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IMPACT OF PHYSICAL ACTIVITY ON PLASMA IL-6 AND TNF-α IN OBESE AND OVERWEIGHT GHANAIANS

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DECLARATION

I hereby declare that this submission is my work towards the MPhil and that, to the best of my knowledge, it contains no material previously published by another person, nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text

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Abstract

Introduction

The national prevalence of obesity and cardiovascular diseases is on the increase. Regular physical activity has been recommended as a preventive or curative option for weight loss and the reduction of the risks of cardiovascular diseases and inflammatory factors associated with them. Obesity has consistently been associated with the risk of cardiovascular diseases with an increase in proinflammatory cytokines such as interleukin 6 and tumour necrotic factor having been identified as intermediaries to the development of these diseases. The current study sought to evaluate physical activity among obese and overweight Ghanaians and whether or not there were any concurrent associated changes in proinflammatory cytokines IL-6 and TNF- α.

Materials and Methods

Seventy four obese and overweight participants from an ongoing international study of obesity, hypertension, and physical activity were contacted, of which sixty agreed to take part in the study. Measurements taken for all participants included blood pressure using a blood pressure monitor height and weight measurements using a stadiometer and a standard balance, waist circumference and hip circumference using a tape measure, fasting blood sugar by Accucheck, and body water and fat composition by bioelectrical impedance analysis. Blood samples were obtained for the measurement of plasma IL-6 and TNF-α with high sensitive ELISAs (R&D systems, USA and MABTECH, AB Sweden respectively). Physical activity was measured using actical accelerometers.

Results

Of the 60 participants that were recruited for the study 51 (85%) were females and 9 (15%) were males. On the basis of physical activity (PA) 37 (61.7%) participants were categorized as 'active', while 23 (38.3%) participants were classified as 'sedentary'. Participants had an average physical activity of 121.9 ± 53.0 activity counts per minute (AvgAC), and this fell within the category of light activity (100 – 1353 counts/min). Females (124.6 ± 56.2 AvgAC) were more active than males (100.6 ± 25.8 AvgAC). Participants in the sedentary group also recorded insignificant (p=0.2) but higher measurements of IL-6 (4.2 ± 4.2pg/ml) than participants in the active group (3.5 ± 2.2pg/ml). Sedentary participants also recorded a higher TNF-α plasma concentration (2.0 ± 1.6pg/ml) than active ones (1.8 ± 1.1pg/ml) but this difference was not significant (p=0.3). The distribution of IL-6 was only marginally associated with physical activity, but this correlation was not significant (0.8). TNF-α correlated negatively (coefficient of correlation = -0.7) with physical activity. This relationship was however not significant (p=0.6).

Conclusion

Obese and overweight participants have high circulating levels of IL-6 and TNF-α. However, due to non-compliance to physical activity recommendations, there is little reduction in the levels of these pro-inflammatory cytokines among obese and overweight Ghanaians.

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List of Abbreviations

AvgAC – Average Activity Count

BIA – Bioelectrical Impedance Analysis

BMI – Body Mass Index

BMR – Basal Metabolic Rate

CVD – Cardiovascular Diseases

DBP – Diastolic Blood Pressure

DLW – Doubly Labelled Water

EDTA - Ethylenediaminetetraacetic Acid

ELISA – Enzyme Linked Immuno-Sorbent Assay

FFM – Fat-Free Mass

FM – Fat Mass

GM-CSF - Granulocyte Macrophage Colony-Stimulating Factor

GPAQ – Global Physical Activity Questionnaire

IL-1 – Interleukin-1

IL-6 - Interleukin-6

INF-γ - Interferon-gamma

IPAQ - International Physical Activity Questionnaire

IRS-1 - Insulin Receptor Substrate 1

KCCR – Kumasi Centre for Collaborative Research in Tropical Medicine

MCP-1 - Monocyte Chemoattractant Protein-1

METS – Modelling the Epidemiologic Transition Study

PA – Physical Activity

PAQ –Physical Activity Questionnaire

PBFM – Percent Body Fat Mass

PBS – Phosphate Buffer Saline

SBP – Systolic Blood Pressure

TBW – Total Body Water

TEE – Total Energy Expenditure

TNF-α – Tumour Necrotic Factor alpha

WHO – World Health Organization

CHAPTER ONE

INTRODUCTION

1.1. Background

Populations all over the world are experiencing rapid increases in the prevalence of obesity (Low *et al.*, 2009). Professional institutions and governments have issued obesity control and prevention guidelines, all of which include recommendations on physical activity (Fletcher, 1996, Pate, 1995). However, if fully implemented, it is not clear if the current recommendations on physical activity would significantly impact the trend in age-related weight gain and its associated health risks.

The energy budget is defined by the direct relationship between energy intake and expenditure. Obesity usually results from an excess of calories consumed as food over calories expended as physical activity. Increases in expenditure would normally be accompanied by increases in intake, and excess intake can be stimulated independently by high caloric density of food, changes in food availability, and eating patterns. As demonstrated repeatedly by investigators in this field, the regulation of energy stores and body composition must be seen as a complex, dynamic process influenced by the interplay of factors that modify both intake and physical activity. An individual ultimately uses both biological stimuli, via the hormones that control satiety, and social cues to achieve energy balance. Whether variations in patterns of activity observed in modern, free-living populations plays a key role in this process or not is unknown. Since recommendations to increase activity energy expenditure to reduce weight gain are at the core of current public health policy this issue deserves careful study.

Under experimental conditions, increases in activity with control of energy intake will lead to weight loss. However, more relevant to prevention and control efforts at the population level is the question of whether variations in day-to-day activities mitigate the tendency to gain weight. By far, the largest number of studies has employed questionnaires. In most analyses, categories of sedentary activities (e.g., hours of TV watching) or leisure time sports have been used as the measures of exposure. Questionnaires are both insensitive and potentially biased instruments. The challenge in this field is clearly to move toward objective measurement tools such as accelerometers. Previous studies (Lavoie *et al*, 2010; Harris *et al*, 2004) have demonstrated little effect of physical activity on interleukin 6 and tumor necrotic factor although their studies employed exercise interventions or did not use objective measurements of physical activity.. Current data on the subject is not entirely convincing.

1.2. Problem Statement

The national prevalence of obesity and cardiovascular diseases is on the increase (Tagoe and Dake, 2011). Regular physical activity has been recommended as a preventive or curative option for weight loss and the reduction of risk factors of cardiovascular diseases. However this recommendation may not be contributing to any significant decreases in the prevalence of obesity and risks of cardiovascular diseases (CVDs). It remains unclear if any increases in physical activity are concurrent to any reductions in obesity and related risks of cardiovascular disease, and if so to what extent. As Ghana progresses through the epidemiologic transition, it is expected that the burden and prevalence of obesity and cardiovascular diseases will continue to increase

1.3. Justification

Physical activity has consistently been associated with lower plasma levels of pro-inflammatory cytokines such as interleukin-6 (IL-6) and Tumour Necrotic Factor - Alpha (TNF-α) (Jankord and Jemiolo, 2004; Pahor *et al* 2004). However, there is little information on whether or not regular physical activity significantly lowers levels of inflammatory markers of obese and overweight individuals. In addition, studies that have measured inflammatory cytokines of obese individuals in relation to physical activity have either been exercise interventions (Friedenreich *et al*, 2011; Pakiz *et al*. 2011) which are deliberate, repetitive, and limited, or use physical activity questionnaires (Colbert *et al*, 2004; Jankord and Jemiolo, 2004), which are not very reliable and objective methods to assess physical activity. The current study will measure the levels of inflammatory cytokines in subjects who will use accelerometers to provide a more accurate measure of physical activity. This will enable obese and overweight participants to be observed naturally.

1.4. Aim

The current study seeks to evaluate the impact of higher levels of physical activity in reducing inflammatory risk factors for CVDs among obese and overweight individuals.

1.5. Objectives

1) To evaluate the concentrations of pro-inflammatory cytokines (TNF-α and IL-6) among obese and overweight participants
2) To evaluate any differences in the concentrations of pro-inflammatory cytokines (TNF-α and IL-6) among active and sedentary obese and overweight groups

3) To evaluate any relationships between physical activity and BMI
4) To evaluate any relationships between physical activity and concentrations of pro-inflammatory cytokines (TNF-α and IL-6) of sedentary and active participants
5) To evaluate the compliance by overweight and obese individuals to physical activity recommendations

1.6. Hypothesis

The current study proposes that active, obese, and overweight, individuals have lower concentrations of inflammatory markers than those of sedentary, obese, and overweight, individuals.

1.7. Expected Outcome

It is expected that the current study will serve as basis for proper assessment of physical activity recommendations for obese and overweight patients.

CHAPTER TWO

LITERATURE REVIEW

2.1. Classification of Obesity

Body mass index (BMI) is defined as the weight of an individual, measured in kilograms, divided by the algebraic square of that individual's height, measured in metres (WHO, 2003). It is a commonly used benchmark indicator that provides a fair identification and assessment of body fat. Based on results for calculations of BMI, individuals are commonly classified as underweight (<18.5 kg/m^2), normal (18.5 kg/m^2 – 24.9 kg/m^2), overweight (≥ 25 kg/m^2), or obese (≥30 kg/m^2). Grades of classification further exist between these principal classifications, as shown in Table 2.1.

Classification	BMI(kg/m^2)	
	Principal cut-off points	Additional cut-off points
Underweight	**<18.50**	**<18.50**
Severe thinness	<16.00	<16.00
Moderate thinness	16.00 - 16.99	16.00 - 16.99
Mild thinness	17.00 - 18.49	17.00 - 18.49
Normal range	18.50 - 24.99	**18.50 - 22.99**
		23.00 - 24.99
Overweight	**≥25.00**	**≥25.00**
Pre-obese	25.00 - 29.99	25.00 - 27.49
		27.50 - 29.99
Obese	**≥30.00**	**≥30.00**
Obese class I	30.00 - 34-99	30.00 - 32.49
		32.50 - 34.99
Obese class II	35.00 - 39.99	35.00 - 37.49
		37.50 - 39.99
Obese class III	≥40.00	≥40.00

Table 2.1. Body Mass Index Classifications; Source: WHO, 2004

2.2. Trends in Obesity

The World Health Organization (2009) estimated in 2005, that 1.6 billion adults (≥15 years old) were overweight, and that at least 400 million of them were obese. Current data indicates that average adult BMI among Africans and Asians ranges from 22 kg/m^2 to 23 kg/m^2, while the average range for adults in North America, Europe, and in some Latin American, North African, and Pacific Island countries ranges from 25 kg/m^2 to 27 kg/m^2 (WHO, 2003).

Globally, the prevalence of obesity has increased. There has been an increase in the average annual prevalence of obesity in both developed and developing countries. In developed countries, this increase has been as low as 0.2% in Italy between 1983 and 2004, to as high as 18.5% in New Zealand between 1997 and 2004 (Low ***et al.***, 2009). In developing countries, this increase similarly has been as low as 0.1% in Estonia between 1994 and 2004, to as high as 35.3% in Saudi Arabia between 1992 and 2000 (Low ***et al.***, 2009). In their reviews of global obesity, Wang and Beydoun (2007) and Wang ***et al.*** (2007) have provided supporting evidence of the increase in the global trends of obesity, with respect to its prevalence. Their studies were conducted in the USA and China respectively. In the review by Wang and Beydoun (2007), 66% of adults (≥20 years old) were overweight or obese and 34.42% were obese between 2003 and 2004. Wang and Beydoun (2007) also reported that the prevalence of obesity in the USA had doubled from 15.1% in 1976 and 1980 to 30.9% in 1999 and 2000. Wang ***et al.*** (2007) reported a similar trend in China of an increase in the prevalence of obesity from 2.9% to 21.8% even though the particular years for which they made their comparisons were not stated in their review. They however, noted an increase in the combined prevalence of obesity and overweight from 14.6% in 1992 to 21.8% in 2002.

WHO projections to 2015 indicate that approximately 2.3 billion adults will be overweight, and more than 700 million of them will be obese (WHO, 2009).

Currently, global obesity prevalence range from less than 5% in China, Japan, and some African countries, to more than 75% in urban parts of Samoa (WHO, 2003). Low *et al.* (2009) have reported that in developing countries, the prevalence of obesity is generally higher among females than among males. In contrast to this distribution in developing countries, Low *et al.* (2009) have also reported that there are no obvious gender differences in adult obesity in developed countries.

2.3. Causes of Obesity

Various factors account for the global distribution of obesity and the increasing trends of its prevalence in both developed and developing countries, and more especially in developing countries where the rate of increase is fast, and coexists with a relatively high prevalence of infectious diseases. These factors include economic growth, modernization, urbanization, and globalization of food markets (WHO, 2003). As incomes increase and human settlements become less rural and more urban, dietary intake of saturated fats and sugars surpasses that of complex carbohydrates (WHO, 2003). Other important nutrients which are less consumed as part of diets include vitamins, minerals, and other micronutrients (WHO, 2003). Concurrently, there has been a significant transition from more physically demanding jobs to less physically demanding ones. Technology in homes, automated transport and less active leisure also account for less physical activity (WHO, 2003).

In recent years, some attempts have been made to study the linkage between genes and obesity. Indeed some genes have been identified (Table 2) which may pre-determine susceptibility to obesity or overweight. It is believed that the interplay of

these genes with the environment (changing patterns of social activities and nutritional transition) is responsible for the global nature of obesity.

Table 2.2: Some genes that have been suggested to play roles in obesity, *Source O'Rahilly and Farooqi (2006)*

locus	population	design	trait	LOD score/*p*-value	candidate genes
2p	Mexican American	extended pedigrees	leptin, fat mass	7.5	POMC
	French Caucasian	sib pairs	leptin	2.7	POMC
	African American	nuclear families	leptin	$p=0.008$	
	US Caucasian	nuclear families	adiponectin	2.7	
3q	Pima Indian	sib pairs	leptin	2.0	Glut2
	African American	nuclear families	BMI	1.8	PI3 kinase
	French Caucasian	sib pairs	BMI	3.9	
	US Caucasian	nuclear families	BMI	3.3	
	Indo-Mauritian	nuclear families	CHD	3.1	
4p	US Caucasian	extended pedigrees	BMI	6.1	PPARGC1 CCKAR
	Mexican American	extended pedigrees	BMI	4.5	
5cen-q	French Caucasian	sib pairs	leptin	2.9	CART
	African American	nuclear families	BMI	1.9	
	US Caucasian	nuclear families	adiponectin	4.1	
6q	French Caucasian	extended pedigrees	BMI	4.0	SIM1, MCHR2 and PC-1
8p	Mexican American	extended pedigrees	leptin, BMI	3.1	beta3
	US Caucasian	nuclear families	BMI	2.0	
10p	Pima Indian	sib pairs	% body fat	2.8	none
	Pima Indian	sib pairs	BMI	3.6	none
	French Caucasian	sib pairs	leptin	4.9	
	US Caucasian	nuclear families	adiponectin	1.9	
	German Caucasian	nuclear families	BMI	2.3	
	US Caucasian	nuclear families	BMI	1.9	
	African American	nuclear families	BMI	2.2	
12q	Canadian	extended pedigrees	abdominal subcutaneous fat by CT	2.88	HNF-1
17p	Mexican American	extended pedigrees	BMI	3.2	Glut4 PPARα
	US Caucasian	nuclear families	leptin	5	
20q	US Caucasian	nuclear families	BMI	3.2	GNAS1
	US Caucasian	nuclear families	BMI	3.0	CEBP-B
	Pima Indian	sib pairs	24 h RQ	3.0	ASIP
	French Caucasian	sib pairs	BMI	1.7	
Xq23	Finnish	extended pedigrees	BMI	3.1	SLC6A14

2.4. Consequences of Obesity

Obesity is a major risk factor associated with certain common chronic, non-infectious diseases. Obese individuals are very likely to suffer from diet-related chronic diseases such as diabetes, cardiovascular disease, hypertension and stroke, and certain forms of cancer (WHO, 2003). Overweight and obesity partly account for adverse metabolic defects on blood pressure, cholesterol, triglycerides, and insulin resistance (WHO, 2003). There are a few inconsistencies between reports of the consequences of obesity and overweight BMIs, partly because different BMI

classifications have been used by many researchers, and partly because the presence of many medical conditions or co-morbidities may be vaguely associated with the medical consequences of obesity.

Obesity accounts for much less severe medical conditions (in terms of mortality) such as chronic musculoskeletal disorders, skin disorders, infertility, and respiratory difficulties, than other medical conditions of much more severity such as cardiovascular disorders, conditions associated with insulin resistance (e.g. type 2 diabetes), and some cancers (WHO, 2003). Approximately 85% of diabetes patients have type 2, and of this percentage majority of them (90%) are either obese or overweight (WHO, 2003). Higher BMIs also account for cancers of body organs such as breasts, colon, prostate, endometroium, kidney, and gall bladder, and also significantly contributes to osteoarthritis (WHO, 2013). In association with smoking, hypertension, and high blood cholesterol, gradually increasing BMIs ($\geq$20 kg/m^2) globally account for 58% of diabetes, 21% of ischemic heart disease, and 8 – 42% of some cancers (WHO, 2002).

2.5. Prevalence and Trends of Obesity in Ghana

The current national prevalence of obesity is 7.5% (WHO, 2011), with regional and socio-demographic variations (Biritwum ***et al.***, (2005). Obesity is more common among females (7.8%) than males (2.8%), and more common among married (5.9%) and previously married, i.e. divorced or widowed (7.6%) than never married (1.6%) (Biritwum ***et al.***, (2005). Biritwum ***et al.***, (2005) has also reported higher prevalence of obesity among employed (employee (9%) than participants who were not working for pay (5.9%), and self-employed (5.0%). In their study, they observed that the prevalence of obesity increased by age up to 60 years and respondents with higher

educational status accounted for more obese individuals. Their findings also demonstrated a general pattern of gradual decline in the regional prevalence of obesity from the south (16.1% in Greater Accra) to northern Ghana (2.2% in Upper West). According to Biritwum ***et al.***, (2005), Ga-Adangbe (14.6%), Ewes (6.6%), and Akans (6%) constitute ethnic groups with the highest prevalence of obesity.

A majority of obesity and other obesity-related studies in Ghana have been conducted in Accra. Examples of such studies include those by Duda ***et al.,*** (2007), and Amoah (2003). Briefly, Duda ***et al.,*** (2007) investigated the prevalence of obesity among women living in Accra, while Amoah's related studies in 2003 evaluated the overall prevalence of overweight and obesity among adults (≥25 years old) living in Accra.

Amoah (2003) has reported the prevalence of overweight and obesity in Accra to be 23.4% and 14.1% respectively. In support of his work, Biritwum ***et al.***, (2005) have also reported the prevalence of overweight and obesity in Accra to be 26.6% and 16.1% respectively. Amoah's observed sex-adjusted prevalence for overweight (27.1%; 17.5%) and obesity (20.2 %; 4.6%) were higher among females than males. He also observed that obesity increased with age until 64 years. Urban, more affluent residents mostly accounted for overweight and obesity. Obesity was highest among the Gas and Akans, and a relatively small prevalence was reported among the Ewes. The prevalence of obesity was also reported by Amoah (2003) to have increased with increased levels of education and sedentary jobs. Duda ***et al.*** (2007) have reported a high prevalence of overweight (27.6%) and obesity (34.7%) among 1237 adult females (≥18 years old) living in Accra. This prevalence was discordant with the self-reported assessments of BMI by female participants. This disparity in observed and perceived prevalence of overweight and obesity is critical and of great concern,

and they have subsequently suggested the need to structure and implement programs to improve the health of women in Accra. In contrast to Amoah's (2003) findings, Duda ***et al.*** (2007) observed a higher mean BMI (27.9 kg/m2 compared to 22.6 kg/m2). There is however no evidence to suggest a significant nutritional and physical activity transition between 2003 and 2007 which may have accounted for the significant increase (on comparison of both studies) in average BMIs. Of particular interest was the observation by Duda ***et al.*** (2007) that overweight and obesity did not have any correlation with measures of affluence, in contrast to socio-economic status-related observations by Amoah (2003).

2.6. Co-morbidities of Obesity in Ghana

In Ghana, co-morbidities of obesity are cardiovascular diseases (CVDs) such as hypertension (Bosu, 2010; Cappucio *et al*, 2006; Agyemang, 2006; Addo *et al*, 2006, Escalona *et al*, 2004; Cappucio *et al*, 2004; Plange-Rhule *et al*, 1999), diabetes (Owiredu *et al*, 2008, Amoah *et al*, 2002), stroke (Wiredu and Nyame, 2001) and hyperlipidaemia (Owiredu *et al*, 2008) have been reported.

2.6.1. Diabetes

The prevalence of diabetes in Ghana is 8.8% (WHO, 2011). The disease condition is most prevalent among the oldest age-group (64 years and older), and more among males (7.7%) than females (5.5%) (Amoah *et al*, 2002). Associations of diabetes include overweight and obesity, increases in age, and hypertension.

2.6.2. Hypertension and Stroke

A review of hypertension in Ghana by Bosu (2010) has shown that the prevalence of hypertension in Ghana ranges from 19% in Kassena-Nankana, Upper East Region (Kunutsor and Powles, 2009) to 48% among women in the Accra Metropolis,

Greater Accra Region (Hill *et al*, 2007). His review however suggests that the overall prevalence of hypertension in Ghana is approximately 29%. Urban populations had higher prevalence than rural ones. Factors associated with hypertension include older age group, over-nutrition, and alcohol consumption.

The percentage (11.1%) of stroke-related deaths has not changed since 1981 (Wiredu and Nyame, 2001). In their study, Wiredu and Nyame (2001) also observed that hypertension accounted (either as a cause or contributor) for the majority (76.7%) of stroke-related deaths.

2.6.3. Dyslipidaemia

In their study of obesity and cardiovascular risk factors in Kumasi, Owiredu *et al*, (2008) observed that apart from diabetes and hypertension, 17% of participants who enrolled in his study had high total serum cholesterol levels (5.00±1.85 mmol/L).

2.7. Physical Activity

2.7.1. Definition of Physical Activity

Physical activity is any body movement that works your muscles and requires more energy than resting. Examples of physical activity include walking, running, and dancing. Even though "physical activity" and "exercise" are commonly used synonymously, exercise is a type of physical activity that's planned and structured, for example weight-lifting. There are four main types of physical activity. These are aerobic, muscle-strengthening, bone-strengthening, and stretching, the most important of them being aerobic activity.

2.7.2. Aerobic Activity

Aerobic activity (also called endurance activity) moves your large muscles, such as those in your arms and legs. Aerobic activity increases heart and breathing rate..

Aerobic activity is classified as either light, moderate, or vigorous. The level of intensity depends on the effort used for that activity. Thus, what is light-intensity activity for one person may be moderate-intensity for another.

2.7.2.1. Light and Moderate Intensity Activities

Light-intensity activities are common daily tasks that don't require much effort. Moderate-intensity activities cause noticeable increases in breathing and heart rate. As a rule-of-thumb moderate-intensity activity is that activity that allows one to talk but not sing.

2.7.2.2. Vigorous Intensity Activities

Vigorous-intensity activities produce larger increases in heart and breathing rate. As a rule-of-thumb, vigorous-intensity activity is one that can't permit one to say more than a few words without pausing to catch their breath.

2.7.3. Benefits of Physical Activity

Physical activity has many health benefits and this includes helping to maintain a healthy weight and reduces the risk of cardiovascular diseases such as diabetes and hypertension. Physically active adults are also at lower risk for depression and declines in cognitive function as they get older. Concentrations of inflammatory mediators such as interleukin-6, TNF-alpha, and C Reactive protein are altered significantly to reduce the risks of inflammatory diseases.

2.7.4. Physical Activity Recommendations for Adults

The guideline advises that some physical activity is better than none. Inactive adults are advised to gradually increase their level of activity. For major health benefits, at least 150 minutes (2 hours and 30 minutes) of moderate-intensity aerobic activity or 75 minutes (1 hour and 15 minutes) of vigorous-intensity aerobic activity each week

is recommended. Another option involves a combination of both. For even more health benefits, 300 minutes (5 hours) of moderate-intensity aerobic activity or 150 minutes (2 hours and 30 minutes) of vigorous-intensity activity each week (or a combination of both) is recommended. All activity must be conducted in bouts of 10 minutes. Muscle-strengthening activities that are moderate or vigorous intensity should be included two or more days a week. These activities should work all of the major muscle groups (legs, hips, back, chest, abdomen, shoulders, and arms). Examples include lifting weights.

2.8. Measurement of Physical Activity

2.8.1. Questionnaires

Self-report methods are the most convenient and cheapest way to collect physical activity data from a large number of people in a short time. Physical activity questionnaires (PAQs) are the most widely used self-report instrument to assess physical activity and have been used extensively in research. Self-report measures include self or interviewer administered (Matthews *et al*, 2002). These include activity diaries or logs, recall questionnaires, quantitative history, and global self report questionnaires. These questionnaires all vary greatly in their detail. Self report instruments may be completed on paper and mailed back or completed on the internet. Interviews may be conducted either face-to-face or via phone.

There is a plethora of PAQs available. These include Baecke Physical Activity Questionnaire, Godin Shepard Leisure Time Questionnaire, Paffenbarger Physical Activity Questionnaire, Bouchard's Activity Diary, and the Previous Day Recall (PDR), the GPAQ, and IPAQ.

2.8.2. Pedometers

These are low-cost motion sensors which are typically worn on a belt or waistband and respond to vertical accelerations of the hip during gait cycles (Welk *et al*, 2000). Pedometers provide data on steps taken, and therefore, really measure walking activity only. Due to this, they will not capture activities such as cycling, swimming, walking on an incline or weight lifting. Walking however is one of the most common forms of physical activity and pedometers readily measure this.

2.8.3. Accelerometry

Accelerometry is a direct measure of acceleration of the body or segments of the body. Accelerometry is the most common objective method used to measure physical activity; it has been used extensively in field settings to monitor activity patterns. Technological advances have resulted in devices that can measure activity accurately, over an extended time period (greater than 7 days), and that are small and discrete for people to wear. The device is enclosed in a case and typically attached to the hip (or lower back, ankle, wrist or thigh) by a strap. Accelerometry has been validated using doubly labelled water, which is the gold standard for assessing physical activity.

2.8.4. Heart Rate Monitoring

Heart rate monitoring is a measure of a direct physiological response to physical activity. The development of minute by minute heart rate monitors with internal capacity for multiple days' storage without displaying heart rate has increased the feasibility of this objective measure of physical activity. A heart rate monitor is commonly configured as a chest strap which is wirelessly connected to a data logger hidden in a watch. The use of electrodes provides an alternative way to obtain heart

rate as it can improve compliance, but is sometimes considered less feasible for individuals.

2.8.5. Direct Observation

Direct observation has been used in children as a criterion method to assess physical activity. The main strength of this method is the detailed contextual information it provides but its subjective nature does not allow intensity or energy expenditure to be assessed. Considerable time and effort is required to conduct observation studies but advances in technology have increased the potential of this method to assess physical activity. The increasing interest in the determinants of physical activity and the social environmental influences of activity in young people has also caused an increase in the use of this method (Trost *et al*, 2007). Typically an observer will watch the children using a specific observational system and record a rating of physical activity level into a laptop computer or coding form.

2.8.6. Doubly Labelled Water

Doubly labelled water (DLW) measures total energy expenditure (TEE) by observing the differential rates of elimination of a bolus dose of the stable isotope tracers ^{2}H (deuterium) and ^{18}O. The tracers used are non-radioactive and occur naturally in all waters (including drinking waters), and therefore completely safe to use in any population. The method has been used in adults, children and infants to measure TEE, in many diverse investigations including the energy costs of clinical conditions, and the energy utilisation of people participating in intensive physical activities under extreme conditions (Trabulsi, J. and D.A. Schoeller, 2001; Johnson *et al*, 1998). It has also been used widely to validate other methods of assessment of dietary and physical activity.

2.8.7. Indirect Calorimetry

Indirect calorimetry provides an estimation of energy expenditure. The experimental protocol used determines which components of energy expenditure are captured, which also depends upon the definition of these components. Basal Metabolic Rate (BMR) is the largest component of total energy expenditure (TEE), typically 60-75% when measured over 24 hours and the thermic effect of food is the smallest at 10%. The remaining component of TEE is energy expenditure due to physical activity (physical activity energy expenditure) and this component is the most variable between individuals but typically constitutes 15-30% of TEE when measured over 24 hours (Johnson and Schoeller, 2001).

2.9. Chronic Inflammation

Chronic inflammation is considered to be inflammation of prolonged duration if the stimulus persists. The presence of macrophages is a sign of chronic inflammation. In addition, lymphocyte accumulation, blood vessel proliferation, tissue fibrosis and necrosis are seen in chronic inflammation (Kumar *et al*. 2005). During chronic inflammation, cytokine interactions promote monocyte chemotaxis towards the site of inflammation. Subsequently, macrophage activating factors such as IFN-γ, MCP-1 and other molecules activate the macrophages while migration inhibition factors, such as GM-CSF and IFN- γ, retain them at the inflammatory site. The macrophages contribute to the inflammatory process by chronically emitting low levels of IL-1 and TNF-α (Park *et al*, 2005). IL-6 is also secreted by macrophages and participates in the humoral response during chronic inflammation. The presence of these factors can modulate the local immune response and therefore, it is possible that an exogenous source of cytokines may amplify the process.

2.9.1. Interleukin 6 (IL-6)

The IL-6 cytokine and its soluble receptor are released from adipose tissue. Secretion of IL-6 is greatest from visceral fat stores with serum levels positively correlated to impaired glucose tolerance, insulin resistance and obesity. In experimental models, the administration of IL-6 leads to hyperlipidaemia, hyperglycaemia and insulin resistance. This occurs through alterations of the insulin receptor (Fernandez-Real and Ricart, 2003). IL-6 decreases adiponectin secretion and inhibits adipogenesis. These actions, along with the other peripheral effects of IL-6, are thought to contribute to obesity and insulin resistance (Kershaw and Flier 2004). Animal studies examining the IL-6 gene suggest that the effect of IL-6 differs between the peripheral and the central nervous system. Centrally, the IL-6 levels are negatively associated with fat mass. Here, there appears to be the prevention of the deleterious effects of raised levels of IL-6 through increased energy expenditure and inhibition of feeding (Wallenius *et al*, 2002). This action supports the role of IL-6 as a central paracrine hormone which prevents additional fat accumulation. The IL-6 cytokine arises from many sources including adipose tissue, fibroblasts, endothelial cells and monocytes. The level of IL-6 is also known to correlate with increasing BMI (Khaodhiar *et al.* 2004). IL-6 may also contribute to the secretion of hepatic triglycerides (very low density lipoprotein) and has been implicated in insulin resistance (Bastard *et al.* 2002), however, these mechanisms are not clear (Antuna-Puente *et al.* 2008). More recently, it was suggested that IL-6 played a role in stimulating lipolysis in white adipose tissue after detecting its release from muscle tissue (Pedersen *et al.* 2004).

2.9.2. Tumour Necrotic Factor-α (TNF-α)

TNF-α is a pro-inflammatory cytokine whose expression and circulating levels are increased with obesity and decreased with weight loss (Maury and Brichard, 2010).

TNF-α has numerous effects in adipose tissue, including the regulation of apoptosis, adipogenesis, lipid metabolism, and insulin signalling (Galic *et al*, 2010; Prins *et al*, 1997). The primary cell-type responsible for the production of TNF-α are macrophages (Trayhurn and Wood, 2004). It is hypothesized that TNF-α is produced by macrophages in response to chemo-attractant signals released by dying adipocytes. Evidence suggests that TNF-α triggers a signalling cascade that induces cell apoptosis, which may be one mechanism by which TNF-α regulates adipose tissue mass (Cawthorn *et al*, 2007, Prins *et al*, 1997). Another TNF-α mediated mechanism that may work in parallel to regulate adiposity involves the regulation of key transcription factors (PPAR-γ2 and C/EBPα) controlling adipogenesis (Cawthorn *et al*, 2007). While such effects suggest that increases in TNF-α may be beneficial, TNF-α has also been shown to induce inflammatory pathways and contribute to obesity-related insulin resistance. An increase in TNF-α promotes the secretion of other pro-inflammatory cytokines and reduces anti-inflammatory cytokines; resulting in an overall pro-inflammatory state. For example, a study conducted by Wang and Trayhurn (2006) found that treating human adipocytes with TNF-α for 24hrs led to significant decreases in adiponectin expression and increases in IL-6 and TNF-α expression. TNF-α has also been found to increase the production of plasminogen activator inhibitor-1, an adipokine linked to cardiovascular disease (Cawthorn and Sethi, 2008). Taken together, these examples demonstrate that TNF-α is a powerful regulator of inflammatory molecules, favouring an overall inflammatory state (Wang and Trayhurn, 2006). Increased TNF-α also promotes insulin resistance via the inhibition of the insulin receptor substrate 1 (IRS-1) signalling pathway. It is believed that TNF-α interferes with insulin signalling by deregulating protein kinases, which subsequently affects the phosphorylation status of IRS-1, leading to

an inability of IRS-1 to associate with the insulin receptor (Hotamisligil *et al*, 1996; Kanety *et al*, 1995). Antibody neutralization studies with TNF-α resulted in a significant increase in glucose uptake in response to insulin (Hotamisligil *et al*, 1993). Moreover, whole-body deletions of TNF-α or its receptors were found to increase insulin sensitivity and glucose tolerance (Uysal *et al*, 1997). Taken together, these works demonstrate a critical role for TNF-α in the regulation of obesity-related pathways.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Sampling Frame and Study Design

This study was a nested case-control study and an ancillary to "Modelling the Epidemiologic Transition Study" (METS), an ongoing study of energy expenditure, hypertension, and diabetes in Ghana, South Africa, Jamaica, Seychelles, and Maywood in the USA. In Ghana, METS was undertaken at Nkwantakese, a peri-urban community, located in the Ashanti region of Ghana. Data collected from METS included demographic and anthropometric data (including, height and weight measurements, blood pressures, fasting blood sugar concentration, and blood and urine samples), activity data and dietary recalls. Participants were eligible to participate if they were aged 25 – 45 years. Individuals with obvious chronic or infectious diseases (including active malaria), pregnant or lactating women, and HIV positive individuals were excluded from the study.

Of a total of 246 METS participants 30% (74) were obese and overweight. From this population, a random sample of 60 obese and overweight participants (confidence = 95%, error margin = 0.05) were recruited for the subsequent study. Participants were categorized into two activity intensity groups as either sedentary (<100 activity counts/min) or active (≥100 activity counts/min) based on raw accelerometer activity count data.

Data obtained for this study included plasma concentrations of interleukin 6 (IL-6) and tumour necrotic factor (TNF-alpha), and activity data by accelerometry.

Data was collected over a period of one month.

3.2. Physical Activity by Accelerometer

Physical activity (PA) was measured using the Actical activity monitor (Respironics /Mini-Mitter, Bend, OR). Previous studies have shown that accelerometer-based activity monitors can discriminate different intensities of activity, making it possible to adequately characterize each of the study participants with regard to overall intensity of PA. Physical activity data was recorded as activity counts per minute (cpm). The activity monitor recorded the intensity, duration and frequency of physical motion through the use of an accelerometer which produced a variable electrical current based on the combination of the amplitude and frequency of motion. Accelerometers are omnidirectional motion sensors that count the vertical and horizontal acceleration of the user. This information was stored within the instrument as activity counts per epoch, or specified subunit of time, e.g., per minute. As the intensity of the activity increased, so did the number of activity counts per epoch. The Actical monitor is lightweight, waterproof and is worn on the wrist, waist or ankle. For this study, the monitor was worn at the waist. Consistent placement of the activity monitor ensured comparability of data.

3.3. Blood Sampling and Processing

Blood samples for plasma were collected by a trained phlebotomist into 6mL vacutainers with EDTA additives (BD Biosciences, San Diego, CA, USA). They were centrifuged for 15 minutes at 1000xg within 30 minutes of collection. Plasma was immediately aliquoted into 2mL cryovials and stored at -80°C.

3.4. Immunochemical Assays

All biochemical assays for IL-6 and TNF-alpha were developed and analysed at the Kumasi Centre for Collaborative Research into Tropical Medicine (KCCR), Kumasi.

IL-6 was measured with a highly sensitive ELISA (R&D systems, Minneapolis, MN, USA) as described by the manufacturer. TNF-alpha was measured with a highly sensitive ELISA (MABTECH AB, Sweden) as described by the manufacturer. Briefly, all reagents and working standards were prepared as directed by the manufacturer for IL-6 ELISA. Any excess microplate strips were removed from the plate frame and returned to the foil pouch that contained the desiccant pack and resealed. 100 µL of Assay Diluent RD1W was then added to each well. Subsequently 100 µL of Standard, sample, or control was added per well and covered with adhesive strip provided. The plate was then incubated for 2 hours at room temperature. After this, each well was aspirated and washed three times with wash buffer (400µL) for a total of four washes. 20µL of IL-6 conjugate was then added to each well, covered with a new adhesive strip, and incubated for 2 hours at room temperature. The aspiration and washing step was then repeated. 200µL of substrate solution was added to each well and incubated for 20 minutes at room temperature and protected from light. Subsequently 50µL of Stop Solution was added to each well and any colour changes observed were noted. The optical density of each well was evaluated within 30 minutes, using a microplate reader set to 540 nm with wavelength correction.

For TNF-alpha a high protein binding ELISA plate was coated with mAb TNF3/4 which was diluted to 2µg/ml in PBS at pH 7.4 by adding 100µL per well and incubated overnight at 4-8 degrees Celsius. The plates were washed twice with 200µL of PBS the next day, blocked with Tween 20 containing 0.1% BSA and incubated for one hour at room temperature. After incubation the plates were subsequently washed five times with PBS that had 0.05% Tween. The TNF standard was prepared by reconstituting contents in vial 4 in 1ml PBS to a concentration of 1µg/ml and left at room temperature for 15 minutes and then vortexed. Later 100µl/well of samples and standards were added and incubated for 2 hours at room

temperature. The plates were washed with PBS containing 0.05% Tween after this step. After this 100µl of mAb TNF5-biotin at 1µg/ml in incubation buffer was added to each well and incubated for one hour at room temperature. The washing step then repeated. Subsequently 100µL of Streptavidin-HRP diluted 1:1000 in incubation buffer was added to each well and incubated at room temperature for one hour and the washing step repeated immediately after. Finally, 100µL of substrate solution was added to each well and the optical densities were read in an ELISA reader.

3.5. Additional METS Measurements

3.5.1. Body Mass Index (BMI)

At the initial clinic visit, the height and weight of all participants were measured. Participants were preferred to be in light clothing and were asked to remove their footwear before their weights and heights were measured. Weights of participants were measured to the nearest 0.1 kg using a standard balance (Health-o-meter, Bridgeview, IL). Heights were also measured to the nearest 0.1 cm using a stadiometer and the heads of participants held in the Frankfort horizontal plane. BMI was subsequently calculated using the formula: $weight / height^2$

3.5.2. Blood Pressure

Blood pressures were measured using the Omron Automatic Digital Blood Pressure Monitor (model HEM-747Ic, Omron Healthcare, Bannockburn, IL, USA). Three pulse and blood pressure readings were taken, with the antecubital fossa at the level of the heart. The averages of the blood pressure readings were then recorded.

3.5.3. Fasting Blood Sugar

Fasting blood sugar was measured on-site with an Accucheck® glucose meter. Briefly, test blood samples were obtained by a finger prick using approved lancets

and the blood was transferred onto Accucheck strips by capillary action. The strips were placed in the Accucheck reader which calculated the glucose level of the participant in mg/dl.

3.5.4. Body Composition by Bioelectrical Impedance Analysis (BIA)

Body composition was assessed in all participants using BIA. BIA measured the impedance to the flow of an applied mild alternating current by body tissues. The measured impedance of body tissues was used to calculate total body water, from which fat-free mass and fat mass were in turn calculated. Briefly, participants were placed in the supine position with their limbs abducted. Current-supplying electrodes were placed on the dorsal surfaces of the right hand and foot at the metacarpals and metatarsals, respectively. Detection electrodes were placed at the pisiform prominence of the right wrist and the anterior surface of the true ankle joint. The single-frequency instrument (BIA Quantum, RJL Systems, Clinton Township, MI) was then attached to electrodes and a generated excitation current of 800 μA at 50kHz was passed through them. The resulting resistance and reactance measurements were recorded and existing BIA equations were used to calculate total body water, fat free mass, fat mass and percent body fat.

3.6. Statistical Analyses

Two statistical packages were used for univariate and multivariate analysis; STATA (version 12) and GraphPad Prism (version 4). Summary statistics were presented as means plus or minus standard deviation (SD), together with minimum and maximum measurements. Concentrations of IL-6 and TNF for both groups of sedentary and active obese and overweight participants were compared using the Students' t-Test.

Simple and multiple regression analyses were also used to evaluate any relationships between physical activity, BMI, and IL-6 and TNF-α.

CHAPTER FOUR

RESULTS

4.1. Demographics (Sex and Age)

Of the 60 participants that were recruited for the study 51 (85%) were females and 9 (15%) were males. On the basis of physical activity (PA) 37 (61.7%) participants were categorized as 'active', while 23 (38.3%) participants were classified as 'sedentary'. Participants' ages ranged from 26 years to 47 years with an average age of 38.0 ± 5.7. Males were slightly older (38.2 ± 6.6) than females (37.6 ± 5.6). Sedentary participants were also slightly older (39.2 ± 5.9) than their active study co-participants (37.2 ± 5.5). However, no significant differences were identified between sexes (p=0.4) or physical activity groups (p=0.09) with respect to age.

4.2. Anthropometrics

4.2.1. Body Mass Index (BMI)

The average height-adjusted weight (BMI) for the study group was 30.0 ± 5.1 kg/m^2. Participants BMIs ranged from 'overweight' (25.1 kg/m^2) to obese class III (49.3 kg/m^2), otherwise referred to as morbidly obese. Forty participants were overweight, while thirty were obese. Females (30.6 ± 5.4 kg/m^2) were significantly heavier (p=0.003) than males (27.1 ± 1.9 kg/m^2). Sedentary participants were also heavier (30.7 ± 5.8 kg/m^2) than active participants (29.6 ± 4.7 kg/m^2). Both physical activity groups did not differ significantly (p=0.2) in BMI.

4.2.2. Blood Pressure (Systolic and Diastolic Blood Pressure)

4.2.2.1. Systolic Blood Pressure (SBP)

The study group had an average systolic blood pressure (SBP) reading of 111.1 ± 13.3 mmHg. The lowest reading was 87.5 mmHg and the highest was 145.8 mmHg. Only 5% (3) of participants had systolic blood pressure readings greater or equal to 140 mmHg. SBP

readings of males (123.3 ± 15.0 mmHg) were significantly higher (p=0.0011) than those of females (108.9 ± 11.9 mmHg). Participants in the sedentary group (113.1 ± 14.6 mmHg) also had higher SBP measurements than those in the active group (109.9 ± 12.5mmHg). There was no significant difference (p=0.2) between the two physical activity groups.

4.2.2.2. Diastolic Blood Pressure (DBP)

For all study participants, diastolic blood pressure (DBP) readings averaged 68.3 ± 10.7 mmHg, which were just within the normal range of 70 mmHg to 85mmHg. Diastolic blood pressure measurements ranged from 49.5 mmHg among female active participants to 97.3 mmHg among males active participants. Males (76.0 ± 15.1 mmHg) had significantly higher (p=0.0088) DBP readings than females (66.9 ± 9.3 mmHg). Sedentary participants had slightly higher (70.1 ± 9.7 mmHg) but insignificant (p=0.1) diastolic blood pressure measurements than active participants.

4.2.3. Body Composition by Bioelectrical Impedance Analysis (Body Water and Fat Composition)

Results of all participants were within the average normal ranges of total body water (TBW), fat mass (FM), fat free mass (FFM) and percent body fat mass (PBFM).

4.2.3.1. Total Body Water (TBW)

Total body water values ranged from 25.3 litres to 45.2 litres with an average of 33.4 ± 5.1 litres. Males (40.4 ± 3.4 litres) had significantly higher (p=0.0000) TBW than females (32.1 ± 4.2 litres). However, sedentary (33.7 ± 5.7 litres) and active (33.2 ± 4.8 litres) participants did not differ significantly (p=0.4) in TBW.

4.2.3.2. Fat-Free Mass (FFM)

The average FFM was 45.7 ± 7.0 kg. Males (55.4 ± 4.7 kg) had significantly higher (p=0.0000) FFM than females (44.0 ± 5.8 kg). Although sedentary participants demonstrated higher FFM (46.1 ± 7.8 kg) than active ones (45.4 ± 6.5 kg), there was no significant difference (p=0.4) between the two physical activity groups.

4.2.3.3. Fat Mass (FM)

Mean fat mass for the study participants was 26.5 ± 10.0 kg. Females (28.3 ± 9.6 kg) had significantly higher (p=0.0003) FM than males (16.4 ± 5.8 kg). Sedentary participants (27.7 ± 10.4 kg) had higher FM than active participants (25.8 ± 9.9 kg). There was no significant difference in FM between the two physical activity groups.

4.2.3.4. Percent Body Fat Mass (PBFM)

PBFM measurements ranged from 16.4% in males, and sedentary participants to 54.1% in females, and sedentary participants. Females (38.4 ± 5.8%) had significantly higher (p=0.0000) PBFM values than males (22.4 ± 5.4). Although sedentary participants had higher PBFM (36.8 ± 8.2%) than active participants (35.5 ± 8.1%), there was no significant difference (p=0.3) between the two activity groups.

4.3. Physical Activity (PA)

Participants had an average physical activity of 121.9 ± 53.0 activity counts per minute (AvgAC), and this fell within the category of light activity (100 – 1353 counts/min). Females (124.6 ± 56.2 AvgAC) were more active than males (100.6 ± 25.8 AvgAC). There was however no significant difference in physical activity with regards to sex. Active participants had an average of 151.14 ± 46.2 AvgAC, with a range of PA values from 100.5 AvgAC to 306.3 AvgAC. Sedentary participants had an average of 74.8 ± 17.1 AvgAC, with a range of PA values from 33.8 AvgAC to 98.0 AvgAC.

4.4. Blood Immunochemistry

4.4.1. IL-6

Plasma concentration of IL-6 was generally elevated among all study participants (3.8 ± 3.1pg/ml). There were higher IL-6 plasma concentrations among females (3.8 ± 3.3pg/ml) than males (3.5 ± 1.3pg/ml). Participants in the sedentary group also recorded higher measurements of IL-6 (4.2 ± 4.2pg/ml) than participants in the active group (3.5 ± 2.2pg/ml).

There was however no significant differences among sexes (p=0.4) and physical activity groups (p=0.2).

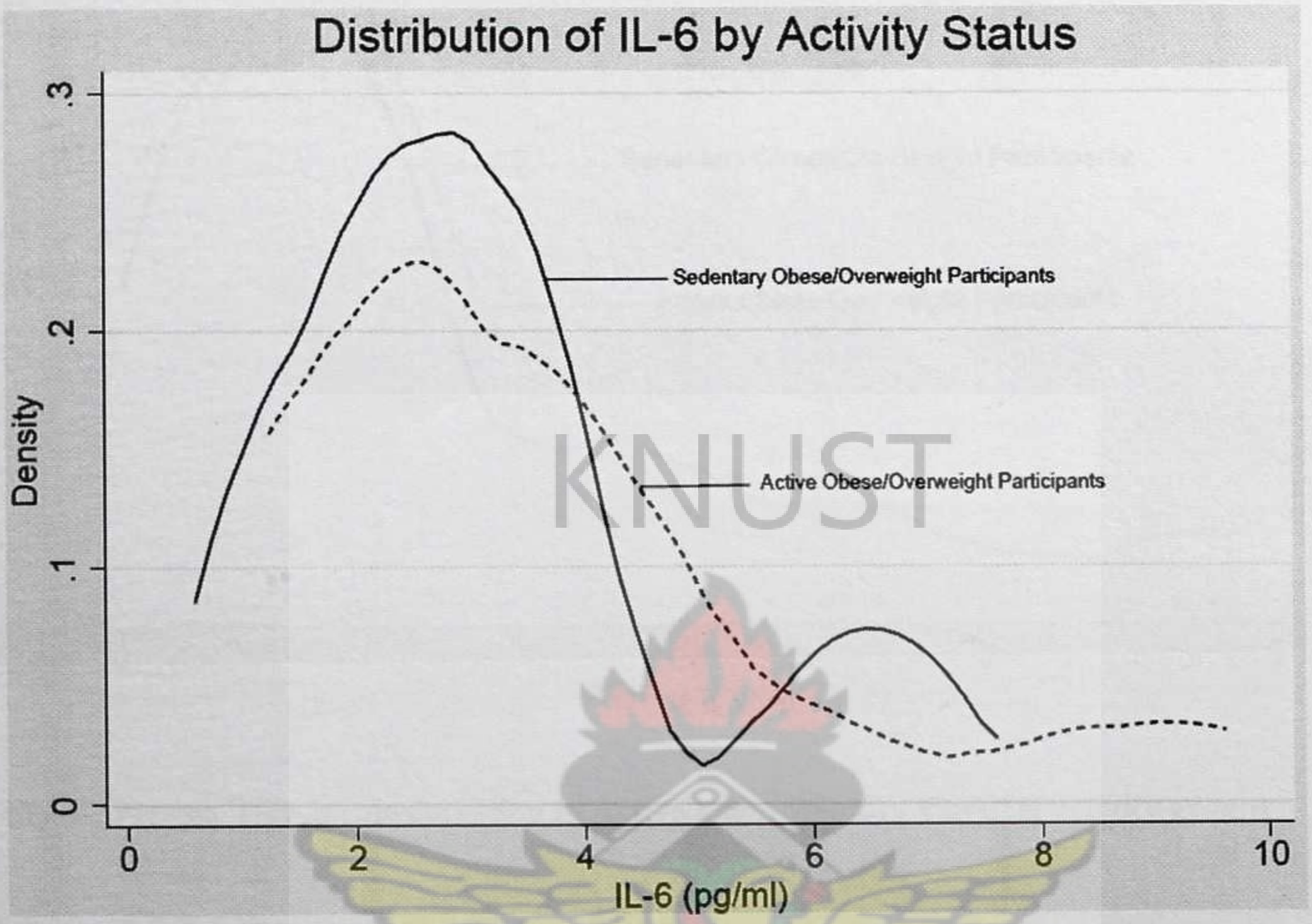

Fig 4.1 Plasma IL-6 concentration in Active and Sedentary Physical Activity groups

4.4.2. TNF-α

Like IL-6, participants demonstrated elevated plasma concentrations (1.9 ± 1.3pg/ml) of TNF-α. There were higher plasma levels of TNF-α among females (2.0 ± 1.3pg/ml) than males (1.2 ± 0.8pg/ml). Sedentary participants also recorded a higher plasma concentration of TNF-α (2.0 ± 1.6pg/ml) than active ones (1.8 ± 1.1pg/ml). There were also no significant differences among sexes (p=0.07) and physical activity groups (p=0.3).

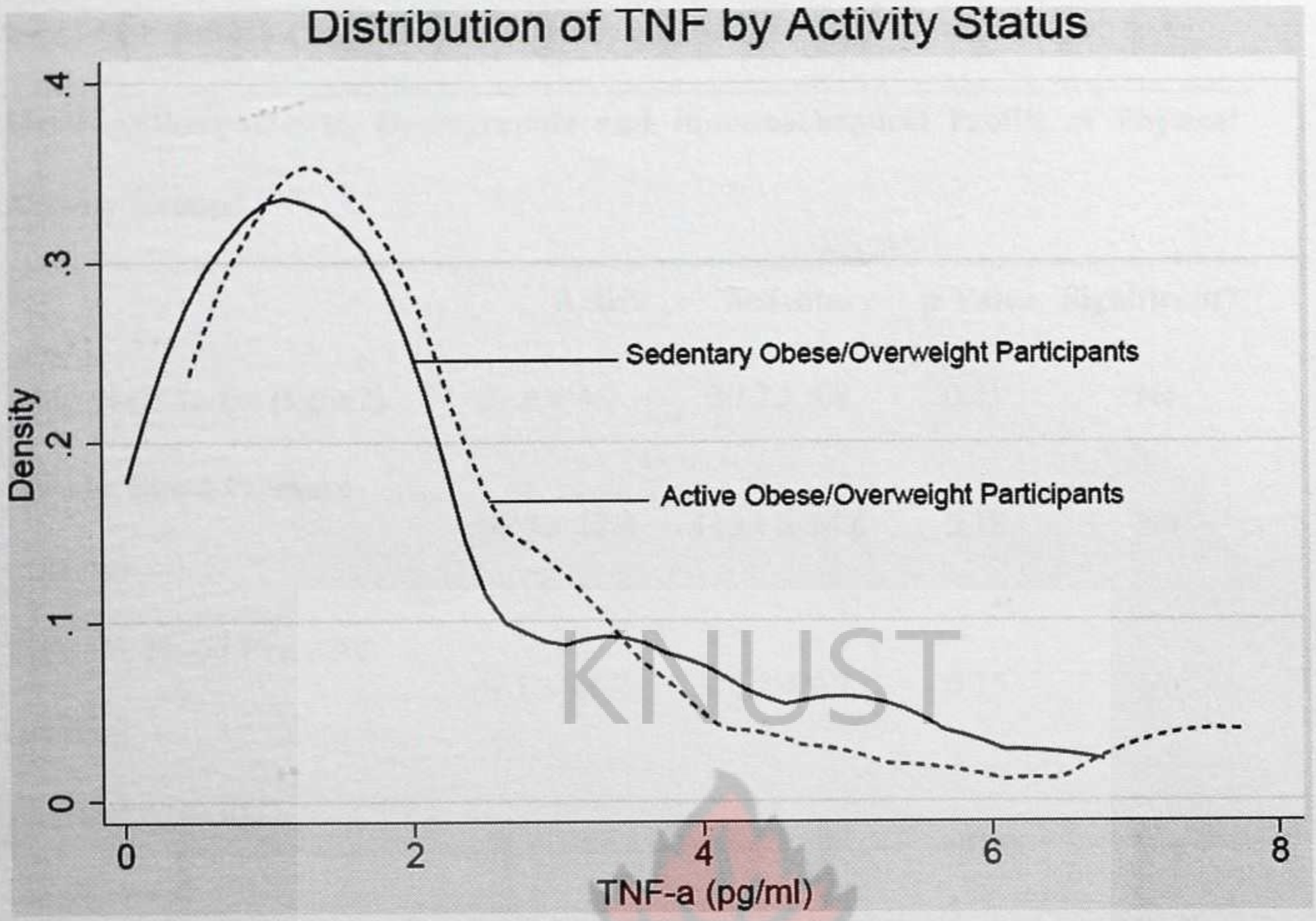

Fig 4.2 Plasma TNF-α concentration in Active and Sedentary Physical activity groups

4.4.3. Fasting Blood Sugar (FBS)

Generally, study participants had fasting blood sugar measurements within the normal-range (5.5 ± 0.6 mmol/l). Males had similar FBS measurements (5.6 ± 0.5 mmol/l) to females (5.5 ± 0.6mmol/l). Fasting blood sugar measurements for active participants (5.5 ± 0.6mmol/l) were also similar to FBS measurements of sedentary participants (5.5 ± 0.6mmol/l). No significant differences were observed between male and females (p=0.3) and active and sedentary groups (p=0.5).

Table 4.1 Summary Characteristics of Study groups

Mean Anthropometric, Demographic and Immunochemical Profile of Physical Activity Groups				
	Active	**Sedentary**	**p-Value**	**Significant?**
Body Mass Index (kg/m2)	29.6 ± 4.7	30.7 ± 5.8	0.21	No
Systolic Blood Pressure (mmHg)	109.9 ± 12.5	113.1 ± 14.6	0.18	No
Diastolic Blood Pressure (mmHg)	67.1 ± 11.2	70.1 ± 9.7	0.15	No
Physical Activity (counts/min)	151.1 ± 46.2	74.8 ± 17.1	**<0.00**	**Yes**
IL-6 (pg/ml)	3.5 ± 2.2	4.2 ± 4.2	0.18	No
TNF-alpha (pg/ml)	1.8 ± 1.1	2.0 ± 1.6	0.34	No
Fasting Blood Sugar (mmol/l)	5.5 ± 0.6	5.5 ± 0.6	0.45	No

4.5. Associations between Body Mass Index (BMI), IL-6, TNF-α, and Physical Activity

Physical activity and BMI were inversely related (coefficient of correlation= -1.9), i.e., the higher the BMI, the lower the physical activity (Fig 4.3). This association was however not significant (p=0.2). The distribution of IL-6 against physical activity was flat and was slightly positively associated (coefficient of correlation = 0.002) (Fig 4.4). This relationship was however not significant (0.8). TNF-α did not exhibit a different distribution with physical activity as was with BMI, i.e. the higher the physical activity, the lower the plasma TNF-α concentrations (coefficient of correlation = -0.7) (Fig 4.5). This relationship was also not significant (p=0.6).

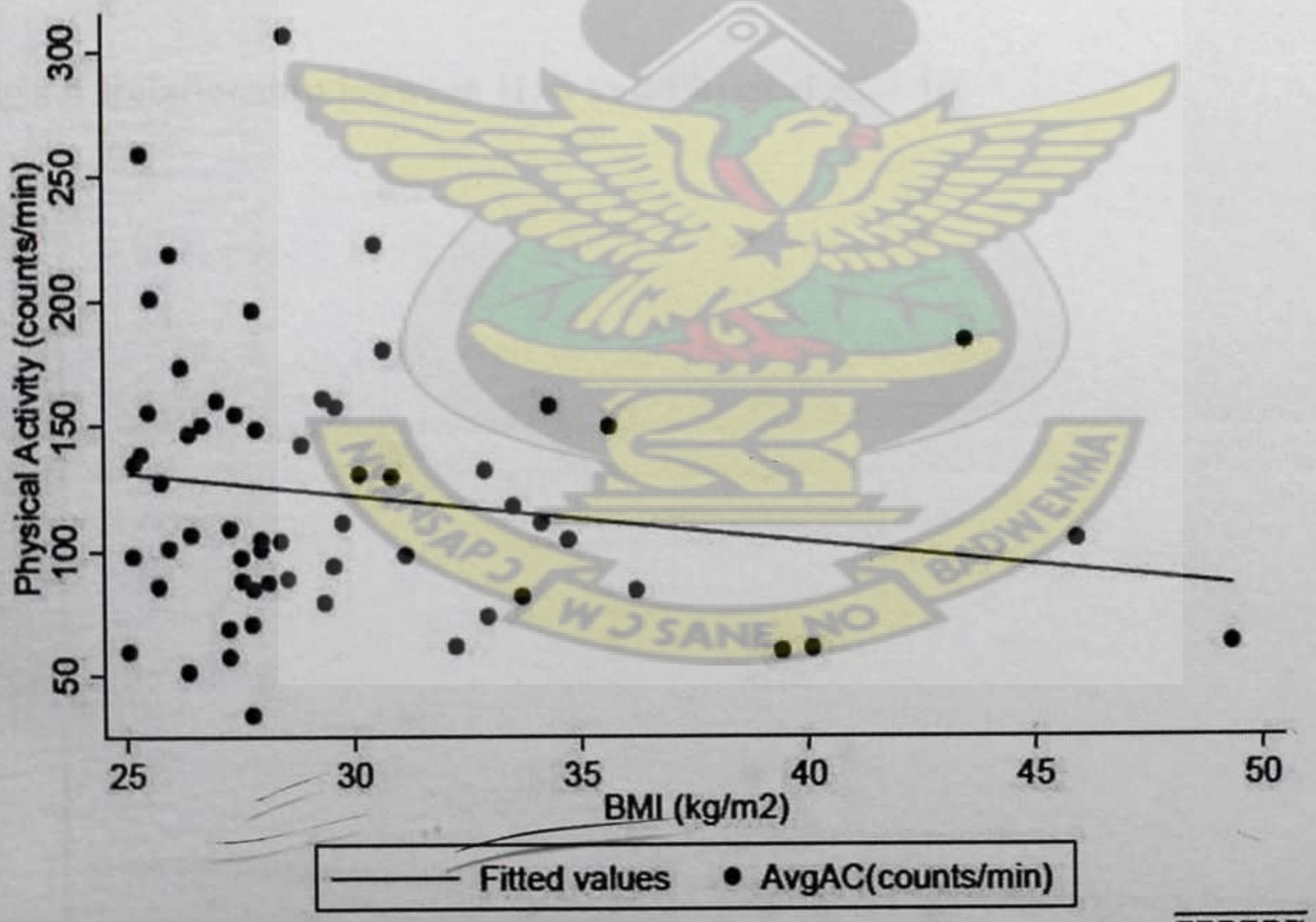

Fig 4.3 Relationship between Body Mass Index and Physical Activity

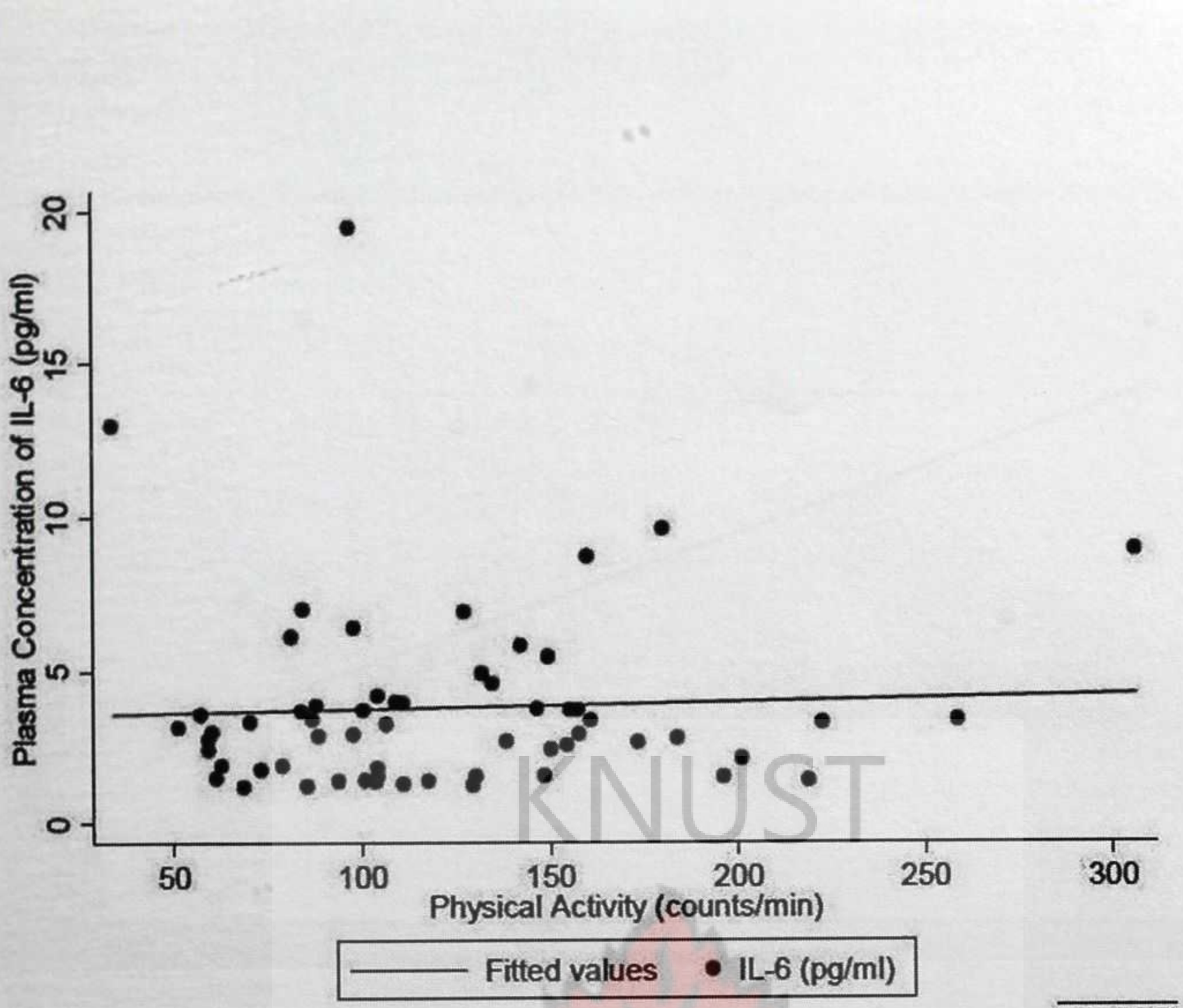

Fig 4.4 Relationship between IL-6 and Physical Activity

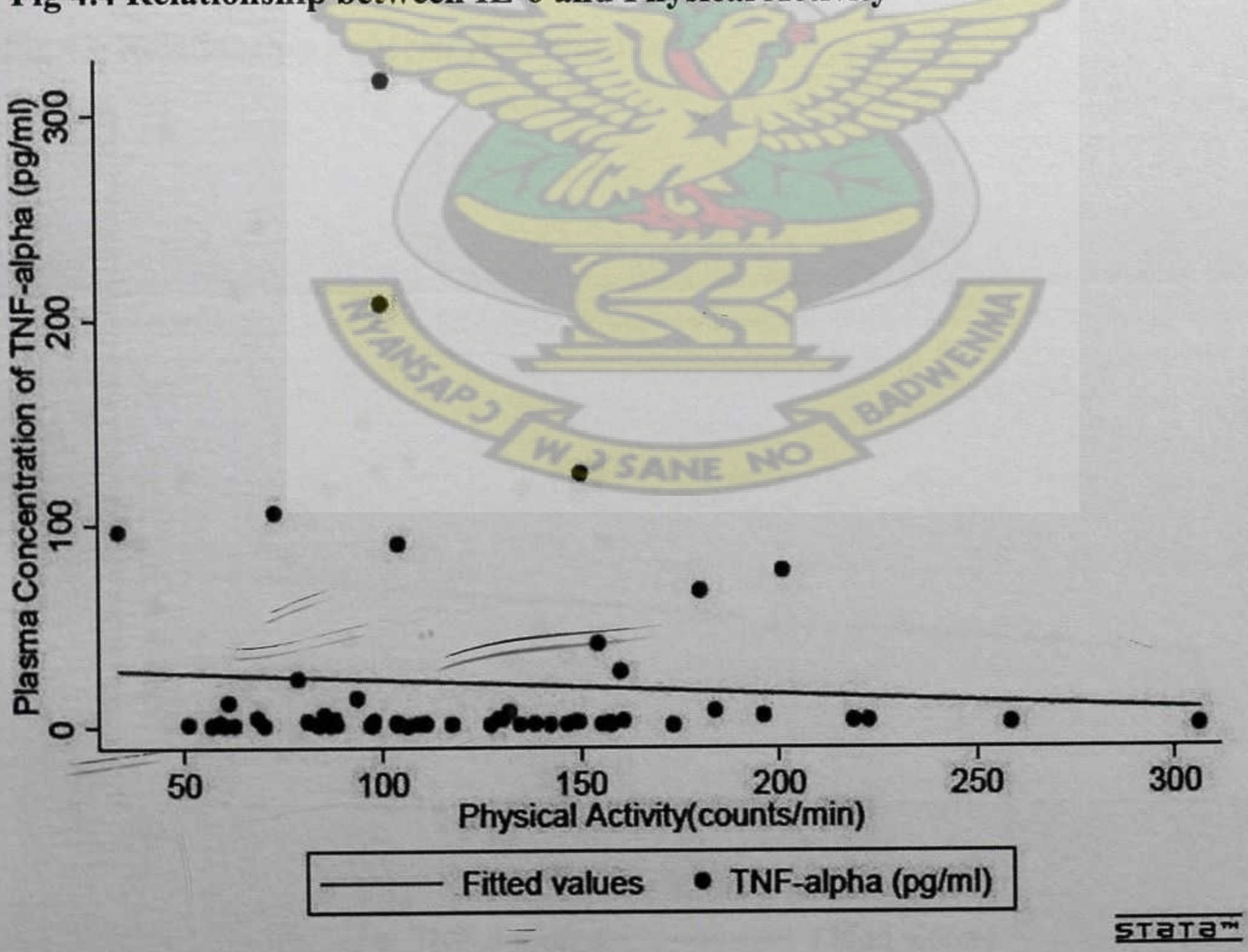

Fig 4.5 Relationship between TNF-α and Physical Activity

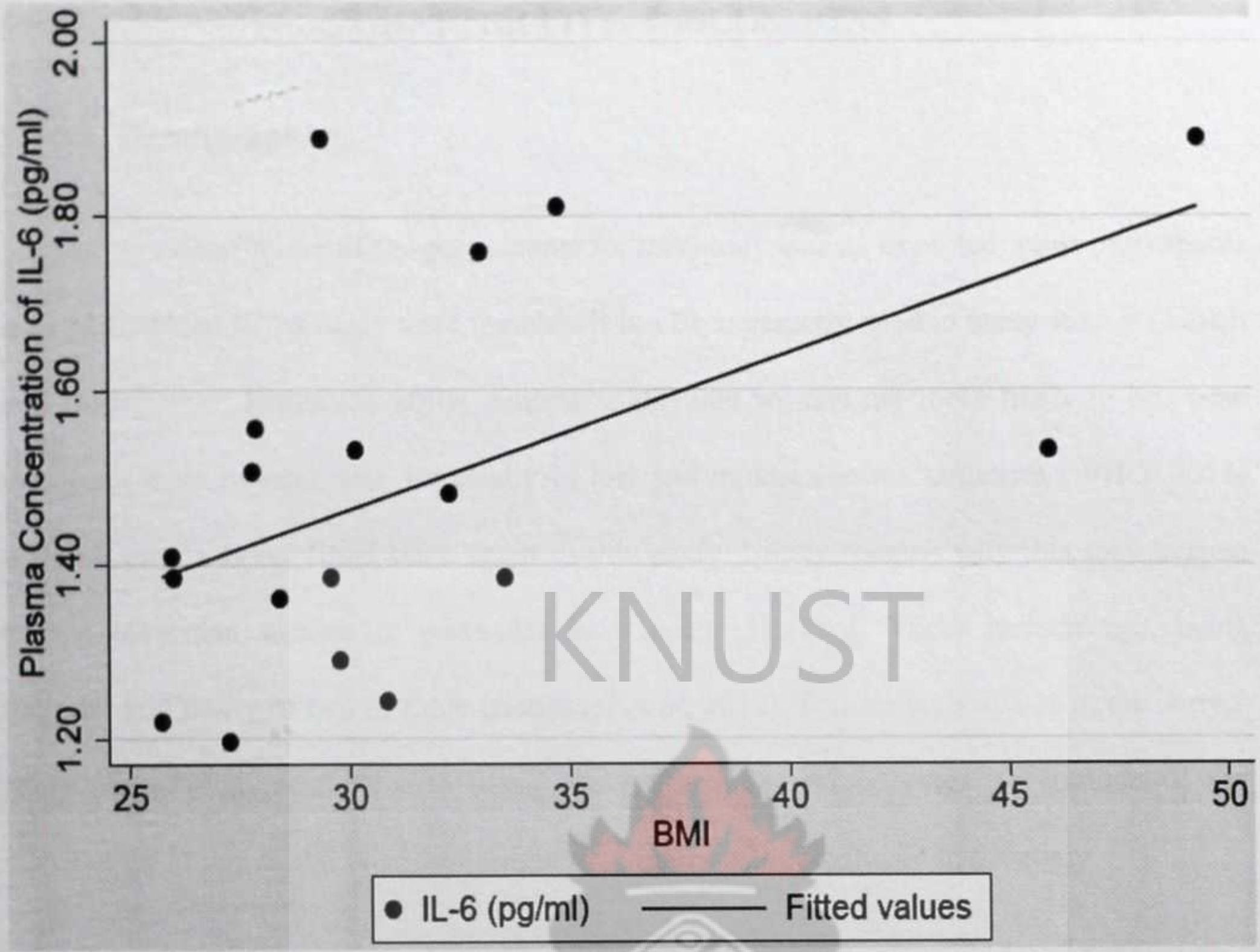

Fig 4.6 Relationship between IL-6 and BMI

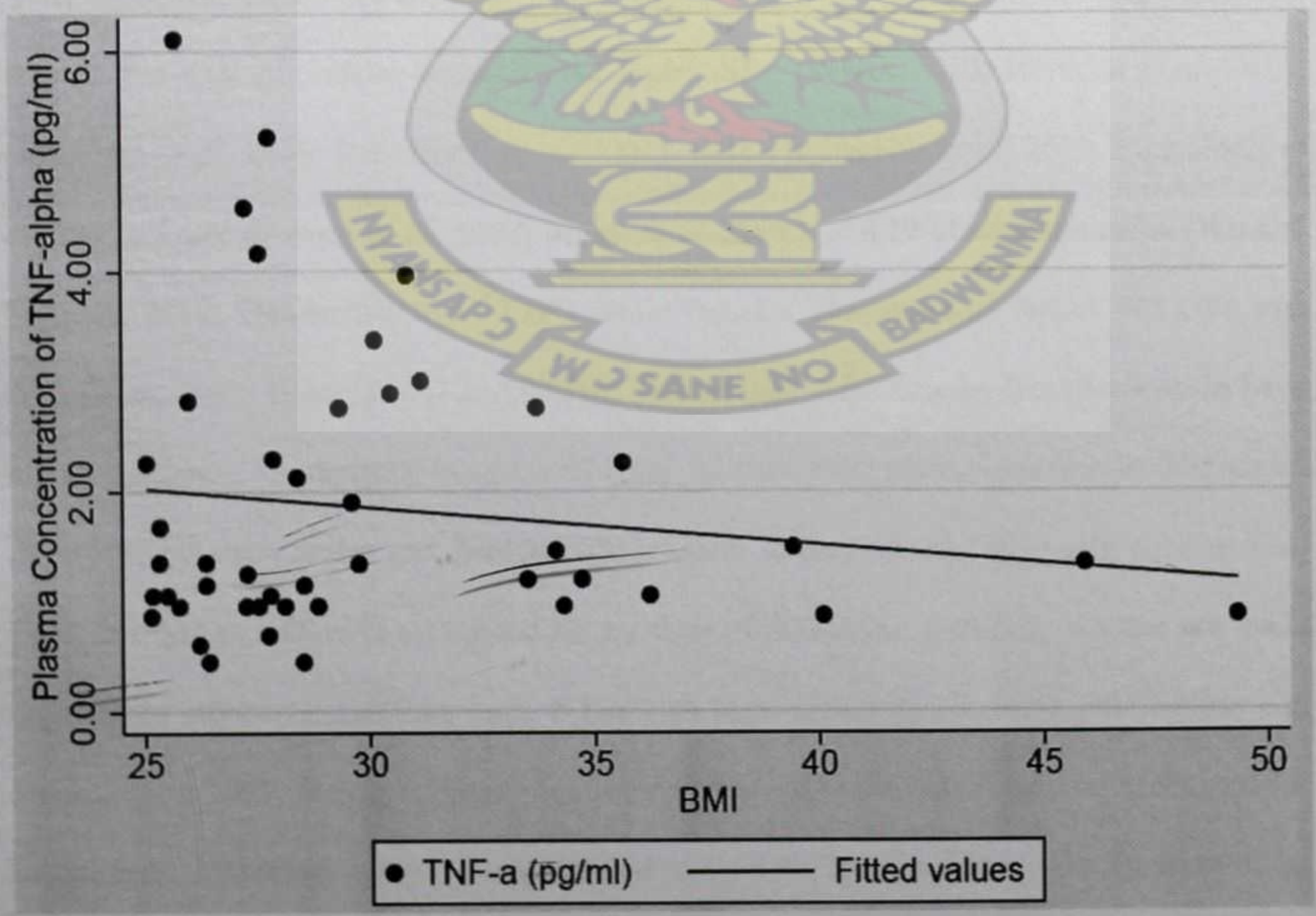

Fig 4.7 Relationship between TNF-α and BMI

CHAPTER FIVE: DISCUSSIONS

5.1. Demographics

Sex and age distribution of the participants for this study was as expected; most participants who participated in the study were females. It has been demonstrated in many studies (Jumah and Duda, 2007; Biritwum, 2005; Amoah, 2003) that women are more likely to be obese than their male counterparts, especially in low and middle income countries (WHO, 2013) and this fact is exemplified once again in this study. Likely reasons why this may happen among Ghanaian women in particular have been identified. These include age, being married, and parity of two or more (Benkeser *et al*, 2012). Female participants in the current study can strongly identify with these reasons, and this corroborates the findings of sex distribution in this study. Also few numbers of males were employed in the study.

5.2. Anthropometrics

Anthropometric measurements that were carried out corroborated the findings of many studies. For example, males demonstrated higher SBP (WHO, 2013; Hermida *et al*, 2013; Oghagbon *et al*, 2009; Reckelhoff *et al*, 2001), DBP (Oghagbon *et al*, 2009; Reckelhoff *et al*, 2001), TBW (Powers *et al*, 2003; Smith *et al*, 2002), and FFM measurements (Wu and Sullivan, 2011; Henderson *et al*, 2007) while females demonstrated higher FM (Wu and O'Sullivan, 2011; Blaak, 2001) and PBFM. These results reiterate the fact that women have higher measures of adiposity in terms of body fat than their male counterparts. The major difference between males and females with respect to measures of adiposity such as FM, FFM, PBFM, and TBW is accounted for by their physiologies; generally women are made up of more adipose tissue than men. It has also been speculated in some publications e.g. Jervase *et al*, 2009, that high blood pressure measurements for males may be accounted for by lifestyle. Increased intake of alcohol and salty diets has been the most attributable or likely causes of higher blood pressure in men.

5.3. Physical Activity

Overall, participants did not meet current recommendations for physical activity. The only attributable reason for this anomaly may be the use of different tools for measurement of physical activity. However, the use of accelerometers rather than conventional and traditional questionnaires provides a more detailed, reliable, and more objective method of assessing physical activity.

Another interesting finding from the study was that women were heavier, but more active than men. The most plausible reason for this is that obese and overweight men were engaged in more sedentary jobs such as driving or sitting at work, while women engaged in more active occupations such as sweeping, bathing the children, cooking and cleaning. However, although these activities involve some movement and considerable physical activity they fell within the category of "light activity".

5.4. Blood Immunochemistry (IL-6, TNF-α, and FBS)

Plasma concentrations of IL-6 and TNF were elevated as expected, because high plasma concentrations of these cytokines have repeatedly been associated with high adiposity (Bahceci *et al*, 2007; Park *et al*, 2005). Findings from the current study suggest that although some participants were active and had high plasma concentrations of both inflammatory cytokines the amount of physical activity they were engaged in was not enough to significantly reduce the concentrations of IL-6 and TNF-α. This also suggests that compliance to physical activity recommendations to reduce the concentrations of these cytokines may be useful. It is thus imperative to conduct further studies to show that compliance to these recommendations proves useful and can be incorporated into national health policies. It is also not surprising that women had higher levels of these inflammatory cytokines than men. This can be accounted for because women had higher measures of adiposity and these cytokines are directly associated, in part, with the degree of adiposity. The insignificant differences between the two physical activity groups with respect to plasma

concentrations of IL-6 and TNF correlates to the insignificant differences in their fat mass and percent body fat mass. High fasting blood sugar levels recorded by males may also be accounted for by lifestyle factors such as irregular eating times and large dietary portion sizes. However, these assumptions may have to be verified in future studies.

5.5. Associations of Physical Activity and BMI with IL-6 and TNF-α

A striking finding was that there was no association between BMI and plasma concentrations of TNF-α, which is in support of a burgeoning theory that TNF-α concentration in plasma does not necessarily match with adiposity or BMI because of the presence of its soluble receptor that is concurrently secreted by adipose tissue into blood (Lavoie *et al*, 2010; Harris *et al*, 2004). This perhaps makes it less accessible to be detected all the TNF-α in plasma. However IL-6 plasma concentrations correlated positively with BMI as observed in other studies.

Although there were significant differences in physical activity between both sedentary and active participants, no associations were found between physical activity and plasma concentrations of IL6 and TNF. What can possibly account for this is the non-compliance of even active participants to physical activity recommendations. In essence, active participants were not engaged in enough physical activity to significantly reduce their plasma levels of inflammatory cytokines IL-6 and TNF.

In addition the associations evaluated by the current study may have come about as a result of limitations of the study including sampling size.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

The current study concludes on the following

1) Obese and overweight participants in the current study have high circulating concentrations of pro-inflammatory cytokines (IL-6 and TNF-α)
2) Participants who were classified as being active engage primarily in light activities, rather than moderate and/or vigorous activity
3) Sedentary and active obese and overweight Ghanaians do not differ significantly in plasma concentrations of IL-6 and TNF-α
4) Among active and sedentary obese and overweight Ghanaians there is no association between BMI and IL-6 and TNF-α. There are also no associations between BMI and physical activity.
5) No one participant is compliant to recommended physical activity guidelines.

The study further recommends the following

1) A larger, more population-based study must be conducted to gather information about physical activity and the inflammatory status of obese and overweight Ghanaians which can be monitored
2) Accelerometers must be used to validate physical activity questionnaires in future studies that evaluate physical activity
3) Weight loss interventions can also incorporate similar protocols as employed in the current study to efficiently monitor progress of weight loss and significant reductions in pro-inflammatory cytokines

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APPENDIX 1

STATA OUTPUT FOR ANALYSIS

Statistics/Data Analysis

User: Albert Lawrence Kwansa

```
      name:  <unnamed>
       log:  C:\Users\METS\Desktop\thesis log 2013.smcl
  log type:  smcl
 opened on:   5 Jan 2013, 21:04:52

. sum age

    Variable |        Obs        Mean    Std. Dev.       Min        Max
-------------+---------------------------------------------------------
         age |         60       37.95    5.699911        26         47

. bysort sex: sum age

-> sex = male

    Variable |        Obs        Mean    Std. Dev.       Min        Max
-------------+---------------------------------------------------------
         age |          9    38.22222    6.629061        26         47

-> sex = female

    Variable |        Obs        Mean    Std. Dev.       Min        Max
-------------+---------------------------------------------------------
         age |         51    37.90196    5.593764        27         47

. log off
      name:  <unnamed>
       log:  C:\Users\METS\Desktop\thesis log 2013.smcl
  log type:  smcl
 paused on:   5 Jan 2013, 21:05:51


      name:  <unnamed>
       log:  C:\Users\METS\Desktop\thesis log 2013.smcl
  log type:  smcl
resumed on:   5 Jan 2013, 21:29:56

. gen sedentary = avgac<100

. label define sedlabel 0 "Active" 1 "Sedentary"
label sedlabel already defined
r(110);

. label define sedenlabel 0 "Active" 1 "Sedentary"

. label values sedentary sedenlabel

. tab sedentary

  sedentary |      Freq.     Percent        Cum.
------------+-----------------------------------
     Active |         37       61.67       61.67
  Sedentary |         23       38.33      100.00
------------+-----------------------------------
      Total |         60      100.00
```

```
9 . sum age

    Variable |       Obs        Mean    Std. Dev.       Min        Max
-------------+--------------------------------------------------------
         age |        60       37.95    5.699911        26         47

10 . bysort sex: sum age

-> sex = male

    Variable |       Obs        Mean    Std. Dev.       Min        Max
-------------+--------------------------------------------------------
         age |         9    38.22222    6.629061        26         47

-> sex = female

    Variable |       Obs        Mean    Std. Dev.       Min        Max
-------------+--------------------------------------------------------
         age |        51    37.90196    5.593764        27         47

11 . rename sedentary physicalactivity

12 . rename physicalactivity pa

13 . bysort pa: sum age

-> pa = Active

    Variable |       Obs        Mean    Std. Dev.       Min        Max
-------------+--------------------------------------------------------
         age |        37    37.18919    5.511795        27         47

-> pa = Sedentary

    Variable |       Obs        Mean    Std. Dev.       Min        Max
-------------+--------------------------------------------------------
         age |        23    39.17391    5.905715        26         47


14 . ttest age, by "pa"
   option by incorrectly specified
   r(198);

15 . log off
      name:  <unnamed>
       log:  C:\Users\METS\Desktop\thesis log 2013.smcl
  log type:  smcl
 paused on:   5 Jan 2013, 21:34:12

      name:  <unnamed>
       log:  C:\Users\METS\Desktop\thesis log 2013.smcl
  log type:  smcl
resumed on:   5 Jan 2013, 21:38:13
```

```
16 . ttest age, by (sex)

Two-sample t test with equal variances

   Grour |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
    male |       9    38.22222    2.209687    6.629061    33.12668    43.31777
  female |      51    37.90196    .7832836    5.593764    36.32869    39.47523
---------+--------------------------------------------------------------------
combined |      60        37.95    .7358553    5.699911    36.47756    39.42244
---------+--------------------------------------------------------------------
    diff |            .3202614    2.078071               -3.83945    4.479973
------------------------------------------------------------------------------
    diff = mean(male) - mean(female)                              t =   0.1541
Ho: diff = 0                                     degrees of freedom =       58

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.5610         Pr(|T| > |t|) = 0.8781          Pr(T > t) = 0.4390

17 . ttest age, by (pa)

Two-sample t test with equal variances

   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
  Active |      37    37.18919    .9061335    5.511795    35.35147    39.02691
Sedentar |      23    39.17391    1.231427    5.905715    36.62009    41.72774
---------+--------------------------------------------------------------------
combined |      60        37.95    .7358553    5.699911    36.47756    39.42244
---------+--------------------------------------------------------------------
    diff |           -1.984724    1.504069               -4.995445    1.025997
------------------------------------------------------------------------------
    diff = mean(Active) - mean(Sedentar)                          t =  -1.3196
Ho: diff = 0                                     degrees of freedom =       58

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.0961         Pr(|T| > |t|) = 0.1922          Pr(T > t) = 0.9039

18 . log off
      name:  <unnamed>
       log:  C:\Users\METS\Desktop\thesis log 2013.smcl
  log type:  smcl
 paused on:   5 Jan 2013, 21:38:55

      name:  <unnamed>
       log:  C:\Users\METS\Desktop\thesis log 2013.smcl
  log type:  smcl
resumed on:   5 Jan 2013, 21:50:55

19 . sum bmi

    Variable |       Obs        Mean    Std. Dev.       Min        Max
-------------+--------------------------------------------------------
         bmi |        60    30.02962    5.143402   25.06777   49.33146
```

```
. bysort sex:sum bmi

-> sex = male

    Variable |        Obs        Mean    Std. Dev.       Min        Max
-------------+---------------------------------------------------------
         bmi |          9    27.05905    1.932922   25.16554   31.17031

-> sex = female

    Variable |        Obs        Mean    Std. Dev.       Min        Max
-------------+---------------------------------------------------------
         bmi |         51    30.55384    5.361898   25.06777   49.33146

. ttest bmi, by (sex)

Two-sample t test with equal variances

   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
    male |       9    27.05905    .6443072    1.932922   25.57327   28.54482
  female |      51    30.55384    .7508158    5.361898   29.04578   32.06189
---------+--------------------------------------------------------------------
combined |      60    30.02962    .6640103    5.143402   28.70094    31.3583
---------+--------------------------------------------------------------------
    diff |           -3.494788    1.81856               -7.135031   .1454546
------------------------------------------------------------------------------
    diff = mean(male) - mean(female)                              t =  -1.9217
Ho: diff = 0                                     degrees of freedom =       58

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.0298         Pr(|T| > |t|) = 0.0596          Pr(T > t) = 0.9702

. bysort pa:sum bmi

-> pa = Active

    Variable |        Obs        Mean    Std. Dev.       Min        Max
-------------+---------------------------------------------------------
         bmi |         37    29.60636    4.701393   25.19679   45.92292

-> pa = Sedentary

    Variable |        Obs        Mean    Std. Dev.       Min        Max
-------------+---------------------------------------------------------
         bmi |         23    30.71051     5.83024   25.06777   49.33146
```

```
23 . ttest bmi, by (pa)

Two-sample t test with equal variances

   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
  Active |      37    29.60636    .7729043    4.701393    28.03884    31.17388
Sedentar |      23    30.71051    1.215689     5.83024    28.18932    33.23169
---------+--------------------------------------------------------------------
combined |      60    30.02962    .6640103    5.143402    28.70094     31.3583
---------+--------------------------------------------------------------------
    diff |           -1.104145    1.369791               -3.846079    1.637789
------------------------------------------------------------------------------
    diff = mean(Active) - mean(Sedentar)                          t =  -0.8061
Ho: diff = 0                                     degrees of freedom =       58

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.2117         Pr(|T| > |t|) = 0.4235          Pr(T > t) = 0.7883

24 . log off
      name:  <unnamed>
       log:  C:\Users\METS\Desktop\thesis log 2013.smcl
  log type:  smcl
 paused on:   5 Jan 2013, 21:54:16

      name:  <unnamed>
       log:  C:\Users\METS\Desktop\thesis log 2013.smcl
  log type:  smcl
resumed on:   5 Jan 2013, 22:04:20

25 . sum avgac

    Variable |       Obs        Mean    Std. Dev.       Min        Max
-------------+--------------------------------------------------------
       avgac |        60    121.8945    53.02188       33.79     306.26

26 . bysort sex:sum avgac

-> sex = male

    Variable |       Obs        Mean    Std. Dev.       Min        Max
-------------+--------------------------------------------------------
       avgac |         9      106.56    25.74676       70.34     155.55

-> sex = female

    Variable |       Obs        Mean    Std. Dev.       Min        Max
-------------+--------------------------------------------------------
       avgac |        51    124.6006    56.22721       33.79     306.26
```

[illegible] . ttest avgac, by (exe)

Two-sample t test with equal variances

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
Male	9	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Female	51	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
combined	60	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
diff		-10.[illegible]	10.[illegible]		-[illegible]	[illegible]

diff = mean(Male) - mean(Female)
Ho: diff = 0 t = -0.9652
degrees of freedom = 58

Ha: diff < 0 Ha: diff != 0 Ha: diff > 0
Pr(T < t) = 0.1768 Pr(|T| > |t|) = 0.3536 Pr(T > t) = 0.8232

[illegible] . bysort pa: sum avgac

-> pa = Active

Variable	Obs	Mean	Std. Dev.	Min	Max
avgac	37	151.1405	44.22199	100.48	308.28

-> pa = Sedentary

Variable	Obs	Mean	Std. Dev.	Min	Max
avgac	23	74.[illegible]	17.[illegible]	33.76	97.88

[illegible] . ttest avgac, by (pa)

Two-sample t test with equal variances

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
Active	37	151.1405	7.[illegible]	44.22199	135.7284	166.5527
Sedentary	23	74.[illegible]	3.[illegible]	17.[illegible]	67.47274	82.2283
combined	60	121.8945	5.[illegible]	39.[illegible]	110.1975	133.5915
diff		76.[illegible]	10.[illegible]		56.14992	96.43012

diff = mean(Active) - mean(Sedentary) t = 7.5813
Ho: diff = 0 degrees of freedom = 58

Ha: diff < 0 Ha: diff != 0 Ha: diff > 0
Pr(T < t) = 1.0000 Pr(|T| > |t|) = 0.0000 Pr(T > t) = 0.0000

```
30 . sum il6
```

Variable	Obs	Mean	Std. Dev.	Min	Max
il6	60	3.780314	3.108336	1.196883	19.4747

-> sex = male

Variable	Obs	Mean	Std. Dev.	Min	Max
il6	9	3.471367	1.343259	1.409465	6.402382

-> sex = female

Variable	Obs	Mean	Std. Dev.	Min	Max
il6	51	3.834834	3.33046	1.196883	19.4747


```
32 . ttest il6, by (sex)
```

Two-sample t test with equal variances

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
male	9	3.471367	.447753	1.343259	2.438847	4.503887
female	51	3.834834	.4663577	3.33046	2.898127	4.771541
combined	60	3.780314	.4012845	3.108336	2.977345	4.583282
diff		-.3634671	1.132463		-2.630338	1.903404

diff = mean(male) - mean(female) t = -0.3210
Ho: diff = 0 degrees of freedom = 58

Ha: diff < 0 Ha: diff != 0 Ha: diff > 0
Pr(T < t) = 0.3747 Pr(|T| > |t|) = 0.7494 Pr(T > t) = 0.6253

```
33 . bysort pa:sum il6
```

-> pa = Active

Variable	Obs	Mean	Std. Dev.	Min	Max
il6	37	3.492285	2.184574	1.243279	9.613507

-> pa = Sedentary

Variable	Obs	Mean	Std. Dev.	Min	Max
il6	23	4.243664	4.211623	1.196883	19.4747

```
34 . ttest il6, by (pa)

Two-sample t test with equal variances

   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
  Active |      37    3.492285    .3591418    2.184574    2.763912    4.220659
Sedentar |      23    4.243664    .8781841    4.211623    2.422421    6.064906
---------+--------------------------------------------------------------------
combined |      60    3.780314    .4012845    3.108336    2.977345    4.583282
---------+--------------------------------------------------------------------
    diff |           -.7513785    .8265687               -2.405935    .9031785
------------------------------------------------------------------------------
    di f = mean(Active) - mean(Sedentar)                          t =  -0.9090
Ho: diff = 0                                     degrees of freedom =       58

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.1835         Pr(|T| > |t|) = 0.3671          Pr(T > t) = 0.8165

35 . sum tnf if tnf<=7

    Variable |       Obs        Mean    Std. Dev.       Min        Max
-------------+--------------------------------------------------------
         tnf |        45    1.851368    1.307062   .4568827    6.10553

36 . bysort sex:sum tnf if tnf<=7

-------------------------------------------------------------------------------
-> sex = male

    Variable |       Obs        Mean    Std. Dev.       Min        Max
-------------+--------------------------------------------------------
         tnf |         7    1.199594    .8447496   .4568827    3.006058

-------------------------------------------------------------------------------
-> sex = female

    Variable |       Obs        Mean    Std. Dev.       Min        Max
-------------+--------------------------------------------------------
         tnf |        38    1.971431    1.349344   .4568827    6.10553

37 . ttest tnf, by (sex) if tnf<=7
option if not allowed
r(198);

38 . log off
      name:  <unnamed>
       log:  C:\Users\METS\Desktop\thesis log 2013.smcl
  log type:  smcl
 paused on:   5 Jan 2013, 22:18:38

      name:  <unnamed>
       log:  C:\Users\METS\Desktop\thesis log 2013.smcl
  log type:  smcl
resumed on:   5 Jan 2013, 22:19:12
```

```
39 . ttest tnf if tnf<=7, by (sex)

Two-sample t test with equal variances

   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
    male |       7    1.199594    .3192853    .8447496    .4183312    1.980857
  female |      38    1.971431    .2188926    1.349344    1.527913    2.414949
---------+--------------------------------------------------------------------
combined |      45    1.851368    .1948453    1.307062    1.458683    2.244052
---------+--------------------------------------------------------------------
    diff |           -.7718367    .5309274               -1.842554    .2988804
------------------------------------------------------------------------------
    diff = mean(male) - mean(female)                              t =  -1.4538
Ho: diff = 0                     degrees of freedom =       43

 Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.0766         Pr(|T| > |t|) = 0.1533          Pr(T > t) = 0.9234

40 . bysort pa:sum tnf if tnf<=7

-------------------------------------------------------------------------------
-> pa = Active

    Variable |       Obs        Mean    Std. Dev.       Min        Max
-------------+--------------------------------------------------------
         tnf |        27    1.782781    1.127407   .4568827   5.218607

-------------------------------------------------------------------------------
-> pa = Sedentary

    Variable |       Obs        Mean    Std. Dev.       Min        Max
-------------+--------------------------------------------------------
         tnf |        18    1.954247    1.568164   .6943536    6.10553

41 . ttest tnf if tnf<=7, by (pa)

Two-sample t test with equal variances

   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
  Active |      27    1.782781    .2169695    1.127407    1.336794    2.228768
Sedentar |      18    1.954247    .3696197    1.568164    1.174418    2.734077
---------+--------------------------------------------------------------------
combined |      45    1.851368    .1948453    1.307062    1.458683    2.244052
---------+--------------------------------------------------------------------
    diff |           -.1714659    .4014738               -.9811149     .638183
------------------------------------------------------------------------------
    diff = mean(Active) - mean(Sedentar)                          t =  -0.4271
Ho: diff = 0                     degrees of freedom =       43

 Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.3357         Pr(|T| > |t|) = 0.6714          Pr(T > t) = 0.6643
```

42 . sum tbw

Variable	Obs	Mean	Std. Dev.	Min	Max
tbw	60	33.35963	5.085593	25.32639	45.20186

-> sex = male

Variable	Obs	Mean	Std. Dev.	Min	Max
tbw	9	40.44606	3.396622	35.07706	45.20186

-> sex = female

Variable	Obs	Mean	Std. Dev.	Min	Max
tbw	51	32.10909	4.24717	25.32639	42.29527

44 . ttest tbw, by (sex)

Two-sample t test with equal variances

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
male	9	40.44606	1.132207	3.396622	37.83518	43.05693
female	51	32.10909	.5947227	4.24717	30.91455	33.30362
combined	60	33.35963	.6565473	5.085593	32.04589	34.67338
diff		8.336967	1.496912		5.340572	11.33336

diff = mean(male) - mean(female) t = 5.5694
Ho: diff = 0 degrees of freedom = 58

Ha: diff < 0 Ha: diff != 0 Ha: diff > 0
Pr(T < t) = 1.0000 Pr(|T| > |t|) = 0.0000 Pr(T > t) = 0.0000

45 . bysort pa:sum tbw

-> pa = Active

Variable	Obs	Mean	Std. Dev.	Min	Max
tbw	37	33.16374	4.76247	26.0103	45.20186

-> pa = Sedentary

Variable	Obs	Mean	Std. Dev.	Min	Max
tbw	23	33.67477	5.663712	25.32639	43.87567

```
46 . ttest tbw, by (pa)

Two-sample t test with equal variances

   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
  Active |      37    33.16374    .7829453    4.76247    31.57585    34.75163
Sedentar |      23    33.67477    1.180966    5.663712   31.22559    36.12394
---------+--------------------------------------------------------------------
combined |      60    33.35963    .6565473    5.085593   32.04589    34.67338
---------+--------------------------------------------------------------------
    diff |           -.5110269    1.360306               -3.233976   2.211922
------------------------------------------------------------------------------
    diff = mean(Active) - mean(Sedentar)                          t =  -0.3757
Ho: diff = 0                                     degrees of freedom =       58

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.3543         Pr(|T| > |t|) = 0.7085          Pr(T > t) = 0.6457

47 . save "C:\Users\METS\Desktop\MPhil Thesis dataset.dta", replace
   file C:\Users\METS\Desktop\MPhil Thesis dataset.dta saved

48 . sum ffm

    Variable |     Obs        Mean    Std. Dev.       Min        Max
-------------+--------------------------------------------------------
         ffm |      60    45.69813    6.966566    34.69368   61.92035

49 . bysort sex:sum ffm

-> sex = male

    Variable |     Obs        Mean    Std. Dev.       Min        Max
-------------+--------------------------------------------------------
         ffm |       9    55.40556    4.652907    48.05076   61.92035

-> sex = female

    Variable |     Obs        Mean    Std. Dev.       Min        Max
-------------+--------------------------------------------------------
         ffm |      51    43.98505    5.818041    34.69368   57.93872

50 . ttest ffm, by (sex)

Two-sample t test with equal variances

   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
    male |       9    55.40556    1.550969    4.652907   51.82902    58.9821
  female |      51    43.98505    .8146887    5.818041    42.3487    45.6214
---------+--------------------------------------------------------------------
combined |      60    45.69813    .8993798    6.966566   43.89847    47.49778
---------+--------------------------------------------------------------------
    diff |            11.4205    2.050564                7.315852    15.52515
------------------------------------------------------------------------------
    diff = mean(male) - mean(female)                              t =   5.5694
Ho: diff = 0                                     degrees of freedom =       58

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 1.0000         Pr(|T| > |t|) = 0.0000          Pr(T > t) = 0.0000
```

```
. bysort pa:sum ffm

-> pa = Active
    Variable |        Obs        Mean    Std. Dev.       Min        Max
-------------+--------------------------------------------------------
         ffm |         37    45.42978    6.523932    35.63055   61.92035

-> pa = Sedentary
    Variable |        Obs        Mean    Std. Dev.       Min        Max
-------------+--------------------------------------------------------
         ffm |         23    46.12982    7.758509    34.69368   60.10366

52 . ttest ffm, by (pa)

Two-sample t test with equal variances

   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
  Active |      37    45.42978    1.072528    6.523932    43.25459   47.60497
Sedentar |      23    46.12982    1.617761    7.758509    42.77479   49.48485
---------+--------------------------------------------------------------------
combined |      60    45.69813    .8993798    6.966566    43.89847   47.49778
---------+--------------------------------------------------------------------
    diff |           -.7000365    1.863433               -4.430104   3.030031
------------------------------------------------------------------------------
    diff = mean(Active) - mean(Sedentar)                          t =  -0.3757
Ho: diff = 0                                     degrees of freedom =       58

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.3543         Pr(|T| > |t|) = 0.7085          Pr(T > t) = 0.6457

53 . sum fm
    Variable |        Obs        Mean    Std. Dev.       Min        Max
-------------+--------------------------------------------------------
          fm |         60    26.49354    10.01863    9.752535   59.48299

54 . bysort sex:sum fm

-> sex = male
    Variable |        Obs        Mean    Std. Dev.       Min        Max
-------------+--------------------------------------------------------
          fm |          9    16.37222    5.758283    9.752535   28.19634

-> sex = female
    Variable |        Obs        Mean    Std. Dev.       Min        Max
-------------+--------------------------------------------------------
          fm |         51    28.27965    9.562499    15.41082   59.48299
```

55 . ttest fm, by (sex)

Two-sample t test with equal variances

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
male	9	16.37222	1.919428	5.758283	11.94601	20.79843
female	51	28.27965	1.339018	9.562499	25.59016	30.96915
combined	60	26.49354	1.293399	10.01863	23.90545	29.08162
diff		-11.90743	3.301857		-18.51682	-5.298047

diff = mean(male) - mean(female) t = -3.6063
Ho: diff = 0 degrees of freedom = 58

Ha: diff < 0 Pr(T < t) = 0.0003 | Ha: diff != 0 Pr(|T| > |t|) = 0.0006 | Ha: diff > 0 Pr(T > t) = 0.9997

56 . bysort pa:sum fm

-> pa = Active

Variable	Obs	Mean	Std. Dev.	Min	Max
fm	37	25.76481	9.870708	12.57965	59.48299

-> pa = Sedentary

Variable	Obs	Mean	Std. Dev.	Min	Max
fm	23	27.66583	10.36435	9.752535	57.80507

57 . ttest fm, by (pa)

Two-sample t test with equal variances

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
Active	37	25.76481	1.622734	9.870708	22.47375	29.05587
Sedentar	23	27.66583	2.161116	10.36435	23.18395	32.14771
combined	60	26.49354	1.293399	10.01863	23.90545	29.08162
diff		-1.901021	2.671428		-7.248465	3.446423

diff = mean(Active) - mean(Sedentar) t = -0.7116
Ho: diff = 0 degrees of freedom = 58

Ha: diff < 0 Pr(T < t) = 0.2398 | Ha: diff != 0 Pr(|T| > |t|) = 0.4796 | Ha: diff > 0 Pr(T > t) = 0.7602

. sum pbfm

Variable	Obs	Mean	Std. Dev.	Min	Max
pbfm	60	35.99158	8.081982	16.36331	54.07397

. bysort sex:sum pbfm

Variable	Obs	Mean	Std. Dev.	Min	Max
pbfm	9	22.39994	5.429539	16.36331	31.93244

-> sex = female

Variable	Obs	Mean	Std. Dev.	Min	Max
pbfm	51	38.3901	5.76534	26.60015	54.07397

. ttest pbfm, by (sex)

Two-sample t test with equal variances

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
male	9	22.39994	1.809846	5.429539	18.22643	26.57346
female	51	38.3901	.807309	5.76534	36.76858	40.01163
combined	60	35.99158	1.043379	8.081982	33.90378	38.07938
diff		-15.99016	2.068141		-20.12999	-11.85033

diff = mean(male) - mean(female) t = -7.7317
Ho: diff = 0 degrees of freedom = 58

Ha: diff < 0 Ha: diff != 0 Ha: diff > 0
Pr(T < t) = 0.0000 Pr(|T| > |t|) = 0.0000 Pr(T > t) = 1.0000

. bysort pa:sum pbfm

-> pa = Active

Variable	Obs	Mean	Std. Dev.	Min	Max
pbfm	37	35.50406	8.093407	16.88543	53.6366

-> pa = Sedentary

Variable	Obs	Mean	Std. Dev.	Min	Max
pbfm	23	36.77585	8.181827	16.36331	54.07397

62 . ttest pbfm, by (pa)

Two-sample t test with equal variances

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
Active	37	35.50406	1.330548	8.093407	32.80559	38.20254
Sedentar	23	36.77585	1.706029	8.181827	33.23776	40.31394
combined	60	35.99158	1.043379	8.081982	33.90378	38.07938
diff		-1.271787	2.157964		-5.591421	3.047848

diff = mean(**Active**) - mean(**Sedentar**) t = -0.5893
Ho: diff = 0 degrees of freedom = 58

Ha: diff < 0 — Pr(T < t) = **0.2790**
Ha: diff != 0 — Pr(|T| > |t|) = **0.5579**
Ha: diff > 0 — Pr(T > t) = **0.7210**

63 . sum msbp

Variable	Obs	Mean	Std. Dev.	Min	Max
msbp	60	111.0792	13.32041	87.5	145.75

64 . bysort sex:sum msbp

-> sex = male

Variable	Obs	Mean	Std. Dev.	Min	Max
msbp	9	123.3056	15.02382	102.75	145.75

-> sex = female

Variable	Obs	Mean	Std. Dev.	Min	Max
msbp	51	108.9216	11.89963	87.5	140

65 . ttest msbp, by (sex)

Two-sample t test with equal variances

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
male	9	123.3056	5.007941	15.02382	111.7572	134.8539
female	51	108.9216	1.666282	11.89963	105.5747	112.2684
combined	60	111.0792	1.719657	13.32041	107.6381	114.5202
diff		14.38399	4.475104		5.426093	23.34188

diff = mean(**male**) - mean(**female**) t = 3.2142
Ho: diff = 0 degrees of freedom = 58

Ha: diff < 0 — Pr(T < t) = **0.9989**
Ha: diff != 0 — Pr(|T| > |t|) = **0.0021**
Ha: diff > 0 — Pr(T > t) = **0.0011**

```
6 . bysort pa:sum msbp

-> pa = Active

    Variable |        Obs        Mean    Std. Dev.       Min        Max
-------------+---------------------------------------------------------
        msbp |         37    109.8514    12.48992        87.5     145.75

-> pa = Sedentary

    Variable |        Obs        Mean    Std. Dev.       Min        Max
-------------+---------------------------------------------------------
        msbp |         23    113.0543    14.62737        88.5     145.75

67 . ttest msbp, by (pa)

Two-sample t test with equal variances

   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
  Active |      37    109.8514     2.05333    12.48992    105.687    114.0157
Sedentar |      23    113.0543    3.050018    14.62737    106.729    119.3797
---------+--------------------------------------------------------------------
combined |      60    111.0792    1.719657    13.32041   107.6381    114.5202
---------+--------------------------------------------------------------------
    diff |           -3.202996    3.542427               -10.29393    3.887942
------------------------------------------------------------------------------
    diff = mean(Active) - mean(Sedentar)                          t =  -0.9042
Ho: diff = 0                     degrees of freedom =       58

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.1848         Pr(|T| > |t|) = 0.3696          Pr(T > t) = 0.8152

68 . sum mdbp

    Variable |        Obs        Mean    Std. Dev.       Min        Max
-------------+---------------------------------------------------------
        mdbp |         60    68.28333    10.69556        49.5      97.25

69 . bysort sex:sum mdbp

-> sex = male

    Variable |        Obs        Mean    Std. Dev.       Min        Max
-------------+---------------------------------------------------------
        mdbp |          9          76    15.05978      55.75      97.25

-> sex = female

    Variable |        Obs        Mean    Std. Dev.       Min        Max
-------------+---------------------------------------------------------
        mdbp |         51    66.92157     9.278401        49.5         91
```

```
. ttest mdbp, by (sex)

Two-sample t test with equal variances

   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
    male |       9          76    5.019926    15.05978    64.42403    87.57597
  female |      51    66.92157    1.299236    9.278401    64.31198    69.53116
---------+--------------------------------------------------------------------
combined |      60    68.28333    1.380791    10.69556    65.52038    71.04629
---------+--------------------------------------------------------------------
    diff |            9.078431    3.713546                1.644961     16.5119
------------------------------------------------------------------------------
    diff = mean(male) - mean(female)                              t =   2.4447
Ho: diff = 0                     degrees of freedom =       58

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.9912         Pr(|T| > |t|) = 0.0176          Pr(T > t) = 0.0088

. bysort pa:sum mdbp

-> pa = Active

    Variable |       Obs        Mean    Std. Dev.       Min        Max
-------------+--------------------------------------------------------
        mdbp |        37    67.14189      11.249        49.5      97.25

-> pa = Sedentary

    Variable |       Obs        Mean    Std. Dev.       Min        Max
-------------+--------------------------------------------------------
        mdbp |        23    70.11957    9.695615       56.5      95.25

. ttest mdbp, by(pa)

Two-sample t test with equal variances

   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
  Active |      37    67.14189    1.849325      11.249    63.39129     70.8925
Sedentar |      23    70.11957    2.021675    9.695615    65.92687    74.31226
---------+--------------------------------------------------------------------
combined |      60    68.28333    1.380791    10.69556    65.52038    71.04629
---------+--------------------------------------------------------------------
    diff |           -2.977673    2.837542               -8.657631    2.702284
------------------------------------------------------------------------------
    diff = mean(Active) - mean(Sedentar)                          t =  -1.0494
Ho: diff = 0                     degrees of freedom =       58

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.1492         Pr(|T| > |t|) = 0.2984          Pr(T > t) = 0.8508
```

. sum glucrslt

Variable	Obs	Mean	Std. Dev.	Min	Max
glucrslt	60	99.36667	10.13797	76	123

. bysort sex:sum glucrslt

-> sex = male

Variable	Obs	Mean	Std. Dev.	Min	Max
glucrslt	9	101.3333	8.602325	86	113

-> sex = female

Variable	Obs	Mean	Std. Dev.	Min	Max
glucrslt	51	99.01961	10.42207	76	123

. ttest glucrslt, by (sex)

... Two-sample t test with equal variances ...

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
male	9	101.3333	2.867442	8.602325	94.721	107.9457
female	51	99.01961	1.459382	10.42207	96.08835	101.9509
combined	60	99.36667	1.308807	10.13797	96.74775	101.9856
diff		2.313725	3.684351		-5.061305	9.688756

diff = mean(male) - mean(female) t = 0.6280
Ho: diff = 0 degrees of freedom = 58

Ha: diff < 0 Ha: diff != 0 Ha: diff > 0
Pr(T < t) = 0.7338 Pr(|T| > |t|) = 0.5325 Pr(T > t) = 0.2662

. bysort pa:sum glucrslt

-> pa = Active

Variable	Obs	Mean	Std. Dev.	Min	Max
glucrslt	37	99.48649	10.0156	81	123

-> pa = Sedentary

Variable	Obs	Mean	Std. Dev.	Min	Max
glucrslt	23	99.17391	10.55571	76	119

```
77 . ttest glucrslt, by (pa)

Two-sample t test with equal variances

   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
  Active |      37     99.48649    1.646555     10.0156    96.14712    102.8259
Sedentar |      23     99.17391    2.201017    10.55571    94.60928    103.7385
---------+--------------------------------------------------------------------
combined |      60     99.36667    1.308807    10.13797    96.74775    101.9856
---------+--------------------------------------------------------------------
    diff |            .3125734    2.714716               -5.121521    5.746668
------------------------------------------------------------------------------
    diff = mean(Active) - mean(Sedentar)                          t =   0.1151
Ho: diff = 0                                     degrees of freedom =       58

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.5456         Pr(|T| > |t|) = 0.9087          Pr(T > t) = 0.4544

78 . save "C:\Users\METS\Desktop\MPhil Thesis dataset.dta", replace
file C:\Users\METS\Desktop\MPhil Thesis dataset.dta saved

79 . log close
      name:  <unnamed>
       log:  C:\Users\METS\Desktop\thesis log 2013.smcl
  log type:  smcl
 closed on:   5 Jan 2013, 22:57:56

      name:  <unnamed>
       log:  C:\Users\METS\Desktop\STATA Tutorials\Log Sheets\thesis log 2013.smcl
  log type:  smcl
 opened on:  19 Jan 2013, 18:05:18

80 . twoway lfit avgac bmi || scatter avgac bmi

81 . save "C:\Users\METS\Desktop\STATA Tutorials\Data Sets\MPhil Thesis dataset.dta", replace
file C:\Users\METS\Desktop\STATA Tutorials\Data Sets\MPhil Thesis dataset.dta saved

82 . log off
      name:  <unnamed>
       log:  C:\Users\METS\Desktop\STATA Tutorials\Log Sheets\thesis log 2013.smcl
  log type:  smcl
 paused on:  19 Jan 2013, 18:05:43
```