# THE EFFECTS OF $\dot{V}O_2$max ON LEVELS OF TESTOSTERONE AS MALES AGE

A Thesis

Presented to

The Faculty of the Department

Of Health and Human Performance

University of Houston

In Partial Fulfillment

Of the Requirements for the Degree of

Masters of Science

By Mohammed S. Rahman

May, 2016

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## ABSTRACT

**Purpose:** The reduction of Testosterone secretion by the gonads is "hypogonadism" and development of age-related hypogonadism is known as "late-onset hypogonadism". Late-onset hypogonadism is characterized by issues such as osteoporosis, Alzheimer's disease, frailty, obesity, cardiac failure and ischemic heart disease. The purpose of this investigation was to determine the impact of $\dot{V}O_2$max on age-related declines in free and bound plasma testosterone levels in healthy men. **Methods:** A total of 45 male subjects of various ethnicities aged 20-62 years participated in this study. Resting blood samples were collected, after which subjects were on cycle ergometer. Subjects started the trail and complete four three-minute heart rate adjusted incremental stages. The $\dot{V}O_2$max of each subject was estimated using a sub-maximal cycling exercise protocol. Free and Bound testosterone was then removed from blood serum samples and measured. **Results:** High levels of aerobic fitness did not offset age-related declines in circulating levels of free testosterone. While significant differences were found amongst the Young population (Low aerobic fitness versus High aerobic fitness) ($p=0.045$, $F=2.912$), this was not relevant to the investigation. **Conclusion:** After controlling for age, aerobic fitness did not prevent the age-associated decline in Testosterone. Taking the results of this study into consideration when creating countermeasures for older adult males with symptoms of hypogonadism, it is suggested that an exercise countermeasure have a combination of endurance and strength training to help mediate levels of testosterone with age.

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## TABLE OF CONTENTS

## LIST OF FIGURES

## LIST OF TABLES

## Introduction

Advances in medicine and technology in the last several decades have made it possible for a longer life span, which has inevitably resulted in the older adult population to be the fastest growing population in the world. Projections indicate that by 2025 the cohort over age 65 will be increasing 3.5 times as rapidly as the total population (Oeppen & Vaupel, 2002). Aging is a continuous and highly complex process that affects almost all organ systems, physiological and molecular. With aging in humans, the male hormone testosterone begins to decline, which progressively has an effect on a micro and macro level. These declines in the testosterone have an adverse effect on quality of life and how life can be enjoyed.

The consequences of the decline of the male hormone have been noted since times of antiquity. Only in recent decades have the underlying mechanism of how deeply rooted this hormone is in the homeostasis of male biology been slowly extricated. The reduction of hormone secretion by the gonads has been dubbed "hypogonadism" and development of hypogonadism with aging is known as "late-onset hypogonadism". Late-onset hypogonadism is characterized by issues such as osteoporosis, Alzheimer's disease, frailty, obesity, cardiac failure and ischemic heart disease, which can lead to the suppression of the hypothalamic-pituitary testicular axis, further exacerbating the declines in testosterone (Stanworth & Jones, 2008). Studies show that there is an association between testosterone insufficiency in older men and increased mortality risk over the following 20 years (Laughlin, Barrett-Connor, & Bergstrom, 2008).

With the prevalence of cardiovascular disease, type 2 diabetes, and osteoporosis being associated with high mortality in the developing and developed countries (Stanworth & Jones, 2008), exacting a great toll on our health care system, there has been a larger push to find noninvasive countermeasure to help attenuate some of these costs. One viable countermeasure being pursued is modulating lifestyle factors, such as increasing physical activity and maintaining fitness to combat declines in testosterone.

The effects of resistance training on levels of testosterone have been thoroughly examined but for our purposes we will focus on the effects of sustained endurance exercise throughout life and the effect it has on levels of testosterone. It is understood that maintaining moderate to high amount of endurance based activity has its benefits. This form of exercise is very beneficial not only for cardiovascular, skeletal and muscular health but also maintaining levels of testosterone (A. C. Hackney, 1989). It has been observed during a short sub-maximal workload (20 min) on a cycle ergometer, testosterone concentrations were significantly increased (A. Hackney, 1996). The effect of prolonged (>60 min) sub maximal exercise on testosterone was studied and it was found that prolonged sub-maximal exercise to exhaustion resulted in an initial rise in testosterone followed by decline as the activity continued (Galbo et al., 1977). Concentrations of testosterone are closely linked to acute program variable domains such as intensity, volume and choice of exercise with diminishing returns as the exercise bout is prolonged.

As males age, free and bioavailable testosterone levels fall by 2%-3% per year, whereas total testosterone levels fall at an average of 1.6% per year (Feldman et al., 2002). With these steady declines in testosterone, it is hypothesized after controlling for age, serum testosterone levels in healthy men will increase with relative increases in $\dot{V}O_2max$.

## Specific Aims

Specific aim 1 – To determine the impact of $\dot{V}O_2$max on age-related declines in free and bound plasma testosterone levels in healthy men.

Hypothesis 1– After controlling for age, serum testosterone levels in healthy men will increase with relative increases in $\dot{V}O_2$max.

Specific aim 2 – To compare the relative effects of age, fitness, and body composition on free and bound plasma testosterone levels in healthy men.

Hypothesis 2 – Fitness will be the strongest factor effecting total testosterone when compared with age and body composition.

## Review of Relevant Literature

### History of testosterone

Steroids are important hormones that are responsible for plethora of activities, from differentiating males and females to assisting in the development and maturity of children. Steroids have myriad other applications and impact on phenotype, physiology and psychology of an individual. The testes, unlike other endocrine organs, are not hidden within the body. Therefore, since quite early in human history, among different cultures the effects of testosterone or the lack there of were understood to some extent (Nieschlag & Nieschlag, 2014).

Historically, the Greeks, the Chinese, and numerous tribes from the Sub Saharan and various other regions used castration to halt the production of testosterone to the body. It was used for different purposes in different times. Castration has been performed as

punishment, to produce obedient slaves and also to preserve the soprano voices of pre-pubesce boys. As it was known that the removal of the testes reproduced the same symptoms as hypogonadism, such as impotence amongst other side effects (Nieschlag & Nieschlag, 2014), medical professionals during those periods were prescribing ingestion of testes to remedy said symptoms and this was dubbed organotherapy (Freeman E.R. et al, 2001). Progressively throughout time, the way testes were ingested was refined and documentation states that testes were being consumed up till the 1920's. It was later found that ingestion of testosterone orally would be inactivated by the first pass effect in the liver. Therefore, all orally administered organotherapy could only be considered as a placebo effect and this type of therapy was ended by the invention of phosphodiesterase (Nieschlag & Nieschlag, 2014).

In 1931, Adolf Butenandt isolated the androgenic steroid androsterone (androstan - 3α-ol-17-one) from 1500 liters of urine, which was processed to obtain 15mg of this first androgen (Hoberman & Yesalis, 1995). In 1935, Ernest Laqueur and his group extracted and isolated 10mg testosterone (androsten-17α-ol-3-one) from 100kg of bull testes, which they found more active than androsterone and named it "testosterone". In the same year, the chemical synthesis of testosterone was published, marked the beginning of modern clinical pharmacology and endocrinology of testosterone and male reproductive physiology (Freeman et al., 2001).

With the isolation of testosterone, soon thereafter it was clinically available, first in the form of pellets, and then as injectable esters. In 1935, 17α-methyl-testosterone was synthesized but was soon disregarded due to its toxicity in the liver (Hoberman & Yesalis, 1995).

## Types of Steroids

There are five major classes of steroid hormone: testosterone (androgen), estradiol (estrogen), progesterone (progestin), cortisol/corticosterone (glucocorticoid), and aldosterone (mineralocorticoids). Testosterone and its more potent metabolite dihydrotestosterone (DHT), progesterone and estradiol are classified as sex steroids, whereas cortisol/corticosterone and aldosterone are collectively referred to as corticosteroids (Jean D. Wilson, Leihy, Shaw, &Renfree, 2002). For the purpose of this study, the main focus is on androgens, their metabolism and the effect it has on the body.

Androgens are the group of hormones that promote the development and maintenance of male sex characteristics, and are largely responsible for the developmental changes that occur during puberty and adolescence. Androgens also exert their effects in many other parts of the body, including muscle, bone, hair follicles, liver, kidneys and the hematopoietic, immune and central nervous systems (Mooradian et al., 1987).

In the male fetus, androgens stimulate the development of the Wolffian ducts and the male external genitalia (Kicman, 2008). During puberty, androgenic effects resulting from increased testicular steroidogenesis are manifested by growth of the testes, external genitalia and the male accessory reproductive glands and secretory activity begins. Thereafter, the secondary sexual characteristics manifested during puberty can be divided into those that are a result of androgenic and anabolic effects. The androgenic effects are the enlargement of the larynx, the growth of terminal hair, increase in sebaceous gland activity and CNS effect. Anabolic effects are the growth of skeletal muscle and bone and the stimulation of linear growth eventually ceasing due to the closure of the epiphysis. In men, androgens are

essential for sustaining reproductive function and they play an important role in maintaining skeletal muscle and bone, cognitive function and a sense of well-being (Kicman, 2008).

One of the main androgens secreted in males is testosterone. It is both an active hormone and a prohormone for the formation of a more active androgen, the 5α-reduced steroid dihydrotesterone (DHT), which acts in the cell nucleus of target tissues such as skin, male accessory glands and the prostate, exerting predominantly androgenic, but also anabolic effects (Jean D. Wilson et al, 2002). Testosterone is 19-carbon steroid formed from cholesterol via a series of enzymatic reactions in the Leydig cells of the testes and adrenal cortex in males (Stojanovic M. & Ostojic S., 2012). The adrenal glands produce very little testosterone but secrete larger amounts of weaker androgens; in particular, dehydroepaindrosterone (DHEA) and androstenedione (Kicman, 2008). Testosterone secretion is under the control of the luteinizing hormone (LH) which is produced by the pituitary gland. Synthesis and release of LH is under control of the hypothalamus through gonadotopin-releasing hormone (GnRH) and inhibited by testosterone via a negative feedback mechanism (Hoffman et al., 2009).

The basic structure of testosterone is composed of 3 cyclodexane rings and 1 cyclopentane ring with methyl group at positions 10 and 13 (Srinivas-Shankar & Wu, 2006). Healthy males produce approximately 4.0-9.0mg of testosterone per day with blood concentrations ranging from 400 to 1000 ng/dL-1 (10.4-34.7 nmol/$L^{-1}$) (Hoffman et al., 2009). Testosterone is carried to target cells through the bloodstream either free (only about 1-3% of circulating testosterone) or bound to carrier protein. Most of the circulating testosterone (50-60%) is bound with high affinity to sex hormone-binding globulin (SHBG),

while a smaller fraction (40-50%) is bound loosely to albumin (Kaufman & Vermeulen, 2005).

**Metabolism and biosynthesis**

Testosterone is synthesized through either Δ-4 or Δ-5 pathway (Broeder, 2003) with the effects of newly synthesized testosterone in humans occurring by way of two main mechanisms: by the activation of the androgen receptor (directly or as 5α-dihydrotestosterone), and by conversion of estradiol through aromatase and activation of estrogen receptors (J. D. Wilson, 1988).

Free testosterone is transported into the cytoplasm of target tissue cells, where it can bind to the androgen receptor (AR), or can be reduced to 5α-dihydrptestpsterone (DHT) by the cytoplasmic enzyme 5-alpha reductase (Hartgens & Kuipers, 2004). DHT binds to the same androgen receptor with greater affinity than testosterone, resulting in a greater androgenic potency that corresponds to about five times that of testosterone (Breiner, Romalo, & Schweikert, 1986). The testosterone receptor or DHT receptor complex undergoes a structural change that allows it to move into the cell nucleus and bind directly to specific nucleotide sequences of the chromosomal deoxyribonucleic acid. The areas of binding are called hormone response elements and influence transcriptional activity of certain genes, producing the anabolic effects (Saartok, Dahlberg, & Gustafsson, 1984).

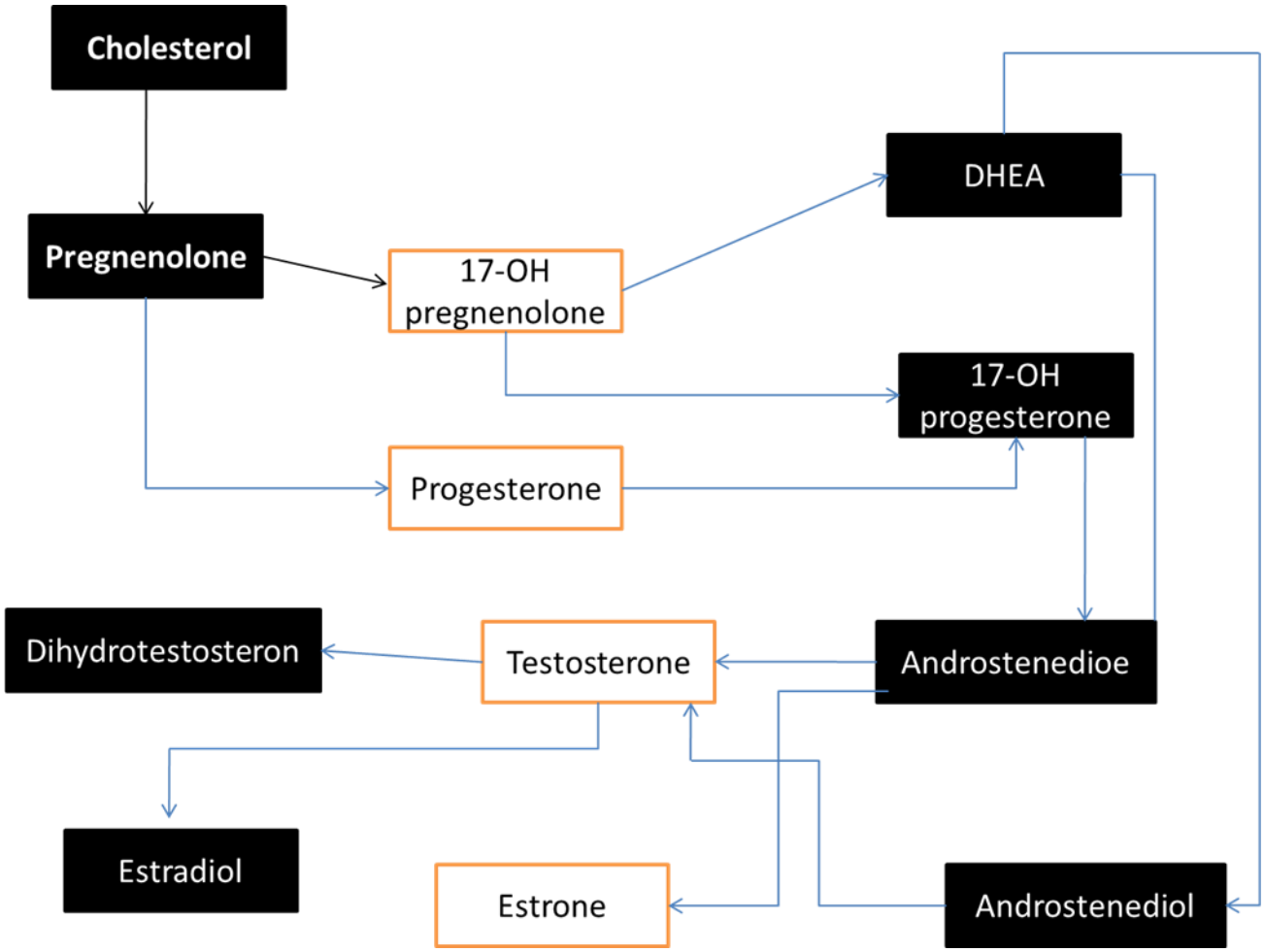


Figure 1 – Androgens biosynthesis from cholesterol to testosterone/dihydrotestosterone and estrogens (estrone and estradiol) via prohormone precursors (e.g. DHEA, androstenedione, androstenediol). Adapted from (Stojanovic M. & Ostojic S., 2012)

The effects of androgens are modulated at cellular level by the steroid-converting enzymes within the particular target tissue. In reproductive target tissues, testosterone can be considered to be a prohormone, being readily converted by 5α-reductase to the more potent androgen DHT. In other tissues, such as adipose tissue and parts of the brain, testosterone is converted by aromatase to the estogen, estadiol. In bone, the mechanism of action of the anabolism of androgens has not been entirely elucidated but both a direct effect and mediated

effect by aromatization to estradiol are important (Lanfranco, Zitzmann, Simoni, & Nieschlag, 2004; Orwoll, 1996).

## Physical fitness

### Resistance exercise effects on testosterone levels

The effects of different forms of resistance exercise and how they modulate the body have been extensively studied over the years; here we will focus on how acute resistance bouts of exercise modulate levels of testosterone in the male body. The association between testosterone and exercise is bidirectional, i.e. testosterone effects exercise and is affected by it. Testosterone responses to resistance exercise depend on factors related to the training session, such as volume, intensity, method, type of muscle contraction, muscle mass involved, as well as factors such as age and the individuals level of training (Lusa & Martins Kruel, 2012). It is generally accepted that endurance training tends to decrease testosterone values than that of physiologically relevant resting values (A. C. Hackney, 1989), whereas strength exercise can increase its basal levels during periods of high volume and high intensity training in response to long or short training periods (Kraemer et al., 1995; Staron et al., 1994).

In general, circulating total testosterone and free testosterone increase immediately after a bout of heavy resistance exercise in men and return to, or below baseline within 30 minutes (Vingren et al., 2010). However, as stated above, the appearance and magnitude of these changes are greatly influenced by the selection among the 'acute program variable domains' (intensity, number of sets, choice of exercise and order of exercise) for the exercise.

## Intensity

Intensity refers to the load or resistance used for a given exercise. There appears to be a relative intensity and volume threshold (total work performed, see section below for "Volume") that must be reached in order to induce a testosterone response (Vingren et al., 2010). When comparing protocols with the same volume but different loads such as 4 sets of 6 repetitions at 52.5% of 1 repetition max (RM) verses three sets of six repetitions at 40% of 1RM during concentric actions and 100% of 1RM during eccentric actions, neither protocol produced an increase in testosterone (Yarrow et al., 2007). A similar study exhibited the effect of altering the intensity while keeping total work constant and intensity reduced had attenuated effects on testosterone (Kraemer et al., 1995). Hence, when the number of repetitions is kept constant, higher intensity and thus higher volume induces a greater testosterone response. Three sets of six repetitions for three lower body exercises at 100% of 6RM but not at 70-76% of 6RM induced a significant increase in testosterone (Vingren et al., 2010).

## Number of sets

This modulator refers to the number of sets performed for each exercise in a resistance exercise session. When total volume is held constant, the number of sets does not appear to influence the acute testosterone response to resistance exercise (Kraemer et al., 1995). It was discovered, when changing the load while keeping volume constant that no alterations in the resistance exercise induced testosterone concentrations (Kraemer et al., 1995). Number of sets does not have a major impact on the effect on testosterone levels as other modulators do but it has been found that after 11 weeks of either failure or not to failure

of resistance exercise sets that there is an increase in resting testosterone levels (Izquierdo et al., 2006).

**Volume**

Volume refers to the total work performed and is often used in the context of resistance exercise to mean (set x number of repetitions x intensity). Volume is a function of several different acute modulators and therefore is not considered and independent acute program variable domain. However, manipulating volume by changing several of its constituents can significantly affect the hormonal response of testosterone (Vingren et al., 2010). Ratamess et al. found that six sets, not one set of ten repetition squats significantly increased total testosterone post-exercise (Ratamess et al., 2005). Twenty sets of 1RM resulted in no change in testosterone, but ten sets of 10RM resulted in a large increase in free and total testosterone. Examination of myriad combinations of sets and repetitions have shown that, in general, a higher volume created a greater testosterone response (Smilios, Pilianidis, Karamouzis, & Tokmakidis, 2003).

**Choice of exercise**

Choice of exercise refers to the specific exercises chosen (i.e. dead lift), the equipment used (i.e. machine or free weight), and how the exercises are performed (i.e. type of muscle action, velocity of movement). Muscle mass plays a pivotal role as a determinant for the occurrence of testosterone increase with resistance exercise. Involvement of small muscle mass, even when exercised vigorously, does not elevate testosterone above resting concentrations (Vingren et al., 2010). Exercise selection, therefore, significantly influences the testosterone response to a resistance exercise session (Migiano et al., 2010). When

resistance exercise induces an increase in testosterone, the magnitude of that increase is also affect by muscle mass involvement i.e. a jump squat protocol increases testosterone concentrations more than a bench press protocol performed by the same participants (Hansen, Kvorning, Kjær, & Sjøgaard, 2001). Larger muscle mass involvement allows for greater total volume, which helps to explain the importance of muscle mass involvement in inducing a testosterone response to resistance exercise.

**Order of exercise**

The order of exercise refers to the sequence of exercises within an exercise session and this order affects the power output and the number of repetitions that can be completed for each session (Vingren et al., 2010). The order of exercise can also affect the timing of the hormonal response to resistance exercise (Spreuwenberg et al., 2006). As stated previously, large muscle mass exercises acutely increase circulating testosterone concentrations, therefore if these exercises are performed in the beginning of the exercise session, muscles used during subsequent exercises will reap the benefits and will perfuse with elevated testosterone concentrations. When biceps exercises were performed after four sets of leg press, the biceps hypertrophied significantly more than when biceps exercised first with no prior exercise (Hansen et al., 2001).

**Acute endurance exercise on testosterone**

In response to a short term sub-maximal workload on a cycle ergometer, testosterone concentrations were significantly increased (20 min of exercise duration). Testosterone concentrations returned to pre-exercise levels after 40 min into the recovery from the exercise bout. During a graded exercise bout (running) to exhaustion, testosterone

concentrations were increased somewhat proportionally to the exercise intensity. Furthermore, peak testosterone responses coincided with the maximal intensity of exercise reached (A. Hackney, 1996). The effect of prolonged (>60 min) sub-maximal exercise on testosterone was studied and it was found that prolonged sub-maximal exercise to exhaustion resulted in an initial rise in testosterone concentrations (during the exercise) followed by decline as the activity continued (Galbo et al, 1977). This and other studies demonstrated that by the end of prolonged sub-maximal exercise the reduction in testosterone could typically be in the magnitude of 25% to 50% if the activity duration was four hours or longer. Testosterone concentrations, did return to a normal range within 24 to 72 hours into the recovery of the activity (A. Hackney, 1996). In these studies, the level of testosterone is affected by the same "acute program variable domains" that was mentioned earlier. The intensity, volume, and choice of exercise having notable effect on production with diminishing returns as the exercise bout is prolonged.

**Chronic endurance exercise on testosterone**

Despite strong evidence for the health benefits of continued endurance exercise into later life, long term strenuous exercise or overtraining syndrome impairs hormone production, including testosterone (A. C. Hackney, Moore, & Brownlee, 2005). Retrospective, cross-sectional comparative studies examining resting blood samples indicate lower circulating testosterone levels in chronically exercise-trained male athletes, specifically those who participate in endurance sporting activities such as, marathon distance running, long-course triathlons, and Olympic distance race walking (A. C. Hackney, 1996; A. C. Hackney, 1989; A. C. Hackney, 2005). The subjects in these studies have typically been exercisers who had been chronically involved with the exercise training aspects of their own

sport for a number of years (greater than 10-15). These studies report the testosterone levels, free and total concentrations, of these men to be only 50%-85% of the levels of comparable age-matched, sedentary men (Lane et al, 2014). These declines in testosterone can lead to side effects within the chronically trained individuals known as "exercise hypogonadal male syndrome", that are similar to symptoms seen in aging population.

There are gaps in the literature, as studies have not looked at chronic exercise training across a wide age range or compare the relative effects of age, fitness and body composition on free and bound plasma testosterone levels in healthy men. This study aims to assist in bridging the gap in the literature.

**Aging:**

**Effects of aging on levels of testosterone**

Hypogonadism – the reduction or absence of hormone secretion or other physiological activity of the gonads. The overall incidence of testosterone deficiency increases with age and approximately one and a half million new cases of deficiency are expected in the US in men aged 40-69 years old (Araujo et al., 2004). The definition set by the European Male Aging Study (EMAS) for this deficiency was the presence of at least three sexual symptoms. Loss of morning erections, low sexual desire and erectile dysfunction, total testosterone (TT) <320 ng/dl (11nmol/l) and free testosterone (fT) <64 pg/ml (220pmol/l) (Stanworth & Jones, 2008). Testosterone deficiency was noted in about 20% of men over 60, 30% over 70 and 50% over 80 years of age (Harman et al., 2001).

The development of hypogonadism with aging is known as late- onset hypogonadism and is characterized by issues such as osteoporosis (Campion & Maricic, 2003), Alzheimer's disease (Moffat et al., 2004), frailty, obesity (Phillips, 1993), cardiac failure (Kontoleon et

al., 2003; Tappler & Katz, 1979) and ischemic heart disease (Barrett-Connor & Khaw, 1988), which can lead to the suppression of the hypothalamic-pituitary testicular axis (HPTA).

Longitudinal studies on aging males have shown that serum testosterone levels decline with age (Feldman et al., 2002; Harman et al., 2001). Free and bioavailable testosterone levels fall by 2%-3% per year, whereas total testosterone levels fall at an average of 1.6% per year. This reduction in free and bioavailable testosterone levels is larger because aging is associated with increase in SHBH (sex hormone binding globulin) levels (Feldman et al., 2002).

## Physiological changes associated with decrease testosterone levels.

### Testosterone and cardiovascular disease

Evidence collected from both clinical studies and surrogate markers show that there is a link between low testosterone and cardiovascular disease (T. H. Jones, 2010; T. Hugh Jones & Saad, 2009). Men with angiographically proven coronary artery disease were shown to have lower levels of testosterone compared with controls (English et al., 2000). Low TT (Total Testosterone) has been associated with central obesity, insulin resistance, hyperglycaemia, dyslipidamia and blood pressure as well as emerging risk factors such as leptin, adiponectin, IL-6 and CRP, all of which have a confounding effect on cardiovascular health (Laughlin et al., 2008). Low circulating T levels are associated with increased risk for major adverse cardiovascular events in hypertensive patients (Vlachopoulos et al., 2013), suggesting that reduced T levels improves risk prediction when combined with the standard risk factors and may represent a critical novel biomarker for the prediction of CVD risk in patients with low T (Ohlsson et al., 2011).

**Effects on bone and osteoporosis**

The pathological loss of bone density and strength is known as osteoporosis. With aging there are higher incidence of bone fractures, most commonly in the hip, vertebra and forearm. Men start to lose bone mass during early adult life and experience an increase in the rate of bone loss with age (Scopacasa et al., 2002). The prevalence of osteoporosis is higher in women but with age increases in both sexes.

Bone mineral density in the aging male population is positively correlated with endogenous androgen levels (Murphy, Khaw, Cassidy, & Compston, 1993; Rucker, Ezzat, Diamandi, Khosravi, & Hanley, 2004). Testosterone levels in young males correlate with bone size, indicating a role in peak bone mass and protection in the future osteoporosis (Lorentzon, Swanson, Andersson, Mellström, & Ohlsson, 2005). Male hypogonadism is a risk factor for hip fracture (Jackson et al 1992), and it was reported that a high prevalence of hypogonadism in a group of male patients with average age 75 years presenting with minimal fractures (Leifke et al., 2005). Several different researchers have reported the positive effect of testosterone treatment on bone mineral density (Behre, Kliesch, Leifke, Link, & Nieschlag, 1997; Katznelson et al., 1996; Snyder et al., 2000) and bone architecture (Benito et al., 2005).

**Testosterone and body composition**

Body composition changes with aging, generally speaking, aging males are prone to loss of muscle mass and gain in fat mass, especially in the form of visceral or central fat (Stanworth & Jones, 2008). A study of community dwelling men aged between 24 and 85 years has confirmed that total and free testosterone levels are inversely correlated with waist

circumference and that testosterone levels are specifically related to this measure of central obesity rather than general obesity (Svartberg et al., 2004). Reductions in free testosterone also correlate with age related decline in fat free mass (muscle mass) and muscle strength (Baumgartner, Waters, Gallagher, Morley, & Garry, 1999; Roy et al., 2002). Another study stated that testosterone levels predict future development of central obesity (Khaw & Barrett-Connor, 1992; Tsai, Boyko, Leonetti, & Fujimoto, 2000). The epidemiological data, taken together, suggest that a hypogonadal state accentuates the loss of muscle and gain in fat mass. Specifically, visceral fat and therefore mimics the changes of normal aging (A. C. Hackney et al., 2005).

**Testosterone and diabetes**

The primary pathology for type 2 diabetes is resistance to insulin, which predates the onset of diabetes by many years, during which time serum insulin is raised to maintain normoglycemia. The prevalence of type 2 diabetes is increasing in the developed/developing world and it is increased with age. Insulin resistance or impaired glucose tolerance also play a part in abnormal lipid profile, central obesity and hypertension, all of which can lead to mortality (Stanworth & Jones, 2008)

Epidemiological studies reveal a connection between testosterone and glucose metabolism. Studies in non-diabetic men have shown an inverse correlation of total or free testosterone with glucose and insulin levels (Haffner, Valdez, Mykkänen, Stern, & Katz, 1994; Simon et al., 1992). Men with testosterone deficiency are at a greater risk of developing type 2 diabetes (Oh, Barrett-Connor, Wedick, & Wingard, 2002). Approximately, 43% of men with type 2 diabetes have reduced total testosterone, while 57% had reduced calculated free testosterone (Grossmann et al., 2008). Hypogonadism and type 2

diabetes are often diagnosed together in the same patient and testosterone deficiency may be more prevalent in men with diabetes, higher BMI, or severely obese (BMI>40) (Dhindsa et al., 2004; D. Kapoor et al, 2006; D Kapoor et al, 2007).

BMI plays a major role when observing how it is a predictor of diabetes, as well as having an effect on lowering testosterone levels. That being said, it is one of the markers in this proposed study, used to compare levels of free and bound testosterone in healthy men.

**Countermeasures (supplementation and treatments)**

The aim of treatment for hypogonadism is to normalize serum testosterone levels and extricate symptoms or pathological states that are due to low testosterone levels. Testosterone therapy, which is the replacement of diminished production, is widely used to as a countermeasure. The exact target testosterone level is a matter of debate, but current literature advocate levels in the mid-lower normal adult range (Stanworth & Jones, 2008). Currently available testosterone preparations in common use include intramuscular injections, subcutaneous pellets, buccal tablets, transdermal gels and patches (D. Kapoor et al., 2006). Testosterone replacement therapy in males increase their bone mineral density of both spine and hip, fat- free mass, erythropoiesis, prostate volume, energy and sexual function (Snyder et al., 2000), insulin resistance and glycemic control in men with type 2 diabetes (D. Kapoor et al., 2006) and improvements in mood and cognition (Stanworth & Jones, 2008).

As with testosterone, aging expresses declines in the functional capacity of the cardiovascular system, resulting in a decrease in maximal $O_2$ uptake capacity ($\dot{V}O_2max$). The decline is multifactorial and has been attributed to biological aging (primary aging), lifestyle

habits (secondary aging) and the development of subclinical and clinically apparent disease (tertiary aging) (Katzel, Sorkin, & Fleg, 2001). The rate of decline in $\dot{V}O_2$max in healthy sedentary men in the Unites States appears to average 10% per decade after age 25 years (Rogers, Hagberg, W. H. Martin, Ehsani, & Holloszy, 1990). A cross sectional comparison of young and older endurance athletes found that a relatively constant level of regular vigorous endurance exercise training reduces the rate of decline in $\dot{V}O_2$max to ~5% per decade (Heath, Hagberg, Ehsani, & Holloszy, 1981). Clearly one component of the decline in $\dot{V}O_2$max with age is a result of the aging process per se.

However, as mentioned above, the effects of hypogonadism on cardiovascular health, body composition, and loss of muscle mass can play a role in the decline of $\dot{V}O_2$max. The level of endurance exercise needed to maintain $\dot{V}O_2$max as we age can offset the production of testosterone but both these factors are interrelated, as nominal testosterone production is required to maintain the body and an extension of that being $\dot{V}O_2$max. It is the aim of this study to ascertain if the effects of a sustained $\dot{V}O_2$max over the lifetime can help offset some of the age-associated declines in testosterone in males. It is hypothesized that there will be a notable effect of sustained lifelong $\dot{V}O_2$max on the serum production of testosterone as males age, after the suppressive actions of aging.

## Methods

### 1. Participants

A total of 45 male subjects of various ethnicities aged 20-62years (mean ± SD: Age= 31 ± 13; BMI = 26.21 ± 4.34) volunteered for this study. Subjects were primarily recruited via flyers on the University of Houston Campus and consisted of student and academic staff.

All subjects completed a life style questionnaire prior to participating in the study. Subjects with excessive alcohol intake (two > drinks per day), smoking within last six months, the use of any known medication known to affect the immune system; or the regular use of ibuprofen and/or aspirin, anti-depressants, medications designed to alter blood pressure or cardiovascular function were rejected for the study. The presence of chronic/debilitating arthritis, central or peripheral nervous disorders, major affective disorder, HIV infection, hepatitis, known cardiovascular disease, having been bedridden in the past three months, or a previous stroke also made subjects ineligible. Before their visit, subjects were instructed to refrain from any form of exercise considered as vigorous for 24 hours prior to their arrival at the laboratory. All subjects gave full written informed consent and ethical approval was attained from the Committee for Protection of Human Services at the University of Houston. The 74 subject's physical characteristics such as age, body mass, BMI, $\dot{V}O_2$max and PA-R are displayed in **Table 1.**

**Table 1.** Subject's characteristics of age, body mass, BMI, PA-R and $\dot{V}O_2$max (mean ± SD)

| | Mean |
|---|---|
| Age (yrs) | 34 ± 14.01 |
| Body Mass (kg) | 82.3 ± 13.87 |
| BMI ($kg/m^{-1}$) | 26.43 ± 3.47 |
| PA-R * | 5 ± 1.84 |
| $\dot{V}O_2$max ($mL.kg^{-1}.min^{-1}$) | 36.67 ± 8.41 |

*Physical activity rating questionnaire of fitness filled out which inquiries about individual's physical activity score, numerical value is aligned with physical activity: infrequent (0-1), moderate (2-3) and vigorous (4-7) exercises (Jackson et al., 1990).

**2. Anthropometric Measurements**

All subjects made a single visit to the lab from 0600 to 1200 local time according to their convenience, to provide a resting blood sample and fill out four questionnaires. The General Physical Activity questionnaire was used to assess the level of subject's physical level, the Life Orientation Test-Revised, Social Diversity Network and Psychological Stress Measure questionnaire were given as well. Height and weight were used to calculate BMI of subjects. Prior to their visit, subjects were instructed to refrain from any vigorous exercise 24 hours prior as well not to ingest any caffeine, food or alcohol two hours prior.

**3. Experimental Procedures**

After the questionnaires were completed, subjects were asked to rest for 5 minutes in seated position before obtaining the initial blood sample. 12ml of blood was drawn from the antecubital vein in 6ml tubes (BD vacutainer, Franklin Lakes, NJ, USA.) coated with lithium heparin to prevent coagulation. Plasma samples were removed from whole blood and stored at -80°C until analysis for free testosterone and bound testosterone by ELISA using a SpectomaxM2 plate reader (Molecular Devices, CA, USA). The procedures for free and bound testosterone were determined in accordance with the manufacturer's instructions (BioCheck Inc, CA, USA).

**4. Estimation of Maximal Oxygen uptake ($\dot{V}O_2max$) and physical activity rating (PA-R)**

The $\dot{V}O_2max$ of each subject was estimated using a sub-maximal cycling exercise protocol (Astrand, 1960). After a brief 10-minute warm-up and successive cool down, subjects started the trail and complete four three-minute heart rate adjusted incremental stages on Trek road bike mounted to an indoor cycling ergometer (Computrainer, RacerMate,

Seattle, WA). The heat rate (HR) of the subjects were continuously measured during the protocol via short range telemetry (Polar Electro Inc, NY, USA) and the average HR during the last minute of every stage was used to calibrate the age adjusted resistance to apply in the following stage. The test was terminated after the twelve-minute mark was reached. Equation provided by Adams and Beam (1998), was used to estimate the $VO_2$ of each subject from the exercise. Prior to exercising, subjects filled out the PA-R which inquiries about individual's physical activity score, numerical value is aligned with physical activity: infrequent (0-1), moderate (2-3) and vigorous (4-7) exercises (Jackson et al., 1990), a $\dot{V}O_2max$ was also estimate from that questionnaire. The $\dot{V}O_2max$ estimated from the questionnaire and the $\dot{V}O_2max$ estimate attained from the sub maximal cycling exercise test is known to have a relationship of $R = 0.88$ ($p < 0.001$) (Jackson et al., 1990).

**5. Bound Testosterone Extraction/Deproteinization**

Serum samples were portioned in 500μL aliquots and diluted 2.5mL of diethyl ether. Following thorough mixing for 2 minutes, the organic phase, containing the dissolved testosterone, was transferred to a glass test tube containing 1mL of distilled water. The aqueous phase containing the sex hormone binding globulin and other binding proteins were discarded. The Tube was then mixed using a handheld vortex for another 2 minutes, and the organic phase was once again transferred to a glass test tube containing 1mL of distilled water. The extraction procedure was repeated an additional three times, before getting dried using a speedvac. The obtained dried precipitates were resuspended in 250μL of Phosphate Buffer Solution and tested following the methods described below to determine total testosterone. Free testosterone concentration was determined using untreated serum samples.

**6. Free Testosterone Assay**

Coated wells were secured in the holder. 10µL of standards, Free and Total testosterone and controls were dispensed into associated wells. 100µL of Testosterone – HRP conjugate reagent was dispensed into each well. After which, 50µL of rabbit anti-Testosterone reagent was dispensed into each well. Thoroughly mixed for 30 seconds. Then the wells were incubated for 90 minutes at 37°C. The microtiter wells were then rinsed and washed 5 times with deionized water. 500µL of TMB reagent was then dispensed into each well and incubated in room temperature for 20 minutes. The reaction was stopped by adding 100µL of stop solution to each well, which is mixed for 30 seconds, after which the absorbance is was ready to be read within 15 minutes at 450nm with a SpectomaxM2 plate reader (Molecular Devices, CA, USA). Bound testosterone concentrations were calculated by subtracting the Total testosterone serum concentrations to the corresponding Free testosterone serum concentrations.

**7. Statistical Analysis**

**Analysis for Specific Aim 1**

In order to determine the impact of $\dot{V}O_2max$ on age-related declines in free and bound testosterone, subjects were stratified into 4 groups. Age of 45 was used to divide all subjects and create "Young" and "Old" groups. Thereafter, the median $\dot{V}O_2max$ of each "Young" and "Old" group was used to further stratify aforementioned groups into "Young/High", "Young/Low", "Old/High" and "Old/Low". A Univariate ANOVA was used to calculate the effect of age and fitness category on free testosterone concentrations. When significant group effects were found, t-tests with Bonferroni corrections will be performed to detect

locations of significance. All values are presented as the mean ± Standard Deviation (SD) and all statistical analysis were performed using "Statistical Package for the Social Sciences" (SPSS v17.0, Chicago, IL, USA). (Statistical significant was set at $P<0.05$)

**Analysis for Specific Aim 2**

In order to compare the relative effects of age, fitness and body composition on free and bound testosterone. Subjects were placed into fitness categories based on ACSM's aerobic fitness table, which accounts for both age and $\dot{V}O_2max$. Categories were titled "Very High", "High", "Good", "Average", "Fair", and "Poor" according to ACSM and were further stratified into tertiles titled "High" (Very High and High), "Average" (Good and Average) and "Poor" (Fair and Poor). A Univariate ANOVA was used to calculate the effect of aerobic fitness, age and body composition on free and bound. When significant group effects were found, t-tests with Bonferroni corrections will be performed to detect locations of significance. All values are presented as the mean ± Standard Deviation (SD) and all statistical analysis were performed using "Statistical Package for the Social Sciences" (SPSS v17.0, Chicago, IL, USA). (Statistical significant was set at $P<0.05$)

## Results

Initial data collection was carried out on a total of 74 male subjects; however, technical difficulties (mostly related to freezer malfunctions) led to the loss of samples from 29 subjects. Consequently, the total subject pool analyzed and presented in this thesis consists of 45 male subjects. The descriptive characteristics of the subjects are presented in **Table2.**

**Table2.** Descriptive information for 45 male subjects represented in mean ± SD. Subjects were stratified into 4 groups. Age of 45 was used to divide all subjects and create "Young" and "Old" groups. Thereafter, the median $\dot{V}O_2$max of each "Young" and "Old" group was used to further stratify aforementioned groups into "Young/High", "Young/Low", "Old/High" and "Old/Low".

| | BMI ($kg/m^{-1}$) | Age(years) | $V.O_2^{max}$($mL.kg^{\cdot}min^{-1}$) | Free Testosterone (ng/ml) | Bound Testosterone (ng/ml) | Total Testosterone (ng/ml) |
|---|---|---|---|---|---|---|
| Young/Low | 27.20 ± 4.06 | 26.06 ± 3.78 | 31.78 ± 4.89 | 14.65 ± 7.20 | 46.62 ± 17.42 | 60.77 ± 23.19 |
| Young/High | 29.06± 4.80 | 29.06 ± 4.8 | 44.12 ± 7.11 | 10.67 ± 5.23 | 38.65± 16.18 | 48.87 ± 18.90 |
| Old/Low | 27.00 ± 2.10 | 58.14 ± 3.97 | 28.09 ± 4.59 | 8.73 ± 5.54 | 39.01 ± 22.16 | 47.74 ± 21.88 |
| Old/High | 25.90 ± 3.75 | 54.28 ± 3.32 | 39.56 ± 4.61 | 7.25 ± 4.61 | 38.73 ± 13.25 | 45.99 ± 14.64 |

**High levels of aerobic fitness do not offset age-related declines in circulating levels of free testosterone.**

Subjects were stratified into 4 groups. Age of 45 was used to divide all subjects and create "Young" and "Old" groups. Thereafter, the median $\dot{V}O_2$max of each "Young" and "Old" group was used to further stratify aforementioned groups into "Young/High", "Young/Low", "Old/High" and "Old/Low" with respective mean ages and $\dot{V}O_2$max: Young/High (29.06 years, 44.12 $mL.kg^{\cdot}min^{-1}$), Young/Low (26.06 years, 46.62 $mL.kg^{\cdot}min^{-1}$), Old/High (54.28 years, 45.99 $mL.kg^{\cdot}min^{-1}$), Old/Low (58.14 years, 39.01 $mL.kg^{\cdot}min^{-1}$). Using a univariate AVONA, we calculated the effect of age and fitness category on free testosterone concentration ($p = 0.045$, $F = 2.912$). Post-hoc analysis (Bonferroni) expressed no differences between the groups, as displayed in **Figures 2-10 and Table 3.**

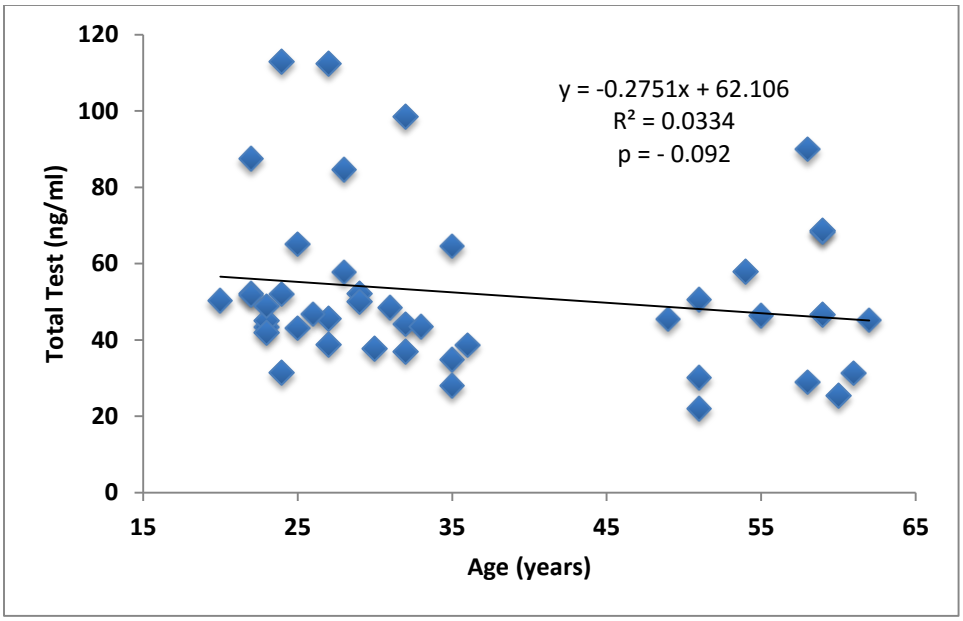


**Figure 2.** Plot expressing relationship between age and total testosterone from cohort of 45 adult males.

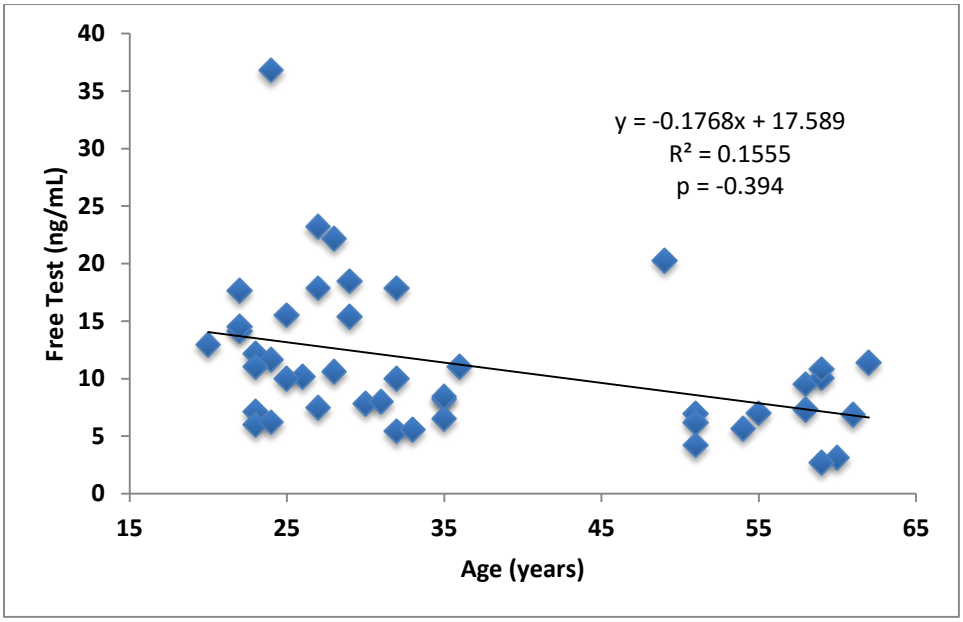


**Figure 3.** Plot expressing relationship between age and free testosterone from cohort of 45 adult males.

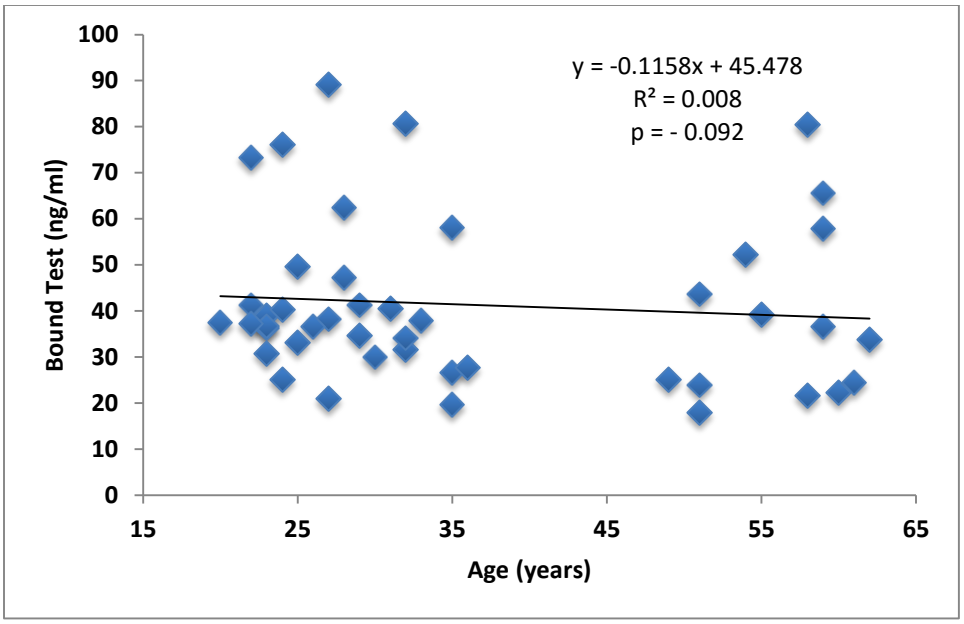


**Figure 4.** Plot expressing relationship between age and bound testosterone from cohort of 45 adult males.

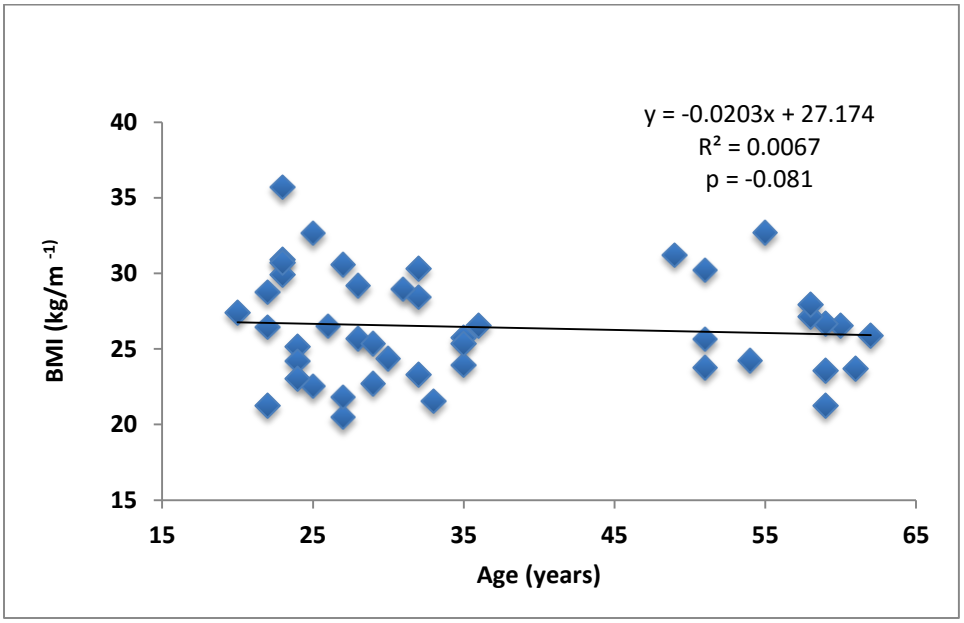


**Figure 5.** Plot expressing relationship between age and BMI from cohort of 45 adult males.

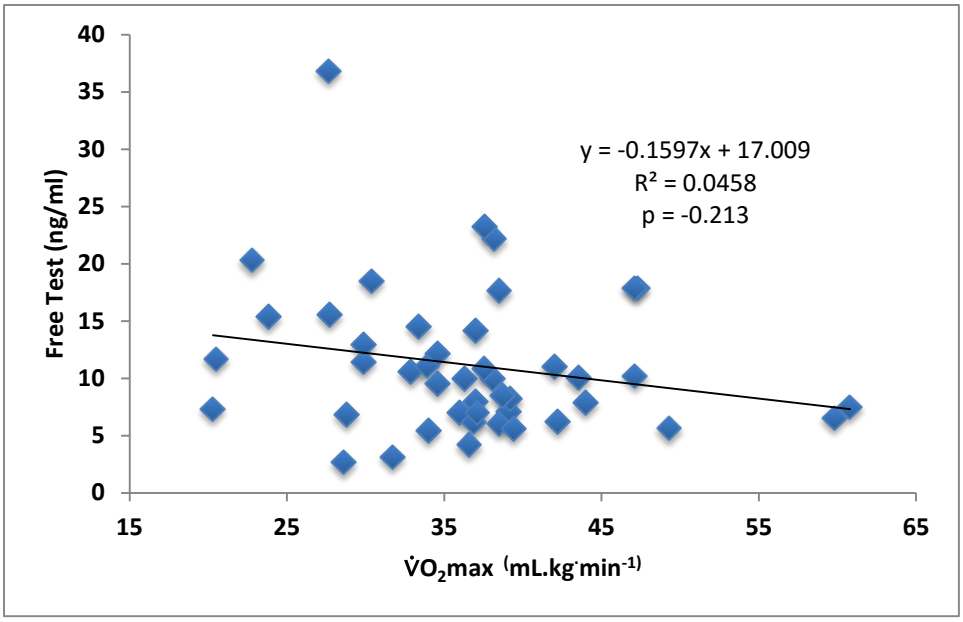


**Figure 6.** Plot expressing relationship between $\dot{V}O_2max$ and free testosterone from cohort of 45 adult males.

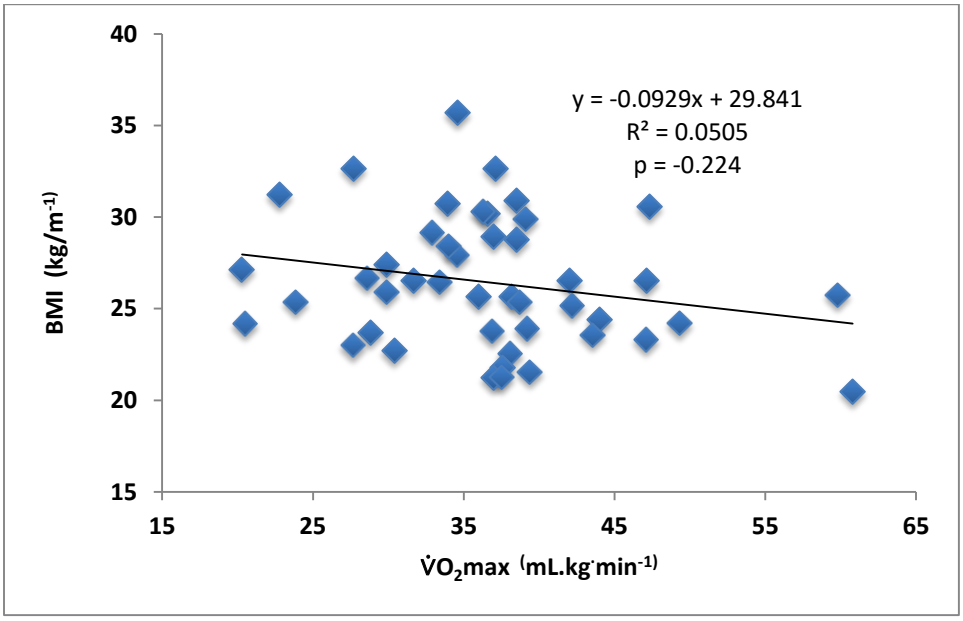


**Figure 7.** Plot expressing relationship between $\dot{V}O_2max$ and BMI from cohort of 45 adult males.

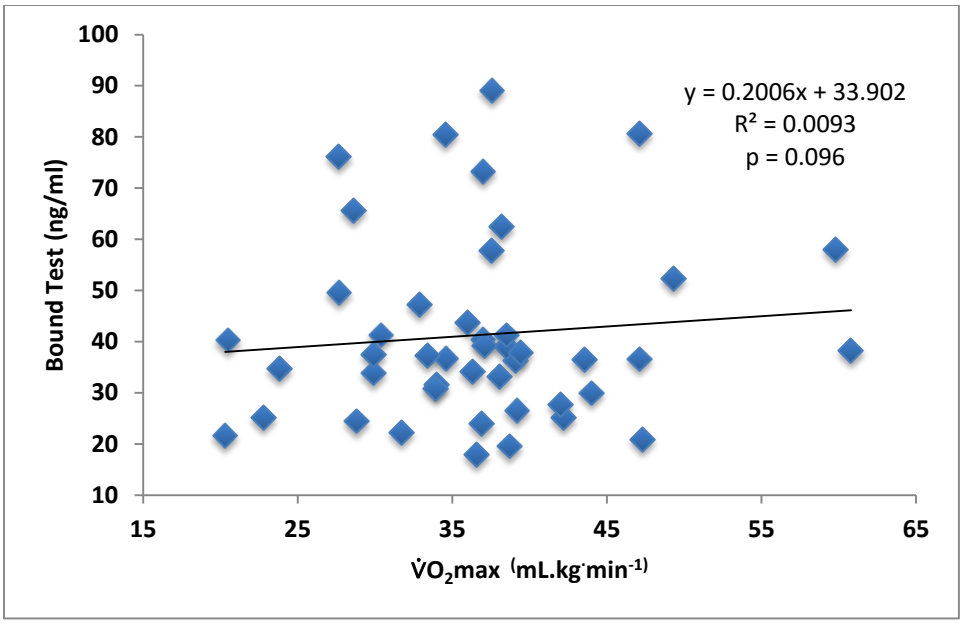


**Figure 8.** Plot expressing relationship between $\dot{V}O_2max$ and bound testosterone from cohort of 45 adult males.

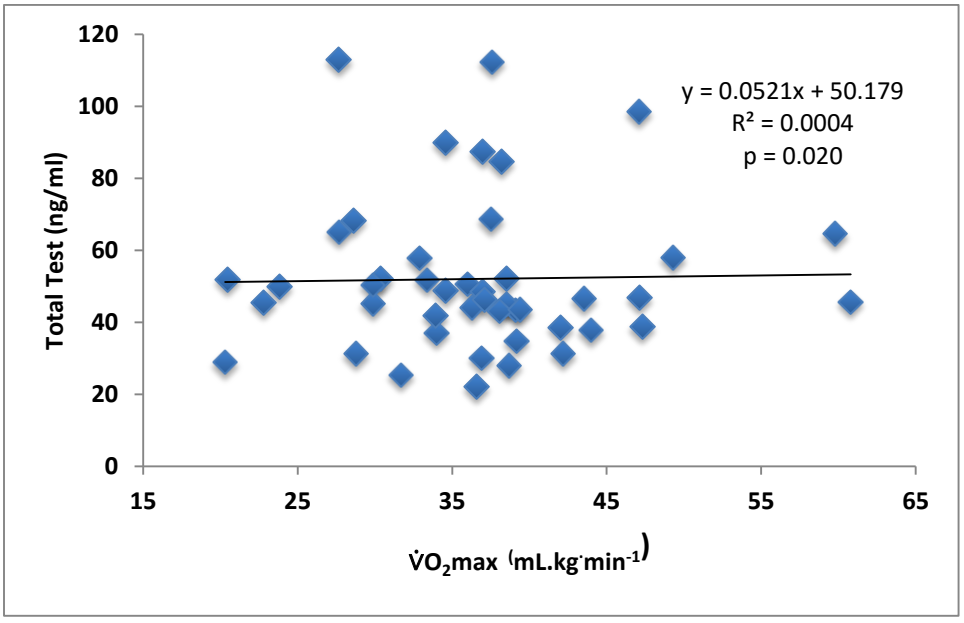


**Figure 9.** Plot expressing relationship between $\dot{V}O_2max$ and total testosterone from cohort of 45 adult males

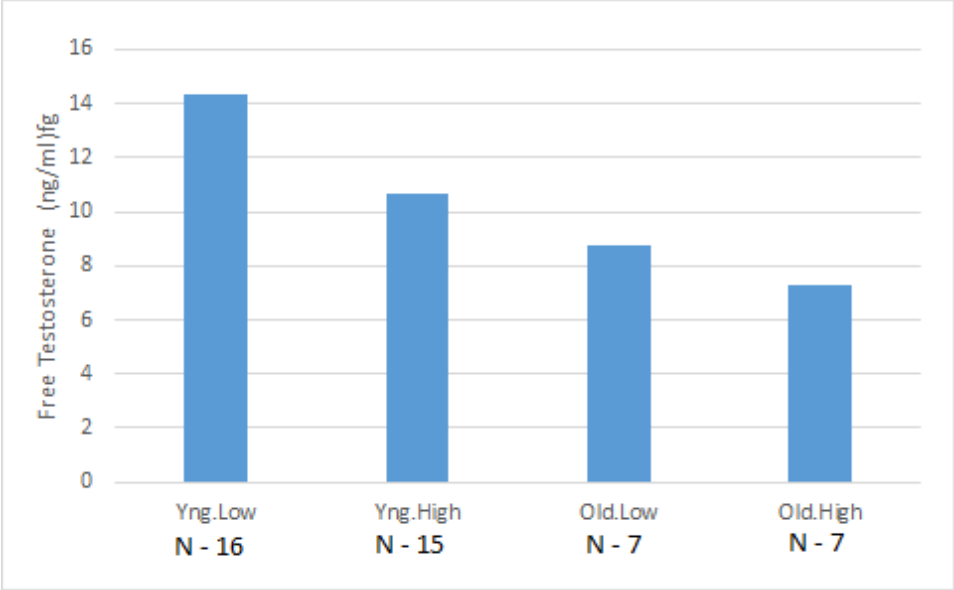


**Figure 10.** Subjects were stratified into 4 groups. Age of 45 was used to divide all subjects and create "Young" and "Old" groups. Thereafter, the median $\dot{V}O_2$max of each "Young" and "Old" group was used to further stratify aforementioned groups into "Young/High", "Young/Low", "Old/High" and "Old/Low". Free testosterone levels contrasted by age and relative $\dot{V}O_2$max (age/ $\dot{V}O_2$max score). Data are mean ± SD (* = $p<0.05$). A one-way ANOVA calculated a main effect ($p = 0.046$) while post-hoc analysis (Bonferroni) located no differences between the groups.

**Table 3.** Subjects were stratified into 4 groups Age of 45 was used to divide all subjects and create "Young" and "Old" groups. Thereafter, the median $\dot{V}O_2$max of each "Young" and "Old" group was used to further stratify aforementioned groups into "Young/High", "Young/Low", "Old/High" and "Old/Low". Testosterone levels (free, bound, and total) and BMI contrasted by age and relative $\dot{V}O_2$max (age/ $\dot{V}O_2$max score). Data are mean ± SD (* = $p<0.05$). A one-way ANOVA calculated a main effect ($p = 0.046$) while post-hoc analysis (Bonferroni) located no differences between the groups.

| | | | | | One-way ANOVA | |
|---|---|---|---|---|---|---|
| | **Young/Low** | **Young/High** | **Old/Low** | **Old/High** | **F Statistic** | **(p value)** |
| **BMI** | ***[N = 16]*** | ***[N = 15]*** | ***[N = 7]*** | ***[N = 7]*** | | |
| Mean | 26.90 | 25.91 | 27.00 | 25.90 | 0.309 | 0.819 |
| SD | ±0.90 | ± 0.93 | ±1.36 | ±1.36 | | |
| **Total Testosterone** | ***[N = 16]*** | ***[N = 15]*** | ***[N = 7]*** | ***[N = 7]*** | | |
| Mean | 59.66 | 48.87 | 47.76 | 45.99 | 1.078 | 0.369 |
| SD | ± 5.32 | ± 5.49 | ± 8.04 | ±8.04 | | |
| **Bound Testosterone** | ***[N = 16]*** | ***[N = 15]*** | ***[N = 7]*** | ***[N = 7]*** | | |
| Mean | 45.72 | 38.73 | 39.01 | 38.78 | 0.507 | 0.679 |
| SD | ± 4.50 | ± 4.64 | ± 6.80 | ± 6.80 | | |
| **Free Testosterone** | ***[N = 16]*** | ***[N = 15]*** | ***[N = 7]*** | ***[N = 7]*** | | |
| Mean | 14.35 | 10.66 | 8.73 | 7.308 | 2.912 | 0.046* |
| SD | ±1.49 | ±1.54 | ±2.25 | ±2.25 | | |

**No difference found in free testosterone between subjects, in each of ACSM's age adjusted aerobic fitness categories**

Subjects were placed into fitness categories based on ACSM's aerobic fitness table, which accounts for both age and $\dot{V}O_2$max. Categories were titled "Very High", "High",

"Good", "Average", "Fair", and "Poor" according to ACSM and were further stratified into tertiles titled "High" (Very High and High), "Average" (Good and Average) and "Poor" (Fair and Poor). A univariate ANOVA showed no effect of aerobic fitness on free testosterone ($p = 0.555$, $F = 0.596$) as displayed in **Table 4**.

Subjects were pooled into two fitness categories: "Low" and "High". Subjects with "Low" fitness had poor, fair or average scores, while those with good, high or very high scores were categorized as having "High" fitness according to ACSM's aerobic fitness table. Subjects with "Low" aerobic fitness had no significant change in circulating free testosterone compared to those with "High" aerobic fitness ($p = 0.181$, $F = 1.849$) as displayed in **Table 5.** There was no effect of ACSM age-related fitness score on fitness category on bound or total testosterone levels ($p>0.05$)

**Table 4.** Subjects pooled into tertiles titled "High", "Average" and "Poor" in compliance to ACSM's aerobic fitness table. Testosterone levels (free, bound, and total) and BMI contrasted by ACSM aerobic fitness score. Data are mean ± SD (* = $p<0.05$). A one-way ANOVA calculated no main effect on (free, bound and total), testosterone or BMI.

| | | | | | **One-way ANOVA** |
|---|---|---|---|---|---|
| | **High** | **Average** | **Poor** | **F - Statistic** | **(p value)** |
| **BMI** | ***[N = 7]*** | ***[N = 24]*** | ***[N =14]*** | | |
| Mean | 24.91 | 26.21 | 27.57 | 1.474 | 0.241 |
| SD | ±3.15 | ±3.48 | ±3.60 | | |
| **Total Testosterone** | ***[N = 7]*** | ***[N = 24]*** | ***[N =14]*** | | |
| Mean | 56.94 | 50.14 | 52.98 | 0.283 | 0.755 |
| SD | ± 20.23 | ±22.41 | ±21.03 | | |
| **Bound Testosterone** | ***[N = 7]*** | ***[N = 24]*** | ***[N =14]*** | | |
| Mean | 46.28 | 40.06 | 40.83 | 0.331 | 0.720 |
| SD | ± 19.38 | ± 19.06 | ±15.08 | | |
| **Free Testosterone** | ***[N = 7]*** | ***[N = 24]*** | ***[N =14]*** | | |
| Mean | 10.85 | 10.34 | 12.68 | 0.596 | 0.555 |
| SD | ±5.11 | ±5.32 | ±8.39 | | |

**Table 5.** Subjects pooled into two fitness categories “Low” and “High”. Subjects with “Low” fitness had poor, fair or average scores, while those with good, high or very high scores categorized as having “High" fitness in compliance with ACSM’s aerobic fitness table. Testosterone levels (free, bound, and total) and BMI contrasted by ACSM aerobic fitness score. Data are mean ± SD (* = $p<0.05$). A one-way ANOVA revealed no main effect on (free, bound and total), testosterone or BMI

| | | | One-way ANOVA | |
|---|---|---|---|---|
| | **High** | **Low** | **F Statistic** | **(p value)** |
| **BMI** | ***[N = 14]*** | ***[N =31]*** | | |
| Mean | 25.63 | 26.80 | 1.049 | 0.312 |
| SD | ±3.49 | ±3.57 | | |
| **Total Testosterone** | ***[N = 14]*** | ***[N =31]*** | | |
| Mean | 49.46 | 53.35 | 0.295 | 0.590 |
| SD | 18.82 | ±23.36 | | |
| **Bound Testosterone** | ***[N = 14]*** | ***[N =31]*** | | |
| Mean | 44.00 | 41.11 | 0.058 | 0.812 |
| SD | ± 17.22 | ±18.80 | | |
| **Free Testosterone** | ***[N =14]*** | ***[N =31]*** | | |
| Mean | 9.25 | 12.00 | 1.849 | 0.181 |
| SD | ±4.17 | ±7.01 | | |

## Discussion

The primary objective of this investigation was to determine the impact of $\dot{V}O_2max$ on age-related declines in free and bound testosterone. The secondary objective was to compare the relative effects of age, fitness and body composition on free and bound testosterone. The first overarching hypothesis was after controlling for age, serum testosterone levels in healthy men will increase with relative increases in $\dot{V}O_2max$. The second overarching hypothesis was that fitness would be would be the strongest factor affecting total testosterone when compared with age and body composition. Although our results showed no statistically significant effect of aerobic fitness on the age-associated decline of testosterone or fitness having an effect on levels of total testosterone, a main effect was found of free testosterone on the groups ($p = 0.046$, $F = 2.912$) while no differences were found within the groups. There are several speculations as to why the outcome of the results did not align with what was hypothesized, but first we will discuss what was analogous with the literature at large.

When observing results of the Old groups, the testosterone levels (free, bound and total) were lower than that of the Young groups. This result was expected as it appears to support the current scientific literature (Feldman et al., 2002; A. C. Hackney, 1989). As males age, there is a decrease of total testosterone levels of an average of 1.6% per year, while free and bioavailable levels fall by 2%-3% per year. This reduction in free and bioavailable levels are higher due to increased levels of SHBG (Feldman et al., 2002; Harman et al., 2001). Higher levels of SHBG will bind with free and bioavailable testosterone, hence reducing testosterone's bioavailability and also concomitantly increasing

the levels of bound testosterone (Stanworth & Jones, 2008). Additionally, it is interesting to note that the differences observed within the Old groups were also congruent with the literature (Feldman et al., 2002; Katzel et al., 2001). Indeed, Old/High exhibited lower free testosterone 7.30 ng/ml ±2.25, bound testosterone 48.78 ng/ml ±6.80 and total testosterone 45.99 ng/ml ±8.04 than the Old/Low group 8.73 ng/ml ±2.25, 39.01 ng/ml ±6.80 and 47.76 ng/ml ±8.04 respectively.

The Young/High group had a lower level of free testosterone 10.66 ng/ml ±1.54 compared to the Young/Low group 14.35 ng/ml ±1.49, bound testosterone 38.73 ng/ml ±4.64 to 45.72 ng/ml ±4.50 which culminated to a total testosterone of 48.87 ng/ml ±6.49 compared to 69.66 ng/ml ±5.32 respectively. The results found here are congruent with the current literature (Nieschlag & Nieschlag, 2014; Zitzmann & Nieschlag, 2001). It is well documented that endurance training can have a prolonged effect on testosterone levels (A. C. Hackney, 1989). Controlled trials involving men undergoing endurance training and control groups of sedentary men give the impression of generally lowered testosterone levels in exercising men (Wheeler, Wall, Belcastro, & Cumming, 1984), yet it is unlikely to be the main underlying cause of the results observed here. Reasons as to why this discrepancy may have occurred are to be further explored below.

One reason that could have affected the outcome may have been the submaximal cycling protocol used to estimate $\dot{V}O_2max$. Subjects that were previously untrained, could have found this protocol to be strenuous and hence considered a bout of moderate to intense exercise session. As shown by Hackney, a short sub-maximal workload on cycle ergometer (20 min of exercise duration) significantly increased testosterone concentrations (A. Hackney, 1996). The choice of exercise should be mentioned as well, as muscle mass plays

a pivotal role as a determinant for occurrence of testosterone increase with exercise (Vingren et al., 2010). The cycling protocol itself could have an effect on testosterone levels, when taking into consideration previous training adaptation or lack thereof of the subjects.

Subjects recruited for the study were given questionnaires (PA-R) to assess their physical activity, however the type of training they routinely performed was not specifically inquired upon. Previous adaptations to strength/endurance training would have had an effect on the outcome measures. Even though disputed, some studies show that resting levels of testosterone are higher in subjects that are chronically strength trained (A. C. Hackney et al., 2005). Subjects in the Young/Low group could have been chronically strength trained and still have a low $\dot{V}O_2max$, which could explain why their free testosterone levels might have been higher than Young/High group. As mentioned previously, chronically trained endurance athletes have lower level of resting testosterone levels, due to the low number of subjects, even if a few subjects in the Young/High group had these exercise induced adaptations there would be an effect on the outcome measures.

Another variable that was not controlled for, which can have a large impact on the outcome is mental stress. The release of cortisol by activation of the hypothalamic-pituitary-adrenal (HPA) axis as a reaction to mental stress is well documented (Craig, Brown, & Everhart, 1989). Increases in cortisol have a suppressive effect on levels of luteinizing hormone (LH). Normal feedback regulation would require LH levels to rise with falling testosterone levels, but with the suppression of LH there is a disruption of the HPA feedback further diminishing the production of testosterone (Craig et al., 1989). Stressful situations as experienced during work, before tournaments or anticipating exams have been shown to decrease testosterone levels (Zitzmann & Nieschlag, 2001). Subjects under increased

psychological stressors from varying life factors during data collection could have had a suppressive effect on the expression of testosterone leading to modulated results.

**Limitations**

This study was an opportunity sample used from a previous data set. Therefore, there were some constraints that were inherently built into it. To attain $\dot{V}O_2$max of subjects a sub-max cycling protocol was used. Higher accuracy would have been attained if a $\dot{V}O_2$max test was used to get exact physiological values. To view the effects of an aerobic regimen on levels of testosterone of males as they age a longitudinal study design would be best suited instead of a cross-sectional study design. The employment of skinfold thickness measurements, bioelectrical impedance, densitometry or dual energy x-ray absorptiometry would have provided more accurate data for body fat than BMI. Initial data collection was carried out on a total of 74 male subjects, out of which 45 subject's data were analyzed. This loss was due to a technical difficulty, specifically a freezer malfunction, which lead to the discarding of 29 subjects. The older subjects were in their middle ages with the oldest being 62 years old; larger differences in testosterone might have been observed if an older subject pool was allocated.

**Future Directions**

To elucidate the effects of aerobic exercise on levels on testosterones as males age, several integral factors mentioned above, which negatively impacted the study need to be modified to attain a more robust view on the outcome. The scale of this experiment needs to be expanded, in part of this expansion, there needs to be a larger acquisition of subjects with

specific inclusion criteria. Subjects who have low $\dot{V}O_2$max and subjects with high $\dot{V}O_2$max should be recruited. Within the high $\dot{V}O_2$max group we should have subjects that are considered fit, as well as chronically trained aerobic athletes, this will lead to a clearer view of what effect is occurring on testosterone levels with age, fitness and body composition. The use of a longitudinal study design to implement an aerobic exercise regimen in which subjects are regularly tested to observe the adaptations and testosterone levels as they age over a period of several years.

**Summary**

The primary objective of this investigation was to determine the impact of $\dot{V}O_2$max on age-related declines in free and bound testosterone. Our hypothesis stated that after controlling for age, serum testosterone levels in healthy men will increase with relative increases in $\dot{V}O_2$max, this was found to be inconclusive. The secondary objective was to compare the relative effects of age, fitness and body composition on free and bound testosterone. We hypothesized that fitness would be the dominant factor affecting total testosterone when compared with age and body composition, this hypothesis was also found to be inconclusive.

The effects of anaerobic and aerobic exercise on the body are well understood. Taking the results of this study into consideration when creating countermeasures for older adult males with symptoms of hypogonadism, it is suggested that they have a combination of endurance and strength training to help mediate levels of testosterone with age. Both forms of exercise have their peaks and downfalls and the combination of both will lead to well-rounded exercise regimen.

As mentioned above there are several variables that may have had a negative effect on the outcome of our results. In future studies aiming to answer the same research questions, if said variables are properly compensated for, a clearer and concise picture may be depicted.

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