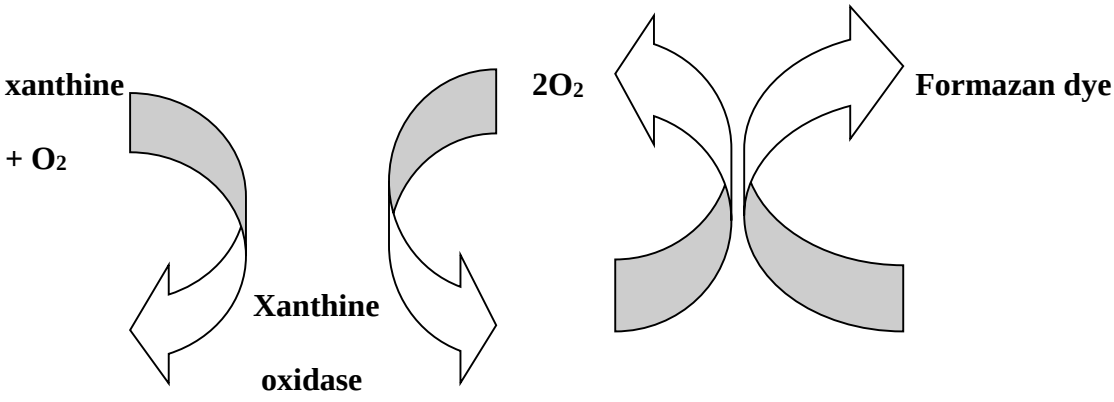
Scores	0	1	2	3	4
Percentage DNA in the tail	<5	5-20	20-40	40-80	>80
Average	2.5	12.5	30	60	90
	0%	6.8%	25%	55%	85%
	0%	12%	28%	65%	97%

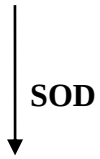
Appendix 7

Superoxide dismutase (SOD) assay

- **Principle**

The assay quantitatively measures SOD activity in a variety of samples. It utilizes a tetrazolium salt for the detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. The superoxide radicals generated convert the tetrazolium salt into a yellow coloured formazan dye with absorbance at 450nm. The SOD in the sample competes with the salt for the superoxide radicals and thereby inhibits the production of the formazan dye. The degree of inhibition corresponds to the activity of SOD. High SOD activity causes a decrease in the superoxide radical concentration by conversion into molecular oxygen and hydrogen peroxide resulting in a decreased pigmentation of the formazan dye.





$O_2 + H_2O$

- **Materials and Reagents**

SOD Assay kit (*Cayman Chemicals, Michigan- USA*)

Deionized water

Centrifuge

Pipette and tips

Eppendorf tubes

96 well microplate

Plate reader

- **Reagent Preparation**

1. **Assay Buffer (10x)**

Dilute 3mls of assay buffer concentrate with 27mls of HPLC-grade water (or de-ionised distilled water) for assaying 96 wells. This final assay buffer should be used to dilute the radical detector. Store at 4^{o}C, this is stable for at least two months.

2. **Sample Buffer (10x)**

Dilute 2mls of sample buffer concentrate with 1.8mls of HPLC-grade water (or de-ionised distilled water) for assaying 96 wells. This is used to prepare the SOD standard and dilute the xanthine oxidase and SOD samples prior to assaying. Store at 4^{o}C, this is stable for at least two months.

3. **Radical Detector**

Prior to use, transfer 50 µl of radical detector to another vial and dilute with 19.95mls of diluted assay buffer for 96 wells. Cover with foil. The diluted radical detector is stable for two hours. Store unused radical detector at -20^{o}C.

4. **SOD Standard**

Dilute 20 µl of the SOD Standard with 1.98ml of sample buffer (dilute) to obtain the SOD stock solution. Take seven clean glass test tubes and mark them A-G. Add the amount of SOD stock and sample buffer (dilute) to each tube as described in manufacturer's manual.

5. **Xanthine Oxidase**

Prior to use, thaw one vial and transfer 50 µl of the supplied enzyme to another vial and dilute with 1.95ml of sample buffer (dilute) for 96 wells. Store on ice. Preparation is table for one hour.

- **Sample preparation**

Plasma and Erythrocyte Lysate

1. Collect blood using an anticoagulant such as heparin, citrate, or EDTA

2. Centrifuge the blood at 700- 1,000 x g for 10 minutes at 4^{o}C. Pipette off the top yellow plasma layer without disturbing the white buffy layer. Store plasma on ice until assaying or freeze at -80^{o}C. The plasma sample is stable for at least one month. Plasma should be diluted 1:5 with Sample buffer before assaying for SOD activity.

3. Remove the white buffy layer (leucocytes) and store at -80^{o}C.

4. Lyse the erythrocytes (red blood cells) in four times its volume of ice- cold HPLC-grade water (or de-ionised distilled water)

5. Centrifuge at 10,000 x g for 15 minutes at 4^{o}C.

6. Collect supernatant (erythrocyte lysate) for assaying and store on ice. Store at -80^{o}C if not assaying same day. Sample is stable for at least one month. The erythrocyte lysate should be diluted 1:100 with sample buffer before assaying or SOD activity.

- **Performing the Assay**

1. **SOD Standard Wells:** Add 200 µl of the diluted Radical Detector and 10 µl of Standard (tubes A-G) per well in the designated wells on the plate.

2. **Sample Wells:** Add 200 µl of the diluted Radical Detector and 10 µl of Sample to the wells.

3. Initiate the reaction by adding 20 µl of diluted Xanthine Oxidase to all the wells you are using. Note: If assaying sample backgrounds, add 20 µl of Sample buffer instead of xanthine oxidase.

4. Carefully shake the 96 - well plate for a few seconds to mix. Cover with the plate cover.

5. Incubate the plate on a shaker for 20 minutes at room temperature. Read the absorbance at 440 - 460 nm using a plate reader.

- **Calculations**

1. Calculate the average absorbance of each standard and sample (if assay was done in duplicates). If assayed, subtract sample absorbance from the sample.

2. Divide standard A's absorbance by itself and divide standard A's absorbance by all the other standards and samples absorbances to yield the linearized rate (LR).

3. Plot the linearized SOD standard rate (LR) (from step 2 above) as a function of final SOD activity (U/ml).

4. Calculate the SOD activity of the samples using the equation obtained from the linear regression of the standard curve by substituting the linearized rate (LR) for each sample.

$$\text{SOD (U/ml)} \left[\left(\frac{\text{sample LR} - \text{y-intercept}}{\text{slope}} \right) \times \frac{0.23\text{ml}}{0.01\text{ml}} \right] \times \text{sample dilution}$$