Body Composition and Blood Measurements of Elite Senior South African Body Builders during a Competitive Season

by
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DECLARATION

I, Dr Riaan Barnard, hereby declare that the work on which this dissertation is based, is my original work (except where acknowledgements indicate otherwise) and that neither the whole work or any part of it has been, is being, or has to be submitted for another degree in this or any other University.

It is being submitted for the degree of Masters in Sports and Exercise Medicine in the School of Medicine in the Faculty of Health Sciences of the University of the Free State, Bloemfontein.

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(Signature)

_____ day of _____2012

CO-WORKERS

The following persons have acted as co-workers during the study:

- Dr Elizabeth Ackermann, as Consulting Haematologist
- Dr Greg Hough, as Consulting Endocrinologist

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CONFLICT OF INTEREST

The author states no conflict of interest.

ABSTRACT

A dearth of literature exists surrounding the sport of Body Building. Anecdotally, when preparing for a competition, most elite body builders in South Africa will go through two totally different phases of training and dieting. The first phase is the bulking- or weight-gaining phase. During this phase, a structured diet with high carbohydrate component and moderate to high fat content will be followed for several months. During the weight-gaining phase, Androgenic-Anabolic Steroid (AAS) substances are used in moderately high doses compared to the pre-contest period. The second phase of training and dieting, is called the pre-contest preparation phase. This is a very intense phase of high volume training that usually starts about 16-13 weeks from the time of the competition. During this phase, extremely strict, structured diets are followed, with each meal being weighed. During the pre-contest phase, a multitude of chemical substances are used to enhance the desired physique – this strategy of using combinations of different classes of drugs, is called “stacking”. This will be the period with the highest AAS substance milligram usage per week.

Very little current information on the profile of these athletes is available to the South African Medical Community, especially the Sports Medicine Community. There exists only a small body of knowledge in the literature on the dosing protocols abused by these athletes and the side effects they incur. Little is known of the usage of high dose AAS amongst the elite, competitive South African Body Building population and the possible side effects. A rare opportunity was presented to the author to study a group of elite level body builders during the 2010 competitive season.

Obtaining participants for this cohort was difficult as these athletes form part of a very secluded group of sportsmen. Though the present cohort was disadvantaged in small cohort size, the opportunity to study such a group in depth will not be readily repeated. This is a novice study – to present, no similar study has been conducted in South Africa.

All the athletes registered with the International Federation of Body Building South Africa were invited to participate in the study. Interested volunteers were asked to contact the researcher. More than 200 invitations were sent out to the existing database – only 19 athletes conveyed their interest in participation. Eventually, only

14 athletes partook in the full protocol. Blood assays were performed on each athlete on 3 different occasions, while anthropometric measurements and blood pressure readings were taken on 4 different occasions over the length of the competitive season. Each individual athlete recorded his AAS abuse, while some athletes provided sample diets as well. Data was captured on Excel spread sheets and forwarded to Department of Biostatistics, University of the Free State, South Africa.

Along with the concomitant abuse of high doses of AAS over extended periods, the present study also found:

- Minimal changes in blood pressure
- Initial decrease in lean mass, followed by rapid increase in lean mass in just one week and failure to maintain that gain over the following weeks
- Disturbed carbohydrate metabolism with increased risk for pre-diabetic status
- Lipid profile changes, with decreased HDL, unchanged Total Cholesterol and decreased LDL
- Liver enzyme changes highly suggestive of AAS-driven adverse effects
- Hypogonadotrophic hypogonadism status
- Very high Androgen Status for the cohort with mean total AAS abuse per week measuring 1638,3 mg, with average AAS cycle lengths of 17.43 weeks.

In conclusion, it should be noted that the present study's cohort differed vastly from cohorts from other studies in the literature, as none of the latter observed cohorts under full pre-contest preparation conditions. It should also furthermore be understood that body builders under full pre-contest preparation will respond differently to the use of special diets, different training strategies and different types of AAS abused, than compared to when they train under normal out-of season conditions.

The author recommends that sports physicians should continuously target their efforts at counselling adolescents and other athletes about the potential long-term harms of AAS abuse, as well as regularly and prudently follow-up on the potential adverse effects that may develop in current AAS abusers. The author further recommends that, if an opportunity to study such a secluded group of body builders would present itself again, it should be immediately fully utilised.

LIST OF FIGURES

LIST OF ABBREVIATIONS

AAS	Androgenic Anabolic Steroids
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BMI	Body Mass Index
CK	Creatine Kinase
DNA	Deoxyribonucleic Acid
DXA	Dual-energy X-ray Absorptiometry
ECW	Extra Cellular Water
FSH	Follicle-stimulating Hormone
GGT	Gamma Glutamyltransferase
GH	Growth Hormone
GnRH	Gonadotrophin Releasing Hormone
HDL	High-Density Lipoprotein
HPTA	Hypothalamus Pituary Testis Axis
HTL	Hepatic Triglyceride Lipase
HTLA	Hepatic Triglyceride Lipase Activity
IFFB SA	International Federation of Body Builders South Africa
IGF-1	Insulin-like Growth Factor-1
IGFBP-4	Insulin-like Growth Factor Binding Protein-4
LBM	Lean Body Mass
LDH	Lactate Dehydrogenase
LDL	Low-Density Lipoprotein
LH	Luteinizing Hormone
LPL	Lipoprotein Lipase
NABBA	National Association of Body Building Athletes
PPT	Post Prandial Triglyceridaemia
SASCOC	South African Sports Confederation and Olympic Committee
SD	Standard Deviation
SHBG	Sex Hormone-Binding Globulin
t½	Half life
TBG	Thyroid-binding Globulin
TG	Triglyceride
TSH	Thyroid-stimulating Hormone

U&E (Creat)	Urea, Electrolytes and Creatinine
U/I	Units International
UFS	University of the Free State
ULN	Upper Limit Normal
WHO	World Health Orginisation
WPF	World Physique Federation

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CHAPTER ONE

Introduction

1.1 INTRODUCTION TO BODY BUILDING

The sport of Body Building has grown in stature over the last two decades. The arrival of the Virgin Active groups of gymnasiums, along with an explosion in the number of sport supplement companies nationally and internationally, delivering a wide variety of sport nutritional products, that have led to more and more athletes joining gymnasiums to condition and train. This has led to more people becoming interested in the sport of Body Building.

There are quite a number of federations and associations in South Africa to which Body Building athletes can affiliate. Each of these bodies has their own sets of rules and competitive divisions. The larger bodies are the National Association of Body Building Athletes (NABBA), World Physique Federation (WPF) and the International Federation of Body Building (IFBB) South Africa. The latter is currently the only recognized governing body for Body Building in South Africa and as governing body, abides to the rules set by SASCOC (South African Sports Confederation and Olympic Committee).

The IFBB SA currently has an annual membership of about a thousand competitive athletes. Any athlete that competes at any IFBB sanctioned show has to pay annual membership fees to the IFBB SA and provide the federation with his or her personal information. This is then added to the IFBB SA database. At the provincial shows, only the top athletes in the different divisions are selected to participate at national level. The national event is usually held during the first or second weekend of September during which an average of 220 athletes compete. Athletes compete in different divisions, such as Novices, Women's Physique, Women's Body Building, Junior- and Senior Men's Division, Masters and Grand Masters. Each division is subdivided into different weight categories. Only the elite of the winners in of all the different divisions are selected to compete internationally, at the World Amateur Body Building Championships, usually held during the first week of November every year.

With the era of professional sportsmen and –women arriving, this has also lead to more athletes becoming involved in the abuse of illegal substances boosting sport performance.

The South African Medical Community has very little knowledge on the types of Androgenic-Anabolic Steroid (AAS) substances and the dosing protocols abused in general amongst the elite, competitive Body Building athletes in South Africa (Millar, 1994). The difficulty in obtaining such information from these athletes is the fact that this is a very secluded group of athletes and those athletes taking such medications to enhance their performance, do so surreptitiously.

This area in Sports Medicine is unique for the following reasons: (Snyder, 2008)

- "Athletes often obtain the medications from sources other than physicians. These preparations are sometimes meant for veterinary use, sometimes from laboratories that are not regulated by government agencies for manufacturing quality.
- Athletes obtain their information about the medications from other athletes, trainers, magazines, underground publications and the Internet.
- Athletes often take several medications in various patterns…. in an attempt to increase the overall effect on performance.
- Athletes discontinue the medications periodically, often to avoid detection when they know they will be tested just before a competition.
- Physicians who see these athletes *are often unaware that they are taking these medications.*
- Physicians' *knowledge of the possible effects of these medications is poor, because the doses and even the medications used have rarely been studied in a controlled fashion."*

1.2 THE AIM OF THE STUDY

The aim of the study is to describe certain physical and biochemical changes in a cohort of elite South African Body Builders during the course of pre-competitive, competition and post-competitive phases, as well as to assess the role of AAS substances in the occurrence of these associated changes.

1.3 GOAL OF THE STUDY

Very little current information on the profile of these athletes is available to the South African Medical Community, especially the Sports Medicine Community. There exists only a small body of knowledge in the literature on the dosing protocols abused by these athletes and the side effects they incur. Little is known of the usage of high dose AAS amongst the elite, competitive South African Body Building population and the possible side effects. An EBSCO worldwide literature search was conducted and no data was found that indicate that a similar study protocol has been followed before.

The goal of the study is therefore to describe certain physical and biochemical characteristics of this population of athletes, to obtain and describe the anecdotal dosages of AAS compounds generally abused and to discuss the possible subsequent adverse changes in measurement (physically and endocrine) that may develop.

1.4 ETHICAL CONSIDERATIONS

It is acknowledged that the involvement of a medical practitioner in a research project involving banned substances may appear as an ethical dilemma. It must therefore be stated that access to this population group was obtained through consultations with athletes who were already using anabolic steroids and approached the researcher in his capacity as medical practitioner for information on possible side effects and long-term physiological damage. Since information on banned substances within this sporting community has not been documented before, it would be imprudent to pass on the opportunity to get insight into this population's substance abuse and the physiological effects thereof. This is a very covert population of sportsmen, constantly abusing illegal substances – a certain level of trust was established between the researcher and the participants, as an intrusion into their world could potentially be harmful. It must, however, be stated clearly that the researcher does not support the abuse of banned substances. All statements appearing to advocate a positive effect resulting from substance abuse is strictly from the viewpoint of the athlete, not the researcher.

CHAPTER TWO

Literature Review

2.1 BODY BUILDING AS A SPORT

A dearth of literature exists surrounding the sport of Body Building. Anecdotally, when preparing for a competition, most elite body builders in South Africa will go through two totally different phases of training and dieting. The first phase is the bulking- or weight-gaining phase. During this phase, a structured diet with high carbohydrate component and moderate to high fat content will be followed for several months. The diet will be followed fairly strictly, but food portions will mostly not be weighed. Training schedules are fairly strict, with heavy weight training and very little aerobic work done. Body skin fold fat % is generally kept between 12-14 %, but some individuals may let that increase to 15-18 %.

During the weight-gaining phase, AAS substances are used in moderately high doses compared to the pre-contest period. Substances of choice are the testosterone esters, especially the longer acting enanthates and cypionates, as well as the testosterone derivatives like boldenone (Equipoise) in its longer ester forms. The oral testosterone derivatives are either 17-alkylated or 17-methylated in order to pass the first liver metabolism. Substances include methandrostenolone (Dianabol), fluoxymesterone (Halotestin), 4-chlorodehdromethyltestosterone (Turinabol). The 19-nortestosterone derivatives, namely nandrolone (Deca-Durabolin) and all its shorter and longer esters are used commonly during this phase. These substances are used in different combinations simultaneously, over periods of 6 weeks to as long as 24 weeks. These periods are commonly referred to as “steroid cycles”.

The second phase of training and dieting, is called the pre-contest preparation phase. This is a very intense phase of high volume training, consisting of numerous protocols of isolation-type exercises, combined with daily aerobic workouts. This phase usually starts about 16-13 weeks from the time of the competition. The length of this phase will be depending on the athlete’s experience at pre-contest prep, his body fat % being less than 12-14 % and his weight category to be competed in. Generally the larger athletes will start their pre-contest preparation at 20 weeks before competition. During this phase, extremely strict, structured diets are followed, with each meal being weighed. Calorie intakes are calculated according to formulas

and the ratios of protein, carbohydrates and fats are changed intermittently. Weekly body fat % assessments are done, followed by calorie adjustments for the following week. High protein-, very low carbohydrate and moderate fat diets are mostly used, but this is highly individualised (refer to Annexure C for more detailed analysis on sample diets).

During the pre-contest phase, a multitude of chemical substances are abused to enhance the desired physique – this strategy of using combinations of different classes of drugs, is called “stacking” (Refer to Annexure B for examples of stacking of different drugs). This will be the period with the highest AAS substance milligram usage per week, in order to have the highest anabolic effect of nitrogen retention to protect the lean body mass gained during the bulking phase, while different ergogenic substances are used for the catabolic process of fat burning. The AAS substances of choice during this phase are usually injectable substances with very short acting esters like the propionates and acetates. Most, if not all of the “cycles” used, will include some form of injectable testosterone, usually combined with the 19-Nortestosterone derivatives Trenbolone, which seems to be a favourite pre-contest body building drug amongst this group of athletes. Athletes will tend to use AAS substances that generally do not aromatize, as this will lead to unwanted estrogen production and subsequent water retention and subcutaneous fat deposit. For this reason, most athletes use the class of substances derived from dehydro-testosterone. These are oxandrolone (Anavar), drostanolone (Masteron), methenolone (Primobolan), mesterolone (Proviron) and stanozolol (Winstrol).

The difference between medically used and recreational abuse, lies in the dosage and interval of administration of these drugs. Medically used AAS is aimed at replacement therapeutic dosages (in hypogonadal men 6-10 mg/d is used), prescribed on a continuous basis, or with regulated intervals of usage. However, the AAS abusers use very complicated drug protocols of different AAS drugs simultaneously, which is increased at dosages as high as 40-100 times more than levels needed to reach physiological homeostasis. The perceived belief for the physiological basis for using the stacking method is to maximise the androgen receptor binding by using different drugs having different binding affinities to the androgen receptor. By 2005, no scientific research clearly showed such an effect through the use of stacking protocols. Stacking would usually continue for periods of 4 to 18 weeks on average, although it has been noted that athletes use stacking protocols continuously for as long as 30 weeks over a competitive season. After the

cessation of such a stacking cycle, athletes would generally take a drug holiday of anything from 1 to 12 months.

2.2 LITERATURE STUDY

2.2.1 Introduction

In a cross-sectional study of 214 male gymnasium members (17-61 years of age, median of 30+/- 9 years), predictors of future Androgenic-Anabolic Steroid (AAS) abuse amongst male gymnasium members were investigated (Snyder, 2008). The study found very alarming statistics. Eighty percent of participants that have never used any AAS substances before, but have used some sport supplementation (mostly creatine) during the preceding 6 months, considered future abuse of AAS substances. Another predictor for considering future abuse of AAS substances was the fact of knowing other current or previous AAS abusers (Dunn, 2009). By the early 1980's, medical literature started showing evidence that as much as one out of every five NCAA Division Athletes has used AAS at some stage in their careers. By the early 1990's the problem had escalated to the extent that the USA passed the Anabolic Steroid Control Act and at that stage it was estimated that over one million people, of which 250 000 were still in high school, used AAS and spent more than $100 million per year purchasing AAS from black market sources (Hall and Hall, 2005; Kicman, 2008).

Two thirds of AAS abusers reported in one study that they started using these drugs by the early age of 16 years. More than 85 % of AAS currently used, is supplied from black market sources. The remainder is attributed from illegal prescription by physicians and from vetinary sources (Hall and Hall, 2005).

In a study conducted by Dias in 2002, his research showed that the average teenager using AAS recreationally would use at least 5 stacking cycles before cessation of drug abuse. Kanayama et al showed in their study conducted in 2003, that 29 % of people, who abuse AAS and opioids, started off with AAS and were then later introduced to the opioids by the same person who initially supplied them with the AAS. These researchers called AAS the "gateway" drug to opioid abuse (Kanayama et al, 2003). In addition, 25 % of AAS abusers shared their needles for injections and have a strong tendency to be involved with other drugs such as cocaine, injectable drugs and marijuana as well (DuRandt et al, 1993).

A literature search was done for a retrospective period of 20 years and only 5 studies on the subject of AAS use/abuse amongst South African athletes could be found – only one such study reviewed the use of AAS amongst Power Lifter athletes.

2.2.2 The different classes of AAS and their proposed mechanisms of action

According to a review article on the classification and mechanisms of action of AAS, AAS are tetra cyclic cyclopenta[a] phenanthrene skeletal compounds that can pass through cell membranes, bind to certain cytoplasmic receptors to for new complexes with DNA, leading to the production of structural proteins with the net result of a positive nitrogen balance. All the different AAS bind directly to only one androgen receptor, which is encoded on the X chromosome and is a 120-kDa cytosolic protein. By the time of this review article in 2005, only one cDNA for the androgen receptor had been identified. Different AAS have different binding affinities to this receptor and it also differs from tissue to tissue in the body. It is postulated that these different binding affinities are what causes the different effects of the varying AAS drugs (Hall and Hall, 2005).

The male body only has a limited number of androgen receptors, which are usually saturated with physiological levels of testosterone. It has long been hypothesized that there must be a secondary mechanism existing whereby AAS facilitates its effects (Wilson, 1988). As mentioned above, one such postulate is the effect AAS have on the up-regulation of IGF-1 (Insulin-like Growth Factor-1) and the down-regulation of IGFBP-4 (Insulin-like Growth Factor Binding Protein-4). Another theory is the blocking of the glucocorticoid receptor directly and displacing cortisol in the process. This effectively blocks the catabolic effects of cortisol (Wilson, 1988). Yet another theory is the down-regulation of the myostatin gene, which negatively regulates muscle growth (Haupt and Rovere, 1984). During Andropause when androgens naturally decrease, myostatin levels increase. It is thus speculated that AAS directly or indirectly suppress the gene expression of myostatin. Haupt and Rovere had yet another theory. They postulated that the use of AAS had a significant psychological effect of euphoria, which in turn allowed athletes to train much harder and more aggressively, as well as recover more rapidly. This leads indirectly to strength and muscular size gains (Haupt and Rovere, 1984).

To date, more than 1000 different testosterone derivatives have been formulated. The biochemical modification has led to the formation of three different categories of AAS (Hall and Hall, 2005).

The ***Class A modification*** entails the esterification of the testosterone ring at the 17-β-hydroxy position with varying carboxylic groups. This modification increases the lipophilic/hydrophobic properties of the AAS, leading to increased androgenic properties and prolonged absorption when administered intramuscularly. The long carbon chain increases the lipid solubility of the AAS molecule, rendering a molecule that needs only to be injected every 2 – 12 weeks. After injection, the Class A drug is then hydrolysed in the body to form molecules metabolically identical to endogenous testosterone. Shortly after the Class A injection, the levels of the drug will peak and then gradually decrease to the time of the next injection. With this type of modification, there are two exceptions, namely methenolone acetate and testosterone undecanoate – they are administered orally and either bypass the portal system, or have a much lower liver metabolism (Hall and Hall, 2005; Coert et al, 1975).

The ***Class B modification*** entails the alkylation of the 17-α-hydroxy position – this leads to the formation of a testosterone derivative which can be taken orally, but that has a much slower hepatic degradation. The Class B drugs have a potency that is weaker than the Class A drugs. They are quite liver toxic and as a group increase the hepatic enzyme production, especially complement 1 inhibitor (Hall and Hall, 2005; Healy et al, 2003).

The ***Class C modification*** is the alkylation modification of the A, B or C rings of the steroid backbone. This modification leads to the formation of a group of AAS drugs that have similar properties as the Class B AAS drugs (oral availability), but that have decreased or non-existing hepatic metabolism. The drugs in the Class C are mainly excreted in urine or faeces either as unmodified, or as metabolites, or as conjugates. In some cases Class C derivatives can also undergo a Class A esterification, forming a new class, called the ***Class AC analogues***, which can be administered orally (Hall and Hall, 2005; Healy et al, 2003).

2.2.3 Body composition changes due to AAS use in competitive male body builders

The use of these substances, diets and exercise regimes as described above, result in marked physical changes in a relatively short period of time (Bhasin et al, 2003).

Andersen et al conducted a study in 1995 where they investigated the eating and weight loss patterns, nutrition and psychological factors in 45 drug-free male competitive body builders. Participants of this study reported cycles of binge eating, weight gain and weight loss over a season. During the same competitive season mean weight loss was recorded as 6.8 kg and mean weight gains as 6.2 kg. As much as 85 % of the participants reported weight gain and 46 % reported binge eating immediately after the competitions. Most (81.15 %) reported a pre-occupancy with food and the preparation thereof. About 30 % to 50 % reported periods of psychological distress during the pre-contest phase, with symptoms of anxiety, short temper and aggressiveness (Andersen et al, 1995).

Kicman and Gower wrote a very comprehensive review article in 2003 on Anabolic Steroids in Sport. They found numerous studies that were conducted in the 1960-1970 period, which concluded that supra-physiological doses of testosterone or any other synthetic AAS had little effect on increasing muscle size and strength. All these reviewed studies had one thing in common – they all lacked adequate control and standardisation (Bhasin et al, 2003; Kicman and Gower, 2003). More recent studies show that the use of AAS can significantly increase size and strength in male athletes, only if they satisfy certain criteria concerning the timing of doses and nutritional factors (Bhasin et al, 1996).

Bhasin et al's study in 2003 standardized the protein and energy intake in their study on 43 experienced weight lifters (Bhasin et al, 2003). Changes in muscle mass were measured with MRI. The volunteers were assigned to one of four groups:

- Placebo with no exercise
- 600 mg Testosterone enanthate per week for ten weeks with no exercise
- Placebo with exercise
- Testosterone with exercise.

The respective exercise groups received controlled, supervised training on 3 days per week. The results showed increase in muscle mass, strength and fat-free mass gains in the placebo with exercise and the testosterone without exercise groups. The group with combined testosterone and exercise made the greatest gains. The effect of combining exercise with supra-physiological testosterone doses was found to be additive. Subsequent work done by them and others show that the increases in fat-free mass, muscle size, strength and power are all highly dose-dependent and will correlate well in linear manner with serum testosterone levels.

Low serum testosterone levels are usually associated with lower fat-free mass (Katznelson, 2000), as seen in the suppression of testosterone levels in young healthy men experimentally by the administration of GnRH (Gonadotropin Releasing Hormone) agonist analogue (Mauras et al, 1998). Their study showed a significant reduction in fat-free mass, with subsequent increase in total fat mass, as well as a decrease in fractional muscle protein synthesis. Brodsky et al in 1996 studied the effect of replacement doses of testosterone in young, healthy, but androgen-deficient men. They assessed the effect on fat-free mass, muscle size and maximal voluntary strength and found that the testosterone replacement doses lead to increases in fractional muscle protein synthesis (Brodsky et al, 1996).

There is a strong belief amongst the body builder community that the effects of androgens on muscle size are dose dependent – at least this was anecdotally observed. For many years no experimental work studied this phenomenon. During the early research work done by Wilson in 1988, it was speculated that androgen receptors in most of the body's tissues were either saturated or down regulated at physiological testosterone concentrations (Wilson, 1988). Observing the anecdotal effects amongst body builders, Wilson thus speculated that there were two separate dose-response curves of testosterone's effect on the androgen receptors. One would be in the hypogonadal range with the maximal response setting in at the lower normal testosterone concentrations. The other's effect would set in at the supra-physiological range and that this effect would be due a separate or different mechanism than what happens at the hypogonadal range (Wilson, 1988).

Studies on the effect of graded testosterone doses on body composition changes, including parameters such as muscle size, strength and power, showed an increase in fat-free mass when using 125-600 mg of testosterone enanthate (Bhasin et al, 2003; Singh et al, 2002). The cohort consisted of 61 eugonadal men between the

ages of 18-35 years. The subjects were randomized into 5 groups, each receiving monthly injections of a long-acting GnRH agonist to fully suppress endogenous testosterone production. Each group then received weekly doses of testosterone enanthate at 25, 50, 125, 300 and 600 mg for a period of 20 weeks. Both the energy and protein intakes were standardized – one of the first studies ever to do so. The results showed a fat-free mass increase only in the 125, 300 and 600 mg groups (+3.4, 5.2 and 7.9 kg respectively). The changes in leg press strength, leg power testing, thigh and quadriceps muscle volumes correlated positively with testosterone concentrations. Fat-free mass correlated negatively (Bhasin et al, 2001; Bhasin et al, 2003).

Muscle hypertrophy seen in the body builders using supra-physiological testosterone doses, were always thought to be due to increases in fractional muscle protein synthesis. Recent studies have however shown that the increases in protein synthesis only probably occur as a secondary effect and may not be the primary or only mechanism by which testosterone administration causes muscle hypertrophy. Sinha-Hikim et al conducted a study to determine if the increase in muscle size is secondary to testosterone administration (Sinha-Hikim et al, 2002). The same protocol was followed as in the Bhasin study. The cohort consisted of 39 men of whom muscle biopsies were obtained from the vastus lateralis muscle. Type II and I muscle fibre cross sectional changes were measured and significantly correlated with total testosterone serum concentrations. The 300 and 600 mg group experienced the greatest increases in the Type I fibre sizes, but what was more significant, was that the 600 mg group also showed significant increases in cross sectional area of Type II fibres. This correlates with what is anecdotally seen amongst body builders in general – the greatest increases in muscle size are mostly seen with testosterone doses beyond 600 mg per week. The relative proportions of the Type I versus Type II fibres did not change significantly amongst any group. The 300 and 600 mg groups had higher myonuclear numbers per fibre and the increases correlated with testosterone concentrations and muscle fibre cross-sectional area (Sinha-Hikim et al, 2002). This study thus confirmed that testosterone-induced increase in muscle volume is due to muscle fibre hypertrophy and not due to hyperplasia as always suspected amongst the body builder community. Subsequently, this study also showed a direct correlation between muscle fibre size and myonuclear number and the authors postulate that this was preceded by a testosterone-induced increase in satellite cell number and their subsequent fusion

with muscle fibres. The mechanisms by which testosterone induces satellite cell number are not known (Sinha-Hikim et al, 2002).

The mechanisms by which androgens induce muscle cell hypertrophy, is still not well understood. In 1995, Urban et al postulated that testosterone stimulates the expression of IGF-I and down-regulates IGFBP-4. The reciprocal changes in IGF-I and IGFBP-4 induced by testosterone administration, thus provide the potential mechanism for an amplified anabolic signal (Urban et al, 1995). During this current literature search, no other postulate for directly testosterone-induced muscle hypertrophy could be found.

Growth Hormone (GH) is also used extensively amongst body builders to induce body composition changes. There is unequivocal evidence that GH induces a protein anabolic effect in healthy adults. In 2003, Heally et al showed that GH significantly reduced whole body protein oxidation, reducing irreversible protein loss and increased protein synthesis rate in trained men. A program of resistance training also increased protein synthesis in muscle, maintaining this effect up to 24 hours after the exercise bout. On a whole body level, the oxidation of protein is also stimulated during exercise, but when GH was administered to individuals engaged in resistance training, the whole body protein oxidation took place at a lower level, thus suggesting that GH has a protein-sparing effect. However, although it has been shown before, that GH causes increase in protein synthesis in untrained men, this effect seems to be lost in highly trained individuals. During 1993, Yarasheki et al administered high-dose GH (40 ug/kg/d for 14 days) to experienced weight lifters and found no change in whole body protein synthesis. This suggests that GH does not induce a high anabolic stimulus in highly trained individuals despite substantial increases in IGF-I levels (Yarasheki et al, 1993).

There is good evidence in the literature that GH increases lean body mass (LBM) (Birzniece et al, 2010). The increase in the LBM is heterogeneous, consisting of an inert compartment of extracellular water (ECW) and a functional cellular compartment of mostly muscle. A systemic review done in 2008 by Liu et al showed increases in LBM of averaging 2.1 kg on the use of GH for periods of 4 weeks (Liu et al, 2008). In 2005, Ehrnborg et al showed after one month of GH administration a decrease in body fat by 7 % and increase in ECW by 10 %. The subsequent increase of 5 % in LBM in this study was due to increases in ECW (Ehrnborg et al, 2005). In a study conducted by Birzniece et al in 2010, GH was administered for 8

weeks and LBM measured by Dual-energy X-ray Absorptiometry (DXA). Averages were found to be 2.15 kg increase in LBM, but there was a concomitant increase in ECW of an average of 2 litres. According to the review article done by Birzniece in 2010, all the data provide strong evidence that the increases seen in LBM after GH administration, is mainly due to fluid increases (Birzniece et al, 2010).

2.2.4 Effects / side effects of AAS to the different organ systems

All AAS substances have some side effects when taken in high doses. Side effects depend upon the structure of the androgen and the conversion of AAS to other metabolic active steroids. Examples are that of the 17-alkylated AAS being hepatotoxic and testosterone that is converted to estradiol via the action of the aromatase-enzyme complex, leading to gynecomastia and other adverse effects.

2.2.4.1 Renal System

In general, renal side effects are very uncommon amongst AAS abusers. Only a few isolated cases have been described in the literature. The main side effect of AAS on renal function is that of slight elevation of serum creatinine (Juhn, 2003). This side effect is aggravated by the concurrent use of creatine supplementation (Maravelias et al, 2005). Some cases of acute renal failure have been described in the literature – all of these cases had the following in common, namely multiple drug stacking, high protein diet, limitation of sodium and water intake, combined with the misuse of the diuretic torasemide (Juhn, 2003; Maravelias et al, 2005).

In rare cases Wilm's tumours have been described, as well as membranoproliferative glomerulonephritis (Maravelias, 2005). AAS also causes sodium retention, which can lead to increases in potassium and hydrogen excretion, eventually leading to metabolic alkalosis and hypokalemia (Modlinski and Fields, 2006).

2.2.4.2 Haematological System

Another common side effect of AAS substance abuse is that of erythrocytosis, largely due to direct androgen stimulation of erythropoiesis. This reaction is mainly driven by the androgen receptor stimulation in renal tissue, leading to the stimulation of erythropoietin production directly. Androgens may also affect the stem cells

directly, perhaps by enhancing the stem cell's responsiveness to erythropoietin (Snyder, 2008).

Castration of the male testicles will lead to a drop of 10 % of red cell mass, with subsequent decrease in red cell diameter and life span. When women are treated with therapeutic doses of testosterone, it leads to an increase in concentration of haemoglobin of about 43 g/l and haematocrit increases by about 11 %. This clearly shows the direct positive effect of AAS on erythropoietin production in renal tissue (Llewellyn, 2006). Under normal circumstances the release of erythropoietin is controlled by hypoxia and the red cell concentration is kept at a certain level by a negative feedback mechanism on the release of erythropoietin.

The British Journal of Sports Medicine published a study where the haematological effects of steroid abuse in a group of 5 power athletes were studied over a period of 26 weeks. The cohort was compared to a group of 6 non-using power athletes. The control group showed no change in haematocrit, but the studied cohort showed average increases in haematocrit of 9.6 % (Llewellyn, 2006).

Steroid abusers are prone to display abnormally high thrombin-anti-thrombin complexes in plasma (Ferenchick et al, 1995). They also showed higher plasma concentrations of prothombin fragments, anti-thrombin III, protein S levels and lower plasma concentrations of tissue plasminogen activator and its inhibitor. These anomalies all contribute to increased risk for clotting disorders. The literature has several case reports of thrombosis, some being fatal, in young strength athletes (Ferenchick et al, 1995).

An increase in the haematocrit and thrombocytes should be regarded as critical, as increased haematocrit values correlate strongly with increased cardiovascular risk and total mortality (Gagnon et al, 1994). The tendency for thrombocytes to more easily aggregate, increases in correlation with testosterone administration. The raised haematocrit and haemoglobin persist for extended periods after the cessation of AAS use (Gagnon et al, 1994). Their study showed haemoglobin to be still well above normal levels up to 16 weeks after cessation; another showed the haemoglobin value returning to normal values only after 5-6 months (Nieminem et al, 1996) and normal red blood count and thrombocytes only after one year (Urhausen et al, 2003).

2.2.4.3 Hepatic System

The hepatic side effects occur most frequently in the Class B AAS with the use of 17-alpha-alkylated substances, as well as to a lesser extent the Class C drugs (Hall and Hall, 2005). These side effects include high serum concentrations of liver enzymes, namely the transaminases Aspartate Amonitransferase (AST) and Alanine Aminotransferase (ALT), as well as Gamma Glutamyltransferase (GGT), the latter usually at a much later and advanced stage, particularly with the use of oxymetholone (Snyder, 2008). The liver toxicity causes hepatocellular and intrahepatic cholestasis, which may eventually lead to liver failure after prolonged exposure. Other side effects include cholestatic jaundice and peliosis hepatitis (Pavlatos et al, 2001), as well as hepatocellular adenoma and hepatocellular carcinoma. Due to disturbed liver function, carbohydrate metabolism can also be influenced, leading to Type II pre-diabetic states (Cohen et al, 1987).

In a literature search done by Kuipers in 1998, he found that some of the longitudinal studies investigating AAS abusers, at times showed contradictory and varying results. A number of these studies showed moderate liver enzyme increase, whereas other studies again showed none at all. The moderately increased liver enzymes would normalise again within a few weeks of AAS abstinence. According to Kuipers, these findings suggest that the enzyme leakage is partly pre-determined by the existing condition of the liver at the time of onset of AAS abuse. As such, those individuals with existing abnormal liver enzymes appear to be at greater risk for liver damage (Kuipers, 1998). In another review article, conducted by Hartgens and Kuipers in 2004, the majority of longitudinal studies reported no changes in liver enzymes due to AAS abuse, although some studies showed elevations in AST and ALT levels within a few weeks of taking oral AAS drugs. In all these reviewed studies, serum GGT and LDH remained unaffected (Hartgens and Kuipers, 2004).

Liver function can be evaluated to examine to what detrimental extent the use of oral 17-alkylated and 17-methylated AAS substances have on the liver. This may serve as an indicator of poor carbohydrate metabolism, lipid metabolism and possible chronic liver inflammation and subsequent liver cirrhosis (Scally, 2008).

Muscularity may cause increases in the transaminases. In such cases, this will usually be shown as an AST/ALT-ratio of $>$ 1.0 (Noakes, 1987). However, in the AAS abusers, it is commonly noted that the ALT values are on average higher than

the AST values – primarily as a manifestation of AAS-induced liver function impairment (Urhausen et al, 2003).

The most commonly used measures of hepatotoxicity include AST, ALT and Lactate Dehydrogenase (LDH) (Kuipers, 1998; Scally, 2008). These values are usually in the range of two and up to three-fold the normal values. These levels are very similar as to those seen as side effects to combined oral contraceptive usage in women (Hartgens et al, 1996; Modlinski and Fields, 2006). The most sensitive marker to hepatobiliary disease and obstruction is the GGT level (Scally, 2008).

The peak in AST, ALT and GGT levels usually would occur after 2-3 weeks of AAS consumption. These levels would return to baseline levels after a few weeks up to three months after AAS abstinence (Hartgens et al, 1996). In the review article by Kutscher et al in 2002, it is stated that the reversible course of liver enzyme elevations explains the reason why athletes opt to abuse these drugs in a cyclic manner. However, when the AAS is administered for at least one month continuously, but generally greater than 2 to 5 months, dose-dependent jaundice and hepatic dysfunction are more likely to develop (Ishak and Zimmerman, 1987; Kutscher et al, 2002). The cyclic administration of AAS can thus reduce the subsequent liver toxicity (Blue and Lombardo, 1999; Kutscher et al, 2002).

Levels of serum liver enzymes are more the indicators of the hepatocyte integrity or cholestasis, rather than liver function itself (Scally, 2008). A decrease in liver functioning mass can be more accurately assessed by changes in clotting times or serum protein levels. There is no single, simple test that can assess overall liver pathology. AST and ALT are the most sensitive indicators of hepatocellular injury, indicating hepatocellular necrosis or inflammation. ALT raises the most dramatically in acute liver disease. The magnitude of the elevation has no prognostic value; neither does it correlate with the degree of liver damage. AST is relatively nonspecific and rises acutely in myocardial infarction, heart failure and muscle injury, but high levels indicate liver cell injury. Most liver diseases will show AST increases that are less than that of the ALT increases (AST / ALT-ratio < 1.0). In AAS abusers, the AST and ALT level increases correlate significantly with the extent (duration and weekly dosage) of AAS abuse (Scally, 2008).

There are a few factors that affect the AST and ALT levels other than liver injury alone (Dufour, 2000). With liver cell membrane damage, both enzymes are released

into the bloodstream in increasing amounts. Liver cell necrosis is not required as prerequisite for the release of the aminotransferases. The extent of liver cell damage and the level of aminotransferases release are poorly correlated (Scally, 2008).

The time of day does not influence the AST levels, but ALT levels can show as much variation as 45 % during the day, being the highest in the afternoon and the lowest at night. AST levels can vary as much as 5-10 % from one day to the next, where ALT can vary as much as 10-30 % (Scally, 2008).

Body Mass Index (BMI) plays a significant role in variations in AST and ALT levels. Both AST and ALT levels can be increased as much as 40-50 % with high BMI. AST levels can increase three-fold with strenuous exercise, whereas ALT levels can be 20 % lower in those who exercise at usual levels than in those who do not exercise or who exercise more strenuously than usual. The effect of exercise is seen more predominantly in men, with women showing less than 10 % differences. The enzyme increases are much higher with strength training (Scally, 2008).

Muscle injury can cause significant increases in AST levels and moderate increases in AST levels, but this usually will correlate well with increases seen in Creatine Kinase (CK) levels. If the striated muscle is the cause of the increase in AST and ALT, the CK levels will also be elevated to the same or even higher degree. CK levels increase very specifically in accordance to the type of contraction executed. In performing isometric concentric muscle contraction, the CK levels will peak within 24-48 hours after the exercise bout, but with eccentric contractions, it would only peak between 3-7 days after the exercise bout. Strength training thus results in a biphasic pattern of CK increases (Scally, 2008).

GGT is present in liver, pancreas and kidney tissues. It is elevated in structural liver damage, biliary tract and common bile duct obstruction, alcohol abuse and drug abuse. GGT is a very sensitive predictor of liver dysfunction and can be elevated with even minor, sub-clinical levels of liver damage. In the past, most reports on AAS induced liver damage, relied on the elevated levels of the aminotransferases enzymes, with total disregard to the influence of muscle damage contributing to the elevated levels. Both AST and ALT levels can increase in response to strenuous weight training protocols, such as the ones followed generally by AAS abusers. The enzyme evaluations done in AAS abusers to ascertain the AAS-induced liver

damage, should also consider the CK and GGT levels as essential elements to distinguish muscle damage from liver damage (Scally, 2008).

In 1999, Dickerman and colleagues conducted a study where they compared the liver enzyme changes in two groups of body builders – the first taking self-directed regimes of AAS and the second group not taking any AAS. Both groups of body builders showed increases in AST, ALT and CK values, whereas the GGT values stayed within normal range in both groups. Dickerman commented that the prior reports on AAS-induced hepatotoxicity based on elevated aminotransferases alone, were overstated, because none of the exercising subjects in their study, including the steroid users, showed any hepatic dysfunction based on the GGT levels. According to Dickerman, over-emphasizing AAS-induced hepatotoxicity when interpreting elevated aminotransferases levels and disregarding muscle damage, is misleading the medical community (Dickerman et al, 1999).

Once liver damage has been caused by AAS abuse, active treatment protocols should steadfastly adhere to the World Health Organization (WHO) guidelines of treatment for drug-induced liver disease. A grading system of abnormality has been developed (0 is least severe, IV is most severe) based on the monitoring of ALT, AST, GGT and Alkaline Phosphatase (ALP) level increases (Scally, 2008). The recommended action is based on the Grade Level.

Grade 0 - enzyme level is the upper limits of normal (ULN) reference range.

Grade I - > ULN up to 2.5 times ULN – continue treatment, but monitor regularly.

Grade II - > 2,5 up to 5 times ULN – should be more closely monitored or managed in a similar manner to those in Grade III.

Grade III - > 5 up to 20 times ULN – the dose should be reduced or interrupted, and cautiously reinstated when enzymes return to normal or to Grade I levels.

Grade IV - > 20 times ULN – should be discontinued permanently.

Upon discontinuation of AAS with continued transaminases elevations, best recommendation is to follow a diagnostic algorithm for a known cause. It is unwise to consider any continued enzyme increases in the absence of a diagnosis, as just being non-significant or even of no concern.

2.2.4.4 Cardiovascular system and lipid metabolism

Cardiac disease has also been associated with high dose AAS use. Cardiac hypertrophy, especially concentric left ventricular hypertrophy, has been reported in the power lifter and body builder population (Urhausen et al, 2003). This has also been found amongst other athletes not using AAS.

The effects of AAS on arterial blood pressure are not clear. The response is most probably dose dependant and usually associated only with diastolic blood pressure increases. The blood pressure increase may also be due to blood volume increase and/or fluid retention (Ferenchick et al, 1995). The diastolic blood pressure will normalize within 6-8 weeks of AAS abstinence and repeated intermittent use of AAS does not have an effect on the diastolic blood pressure during periods of abstinence (Kuipers, 1998).

A well-documented risk factor is the effect of the 17-alpha-alkylated androgens administered orally, which cause a decrease in High-Density Lipoprotein (HDL) cholesterol and an increase in Low-Density Lipoprotein (LDL) cholesterol levels (Cohen et al, 1987; Friedl et al, 1990; Thompson, 1989). If the decrease of HDL levels exceeds 15 % in the general population not using AAS substances, it is usually seen as a predictor of increased risk of coronary heart disease (Thompson, 1989).

A number of studies (Cohen et al, 1987; Friedl et al, 1990; Gordon et al, 1977; Hartgens et al, 1996; Hislop et al, 2001; Nieminem et al, 1996) are available in the literature to show the detrimental effect that AAS substances have on lipid profiles. Studies have found the changes to be a possible cardiovascular risk predictor later in life:

*"Serum lipoprotein profiles were measured in nine male and three female **power lifters** who were taking anabolic steroids. Male steroid users had higher total serum cholesterol, lower HDL-C, and lower HDL-Apo protein A-I (apoA-I) levels than weight-trained reference group that did not use steroids. Female steroid users*

showed similar trends. Mean serum HDL-C and HDL-C to total cholesterol ratio were lower in male steroid users than in a young male South African population at high risk for atherosclerosis. The ratio of HDL3-C was higher in steroid users than in the reference group. Ratios of apoA-I to apoA-II were similar in the two groups. These unfavourable lipid profiles suggest that male and female steroid users may face an increased risk of coronary artery disease" (Cohen et al, 1987).

However, all these studies were been done on individuals or groups of athletes that were not in pre-contest diet phases. As can be noted in the above quoted study, weight lifters and not body builders were studied. These two groups of athletes differ considerably as far as diet and exercise protocols, as well as drug regimes are concerned.

Most of the above mentioned studies conducted on cardiovascular risk factors with the abuse of AAS, showed increased levels in total cholesterol, a decline in HDL cholesterol (in most cases well below the normal range), and LDL cholesterol showed variable responses from slight increases to no change at all. It also seems that the response to total cholesterol changes is related to the type of exercise done. When the greater deal of exercise performed consists of aerobic exercises, the cholesterol increasing effects of the AAS is counter-balanced by an exercise-induced decreasing effect on cholesterol, which may even result in a net decline in total cholesterol. Aerobic exercise however does not seem to be able to offset the AAS induced decline in HDL (Kuipers, 1998).

The precise effect of AAS on LDL is not known yet (Kuipers, 1998). AAS influence the hepatic triglyceride lipase (HTL) and lipoprotein lipase (LPL) enzymes. HTL is primarily responsible for the clearance of HDL cholesterol, while LPL clears glycerol and free fatty acids. AAS stimulate the increased effect of HTL, resulting in increased clearance of HDL, leading to the decreased serum HDL levels (Kuipers, 1998).

Testosterone readily aromatizes to 17b-estradiol, while the orally active class of drugs such as the 17a-methyltestosterones do not form potent estrogens (Friedl et al, 1990; Hall and Hall, 2005; Kicman, 2008). As estrogens decrease Hepatic Triglyceride Lipase Activity (HTLA) and androgens increase HTLA – thus each producing inverse effects on HDL cholesterol – the net effect of AAS abuse on HDL may be related to the metabolic end path of the particular AAS, particularly to which

degree the AAS will aromatize to form potent estrogens (Friedl et al, 1990). The higher the HTLA, the lower the HDL will drop. When Testosterone enanthate is administered in pharmaceutical doses alone, the expected androgenic induction of HTLA is counterbalanced by the aromatization of the Testosterone enanthate to increased levels of 17b-estradiol – resulting in no change in the HDL cholesterol levels. The 17-alkylated androgen stimulated increase in HTLA activity precedes, as well as appears to cause the reduction in HDL cholesterol. Methyl testosterone, which is not aromatized to any form of potent oestrogen, will cause a decrease in HDL to about 50 % of baseline. Even lower levels in HDL cholesterol have been reported when AAS abusers use combinations of parenteral and oral 17-alkylated androgens (Friedl et al, 1990).

Pharmacological administration of Testosterone enanthate alone does not necessarily change the lipoprotein profile to a more atherogenic picture (Gordon et al, 1977). Gordon et al's study showed that, if HDL cholesterol changes alone were used for cardiovascular risk stratification, those athletes using 17-alkylated AAS would be at a two- to three fold higher risk for coronary artery disease, than those athletes using only Testosterone enanthate (or any other AAS that would easily aromatize to form potent estrogens).

In a South African study, the effects of androgen manipulation on Postprandial Triglyceridaemia (PPT), LDL particle size and lipoprotein (a) in men were observed (Hislop et al, 2001). The cohort consisted of three groups: male bodybuilders self-administering AAS, healthy men whose testosterone concentrations were suppressed with the GnRH agonist triptorelin, and a separate control group not receiving any hormonal treatment. The researchers found that androgen supplementation could reduce PPT, especially in individuals having existing elevated PPT levels, but that androgen suppression did not have any effect on the PPT levels. The LDL particle size or the LDL concentrations were not influenced by androgen manipulation. The HDL cholesterol and lipoprotein (a) levels were markedly influenced by androgen manipulation – androgen suppression increased the levels, while androgen supplementation decreased both levels. Decreased lipoprotein (a) levels may be an anti-atherogenic effect of androgen hormones. When LDL particle size is increased and PPT is reduced, this may have further anti-atherogenic effects of AAS abuse in individuals who are predisposed to atherogenic dyslipidaemia. Thus, apart from lowering HDL concentrations, no other potentially

atherogenic effects of endogenous androgens or AAS were seen (Hislop et al, 2001).

A study reviewing the body composition changes, cardiovascular risk factors and liver function in long-term AAS abusers three months after drug withdrawal found that elevated blood pressure levels returned to baseline levels on average six weeks after drug withdrawal (Hartgens et al, 1996). This was independent of the number of AAS courses used before. After drug withdrawal, the changes in serum lipids and lipoproteins seem to return rapidly to within baseline values within several weeks or months. At three months after drug withdrawal no abnormalities were seen. Hartgens' data suggests that the use of successive cycles is not associated with any long-term unfavourable effects on the cardiovascular system, as long as the drug withdrawal period is at least three months.

2.2.4.5 Endocrine system

Taking baseline s-Cortisol levels early on in the pre-contest phase and then reviewing these levels again at a set period during the post-competitive phase can evaluate adrenal function (Modlinski and Fields, 2006).

Androgens used in high doses suppress gonadotrophin secretion, leading to the suppression of endogenous testicular function with decreased levels of circulating testosterone and decreased sperm production (Modlinski and Fields, 2006). Spermatogenesis and fertility are diminished, but the sperm count will usually return to normal within 75-90 days after the last testosterone esters have cleared the body. Gonadotrophin and testosterone secretion can be suppressed for much longer (Kicman and Gower, 2003; Kuipers, 1998; Modlinski and Fields, 2006). Depending on what substances are used, normal testosterone levels will reappear anything from 6 weeks to as long as nine months after drug withdrawal. As little as 100 mg of nandrolone decanoate suppress endogenous testosterone levels to near zero within 4 days of administration, and continues its suppressive effect up to nine months (Roberts and Clapp, 2005). The hormonal status of hypogonadotrophic hypogonadism, the combination of decreased serum concentrations of Follicle-stimulating Hormone (FSH), Luteinizing Hormone (LH) and testosterone, is a common side effect after long-term high dose AAS abuse (Kicman and Gower, 2003; Kuipers, 1998). Various studies have suggested that using more than one AAS at one time will lead to a much stronger inhibition of the gonadal function, than

using only one AAS alone. The changes in fertility will usually reverse fully after several months of AAS abstention. In most cases the hypogonadism lasted more than 12 weeks (Kuipers, 1998).

The suppressive effect of exogenous testosterone- and derivatives administration on the athletes' Hypothalamic-Pituatary-Gonadal Axis (HPTA) can be evaluated by the LH-, FSH- and Total Testosterone assays (Kicman and Gower, 2003; Modlinski and Fields, 2006). Most athletes will use some form of testosterone administration during the mass-gaining phase preceding the pre-contest preparation phase. Some athletes will stop using the testosterone doses several weeks before commencing the pre-contest phase, while others will stay on testosterone continuously. If base line levels of Total Testosterone blood samples were to be taken at the normal 16-week pre-contest period, some athletes will have very high and other very low levels of measured testosterone. This could have led to false base line levels and influence comparison at a later stage during the study. For this reason, the present study only measured the testosterone levels after 4-6 weeks post-competition to assess the level of possible hypogonadism that might have developed when exogenous testosterone administration was ceased for the period of 4-6 weeks post-competition.

2.2.4.6 Soft tissue side effects

The use of AAS can lead to a paradoxical effect on soft tissues. Though muscle and bone strength are increased by AAS use, the abuse of high-dose AAS may lead to decreased tendon strength, caused by collagen fibre dysplasia (Maravelias, 2005). Athletes who abuse high-dose AAS to gain strength are thus at increased risk for short-term, as well as long-term disabling tendon ruptures. There are several studies that have documented rare tendon ruptures of the triceps, biceps and bilateral quadriceps in AAS abusers (David et al, 1995; Stannard and Bucknell, 1993; Visuri and Lindhohm, 1994).

2.2.4.7 Gynecomastia

The feminization of breast tissue in AAS abusers is a very common phenomenon (Kicman and Gower, 2003; Modlinski and Fields, 2006; Roberts and Clapp, 2005). The excessive circulating testosterone undergoes enzymatic aromatisation to form estrogen (Kuipers, 1998; Modlinski and Fields, 2006). The estrogen then attaches to

the receptors in the breast tissue, leading to the stimulation of ductal growth. Both estrogen and progesterone has stimulatory effects on the production of Human Growth Hormone. The resulting increased IGF-1 levels lead to the stimulation of the IGF-1 receptors in the stromal breast tissue, thus leading to the proliferation of differentiated breast tissue. (Roberts and Clapp, 2005)

Some cases of AAS abusers have gynecomastia to the degree needing surgical removal (Kuipers, 1998; Roberts and Clapp, 2005). Lately the use of tamoxifen (selective estrogen receptor modulator) and letrozole (aromatase inhibitors) as pharmaceutical treatment for existing gynecomastia, as well as preventative treatment, has become very popular amongst AAS abusers. (Roberts and Clapp, 2005)

2.2.4.8 Additional side effects

In the literature sleep apnoea has also been reported, which follows due to AAS stimulated increase in haematocrit, leading to blood stasis and thrombosis (Kuipers, 1998).

Thyroid function may also be affected by AAS abuse. Thyroid Stimulating Hormone (TSH) is decreased by concurrent AAS abuse. This may also be accompanied by Thyroid Binding Globulin (TBG) decreases (Cohen et al, 1987).

Other side effects listed include stunted growth, acne vulgaris (Maravelias et al, 2005), male pattern baldness, benign prostate hypertrophy, suppressed immune system, sterility in both males and females, and psychological effects of euphoria and aggressiveness (commonly called “Roid Rage”) (Pope and Katz, 1990).

CHAPTER THREE

Methodology

3.1 TYPE OF STUDY

The type of study conducted, was an *Observational Type* of study – *Analytical: prospective cohort analytical.*

3.2 ETHICAL CONDUCT

Permission to do the study was obtained from the International Federation of Body Builders (IFBB) South Africa. In addition, the study protocol was drawn up and submitted for approval to the Ethics Committee of the Faculty of Health Sciences, University of the Free State, South Africa (Ethical approval number 152/09).

Informed consent to participate in the study was obtained voluntarily from all participants. A confidentiality agreement was signed with each participant that wanted to, after which a case number was assigned to each participant. The memorandum for the confidentiality / non-disclosure agreement was drawn up by registered attorneys. An information document containing all relevant information concerning the study was provided to each participant.

All participants were informed of their blood results. Any abnormal results were forwarded to the participant's respective General Practitioners of choice, or were in most cases conveyed to each participant personally by the researcher. In some cases results were included in an information letter, addressed to the participant's General Practitioners of choice. The study protocol was designed in such a manner that, if any problem were to arise during the course of the study, the athlete was immediately referred to his own General Practitioner with an information letter discussing his condition / problem. At the conclusion of the research study, none of the participants had to be referred in such a manner.

3.3 SAMPLE / STUDY PARTICIPANTS

The cohort that was studied was compiled from Elite-level Competitive Body Builders in South Africa, competing in the Senior Men's Divisions of the South African Body Building Championships in 2010, including participants from all weight categories.

3.3.1 Sample selection

All athletes that competed in the above competition, as well as the preliminary rounds, were eligible to be included in the study.

3.3.2 Three options for sample selection were available

All athletes registered with the International Federation of Body Building South Africa were to be invited to participate in the study. Interested volunteers were asked to contact the researcher. If the response were to render a group of greater than 20 athletes, random selection were then to be applied according to the *simple random sampling principle.* If this method failed to deliver at least 20 participants, volunteers were to be invited to participate via a letter placed in "Muscle Evolution" magazine, which in 2010 had a readership of approximately 10 000.

If the latter method leads to a sample group of more than 20 participants, subsequently simple random sampling was to be applied. If 20 volunteers could not be recruited by these two methods, non-random sampling were to be used, using the *snowballing / networking type of method*, which was used for *hidden or rare populations* (Joubert, Katezenellenbogen, 2007). This principle used the method of contacting specific athletes personally and asking them to participate, as well as asking them to assist in obtaining more prospective participants / volunteers known to them.

Participants had to fulfil the following inclusion criteria:

1. Male athletes only
2. Age of 22 years or older, as this constituted the "Senior Body Builder Divisions".
3. Levels of participation were to be at Provincial and National levels.

Volunteers were excluded if they met any of the following criteria:

1. Any athlete that want to withdraw from the study at any stage
2. Those athletes who were medically advised not to partake due to existing pathology seen on their initial blood assays.

The sample size was dependent on the number of volunteers. Initially a sample size of 20 participants was envisaged. The size of the population competing at National level differs from year to year. The average number of contestants that would fulfil the above criteria usually would be between 100 – 120 athletes. Initially 22 athletes showed interest in participation after the first method of sampling was followed. The researcher then contacted each of these athletes personally. Information documents and confidentiality agreements were posted to each of the cohort. Finally, only 19 athletes returned their signed confidentiality agreements. Two of these athletes participated in the pilot study and their results were subsequently included in the final group of 14 athletes. All of these athletes fulfilled the criteria to participate.

Of the initial group of 19 participants, 5 athletes had to withdraw for different reasons at different stages of the research protocol. These were as follows:

- Two athletes withdrew due to financial constraints in preparing for the competitive season. Both decided to compete in 2011.
- One athlete withdrew due to personal problems in his marriage (divorce).
- One athlete developed pneumonia and had to withdraw.
- One athlete developed a severe back injury and had to withdraw for the 2010 season.

The remainder of the cohort consisted of the two athletes in the pilot study, as well as 12 more athletes, who all continued for the rest of the season. Twelve of the final cohort of 14 athletes finished the full protocol up to and including the third sampling session. One athlete had to withdraw after competing, but before the third sampling – this was due to pending surgery for hiatus hernia. The other athlete that could not complete the full protocol could not attend the third sampling session, as he was overseas for business at that time.

3.4 MEASUREMENTS

3.4.1 Participants

Once the athletes were randomly selected, each individual was contacted in person by the researcher. Contact numbers for each participant were supplied via the database of the IFBB SA.

During this first contact session, each participant's contact details, such as cell phone numbers, e-mail addresses, work telephone numbers and physical / postal addresses were obtained. The following documents were in due course posted to each participant:

- Information Document regarding Research Study
- Consent to participate in Research Study
- Non-disclosure / Confidentiality Agreement
- Copies of each of the three blood requisition forms, stating the address of the closest AMPATH facility either to their respective residential- or work address
- Copies of the Excel Spread sheets on which each athlete was to supply their weekly AAS substance doses
- An empty envelope, addressed to the researcher, with postage stamps included.

Once the athlete signed the consent forms and the confidentiality agreement, they were placed in the empty envelope provided by the researcher and returned.

On each occasion of blood sampling, the correct blood requisition forms were taken to the nominated AMPATH facility. The registered Professional Nurse acting as the venesectionist recorded each athlete's blood pressure – this was done in the sitting position after five minute's rest and before the blood was sampled. The blood pressure measurements were recorded by using a standardized mercury barometer, issued with the correct cuff size. All the necessary details were recorded on the correct blood requisition form. The form was then scanned into AMPATH's main computer for data capturing, as well as faxed to the researcher. Each athlete was asked to nominate his preferred method of returning the weekly Excel Spread sheets (fax / scan and e-mail / postage).

Registered Professional Trainers trained in the field techniques of skin fold measurements at any of the Virgin Active or similar facilities recorded the body weight and skin fold fat % measurements. Ideally biokineticists should have been used to conduct the skin fold measurements, but unfortunately not all participants had such a service available to them. Weight was measured by using electronic scales that were regularly calibrated. Body weight and body fat % measurements using skin fold fat thickness, are recognized field techniques in assessing body composition changes accurately. They are non-invasive and cost effective. (Wilmore, Costill, Kenney, 2008). Each athlete was asked to use the same Personal Trainer every time for the measurements in order to minimize individual inter-observer measurement error in taking the skin fold fat %. Skin fold fat % measurements were preferably taken at least 48 hours or more after the last intense leg training session in order to minimize false measurement due to subcutaneous water retention.

On the day of competition, every participant was weighed on the same calibrated electronic scale in accordance to the international standards set by the IFFB for weigh-in protocols. The same electronic scale was used on every competition event throughout the remainder of the competitive season. Ideally the second blood sample should have been taken at the time of the competition, when adverse effects probably would have been most pronounced, but this was impossible due to the following reasons:

- Not all classes competed on the same day or at the same venue, making the logistics of sampling difficult
- All athletes prepared for the competition backstage in the same dressing area; thus privacy was a potential problem
- The identity of the participants in the cohort was protected at all times – sampling blood backstage could have potentially exposed their participation in the present study.

3.4.2 Measurement protocol

Blood sampling and testing were provided by AMPATH. AMPATH is a SANAS accredited laboratory with accreditation number ISO 15189:2003. The blood sampling and testing were registered under Academic Projects: Dr R Barnard Steroids, project code: c55343.

PRE-CONTEST PHASE

Personal Trainers	Skin Fold Fat % Body Weight	Send to Researcher via preferred method
AMPATH	Blood Pressure Reading Fasting Blood Glucose Level Fasting Lipid Profile Full Blood Count 08h00 Serum Cortisol Level Liver Enzymes U&E (Creat)	AMPATH main frame ↓ Haematologist co-worker ↓ Fax to Researcher
Athletes Continues until day of competition	Excel Spread sheets	Send to Researcher via preferred method

↓

COMPETITION DAY

Personal Trainers	Body Weight Skin Fold Fat %	Send to Researcher via preferred method

↓

ONE WEEK POST-COMPETITION

Personal Trainers	Body Weight Skin Fold Fat %	Send to Researcher via preferred method
AMPATH	Blood Pressure Reading Fasting Glucose Level Fasting Lipid Profile Liver Enzymes Full Blood Count U&E (Creat)	Same as with pre-contest measurements

↓

FOUR TO SIX WEEKS AFTER COMPETITION

Personal Trainers	Body Weight Skin Fold Fat %	Same as above
AMPATH	Blood Pressure Reading Total Testosterone Level LH, FSH, Estradiol Level 08h00 Cortisol Level	Same procedure as above

The different testing methods and apparatus used by AMPATH Labs during the testing of the different blood samples and the reference ranges are listed below.

Test	**Instrument**	**Reference Range**
ALT	Modular P800	< 50 U/L
ALP	Modular P800	40 – 130 U/L
AST	Modular P800	< 38 U/L
Cholesterol	Modular P800	2.8 – 4.9 mmol/L
Cortisol	Modular E170	143 – 651 nmol/l
Creatinine	Modular P800	64 – 104 umol/L
FSH	Modular E170	1.4 – 13.6 IU/ml
LH	Modular E170	1.3 – 10.10 IU/ml
Estradiol	Modular E170	25 – 107 pmol/l
GGT	Modular P800	< 60 U/L
Glucose	Modular P800	3.9 - 6.0 mmol/L
HDL-Cholesterol	Modular P800	1.0 - 1.6 mmol/L
LDL-Cholesterol	Modular P800	1.6 - 2.9 mmol/L
SHBG	Modular E170	11.4 – 52.3 nmol/l
Testosterone	Modular E170	180 – 536 pmol/l
Triglycerides	Modular P800	0.5 - 1.6 mmol/L
Urea	Modular P800	1.7 - 8.3 mmol/L
HB	ADVIA 2120	13.0 – 17.0 g/dl
RBC	ADVIA 2120	4.50-5.50 x 10^12/l
HCT	ADVIA 2120	40.0 – 50.0 %

3.5 METHODOLOGICAL / MEASUREMENT ERRORS

Possible errors that could have been included into the study were:

1. Inter-observer measurement error with measuring of body fat %. This was minimized throughout the study by making use of accredited and registered Professional Trainers to obtain the measurements.

2. Subject drop out. Through continuous personal contact with all the athletes, they were continuously motivated to adhere to the protocol. Any problems that subjects

might have incurred that could have led to possible drop out were timeously discussed with the researcher.

3. Subject compliance with providing correct data. Poor compliance was minimized by clearly, in layman's terms, providing an information document that described how to fill in data sheets and providing a good infrastructure for collecting the data sheets from the subjects via fax, scan and e-mail or postage.

4. Subjects not 10 hours fasting before blood sampling. This was prevented by clearly explaining, in layman's terms, the protocol for fasting prior to blood sampling. This was done via the information documents supplied to the subjects, as well as during personal contact with the subjects.

5. Using volunteers as subjects. It was also taken into account that the study relied largely on the participation of volunteers. Volunteers might not have had adhered as strict to protocols, as what test subjects in a controlled environment would have done. Another problem that arose with the use of volunteers was that they could systematically differ from those persons that did not volunteer.

3.6 PILOT STUDY

For the 2010 season most competitions were run from August to mid-November. Usually most athletes would start their pre-contest preparations 12-16 weeks from the date of competition. A pilot study was thus run from the beginning of October 2009 to the end of January 2010. It was planned to only use two athletes for the pilot study and to include this obtained data in the main study, only if no adaptations to the protocol were needed. Subsequently both these participants' results were included into the final data recorded in a cohort totalling 14 athletes.

3.7 ANALYSIS OF THE DATA

Blood results were interpreted with the assistance of the two co-workers assisting with the research project.

Statistical analyses were done by the Department of Biostatistics, University of the Free State, South Africa. Results were summarised by mean and standard deviations. The collected data was captured onto Excel spread sheets and

forwarded to the Department of Biostatistics for further statistical analyses. A Wilcoxon Signed Rank Test for dependent, non-parametric interval data were used to test for significant differences between separate testing occasions for each variable. Where cases were missing within a data range, these participants' data were excluded from the analysis for that specific comparison.

3.8 LITERATURE SEARCH

A literature search was conducted with the use of Science Direct, EBSCO Host and Google Scholar.

CHAPTER 4
Results

4.1. INTRODUCTION

In this chapter, the results of certain physical measurements and blood tests of bodybuilders at various stages of the periodization cycle are presented. Data was captured over the full period of pre-contest preparation, according to the protocols described in Chapter 3, Section 3.4. The collected data was then processed in Excel.

Participants underwent blood tests and were physically measured on four different occasions. The first physical measurements and first blood tests were done at the onset of individual participant's pre-contest season. The second physical measurements were taken on the day of the competition. The third physical measurements and second blood tests were conducted one week after the competition. The fourth physical measurements and third blood tests were taken at six weeks after the competition.

It should be noted from the graphs within Chapter 4 that 14 athletes started the protocol, but one athlete could not contribute towards the data collection at one week, as well as six weeks post-competition (due to surgery) and that another athlete could not contribute towards the data collection at six weeks post-competition (due to being unavailable at the time of data collection). For this reason, the number of participants whose data is included in each graph will be provided for each respective graph. The results will be discussed under the headings of blood pressure, body composition and blood profiles.

4.2 BLOOD PRESSURE

Blood pressure readings were taken on three separate occasions as indicated in Figure 4.1

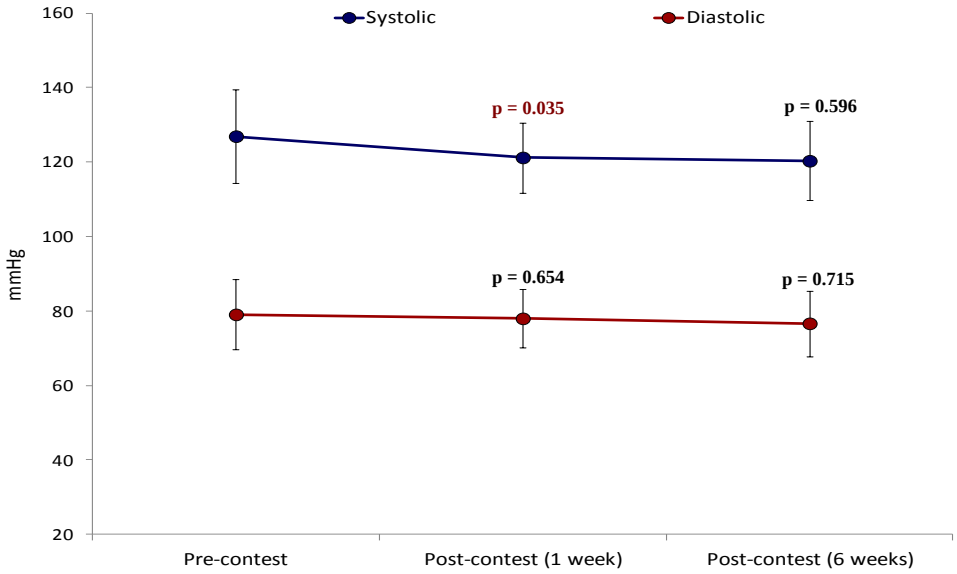
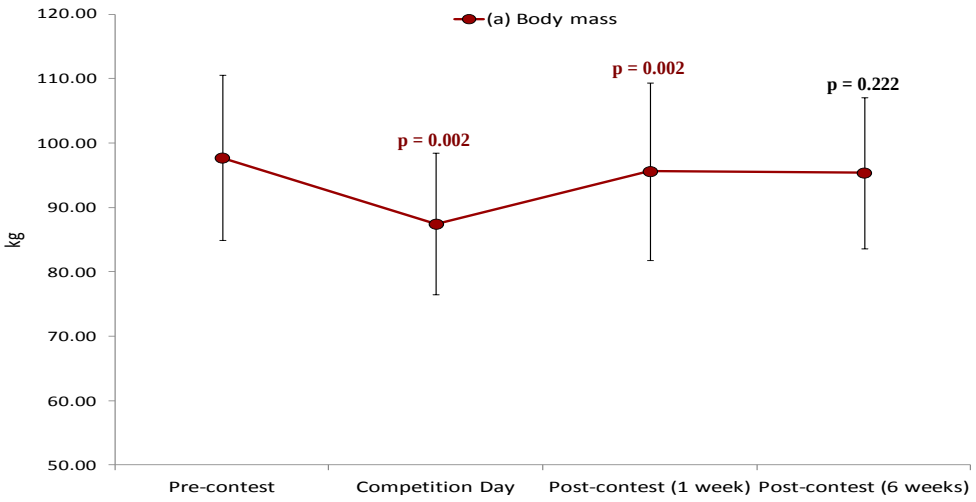


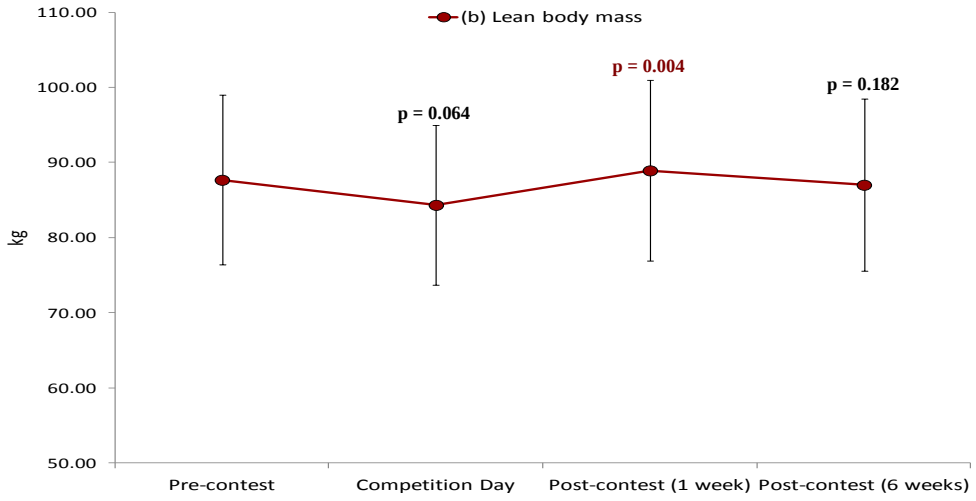
Figure 4.1 Average ± standard deviation of systolic and diastolic blood pressures at pre-contest (n = 14), 1 week after contest (n = 14) and 6 weeks after contest (n = 12).

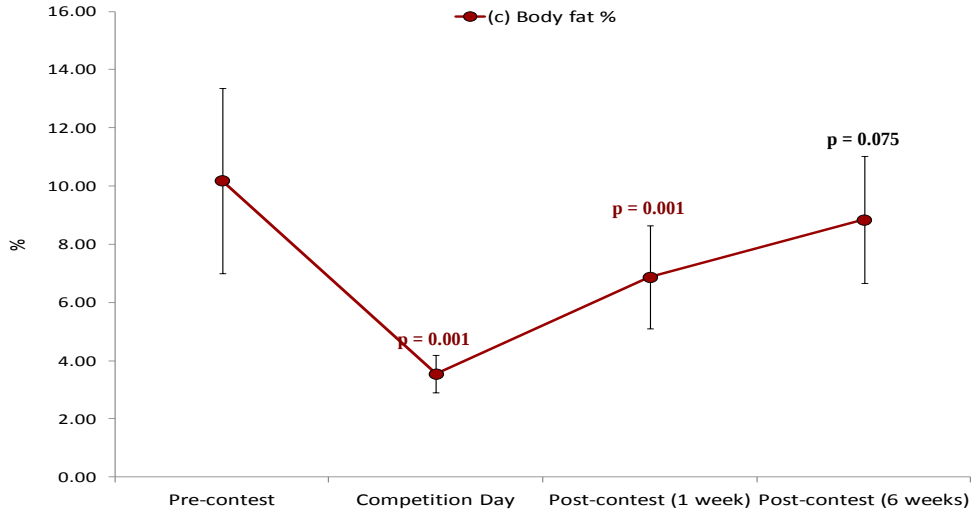
Figure 4.1 illustrates that the mean blood pressure readings for the cohort were normotensive at each day of data collection. There were two athletes who met the criteria for Stage I elevated blood pressure and both these athletes' readings were high (146/87 and 145/95 mmHg) only on the first occasion of data collection.

4.3 BODY COMPOSITION

Body mass and skin folds were measured to determine fat percentage, as presented in Figure 4.2 (a) – (c).







<u>Figure 4.2 (a) - (c)</u> Average ± SD for body mass, lean body mass and body fat percentage at pre-contest (n = 14), competition day (n = 13), 1 week after contest (n = 14) and 6 weeks after contest (n = 12).

Figure 4.2 (a) illustrates the change in the mean total body mass for the cohort over the competitive period. At the onset of the pre-contest season the average body mass for the cohort measured 97.71 ± 12.84 kg. On competition day, the average body mass decreased to 87.45 ± 11.00 kg. One week after the competition day, the average body mass for the cohort increased rapidly to 95.61 ± 13.77 kg. At the next measurement 5 weeks later, the average body mass for the cohort stabilised at 95.38 ± 11.72 kg. There was a statistically significant drop in the mean pre-contest body mass compared to the measurements on competition day ($p = 0.002$). The mean body mass loss was significantly regained to close to mean pre-contest values ($p = 0.002$) – however in a much shorter time interval of only one week. Thereafter the body mass stayed stable with very little increase over the following five-week period.

Figure 4.2 (b) illustrates the change in the mean lean body mass for the cohort over the competitive season. The drop in lean body mass over the pre-contest period was much less compared to the comparative drop of the mean body mass during the same period, but still statistically significant. This was then followed by a sudden statistically significant gain in lean body mass over a relatively short period of 7 days post-competition on average to a level higher that at the beginning of the pre-contest period ($p = 0.004$). The following 5 weeks were characterised by a gradual drop in lean body mass back to a level just above that of the beginning of the pre-contest season.

Figure 4.2 (c) illustrates the change in mean body fat percentage over the competitive season. At the onset of the pre-contest period, a mean value of 10.2 % was recorded for the cohort. This was followed by a gradual decrease in fat percentage to reach very low mean levels of 3.5 % on the competition day. These changes were statistically significant ($p = 0.001$). A sudden sharp increase in fat percentage occurred over the next 7 days after the competition day to settle at mean measurement of 6.9 %. This increase was also statistically significant ($p = 0.001$). Over the following 5 weeks, only a further gradual increase was documented with the mean body fat percentage for the cohort measured at 8.8 % average. At the 6-week post-contest period, the cohort's mean body fat percentage was still lower than at the onset of the pre-contest period.

4.4 BLOOD PROFILES

Several blood profiles of the participants in the cohort were measured on three different occasions, in order to assess the effects of AAS consumption on different body systems.

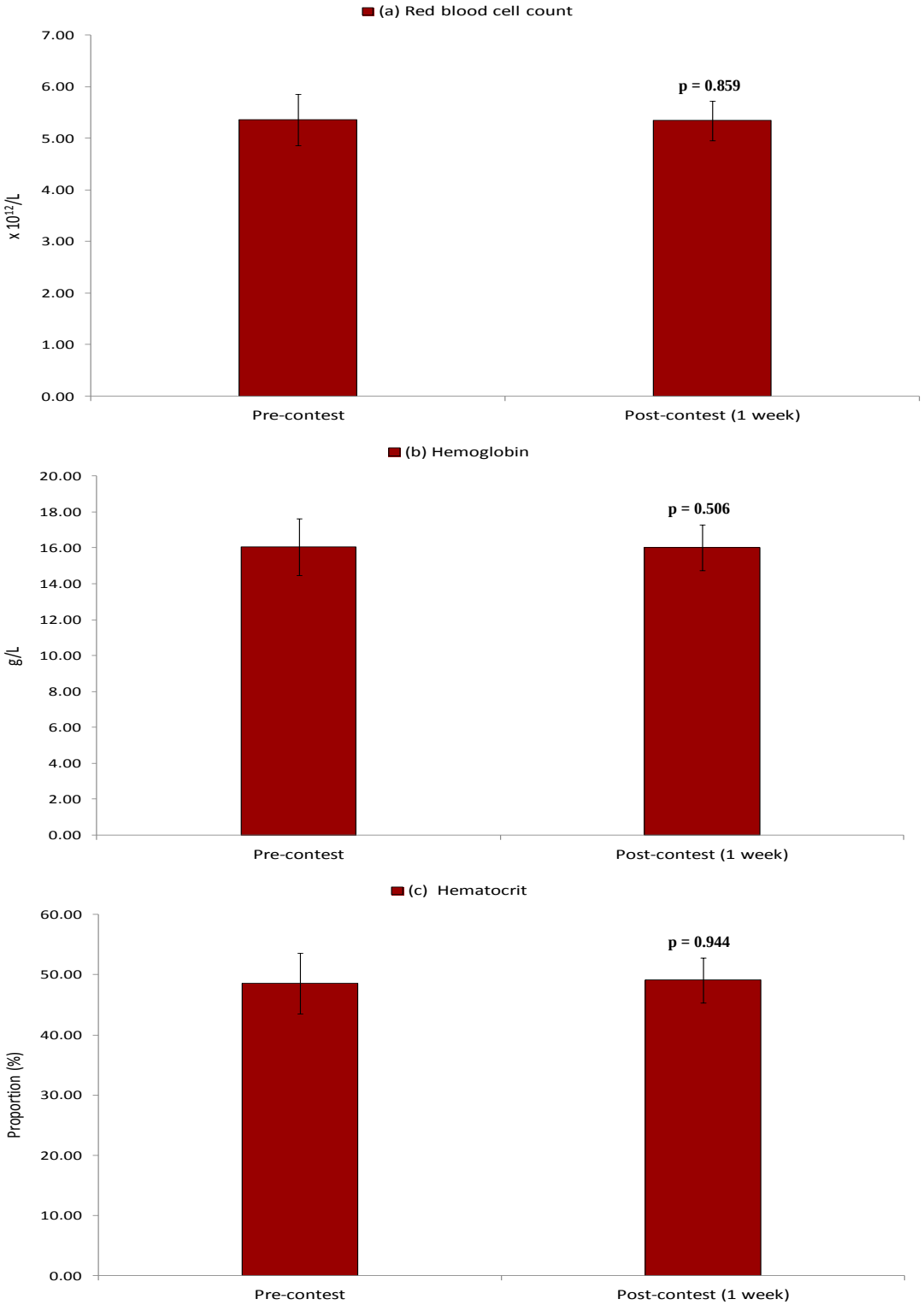
4.4.1 Full blood count

Only changes in three of the Full Blood Count parameters were noted, namely Red Blood Cell Count, Haemoglobin and Haematocrit. These changes are associated with the effects of AAS on erythropoiesis.

Figure 4.3.1 (a) illustrates the changes in the mean red blood cell count for the cohort, measured on two different occasions. The mean pre-contest value measured 5.30 x 10*12 ± 0.50/l, with the one-week post-competition value being 5.34 x 10*12 ± 0.38/l. These measurements did not show any statistically significant changes.

Figure 4.3.1 (b) illustrates the changes in the mean haemoglobin values for the cohort, measured on two different occasions throughout the competitive season. The mean pre-contest value was recorded at a value of 16.06 ± 1.57 g/dl and the one-week post-competition value 16.01 ± 1.27 g/dl. These two measurements did not show any statistically significant differences either.

Figure 4.3.1 (c) illustrates the changes in the mean haematocrit values for the cohort, as measured on two different occasions. The mean pre-contest value measured 48.54 ± 5.04 % and the one-week post-competition value measured 49.09 ± 3.73 %. There was no statistically significant difference between these two measurements.



<u>Figure 4.3.1 (a) - (c)</u> Average ± standard deviation for red blood cell count, haemoglobin and haematocrit at pre-contest (n = 14) and 1 week post-contest (n = 13).

4.4.2 Fasting glucose

Fasting blood glucose levels were investigated in order to establish the effect of AAS consumption on carbohydrate metabolism and the risk of developing possible Diabetes Mellitus Type II glucose intolerance.

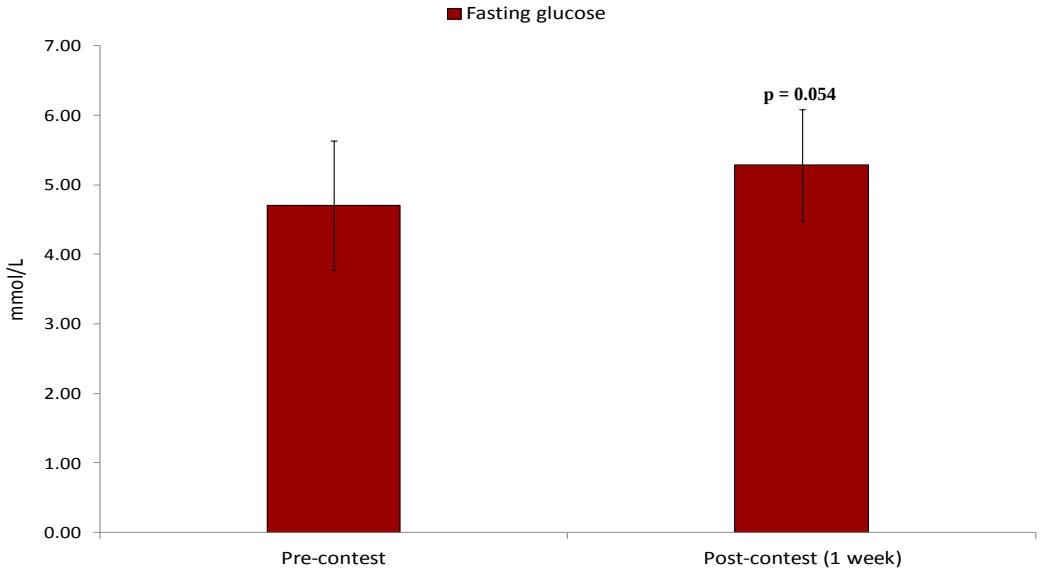


Figure 4.3.2 Fasting blood glucose levels at pre-contest (n = 14) and 1 week post-competition (n = 14).

Figure 4.3.2 illustrates the changes in mean fasting blood glucose levels, as tested on two different occasions. The mean pre-contest glucose level for the cohort was 4.70 ± 0.93 mmol/l and the one-week post-competition mean fasting blood glucose value was measured 5.29 ± 0.80 mmol/l (SD = 0.80 mmol/l). However, the difference in the mean values on the two different measurement occasions was not statistically significant (p = 0.054). However, the clinical significance of these changes will be discussed in chapter 5.

4.4.3 Lipid profiles

Full blood lipid profiles, consisting of Total Cholesterol level, Triglyceride level, HDL level and LDL level, were taken on two separate occasions. These were collected after a preceding 10-hour fasting period.

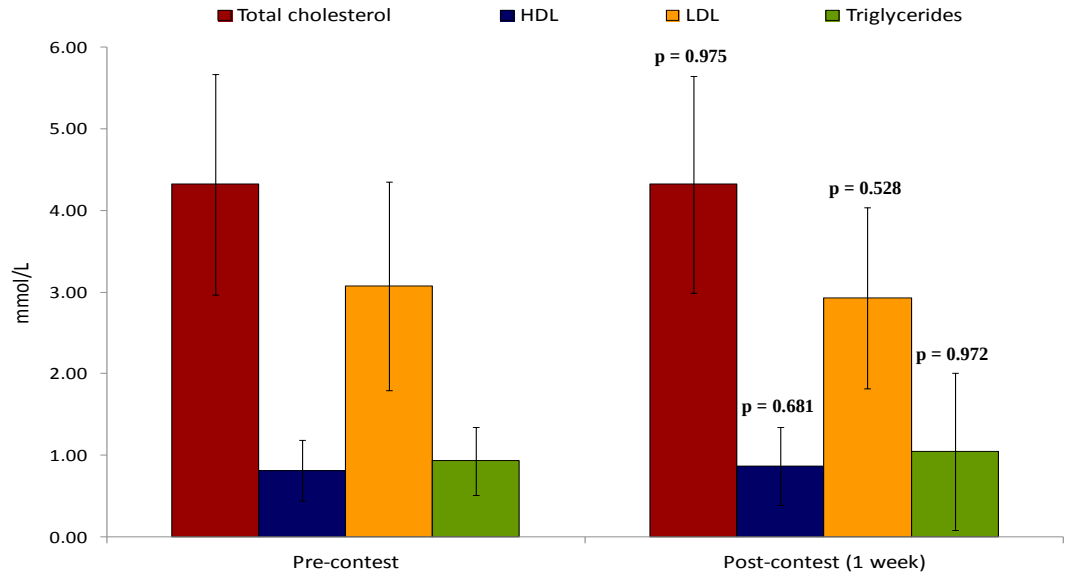


Figure 4.3.3.1 Total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides at pre-contest (n = 14) and 1 week post-contest (n = 14).

Figure 4.3.3.1 illustrates the change in the mean lipid profile for the cohort over a competitive season. The mean Total Cholesterol levels for the cohort were unchanged from the onset of the pre-contest values compared to that of the post – competition values (4.32 mmol/l). The mean HDL level showed an increase of average 6.2 % from the pre-contest period to the post-competition period (0.81 mmol/l vs. 0.86 mmol/l), even though the mean HDL levels for the cohort were below that of the normal range lower level. The mean LDL levels were increased at the onset of the pre-contest period with average for the cohort of 6.2 % above the upper range value (2.9 mmol/l). The mean LDL decreased over the pre-contest period to an average equal to that of the upper range levels (3.08 mmol/l vs. 2.93 mmol/l), thus averaging a decrease of 5.1 %. There was an increase of 11.2 % in the mean Triglyceride levels from the pre-contest period to post-competitive period (0.93 mmol/l vs. 1.04 mmol/l). These changes in mean values were still within the normal range of Triglyceride levels. The changes in mean total cholesterol, HDL,

LDL and triglyceride levels were noted but not statistically significant. The clinical significance of these changes will be discussed in chapter 5.

The Total Cholesterol/HDL ratio was used in risk stratification for cardiovascular disease. Values of between 4 and 5 were stratified as moderate risk and values above 5 stratified as high risk for cardiovascular disease.

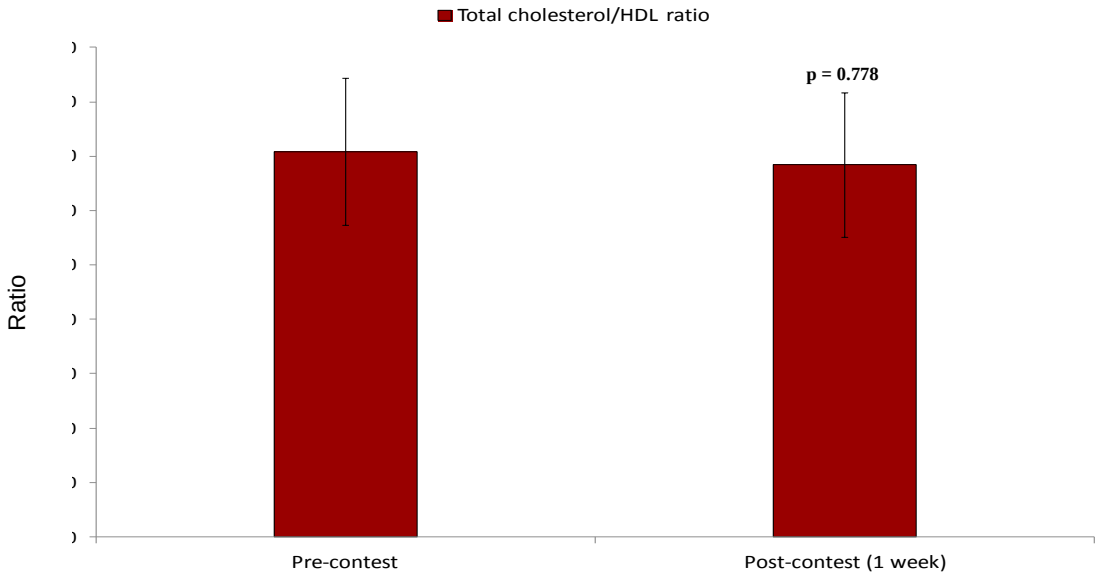
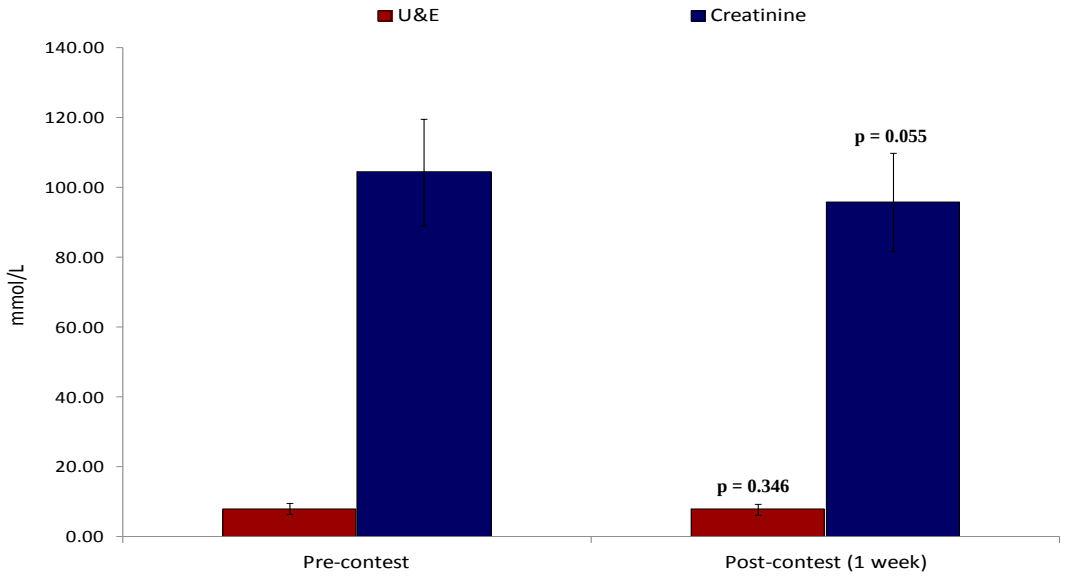


Figure 4.3.3.2 Average ± standard deviation for the Total Cholesterol to high-density lipoprotein (HDL) ratio at pre-contest (n = 14) and 1 week post-contest (n = 14).

Figure 4.3.3.2 illustrates the mean average Total Cholesterol to HDL ratio for the cohort over the competitive season. On both occasions of measurement, the values stratified into the high-risk group for cardiovascular disease. However, the ratio showed a decrease from the pre-contest to the post-competition period of average 3.65 % (7.09 vs. 6.84). These changes were not statistically significant, but may have clinical significance.

4.4.4 Kidney function

Urea, electrolytes and creatinine levels were obtained to evaluate kidney function on two different occasions. Minimal changes in electrolytes, of which none were pathological, were seen within the whole cohort. Kidney function changes (hydration status) were thus related to urea and creatinine changes.



<u>Figure 4.3.4</u> Average ± standard deviation for urea & electrolytes (U & E) and creatinine at pre-contest (n = 14) and 1 week post-contest (n = 14).

Figure 4.3.4 illustrates the changes in the mean urea and creatinine levels for the cohort over the competitive season. Statistically there were non-significant changes to the urea levels between the pre-contest and post-competition period (mean average decrease of 2 %). Even though the mean pre-contest creatinine levels for the cohort were equal to the upper limit of normal range (104 mmol/l), 6 participants had levels increased above the normal range upper limit. At the post-competition measurement, the mean creatinine value for the cohort decreased by 8.9 % to 95.86 mmol/l, despite 3 participants still having values above the normal range upper limit. However, these changes were not statistically significant (p=0.055).

4.4.5 Liver enzymes

Liver enzyme testing was conducted over the pre-contest season, until the first week after the competition. The effects of exercise and AAS on liver enzymes were evaluated by the testing of the 4 enzymes listed below in Figure 4.3.4.

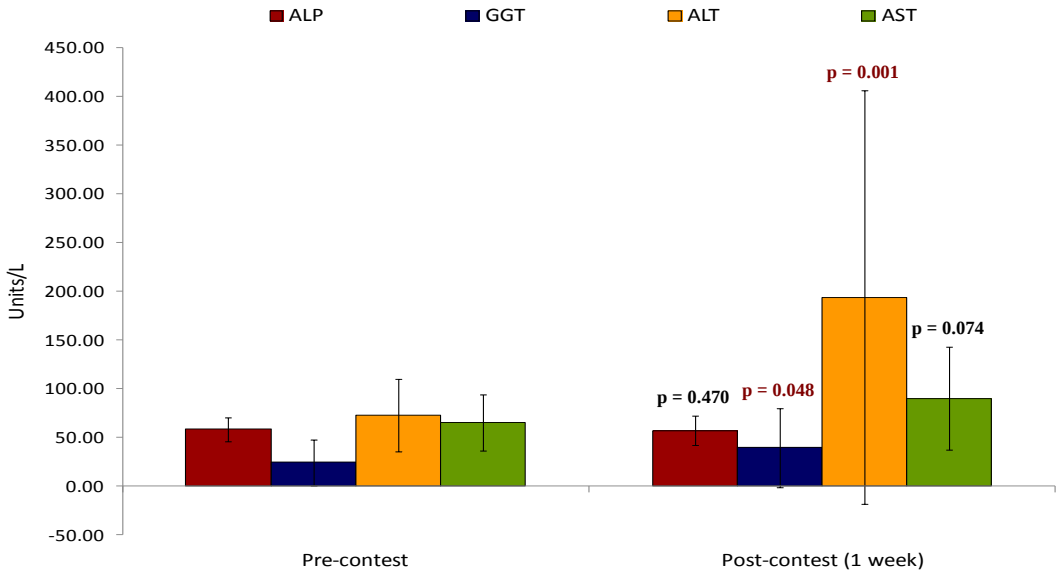


Figure 4.3.5 *Average ± standard deviation for Alkaline phosphatise (ALP), gamma-Glutamyltransferase (GGT), Alanine aminotranferase (ALT) and Aspertate aminotransferase (AST) at pre-contest (n = 14) and 1 week post-contest (n = 14).*

Figure 4.3.5 illustrates the changes in the mean values of ALP, GGT, ALT and AST, across the whole cohort for the period ranging from pre-contest to one week after the competition. The ALP values showed a statistically non-significant decrease of 2 % in pre-competition to one-week post-competition values (58 U/l vs. 56.86 U/l). However, on both occasions, the values were within the normal range. The mean GGT levels for the cohort showed a statistically significant increase (p = 0.048) of 60.96 % (24.36 U/l vs. 39.21 U/l), even though these values were still less than the upper limit normal range. One participant had an increase in GGT levels at the onset of the pre-contest phase, which then returned to within normal range at one-week post-competition measurements. Only one athlete in the cohort showed severely increased levels of GGT at the one-week post-competition measurements.

ALT levels showed highly significant increases for most of the participants in the cohort (p = 0.001). The mean ALT level at onset of the pre-contest phase (72.36 U/l), showed an increase of 44.7 % above the upper limit of normal range (50 U/l). The mean values at the post-competition measurement were 286 % higher than compared to the mean onset values, and 387.86 % higher than the upper limit of normal range (72.36 U/l vs. 193.93 u/L and 50 U/l vs. 193.93 U/l). One particular participant recorded post-competition levels of 772 U/l – 15.44 times higher than the upper limit normal range.

Mean AST levels showed a similar trait to that of the ALT values. Most of the cohort's participants had higher AST levels than the upper limit normal range at all three measurement times. The mean AST level at the onset of the pre-contest period, measured 65.21 U/l, which was 71.6 % higher than the upper limit normal range (38 U/l). There was a subsequent increase in mean AST value of 37.9 % compared to the initial mean value, but showed an increase of 236.65 % when compared to the upper limit normal range (89.93 U/l vs. 65.21 U/l and 89.93 U/l vs. 38 U/l).

4.4.6 Hormonal profiles

Cortisol levels were measured in order to evaluate the changes in the adrenal function of the cohort. Measurements were taken precisely at 08h00 for all participants in the cohort, on both occasions.

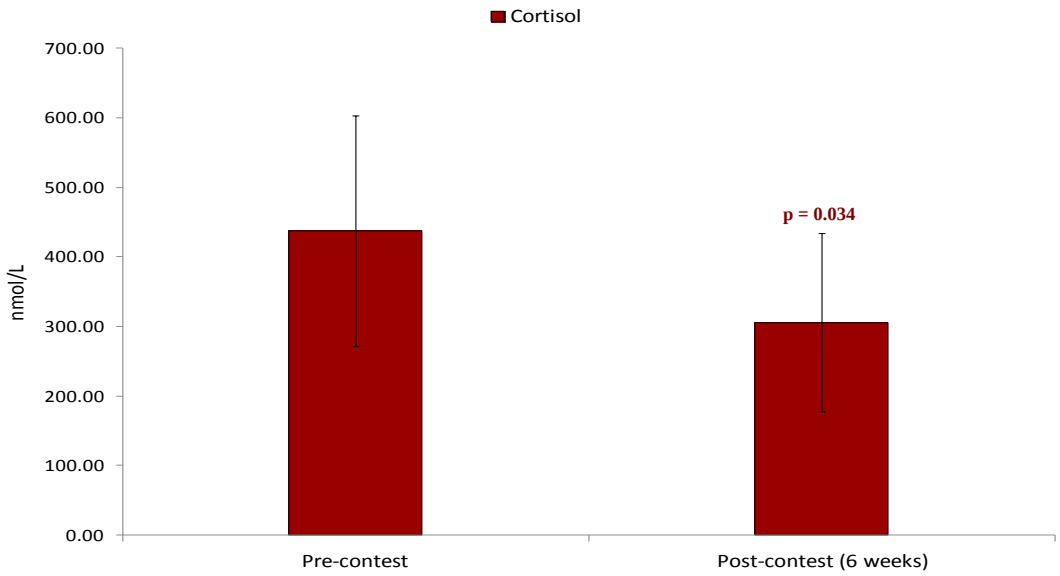
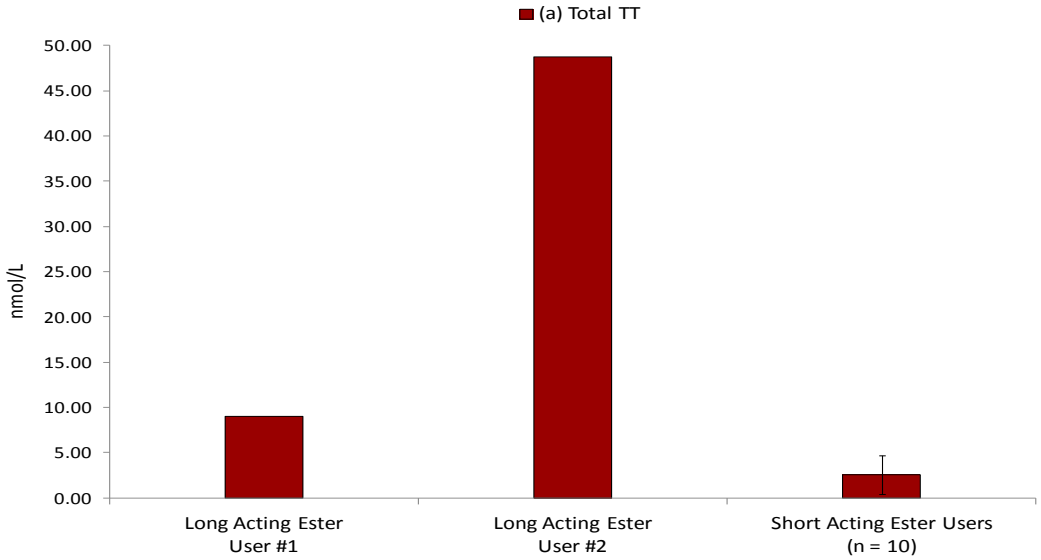


Figure 4.3.6.1 Average ± standard deviation for 08h00 cortisol levels at pre-contest (n = 14) and 6 weeks post-contest (n = 12).

Figure 4.3.6.1 illustrates the changes in the mean cortisol levels for the cohort over the competitive season. On both occasions of measurement, both mean values were within normal range (143-651 mmol/l). There was a statistically significant (p = 0.034) mean value decrease of 43 % from the pre-contest measurement compared to the six-week post-competition measurement (437.36 mmol/l vs. 305.75 mmol/l). Only two participants showed increase values above the norm at the onset of the pre-contest period – this was probably secondary to recent surgical interventions.

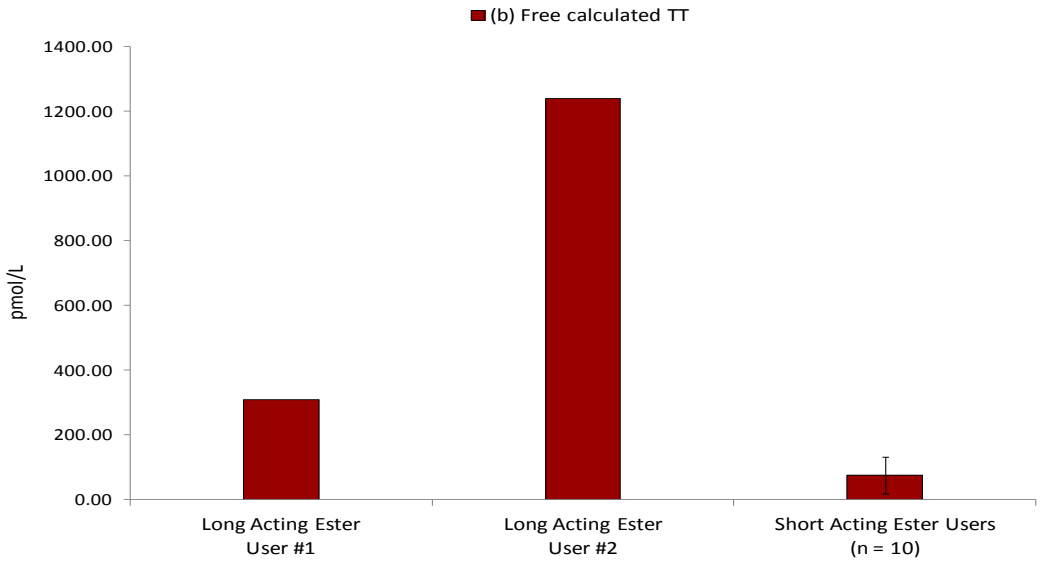
Two participants showed values lower than the lower limit normal range, at the post-competition measurement.



<u>Figure 4.3.6.2 (a)</u> Total Testosterone profiles of long acting (n = 2) and short acting (n = 10) ester users at 6 weeks post-contest.

Figure 4.3.6.2 (a) illustrates the mean value of total testosterone for the cohort. The hormone profiles were investigated on only one occasion, that being six (6) weeks post-competition. The reason for this was that most of the participants were either on AAS at the start of the pre-contest period, or had just finished a previous course of AAS recently. That would have rendered a wide array of different results. All participants uniformly abstained from using any AAS in the 6 weeks following the competition.

The mean value for total testosterone was 6.93 mmol/l. Two participants used long-acting AAS around competition, which were probably still active at the time of testing. Subsequently, their higher total testosterone values increased the cohort's overall mean average. These values were excluded to calculate the mean average for the remaining measured participants, a uniform group with a history of using short-acting ASS around competition. The mean serum testosterone level was then 3.14 mmol/l, which was much less than the lower limit normal range (8.0 mmol/l – 27 mmol/l), thus confirming the expected hypogonadism status of the cohort 6 weeks after the cessation of AAS abuse.

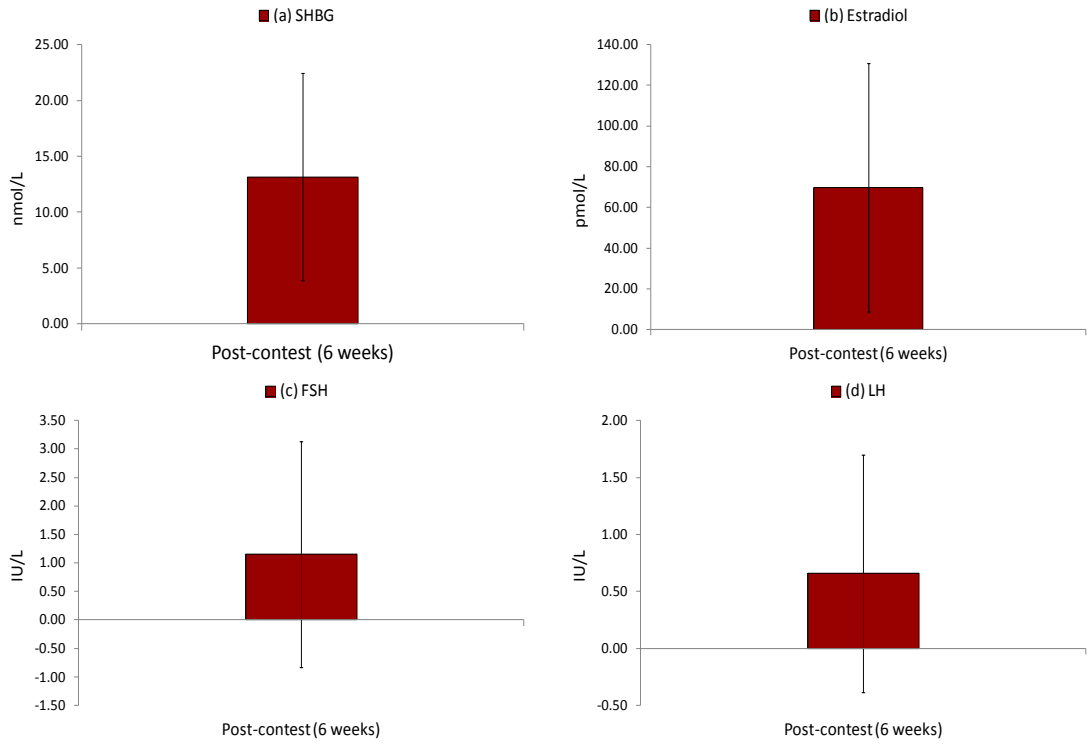


<u>Figure 4.3.6.2 (b)</u> Free calculated Testosterone profiles of long acting (n = 2) and short acting (n = 10) ester users at 6 weeks post-contest.

Figure 4.3.6.2 (b) illustrates the mean free calculated testosterone levels for the cohort. The mean value was 190.75 pmol/l, being just above the lower limit normal range of 180 pmol/l. Again, excluding the two participants on the long-acting AAS, the average value for the remainder of the uniform group of participants on short-acting esters, would have been 95.36 pmol/l, statistically much lower than the lower limit normal range (180 pmol/l).

Figure 4.3.6.3 (a) illustrates the mean average value of SHBG for the cohort. The normal range for this investigation is 11.4 nmol/l – 52.3 nmol/l. The mean value for the cohort was 13.15 nmol/l. Again in this measurement, 8 participants had very low levels statistically lower than the lower limit normal range, but 3 participants had average high levels of SHBG, thus increasing the mean value to within the lower quadrant of normal range.

Figure 4.3.6.3 (b) illustrates the mean estradiol level for the cohort. The normal range for this investigation is 25 pmol/l – 107 pmol/l. The mean value for the cohort was 83.80 pmol/l. Only one participant in the cohort presented with an increased level of estradiol (235 pmol/l).



<u>Figure 4.3.6.3 (a) – (d)</u> Average ± standard deviation sex hormone-binding globulin (SHBG), estradiol, follicle stimulating hormone (FSH) and luteinizing hormone (LH) at 6 week post-contest (n = 12)

Figure 4.3.6.3 (c) illustrates the mean value for FSH for the cohort. The normal range for this investigation is 1.4 IU/l – 13.6 IU/l. The mean value for the cohort was 1.15 IU/l, confirming the hypogonadotrophic status of the cohort in general. Only 3 participants had values within the normal range, but at the lower levels of normal range. The remainder of the cohort had levels varying from extremely low to immeasurable.

Figure 4.3.6.3 (d) illustrates the mean value for LH for the cohort. The normal range for this investigation is 1.3 IU/l – 10.10 IU/l. The mean value for the cohort was 0.72 IU/l, again confirming the hypogonadotrophic status of the cohort in general. Again only 3 participants had measurement values within normal range, with the remainder of the cohort having extremely low or immeasurable values.

This Chapter presented the results of the mean measurements for the different blood assays assessed over the competitive season and will be discussed further in Chapter 5.

CHAPTER FIVE

Discussion

The present study has the disadvantage of small cohort size, but knowing that the opportunity to study such a secluded group of athletes will not be readily repeated, the findings were worth recording.

5.1 BLOOD PRESSURE CHANGES

The mean blood pressure readings for the cohort were normotensive at each of the data collection occasions. Individually, only two athletes showed elevated levels of blood pressure, falling into the Stage I category of Hypertension, of which one athlete had elevated systolic pressure and the other both elevated systolic and diastolic blood pressure. According to the literature (Ferenchick et al, 1995), the effects of Androgenic-Anabolic Steroids (AAS) on arterial blood pressure are not very clear. According to Ferenchick, most studies showed that the response was dose-dependent and usually only associated with isolated increases in diastolic blood pressure. It has been postulated in the literature that the increased diastolic pressure is secondary to increases in blood volume, viscosity, fluid retention, or even a combination of all of these factors (Ferenchick et al, 1995).

Results from the present study suggest the opposite to what has been found in other studies in the literature search (Ferenchick et al, 1995; Kuipers, 1998). There was a definite tendency for the mean systolic blood pressure to decrease over the length of the study, despite the abuse of varying amounts of supra-physiological doses of AAS. The average systolic pressure for the cohort at the onset of the study was 127 mmHg, at one week after the competition 121 mmHg and at the 6 weeks after the competition 120 mmHg. The average diastolic pressure varied very little for the same measurement intervals (79 mmHg; 78 mmHg and 77 mmHg respectively). Usually, the diastolic blood pressures would normalize within 6-8 weeks of AAS abstinence (Kuipers, 1998), independent of the number of previously used courses of AAS (Hartgens et al, 1996).

Meta-analyses on the effects of exercise on the improvement in hypertension, concluded that the estimated decreases in systolic blood pressure of 6-8 mmHg and diastolic blood pressure of 5-6 mmHg could be expected at doses of exercise of optimally 90 minutes per week at intensities of 40-70 % of age predicted maximal

heart rate (Standards of Medical Care in Diabetes, 2009). This is in keeping with the findings from the present study. Therefore, the lack in elevated blood pressures presented by the participants from the present study may potentially be as a result of their pre-contest training regimes which typically consist of as much as 60-90 minutes of high intensity aerobic exercise daily for the last six weeks prior to the competition. These high doses of aerobic exercise could well have counteracted the expected blood pressure elevating side effects of the supra-physiological doses of AAS generally abused by body builders.

Competitive body builders abuse diuretics for only short periods of times, generally administering a variety of diuretics, starting from 72 hours before the competition and continuing to just a few hours before competing. In the present study, no blood pressure readings were taken on the competition day, thus nullifying the blood pressure lowering effects that diuretic abuse could have had on the cohort's subsequent post-competition blood pressure readings.

5.2 BODY COMPOSITION CHANGES

At the onset of the pre-contest season the average body mass for the cohort measured 97.71 $\pm$ 12.84 kg. The cohort thus included participants ranging vastly in body mass due to the different competitive classes. On the competition day the average body mass decreased significantly ($p = 0.002$) to 87.45 $\pm$ 11 kg (Annexure A). Thus the average body mass loss over the pre-contest phase was 10.26 kg. This differs from the findings from the study done in 1995 by Andersen et al, who demonstrated a mean weight loss of only 6.8 kg over the competitive season. This discrepancy may be attributed to the cohorts of the Andersen study and the present study differing in the number of participants entering the heavier competitive categories, lending itself towards the possibility that the heavier athletes had greater potential to lose more weight while decreasing their skin fold fat % accordingly.

One week after the competition day, the average body mass for the cohort increased rapidly to 95.61 $\pm$ 13.77 kg – an average increase of 8.16 kg in only 7 days, which was found to be statistically significant ($p = 0.002$). Andersen and colleagues showed an average post-contest body mass increase of 6.2 kg. In addition, 85 % of the participants measured weight gains directly after the competitions, while 46 % reported binge eating (Andersen et al, 1995). One participant from the present study had a decrease in body mass, while the rest of

the cohort displayed statistically significant (p = 0.002) body mass gains (Annexure A). The rapid gain in body mass may potentially be attributed to binge eating, which was reported by approximately halve of the participants in the present study, corresponding well to the findings from Andersen and colleagues. Despite binge eating being a prudent explanation for the rapid body mass gains in some of the participants, the abuse of diuretics, evidenced by some participants disturbed renal functions, also played a role in the weight loss and that the following rapid rehydration and plasma volumisation during the first week after competitions, are contributing factors to the rapid weight gains (Andersen et al, 1995).

At six weeks post-competition, the cohort's average body mass stabilised with very little changes in measured weight. The results showed similar decrease and increase trends in the average lean mass for the cohort up to the end of the first week post-competition, though these changes were less prominent than compared to the body mass changes. The only significant (p = 0.004) change were noted following the first week after competition day, with the participants presenting a significant increase in lean body mass to levels higher than the pre-contest average. However, this increase was not retained, evidenced by the gradual drop in lean body mass over the following 5 weeks to return to levels close to that of the beginning of the pre-contest season. Twelve of the 14 participants abused short-acting AAS esters with very short half-lives (t½) – thus leading to very little anabolic support, especially towards the end of this period (Annexure A). Similar results to the current findings could nowhere be found in the literature search. The two athletes, who did use the longer-acting esters, maintained their respective lean mass gains much better over this period (see Annexure A for results on athlete numbers one and fourteen, as well as their respective recorded AAS cycles in Annexure B).

The mean body fat percentage decreased significantly (p = 0.001) over the period leading up to the competition, with the average decrease for the cohort being 6.7 %. A very sudden, statistically significant (p = 0.001) increase in mean body fat percentage of 3.4 % occurred in the first 7 days following the competition. No explanation for this phenomenon could be found in the literature. The author postulates that this sudden increase in skin fold fat % might have been caused by the accumulation of water in the subcutaneous fat tissue compartment. The following 5 weeks showed a further gradual increase of 1.9 % in the mean body fat percentages. The abuse of AAS can significantly increase the body mass and

muscle circumferences in male athletes, only if they satisfy certain criteria concerning the timing of doses and nutritional factors (Haupt and Rovere, 1984). Increases in muscle size and fat-free mass resulting from AAS abuse are highly dose-dependent and show linear correlation well with serum testosterone levels (Bhasin et al, 1996). During periods of hypogonadism, there is a significant reduction in fat-free mass, with a subsequent increase in total fat mass, as well as a decrease in fractional muscle protein synthesis (Maurus et al, 1998). Similar findings were seen in 12 of the 14 participants in this study – directly related to their hypogonadotrophic hypogonadodistic status, caused by their abuse of short-acting esters around competition time (Section 4.4.6 and Annexures A and B). Fractional protein synthesis will return to normal once replacement dosages of testosterone are administered (Brodsky et al, 1996).

The body composition changes are also influenced by graded testosterone doses. Bhasin and colleagues, as well as Sing and colleagues, both conducted studies on body composition changes with varying testosterone doses administered weekly (Bhasin et al, 1996; Singh et al, 2002). The average fat-free mass increased (125 mg + 3.4 kg; 300 mg + 5.2 kg and 600 mg + 7.9 kg) in accordance to increasing testosterone doses. This also correlates well with what is seen anecdotally amongst the general body builder population – the greatest increases in muscle size are mostly seen with testosterone doses beyond 600 mg per week (Sinha-Hikim et al, 2002). However, these findings do not correlate well with the results measured in the present study's cohort. The doses of testosterone reported used anecdotally by participants in the present study, varied widely from 300 mg weekly to in excess of 1000 mg weekly (Annexure B). Despite these high doses, lean mass decreased on average by 3.34 kg during the pre-contest period of several weeks, just to increase again rapidly with average of 4.59 kg in the 7 days following the current cohort's competition days (Annexure A). In both the Bhasin- and Singh studies, energy and protein intake were standardized adequate enough to promote anabolism. In the current study, this was impossible to achieve, as every participant followed his own calorie intake and exercise (aerobic and resistance training) plan in order to achieve very low body skin fold fat percentages.

The changes in the body composition of the present study's cohort thus clearly differ from other studies where energy and protein intake, as well as testosterone and exercise doses, were standardized. Differences in findings may have resulted from

the fact that the participants from this study obtained their (different) steroids from illicit sources.

5.3 HAEMATOLOGICAL CHANGES

In the present study, the only consistent changes noted in the cohort's Full Blood Count measurements, were the AAS associated effects on the Red Blood Cell Count, Haemoglobin and Haematocrit. Even after lengthy exposure to supra-physiological doses of AAS, only minimal changes in these three parameters were noted. None of these changes showed any statistical significance.

Based on previous research where increased Haematocrit values correlated strongly with increased cardiovascular risk and increased mortality, it was expected that increases in Haematocrit values would be present and persist for extended periods even after the cessation of AAS abuse (Gagnon et al, 1994). Haemoglobin values can return to normal only after 5-6 months (Nieminem et al, 1996) and normal red blood count and thrombocytes only after one year (Urhausen et al, 2003). However, none of these tendencies were noted amongst the participants in the present study's cohort.

As no blood tests were conducted on the day of the competition, the influence that the preceding short-term diuretic abuse would have had on the Haematocrit values of the cohort, were nullified.

5.4 CARBOHYDRATE METABOLISM CHANGES

Fasting blood glucose levels were measured on two different occasions, the initial measurement at the onset of the pre-contest season in order to establish a mean baseline, and the second measurement one week after the competition, in order to establish the effect of lengthy exposure to supra-physiological doses of AAS on carbohydrate metabolism. The difference in the mean values on the two different occasions was not statistically significant ($p = 0.054$), since the mean increase between the two values was only 0.59 mmol/l. During the initial sampling only 2 of the 14 participants (both with blood glucose of 6.1 mmol/l) presented with fasting blood glucose levels fulfilling the criteria for pre-diabetic status (5.6 - 6.9 mmol/l). One of these participants continued to have raised fasting blood glucose levels at the time of the second measurement (5.8 mmol/l), while 2 more participants

presented with measurements between 5.5 – 6.9 mmol/l, and one more candidate with measurements fulfilling criteria for diabetic status (7.1 mmol/l) (Annexure A). It should however be noted that these measurements were isolated measurements and were not recorded along with the symptoms of Diabetes Mellitus. The criteria for Diabetes Mellitus include symptoms or repeated testing, ideally confirmed by a Fasting Blood Glucose Tolerance Test.

Due to the disturbed liver function after AAS abuse, carbohydrate metabolism can be influenced, leading to Type II Pre-diabetic status (Cohen et al, 1986). A further risk factor for AAS associated pre-diabetic status, is adults with a BMI of $>$ 25, combined with HDL levels of $\leq$ 0.9 mmol/l (Standards of Medical Care in Diabetes, 2009). All the participants in the cohort had BMI $>$ 25, with 10 of the 14 participants presenting with HDL $\leq$ 0.9 mmol/l (mean average = 0.86 mmol/l) at the time of the second measurement (Annexure A). Thus, 71.4 % of the participants were at higher risk for developing pre-diabetic status, but not all of them progressed to such. In the prevention of diabetes, any program that incorporates weight loss (5-10 % of body weight) and that delivers physical activity (aerobic and resistance training) of $>$ 150 minutes per week, will increase insulin sensitivity and lead to lower incidence of pre-diabetic status (Standards of Medical Care in Diabetes, 2009). However, despite the fact that all the participants in the cohort adhered to such a program, some participants still displayed an increased risk for pre-diabetic status. It is unclear whether this observation was solely due to the known side effects of AAS (Snyder, 2008), as other participants did not show the same tendency (Annexure A). Consequently, in the present study, the degree to which AAS abuse contributed to the development of pre-diabetic status in some participants is unclear and needs future investigation.

5.5 LIPID PROFILE CHANGES

The mean Total Cholesterol levels for the cohort were unchanged over the two separate sampling occasions. Although the mean HDL levels showed an increase of 6.2 % over the pre-contest period, the mean post-competitive measurement was below the normal range lower level. Average LDL levels (3.08 $\pm$ 1.28 mmol/l) above normal values (1.60 – 2.90 mmol/l) were measured at the onset, but this showed a decrease of 5.1 % at the time of the second sampling, equalling the upper level of

normal range. The mean Triglyceride levels showed an 11.2 % increase, though this was still within the normal range parameters. The changes in the mean values for the different lipid profile components were statistically insignificant, but do have clinical relevance.

Thompson showed in 1989 a decrease in HDL levels and an increase in the LDL levels when the 17-alpha-alkylated AAS were administered orally, as well as a decrease of ≥ 15 % in HDL levels being a strong predictor of increased risk for coronary heart disease (Snyder, 2008). Earlier studies on the effect of AAS abuse on lipid profiles were conducted on cohorts consisting of power lifters or body builders that were not in pre-contest diet phases (Cohen et al, 1986). The results subsequently showed increases in Total Cholesterol, decreases in HDL and variable changes in LDL levels. However, the precise effect of AAS abuse on LDL levels is not known (Kuipers, 1998).

When the greater deal of exercise performed consists of aerobic exercise, the cholesterol increasing side-effects of AAS abuse is counter-balanced by an exercise-induced decreasing effect, which may result in unchanged or even decreased total cholesterol levels (Kuipers, 1998), as was the case in present cohort being studied. The participants would generally engage in training protocols consisting of aerobic training of 45-90 minutes per day, with resistance training sessions rarely more than 60-75 minutes per day on average.

Aerobic exercise alone however does not seem to be able to offset the AAS induced decline in HDL (Kuipers, 1998), as also observed in the present cohort showing 6 participants with decline in HDL levels and 8 participants with increased HDL levels after lengthy periods of AAS abuse, despite all participants engaging in high levels of aerobic activity. This finding might have been influenced by liver function and AAS choices. AAS influence the HTL and LPL enzyme systems, with HTL primarily responsible for clearance of HDL and LPL for the clearance of glycerol and free fatty acids (Kuipers, 1998). AAS have a direct increased stimulatory effect on HTL activity, resulting in an increased hepatic clearance of HDL, thus decreasing serum HDL levels (Kuipers, 1998).

The effect on the HDL levels is further influenced by whether or not the particular AAS abused will aromatize to potent estrogens or not. Potent estrogens decrease HTL activity, thus increasing HDL levels (Friedl et al, 1990). When testosterone

enanthate alone is administered, the increasing effect of the androgen on HTL activity is counter-balanced by the decreasing effect on HTL activity by the production of the potent estrogen 17b-estradiol, in response to the aromatisation of testosterone. However, the oral and parenteral 17-alkylated AAS do not aromatise, thus leading to unopposed excessively increased HTL activity – resulting in severely decreased HDL levels (Friedl et al, 1990).

If HDL changes alone were used for cardiovascular risk stratification, those athletes using 17-alkylated AAS would have a two- to three fold higher risk for coronary artery disease compared to those athletes using testosterone in different ester forms alone (Gordon et al, 1977). Viewing the different individual AAS cycles in Annexure B will show that none of the participants used only testosterone in its different ester forms alone – all the cycles combined different 17-alkylated AAS with baseline testosterone. The positive influence of the potent estrogens formed during aromatization, is further decreased by the concomitant use of estrogen blocking agents (Selective Estrogen Receptor Modulators and Aromatase Inhibitors). Twelve of the participants in the studied cohort used one or more method of estrogen blockage (Annexure B).

The inconsistent findings in the participants' HDL levels and changes could be attributed to a number of factors, including the following:

- Type of diet followed
- Amount, frequency and intensity of aerobic exercise performed
- Combination of AAS abused
- Doses of 17-alkylated AAS abused
- Concomitant use of estrogen blockage
- Genetics

The Total Cholesterol/HDL ratio is used in risk stratification for cardiovascular disease. On both occasions of measurement, the mean values stratified the cohort into the high-risk group for cardiovascular disease. However, the ratio showed an average decrease of 3.65 % over the pre-contest period. These changes were statistically insignificant, but clinically it implies that the cardiovascular risk decreased despite the abuse of high dose AAS for extended periods. Hislop et al (2001) showed that androgen supplementation decreased both HDL and lipoprotein (a) levels – the latter being an anti-atherogenic effect of the androgens. When the LDL particle size is increased and post-prandial triglyceridaemia is reduced, it may have further anti-atherogenic effects in AAS abusers. Thus, apart from lowering HDL

concentrations, no other potentially atherogenic effects are seen in AAS administration (Hartgens et al, 1996). In the present study's cohort, the decrease in the Total Cholesterol/HDL ratio is probably multi-factorial.

5.6 KIDNEY FUNCTION CHANGES

Urea, electrolytes and creatinine were evaluated on two different occasions, the latter after extended periods of high dose AAS exposure. Minimal, non-pathological changes in electrolytes were seen. Hydration status was thus related to changes in urea and creatinine. Statistically insignificant changes in urea levels were recorded. The mean pre-contest creatinine levels were equal to upper level normal range, though 6 participants had levels raised above the normal range. Statistically insignificant ($p = 0.055$) decrease of 8.9 % followed over the pre-contest period, with 3 participants still having values above normal range upper limit.

Renal side effects are very uncommon amongst AAS abusers, with only a few isolated cases described in the literature. The main side effect of AAS on renal function is that of slight elevation of serum creatinine levels (Juhn, 2003). This correlates well with the findings in the present studied cohort. The acute renal failure cases described, all had the following in common:

- Multiple drug stacking
- High protein diets
- Limitation of sodium and water intake
- Misuse of the diuretic torasemide

Annexure B confirms the multiple drug stacking, while Annexure C shows samples of high protein diets. Anecdotally all the participants in the cohort conveyed to the researcher, that they limited their sodium and water intake the last 72 hours before competitions, as well as abuse diuretics. Despite this, none of the participants showed profiles one week after the competition that fulfilled the criteria for acute renal failure.

5.7 LIVER ENZYME CHANGES

The effects of exercise and AAS abuse on liver enzymes were evaluated by testing the 6 liver enzymes ALP, GGT, ALT, AST, CK and LDH on two separate occasions. The CK, LDH and ALP levels showed minimal non-pathological changes on both

occasions, with levels for all the participants at both occasions being within the normal range. However, the mean GGT levels showed a statistically significant increase ($p = 0.048$) of 60.96 %, even though these mean levels were still within normal range. One participant had increased GGT at onset of the study, but this returned to normal on the second sampling occasion. Only one athlete showed severely increased GGT levels after lengthy period of AAS exposure.

ALT levels showed statistically significant ($p = 0.001$) increases for the participants, with mean value increases of 387.86 % higher than upper limit normal range. Most participants started the pre-contest phase with existing increased ALT levels (Annexure A). The mean AST levels showed a similar trait to that of the ALT levels.
Hepatic side effects due to AAS abuse occur most commonly in the Class B and to lesser extent Class C drugs (Section 2.2.2). All the participants in the cohort abused drugs from these two classes at high doses for extended periods of time (Annexure B). As seen in previous literature, these two classes in particular are known to cause increases in AST and ALT, with increases in GGT at later and advanced stages (Snyder, 2008). The amount of enzyme leakage is partly pre-determined by the existing condition of the liver at the onset of AAS abuse, with the greatest risk for liver damage in those athletes with existing liver damage (Kuipers, 1998). In the present cohort most of the participants started off with either increased AST or ALT levels, with only one participant with increased GGT levels.

Muscularity may increase the transaminases, usually with an AST/ALT-ratio of > 1.0 (Noakes, 1987). In the present cohort studied, the mean AST/ALT-ratio at the onset of the pre-contest period measured = 0.90. On the second occasion of measurement, this ratio measured = 0.46. This thus reflects a much larger increase in the mean ALT values measured at the one-week post-competition period, than the mean values measured for AST. In AAS abusers, it is commonly noted that the ALT values are on average higher that the AST values, which is indicative as a primary manifestation of AAS-induced liver function impairment (Urhausen et al, 2003).

While the GGT level is the most sensitive marker of hepatic cholestasis (which is indicative of structural damage to the liver) - AST and ALT levels are the most sensitive markers of hepatocellular injury, indicating hepatocellular necrosis and inflammation (Scally, 2008). Hepatocellular injury and not necessarily cell death, is the trigger for the release of the aminotransferases. The peak in AST, ALT and GGT

levels would usually be seen at 2-3 weeks after the onset of AAS consumption (Hartgens et al, 1996). These levels would return to baseline after only a few weeks (average 6 weeks) up to three months after AAS abstinence (Hartgens et al, 1996). In AAS abusers the AST and ALT level increases correlate significantly with the extent (duration and weekly dosage) of AAS abuse (Scally, 2008).

Factors other than liver injury can also influence AST and ALT levels (Dufour et al, 2000). The time of day has no influence on AST levels, but ALT levels can fluctuate with as much as 45 % during a day, with levels highest in the afternoon. In this study, blood sampling was thus always undertaken in the early morning in order to minimize the effect of the time of day. According to the review article done by Scally, AST levels can vary from day to day (5-10 %), but less than the variation in ALT (10-30 %). A high BMI can increase both AST and ALT levels with as much as 40-50 % above baseline values. This is seen in normal individuals, who do not abuse AAS, engaging in resistance and aerobic training protocols. In this study, 8 participants presented at the onset of the pre-contest season with AST levels falling within the 40-50 % above baseline levels, while 2 athletes had levels lower than upper limit normal (ULN) and 3 participants had AST levels more than twice, but less than 3 times the ULN. Only one athlete presented with AST levels above 3 times ULN. AST levels can increase three-fold with strenuous exercise, but ALT levels can decrease by 20 % when training at normal levels (Scally, 2008). In the present cohort, the raised AST levels at the onset of the pre-contest period, may have been due to the participants engaging in regular resistance training, as well as all having high BMI values, with muscularity usually causing AST/ALT-ratios $>$ 1.0 (Noakes, 1987). At the one-week post-competition measurement, 4 participants presented with AST levels lower than at the onset of the pre-contest period – no explanation could be found for this. The enzyme increases are predominantly in men and much higher increases are seen when strength training is performed (Scally, 2008). Muscle injury can significantly increase AST levels, but only modest ALT level increases are noted. This is usually accompanied by much higher levels of increase in CK values (Dufour et al, 2000). The changes seen in liver enzymes due to muscle injury were not seen in the present study.

As both AST and ALT levels can increase in response to strenuous training protocols as reported by the participants in this study, the enzyme evaluations done to ascertain the AAS-induced liver damage, should also consider CK and GGT

levels. When the enzyme level changes of the individual participants are viewed separately, the following is noted (Annexure A):

- Three participants' GGT levels decreased after AAS exposure, while 11 participants' levels increased
- Four participants' AST levels decreased, while 10 participants' levels increased
- All 14 participants' ALT levels increased
- The ALT increases were proportionally much higher than the AST increases
- None of the participants' CK or LDH levels was increased.

Though strenuous resistance training, possible muscle damage and high BMI values might have been influencing factors to the enzyme changes seen within the cohort, they were contributing to a much lesser extent than the AAS contribution. The main cause for the liver enzyme changes in the present cohort, seem to have been AAS-driven, evidenced by the increases seen in GGT and the disproportionate increases in ALT values compared to AST values increases (Urhausen et al, 2003). When using the WHO Grading system for drug-induced liver damage and evaluating the mean liver enzyme values for the present cohort, the cohort graded overall into Grade II or less. Individually, only two individual participants fell into the Grade III level for AAS-induced liver damage, while the remainder of the participants fell mostly into the Grade II level.

5.8 HORMONE PROFILE CHANGES

Cortisol levels were taken initially at the onset of the study, in order to establish baseline adrenal function. Levels were repeated again at 6 weeks after the competition. Anecdotally there seems to be a strong belief amongst the body builder population that cortisol levels will greatly increase when testosterone levels are very low during the phase directly after competitions. However, in the current study on both occasions of measurement, both mean values were still within normal range and there was a statistically significant ($p = 0.034$) decrease in the mean cortisol values for the cohort towards the end of the study. There were even two participants showing cortisol levels below the lower level of normal range, despite these participants also measuring low levels of total- and free calculated testosterone (Annexure A).

Post-competition mean total testosterone values were also decreased below the lower levels of the normal range. Two participants used long-acting AAS esters during the final weeks before competition – these esters with their long t1/2 extended exogenous testosterone release into the system, rendering high measured total- and free calculated testosterone levels at the 6 weeks post-competition period. This falsely increased the mean value for the cohort, as the rest of the cohort engaged uniformly only in the use of short-acting esters with t1/2 of 2-3 days. The latter cleared the systems of the remainder of the participants at 7 times t1/2 values, well ahead of the measurement occasion of 6 weeks later. The uniform group of the participants using short-acting esters, measured mean total testosterone levels of only 3.14 mmol/l and free calculated testosterone levels of 95.36 pmol/l – both these values being well below the lower limit of normal ranges. This confirmed the hypogonadism status within this uniform group. When androgens are used in high doses continuously for lengthy periods, gonadotrophin secretion is suppressed, leading to the cessation of endogenous testosterone secretion and sperm production to cease (Kicman and Gower, 2003). Sperm counts will usually return to normal levels within 75-90 days after the last longest-acting ester had cleared the system. Gonadotrophin and testosterone levels can continue to be suppressed for much longer (Kicman and Gower, 2003; Kuipers, 1998; Modlinski and Fields, 2006), depending on the dosage and what particular AAS were abused (Roberts and Clapp, 2005).

The mean average SHBG value for the present cohort was at the lower end of the normal range. However, 8 participants had extremely low levels of SHBG, while 3 participants presented with SHBG levels above ULN. This finding is unexplainable, as 2 of the participants whose values were increased, abused similar AAS drug protocols than those who presented with extremely low SHBG levels (Annexure A & B).

The mean estradiol levels for the cohort were found to be within the normal range, with only one participant presenting with measured levels above ULN. Both the mean values for the FSH and LH levels were lower than the lower limit normal range, confirming the hypogonadotrophic status of the cohort in general. In both cases only 3 participants had values falling within the lower levels of normal range.

Hypogonadotrophic Hypogonadism presents as the combination of simultaneously lowered FSH, LH and testosterone (Kicman and Gower, 2003; Modlinski and Fields,

2006). This is a common side effect after high dose extended abuse of AAS (Kicman and Gower, 2003; Kuipers, 1998; Urhausen et al, 2003). When abusing more than one AAS at a time, the inhibition of gonadal function is stronger. In most cases the hypogonadism can last more than 12 weeks after the cessation of AAS abuse (Kuipers, 1998).

5.9 AAS CYCLE LENGTH AND DOSAGES

The Androgen status of the cohort was not controlled, as ethical considerations precluded the investigator's involvement in drug administration. For this reason, the information on AAS abuse and cycles were anecdotally obtained from the present cohort's athletes who were already abusing self-sourced AAS.

Dosages of the different AAS drugs abused, as well as the cycle lengths, were recorded for each participant in the cohort (Annexure B). Most of the participants abused AAS drugs sourced from illicit underground steroid sources, some participants sourced some of their AAS drugs from legal pharmacies, while one particular participant only used pharmaceutical grade AAS drugs sourced from legal pharmacies. This participant used drugs averaging doses similar to that of the rest of the cohort. The body composition and blood profile changes in this athlete's case were not much different to that of the rest of the cohort's participants.

The mean AAS cycle length for the cohort was 17.43 weeks, with the shortest cycle of 12 weeks and the longest 25 weeks. The mean weekly injectable AAS abuse for the cohort measured 1179.81 mg, with the lowest average 333.3 mg per week and the highest 2564.7 mg per week. The mean weekly oral AAS consumption for the cohort measured 573.47 mg, with the lowest average 180 mg per week and the highest average 1144.7 mg per week. The mean total AAS abuse per week for the cohort measured 1638.3 mg, with the lowest average 453.5 mg per week and the highest average 3421.2 mg per week. Though it might have been expected that the heavier athletes would use higher doses, it was not the case in the present study. Some of the higher weekly doses were consumed by athletes competing between the 80kg to 100kg weight categories. One particular athlete in the 90kg to 100kg category, consumed on average close to twice the doses of AAS used by the athletes competing in the over 100kg category.

It is thus clear from the present study's findings that some of the observations correlate well with observations from other studies in the literature, but that some of the findings differ from what has been documented before in the literature. However, it should be noted that the present study's cohort differed vastly from cohorts from other studies in the literature, as none of the latter observed cohorts under full pre-contest preparation conditions. In conclusion, it should be understood that body builders under full pre-contest preparation will respond differently to the use of special diets, different training strategies and different types of AAS abused, than compared to when they train under normal out-of season conditions.

5.10 ADVERSE EFFECTS OF AAS

AAS are effective in enhancing athletic performance (Kuipers, 1998), however the abuse of such drugs can lead to adverse effects, which may jeopardize health. AAS can display a wide range of physiological effects, due to androgen receptors being distributed throughout the human body, including skeletal muscle, skin, scalp, liver, heart, prostate, brain, nervous system, bone, adipose and kidney tissue. Thus, abusing high doses of AAS, can lead to increases in numerous physiological activities in the body aside from just building muscle tissue (Llewellyn, 2006).

Many of the adverse effects of AAS abuse can be very difficult to recognize without any thorough medical examination (Kicman, 2008). In the present study, none of the participants were aware of the side effects incurred to their cardiovascular, renal, hepatic or endocrine systems, were it not for the blood assays being done on them. These damaging adverse effects are insidious, as the athletes are unaware of the long-term detrimental effects, especially to their cardiovascular systems.

With exception of the possible psychological changes, no adverse effects of acute over-dosage with AAS in healthy adults have been reported in the literature (Kicman and Gower, 2003). With chronic administration, adverse effects at even normal pharmacological doses have been well documented in the literature. One of the problems with athletes, especially body builders, is that they use multiple drug stacking in doses several times to that what is needed for stable therapeutic levels (Kuipers, 1998; Annexure B). The frequency and severity of side effects can then become variable, as this depends on the sex, the dose, the duration of administration and whether 17a-alkylated drugs are used or not (Kicman, 2008). The present study indicated similar findings, especially as far as the hepatic side effects

were concerned. Life-threatening liver damage associated with death has been documented on several occasions in the literature (Kicman, 2008).

The risk for adverse effects can be augmented in body builders, as they are known for taking numerous different drugs at doses many times that of indicated therapeutic doses over extended and sustained periods of time. These findings were clearly demonstrated in the present study where undesirable changes in blood profiles were directly linked to the particular participant's AAS doses and length of cycle (Chapter 4; Annexure B).

AAS abusers and potential users should thus at all times be aware of the adverse effects associated with AAS administration, as these effects may be present without any obvious warning signs (Maravelias et al, 2005).

CHAPTER SIX

Conclusions and Recommendations

Obtaining participants for the present study's cohort was difficult, as these athletes form part of a very secluded group of sportsmen. Though the present cohort was disadvantaged by a small cohort size, the opportunity to study such a group in depth will not be readily repeated. This is a novice study – to present, no similar study has been conducted in South Africa. The researcher recommends that, if such an opportunity would present itself again, it should be fully utilised.

The present study showed that, despite high doses of AAS that were abused, blood pressure could be fairly well controlled, due to the high volumes of aerobic training that were combined with the normal resistance training protocols generally followed by body builders. The results from this study also suggests that the pre-contest body builder loses fat stores, as well as lean mass simultaneously, when preparing for a competition – this despite very high doses of AAS abused. It should be noted that the literature generally associates AAS use or abuse with the acquisition of lean mass. However, these studies used standardised energy intakes, exercise doses and testosterone administration, with participants mostly not under the same strenuous physiological strain under which the competitive body builder prepares for a competition. In the present study's cohort, the AAS doses were not standardised, as the drugs were mostly sourced from illicit origin – this may have contributed to results differing from that found in the studies using standardised protocols.

Due to the ethical considerations, the different AAS drugs used by the participants could not be provided in standardised doses to them. Thus, content of the oral and injectables used in the present study, was unknown. It may be feasible in future studies to ask participants to willingly provide some of these preparations for analytical biochemistry in an accredited laboratory – a grant to finance such an endeavour could be applied for.

A number of risk factors for disturbed carbohydrate metabolism were noted – 71,4 % of the participants had BMI $>$ 25, combined with HDL levels $<$ 0.9 mmol/l. The degree to which AAS abuse contributed to the development of pre-diabetic status in some participants is unclear and needs future investigation. The unchanged or even decreased cholesterol levels observed in the present study were due to participants performing a greater deal of aerobic exercises, probably counter-balancing the

cholesterol increasing side effects of the AAS abuse. However, the AAS induced decrease in HDL levels was not counter-balanced by the high aerobic exercise doses. The extent to which disturbed liver function, the AAS choices abused and concomitant abuse of estrogen blocking agents contribute towards these findings, are unclear and needs further investigation. The finding that the Total Cholesterol/HDL-ratio decreases over the length of the study, implying a decrease in cardiovascular risk despite the abuse of high doses AAS for extended periods of time, may render a false sense of security to the general body builder abusing AAS. It should be noted this decrease was probably multi-factorial and that the full extent of high dose AAS abuse continuously or intermittently for extended periods of time over many years, have not been fully investigated as yet. If the opportunity arises, future longitudinal studies should especially investigate the clinical significance of repeatedly impaired blood lipid profiles in AAS abusers.

The researcher concluded that the main cause for the liver enzyme changes in the cohort, seem to have been AAS-driven, evidenced by the increase seen in GGT and the disproportionate increase in ALT values compared to AST values increase. At the one-week post-competition measurement, 4 participants presented with AST levels lower than at the onset of the pre-contest period – the researcher could not find any explanation for this. Physicians engaged in the long-term follow-up of body builders, should steadfastly adhere to the WHO guidelines for the treatment of drug-induced liver disease. It would be unwise to consider any enzyme increases in the absence of a diagnosis as being non-significant. The researcher suggests regular follow-up of liver enzymes in all AAS abusers.

In the present study, a uniform group of participants were identified, whom all used short-acting AAS esters around the time of competition – they all had hypogonadotrophic hypogonadism (the combination of lowered FSH, LH and testosterone levels), which contributed towards them gradually losing lean mass in the weeks after the competition. The Androgen Status of the cohort was difficult to control, as ethical considerations precluded the investigator's involvement in drug administration. For this reason, only anecdotal information was recorded. The mean injectable AAS weekly abuse was recorded to be 1179.81 mg; the mean oral AAS weekly abuse was 573.47 mg, while the mean total AAS abuse per week measured 1638.3 mg.

It should be noted that the present study's cohort differed vastly from cohorts from other studies in the literature, as none of the latter observed cohorts under full pre-contest preparation conditions. It should also furthermore be understood that body builders under full pre-contest preparation will respond differently to the use of special diets, different training strategies and different types of AAS abused, than compared to when they train under normal out-of season conditions.

Due to the lack of any longitudinal studies on the adverse effects of long-term AAS abuse, the long-term implications of AAS abuse on health is still unknown. Mostly, the emphasis of the sports medicine literature is on the methods of detection of the newest medications that athletes are currently using. The South African Medical Community has very little knowledge on the types of AAS substances and the dosing protocols abused in general amongst the elite, competitive Body Building athletes in South Africa.

The potential physiological, psychological and health implications due to the AAS abuse amongst body builders are factors that warrant continuous future attention by investigators, as well as sports physicians. The sports physicians should continuously target their efforts at counselling adolescents and other athletes about the potential long-term harms of AAS abuse, as well as regularly and prudently follow-up on the potential adverse effects that may develop in current AAS abusers.

ANNEXURE A – GRAPHS OF INDIVIDUAL RESULTS

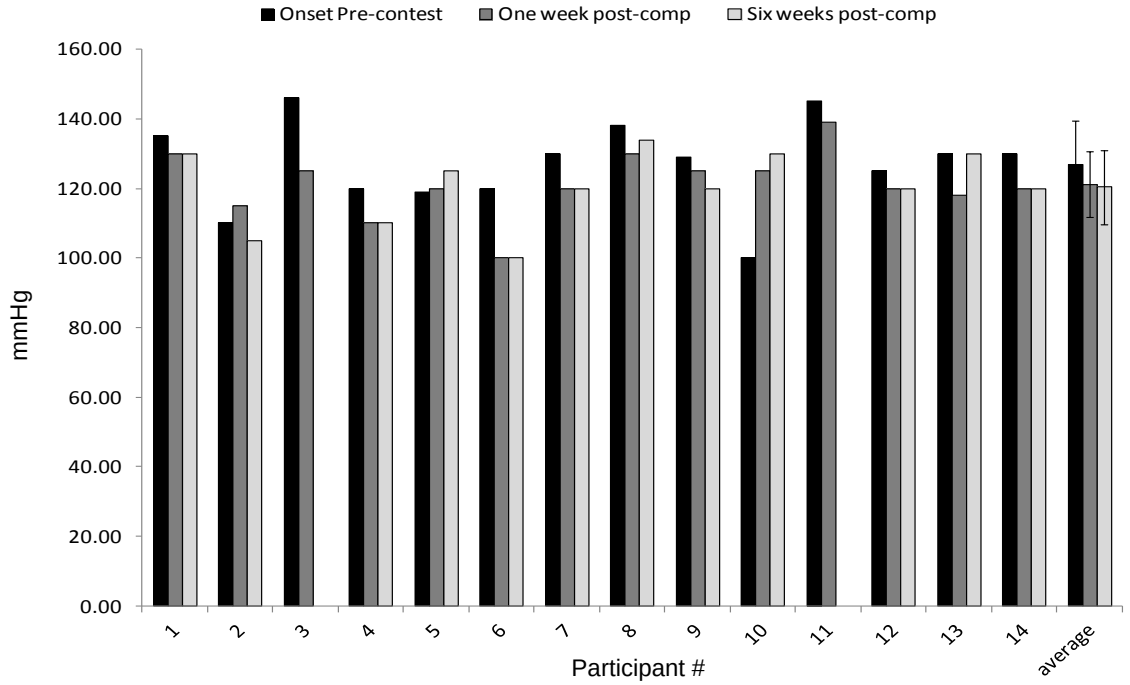


Figure A.1 Individual SBP results

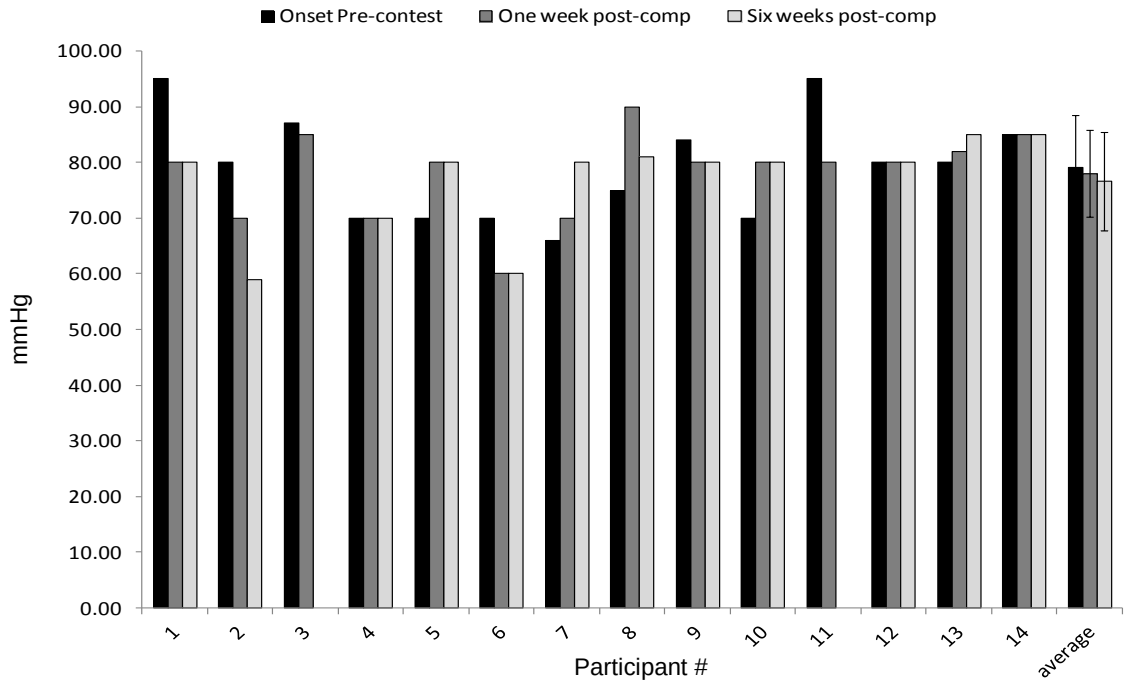


Figure A.2 Individual DBP results

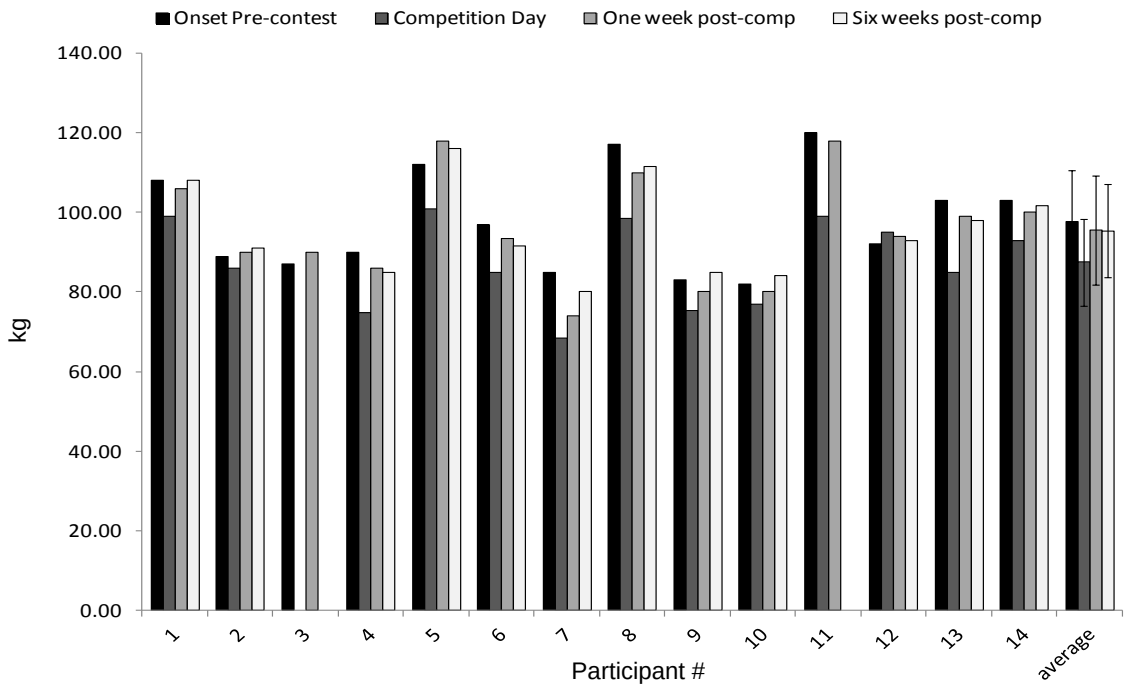


Figure A.3 Individual body mass results

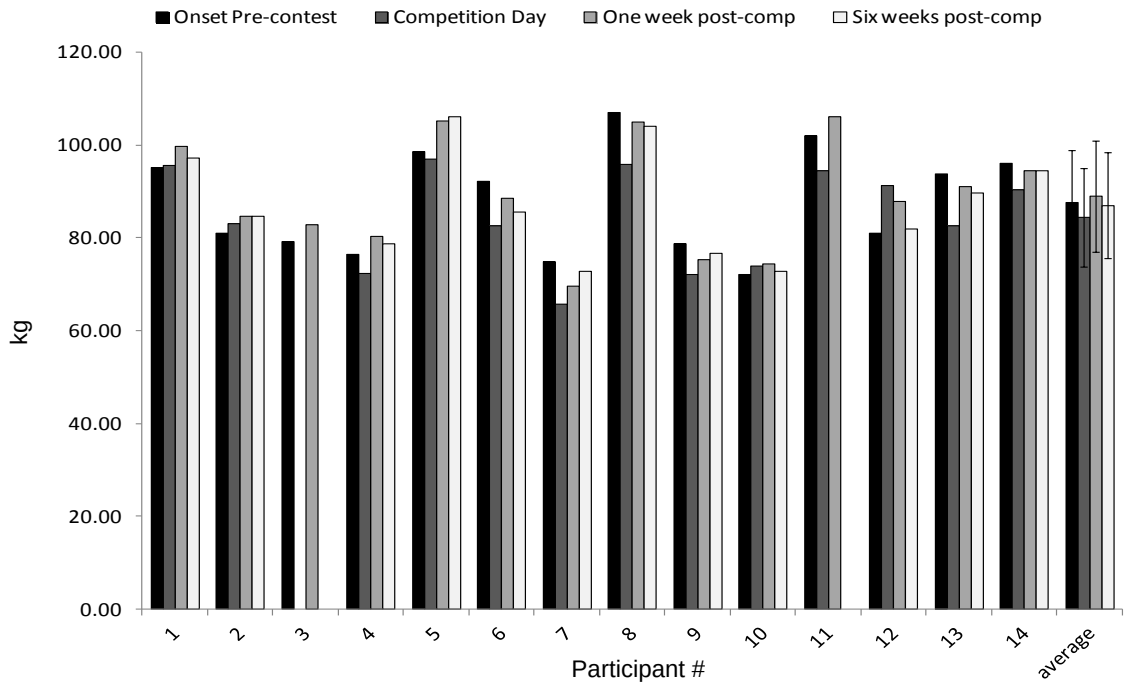


Figure A.4 Individual lean body mass results

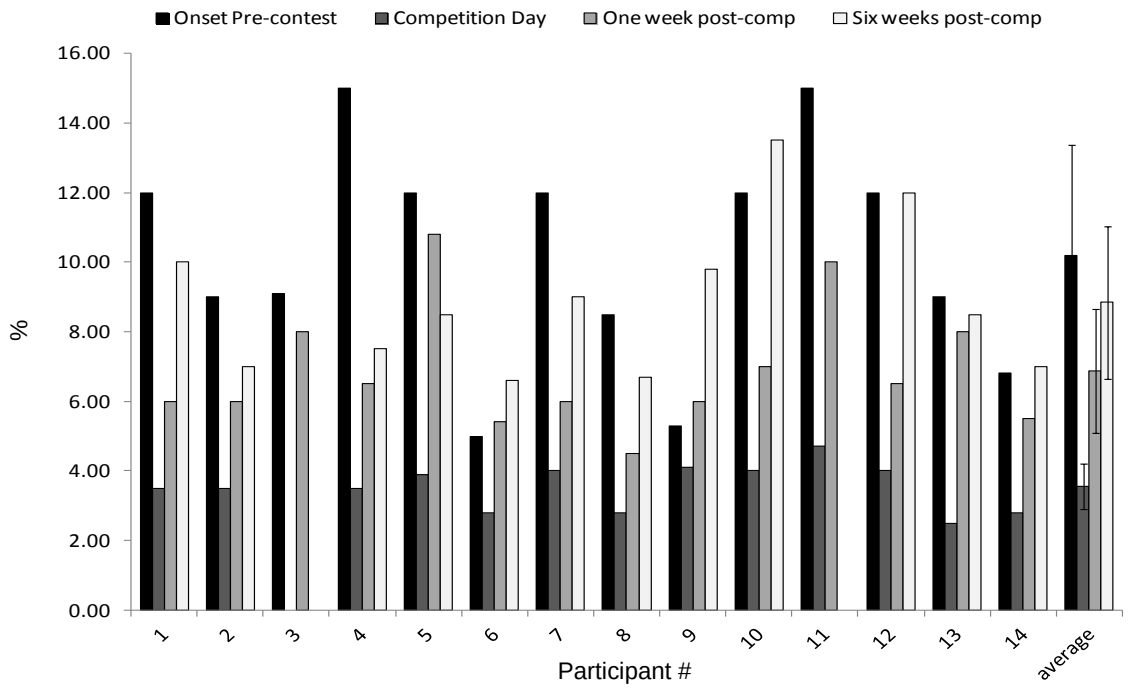


Figure A.5 Individual fat percentage results

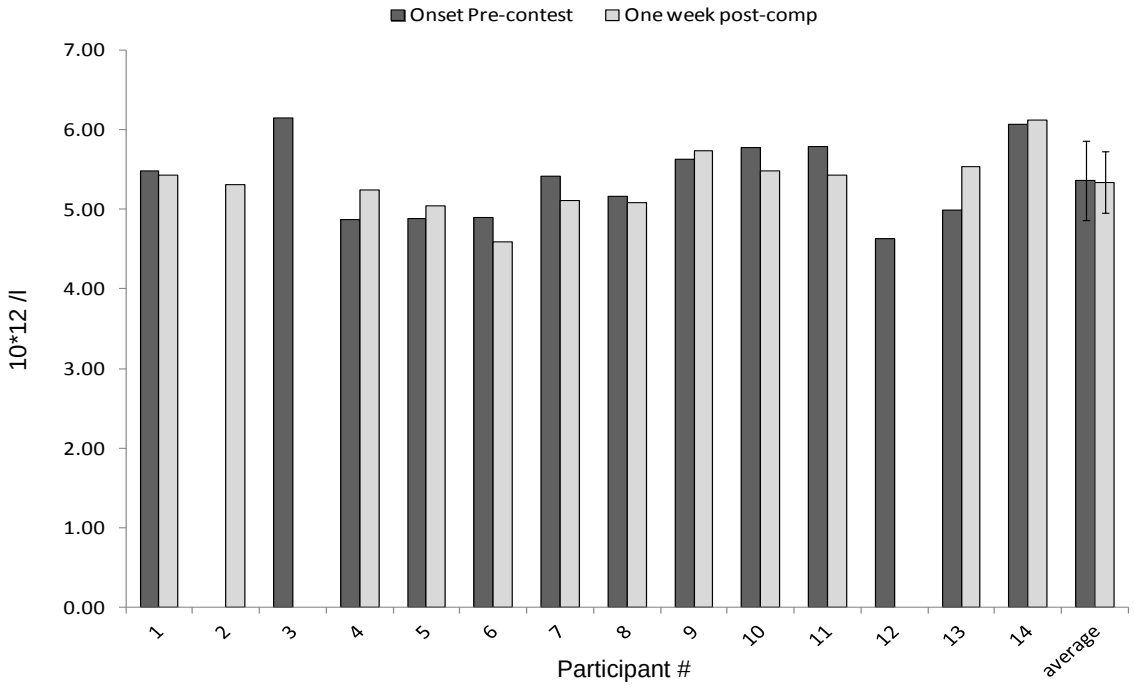


Figure A.6 Individual red blood count results

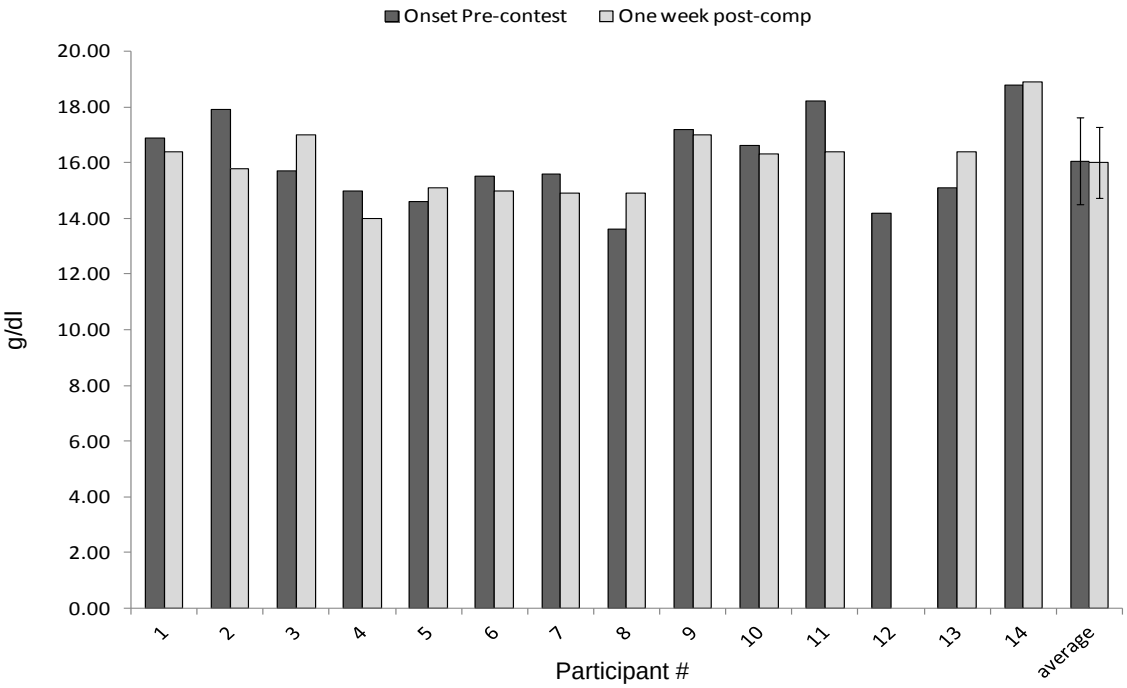


Figure A.7 Individual haemoglobin results

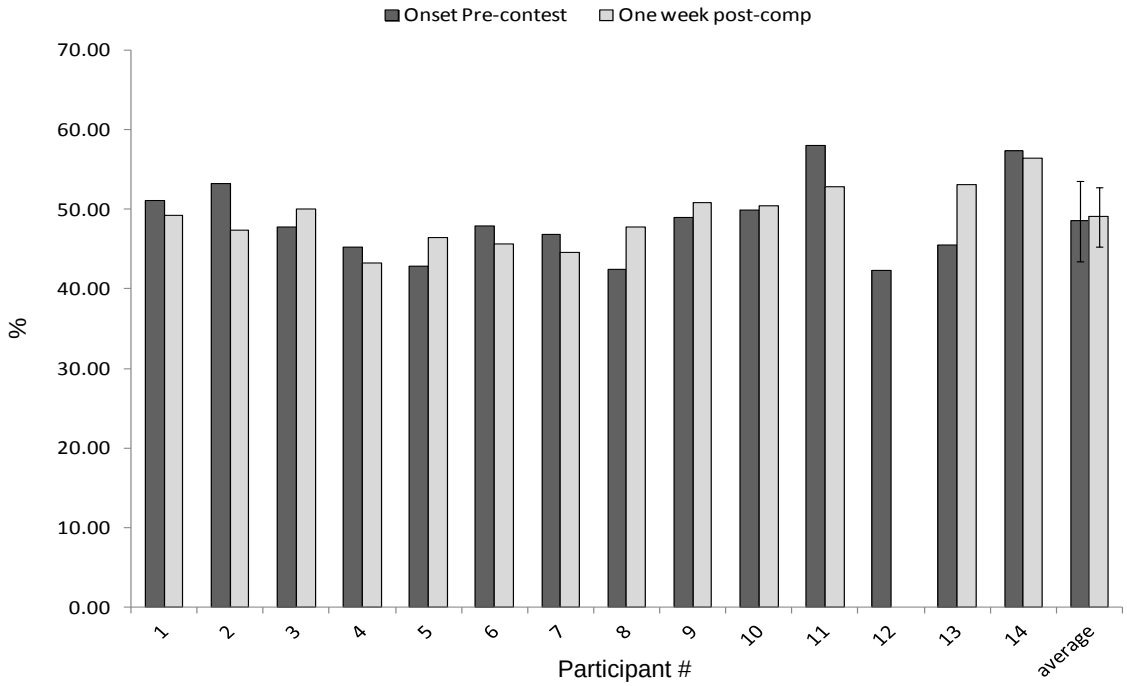


Figure A.8 Individual haematocrit results

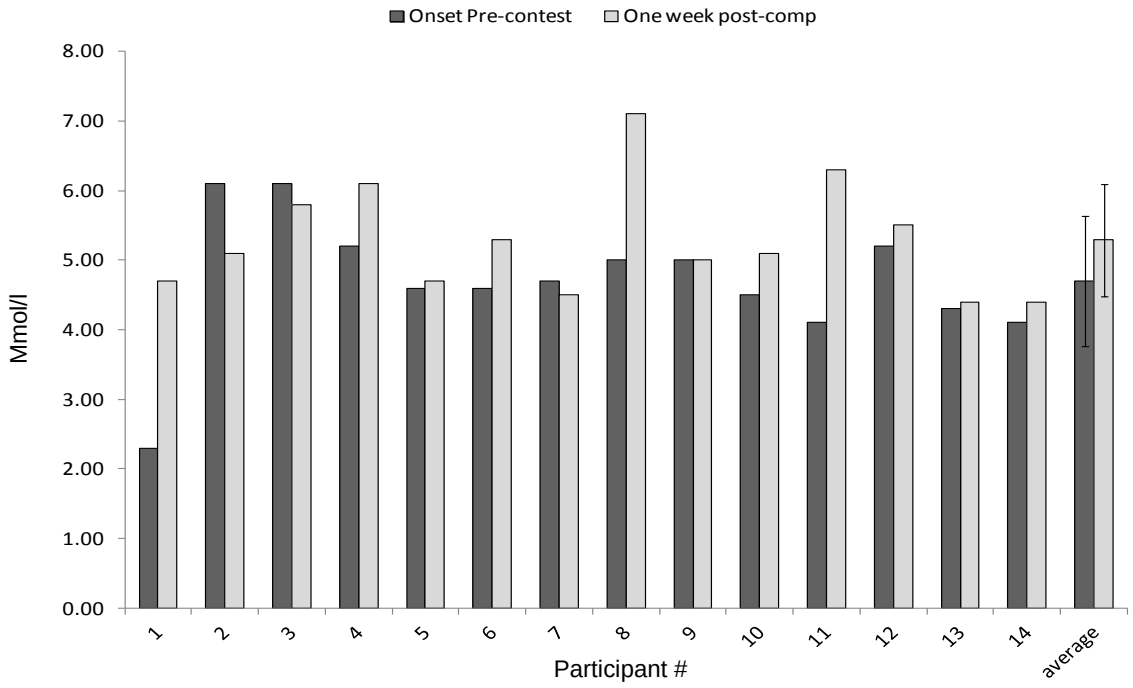


Figure A.9 Individual glucose results

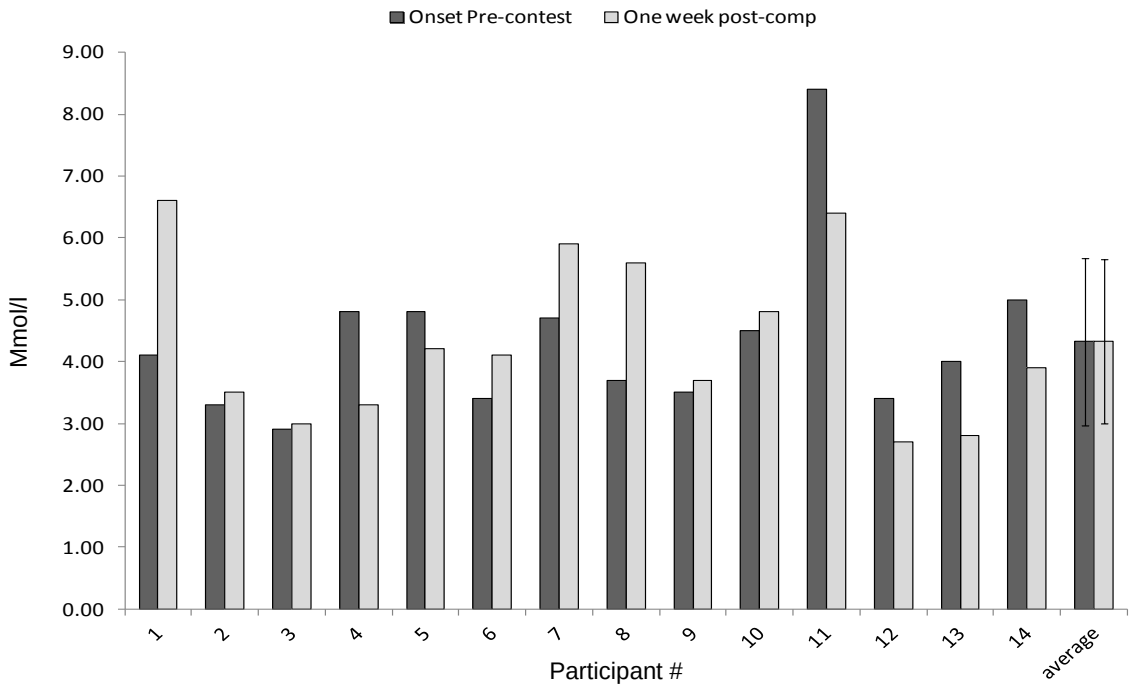


Figure A.10 Individual total cholesterol results

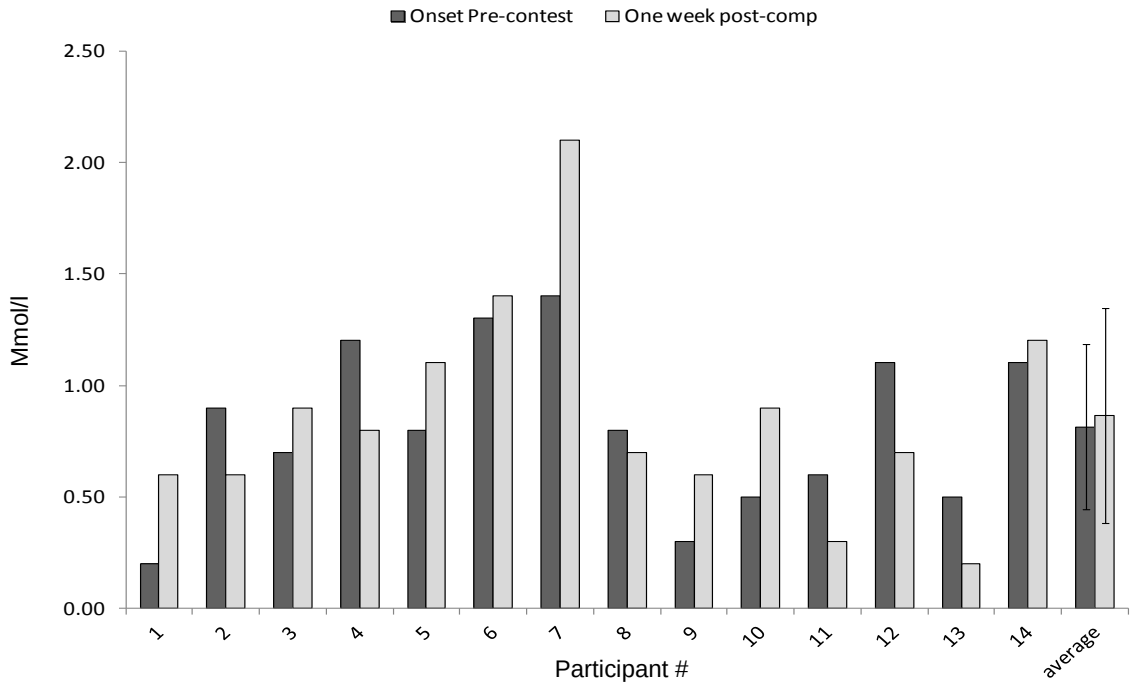


Figure A.11 Individual HDL results

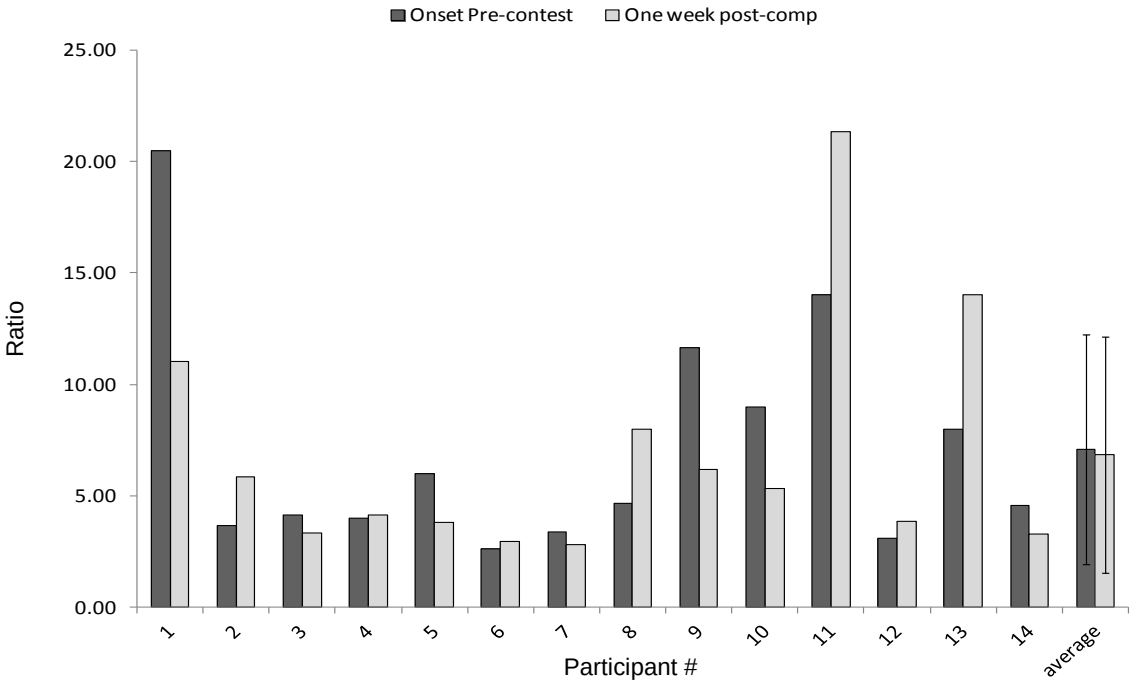


Figure A.12 Individual total cholesterol/HDL ratio results

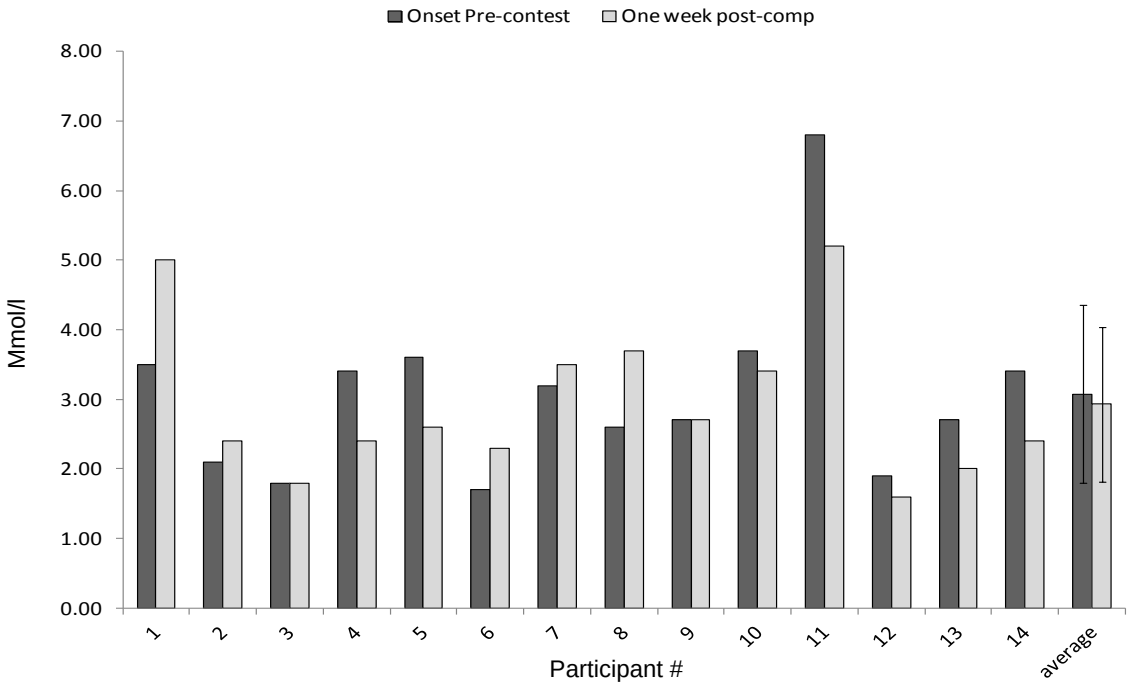


Figure A.13 Individual LDL results

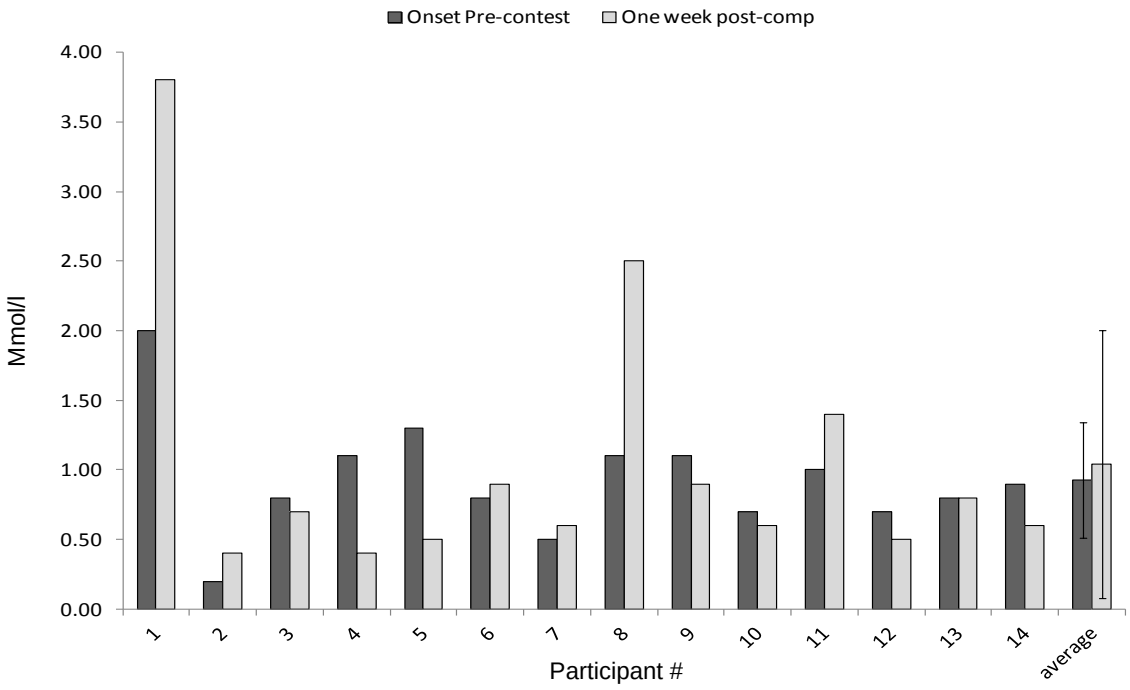


Figure A.14 Individual triglyceride results

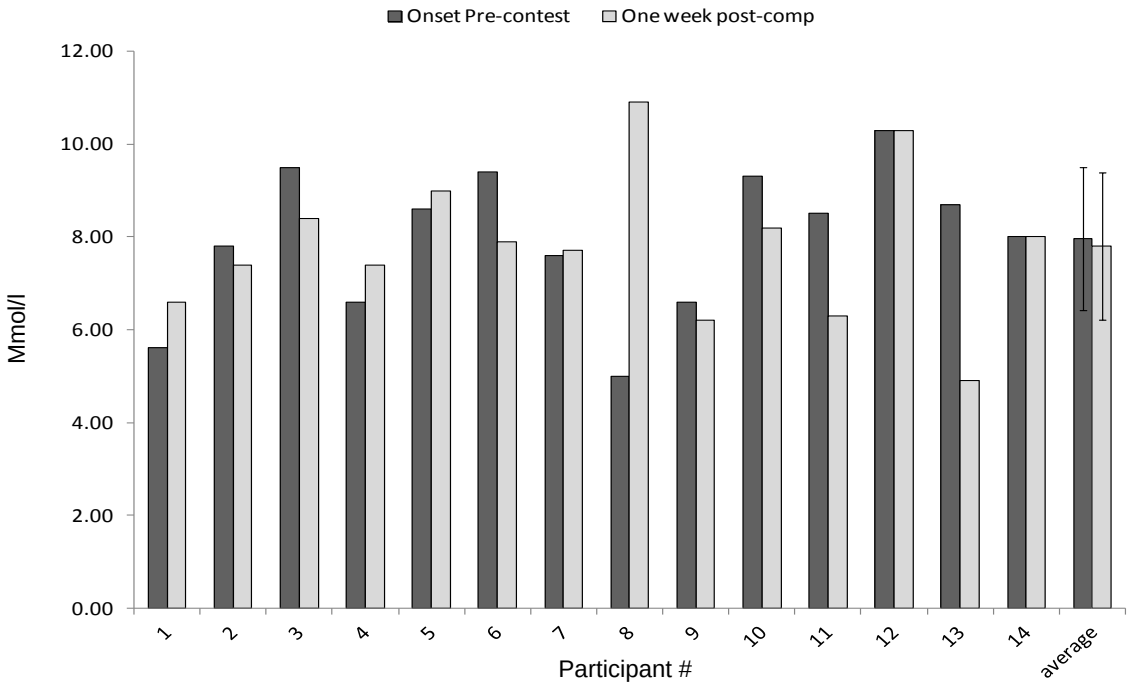


Figure A.15 Individual Urea results

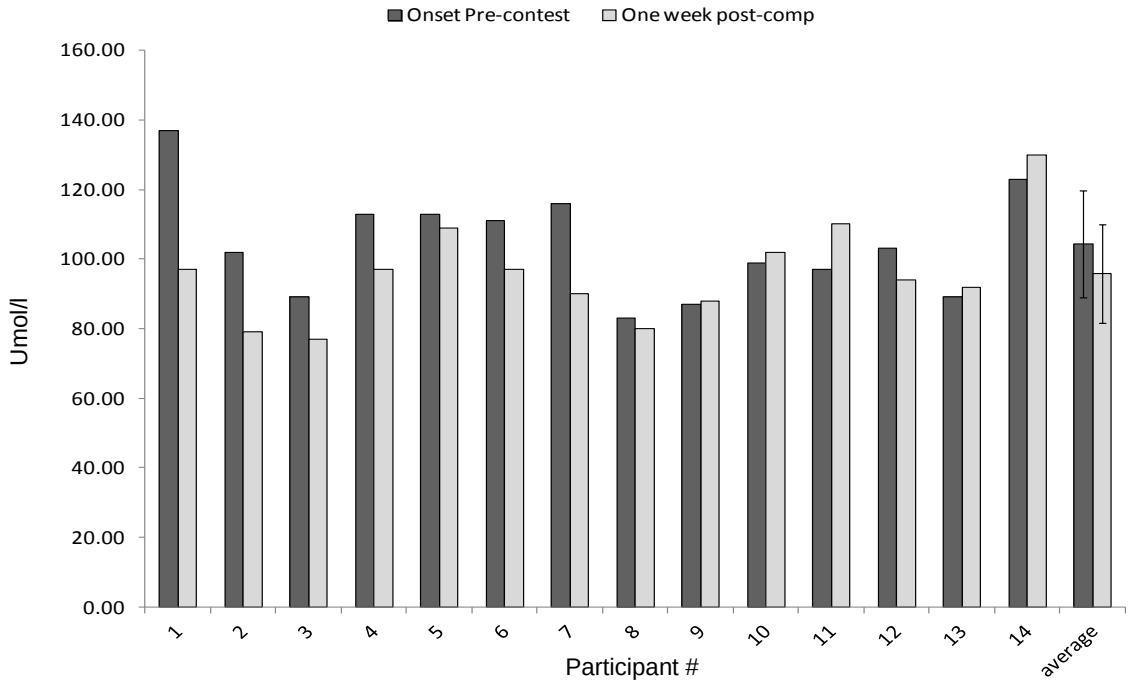


Figure A.16 Individual creatinine results

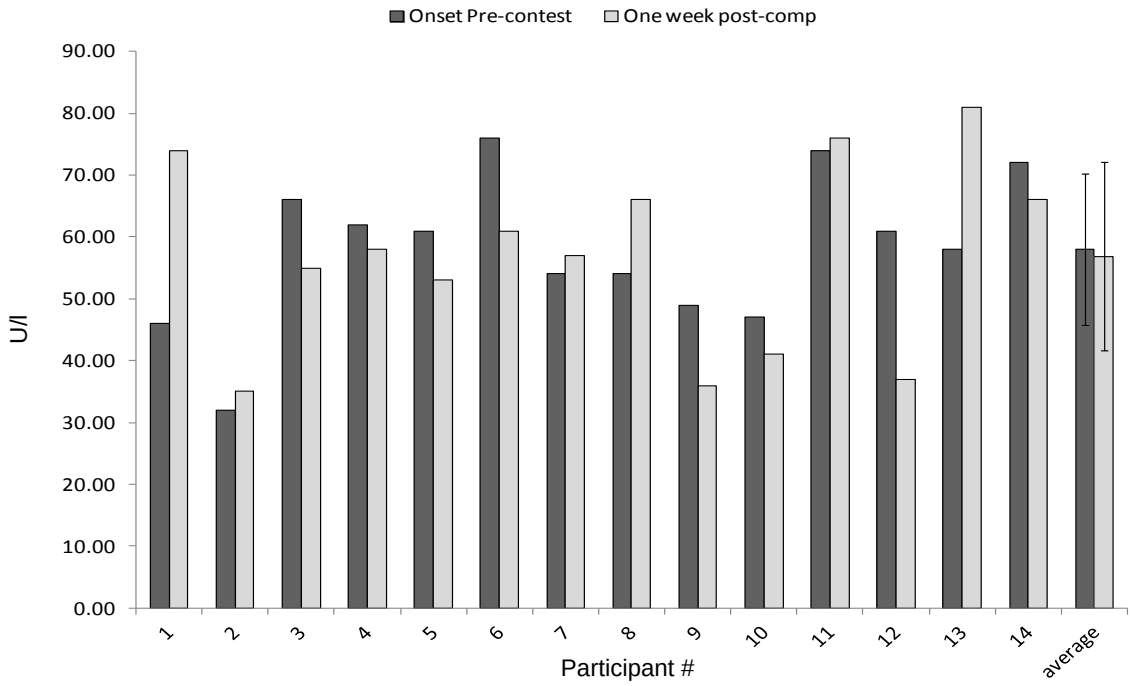


Figure A.17 Individual ALP results

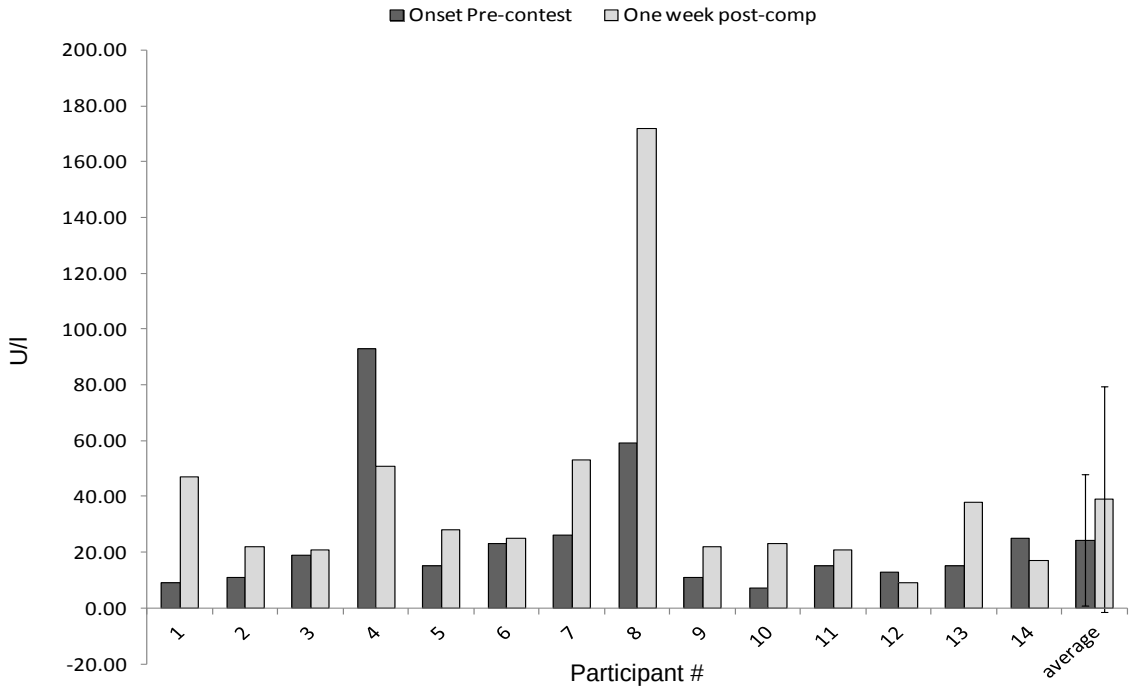


Figure A.18 Individual GGT results

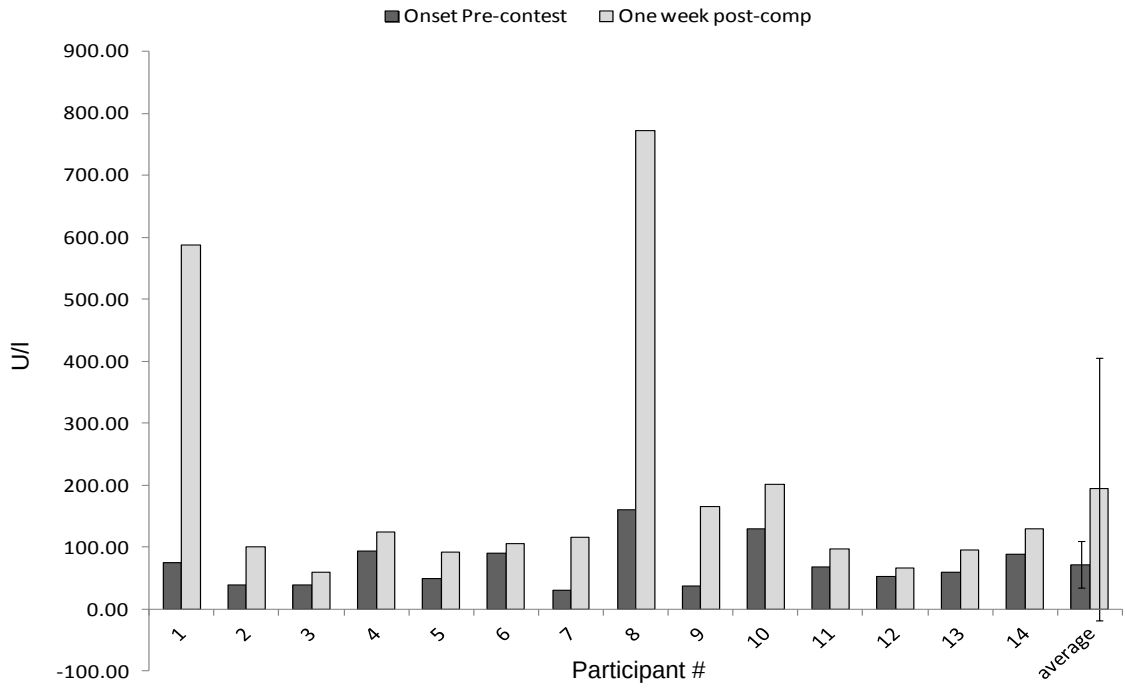


Figure A.19 Individual ALT results

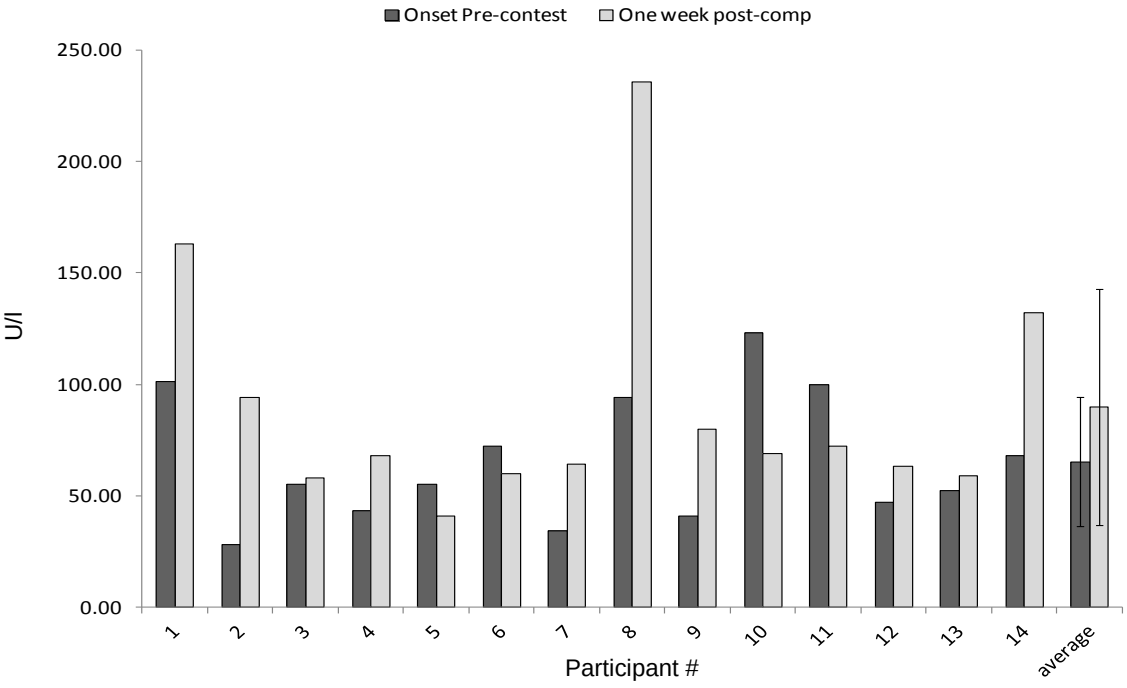


Figure A.20 Individual AST results

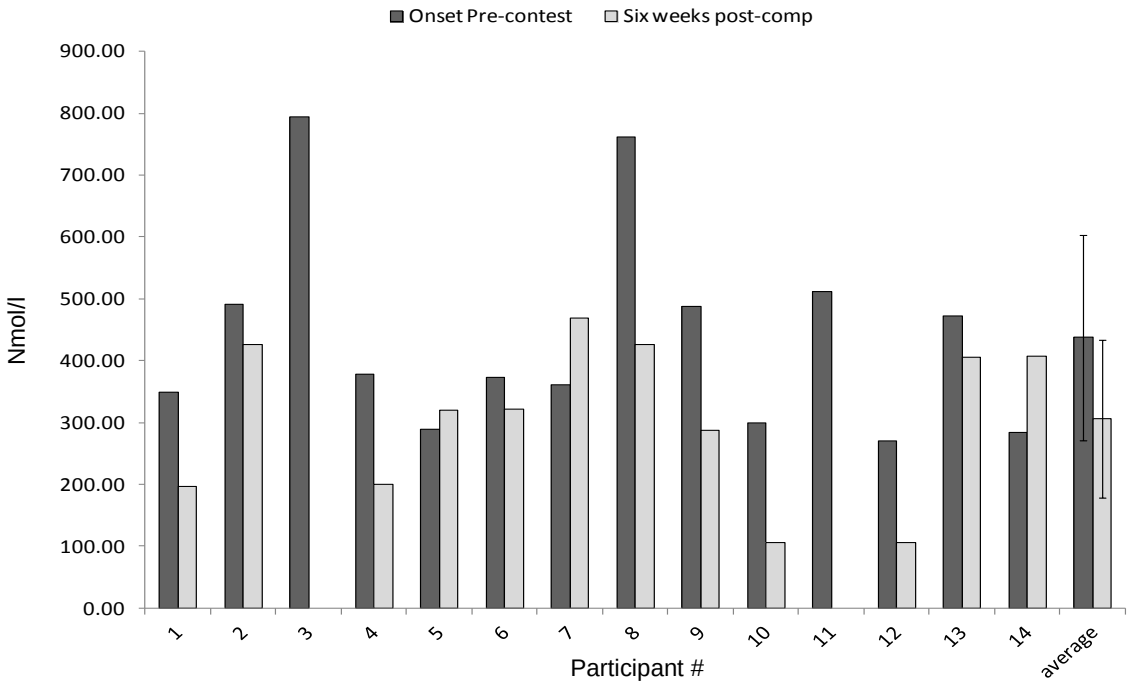


Figure A.21 Individual cortisol results

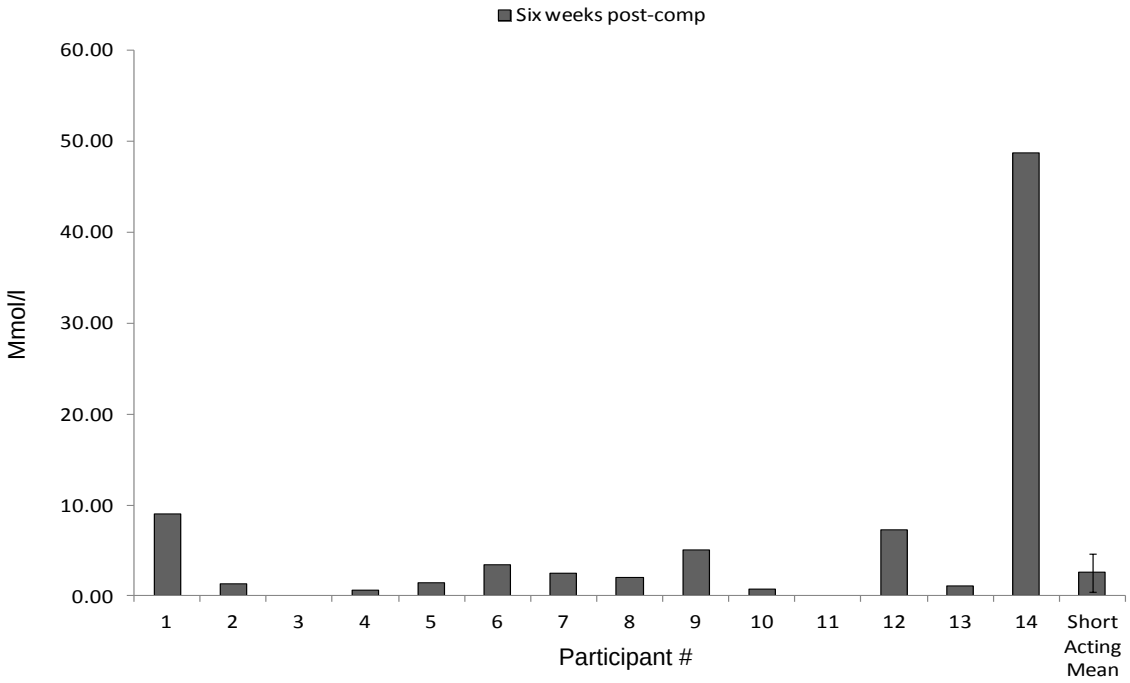


Figure A.22 Individual testosterone results

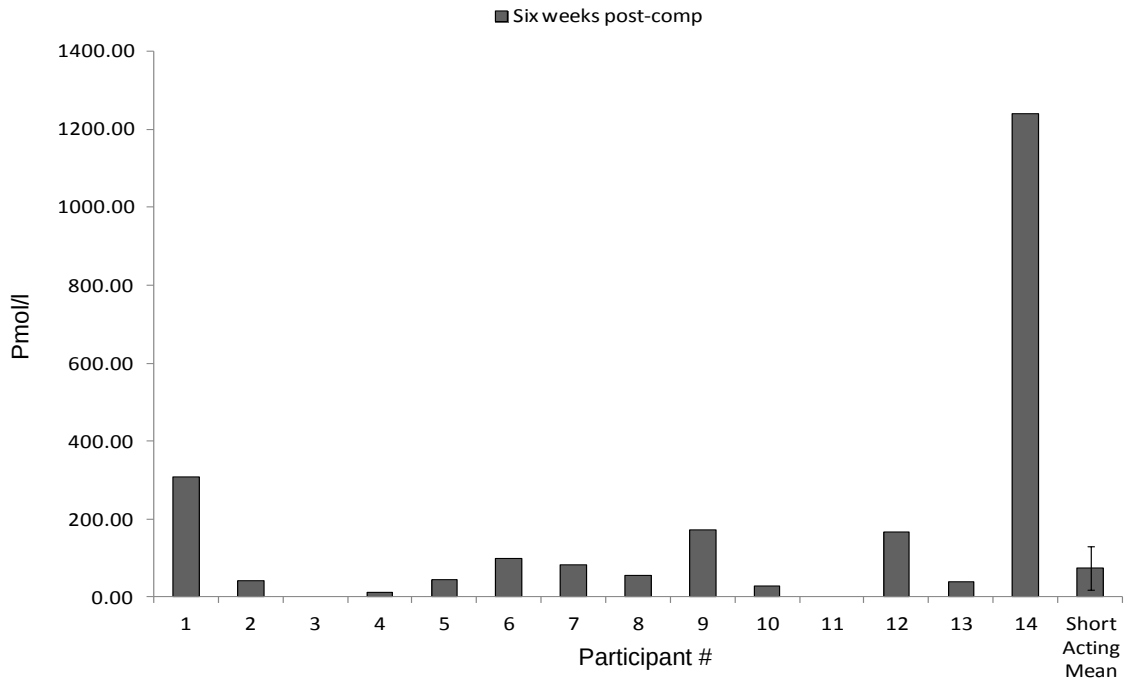


Figure A.23 Individual free calculated testosterone results

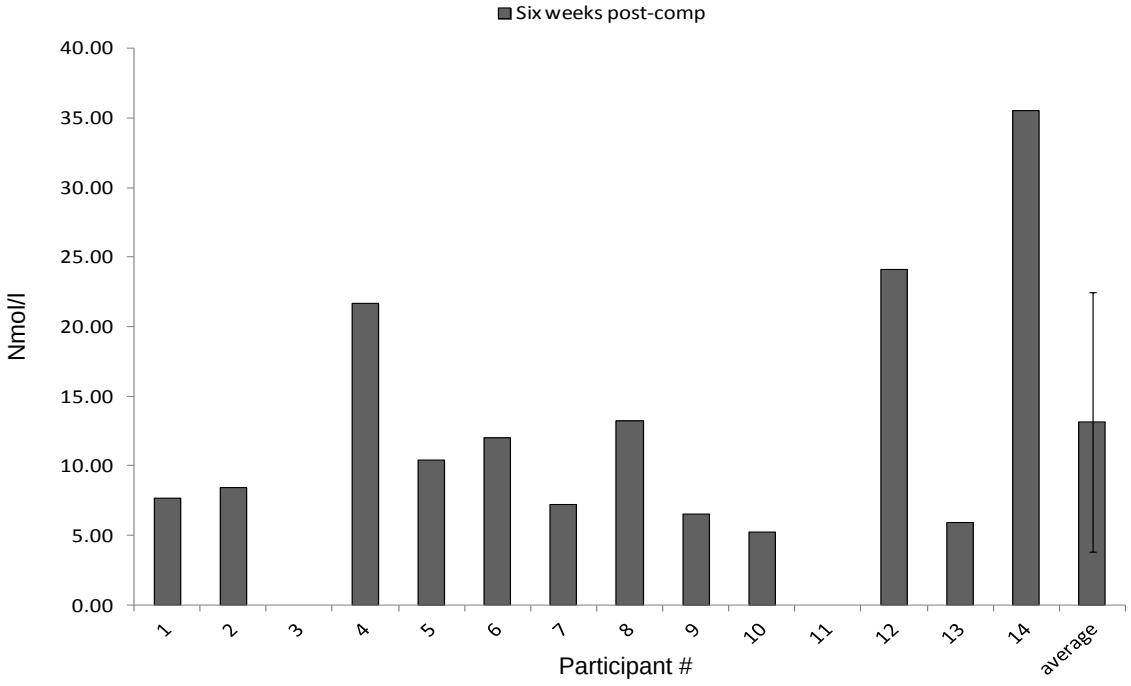


Figure A.24 Individual SHBG results

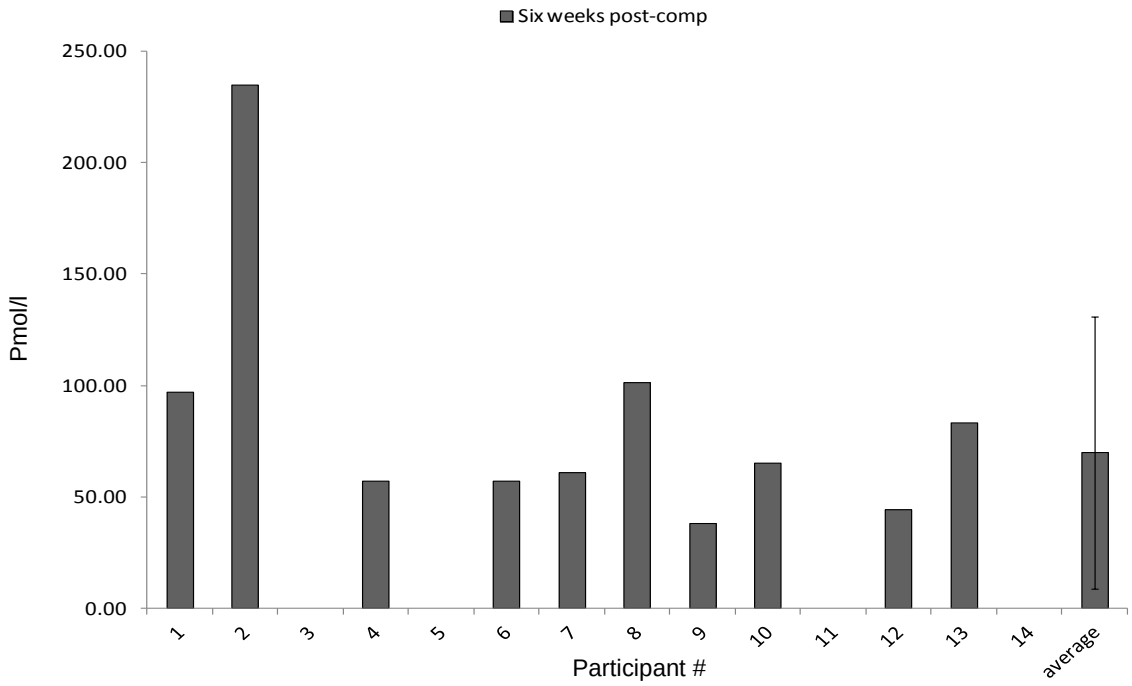


Figure A.25 Individual estradiol results

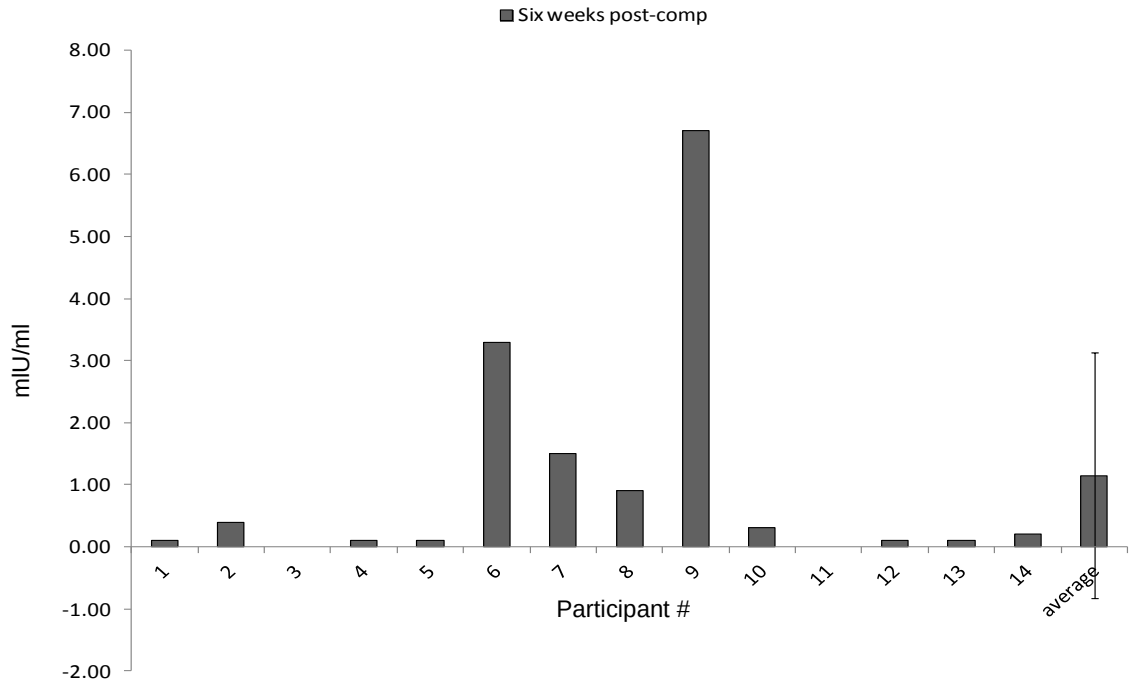


Figure A.26 Individual FSH results

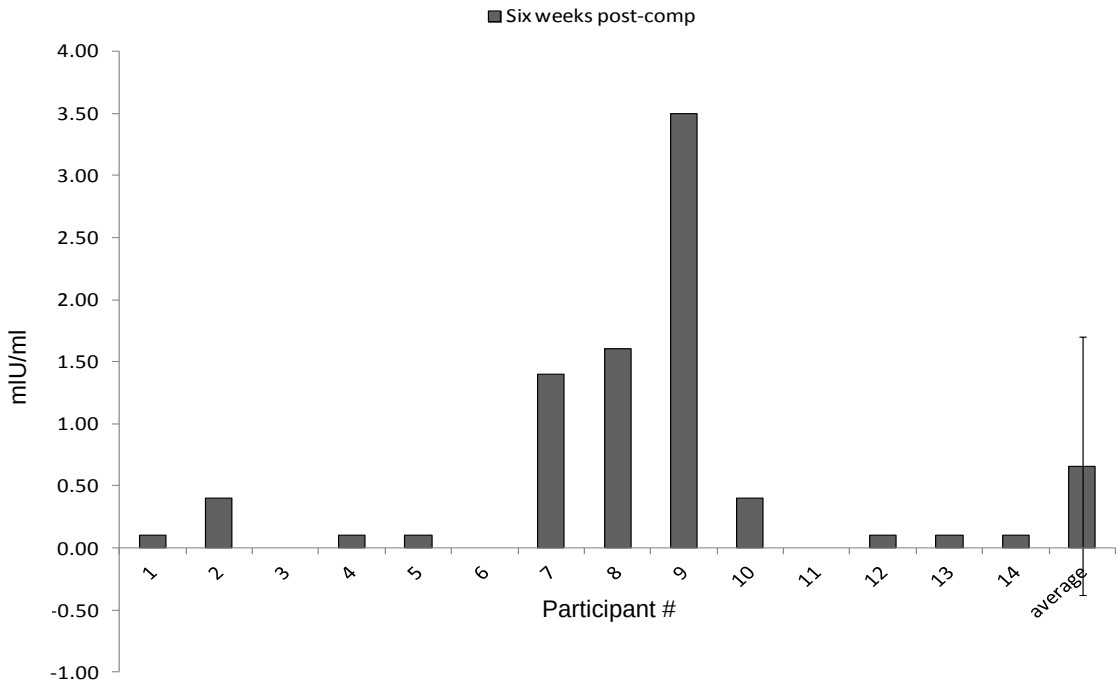


Figure A.27 Individual LH results

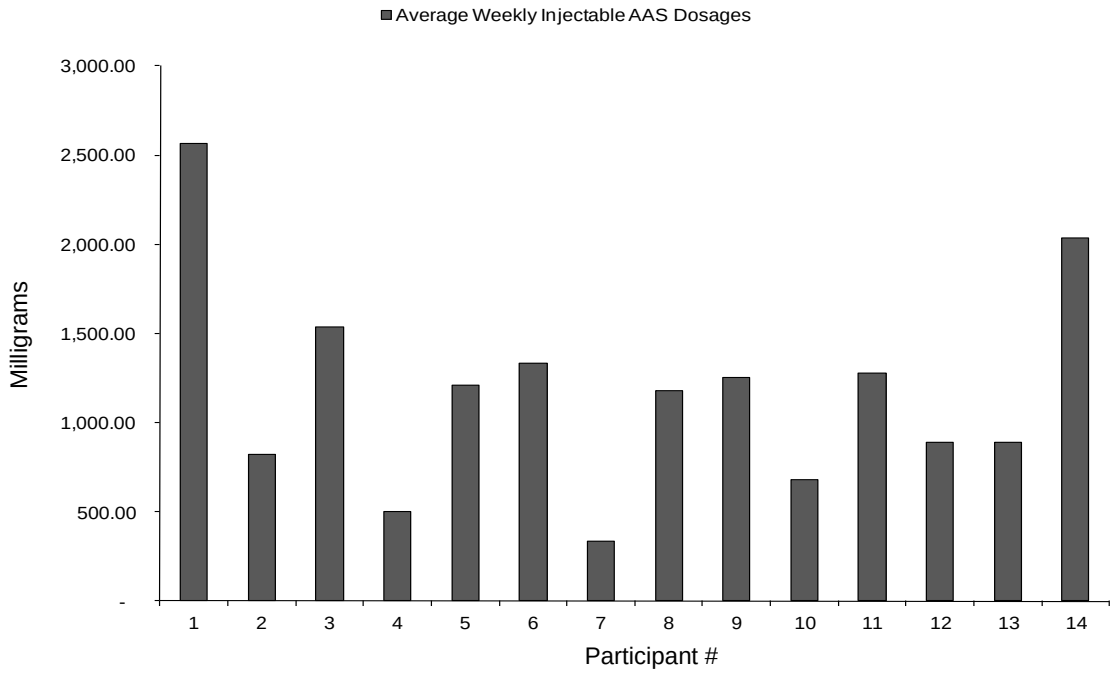


Figure A.28 Individual weekly average injectable AAS dosages

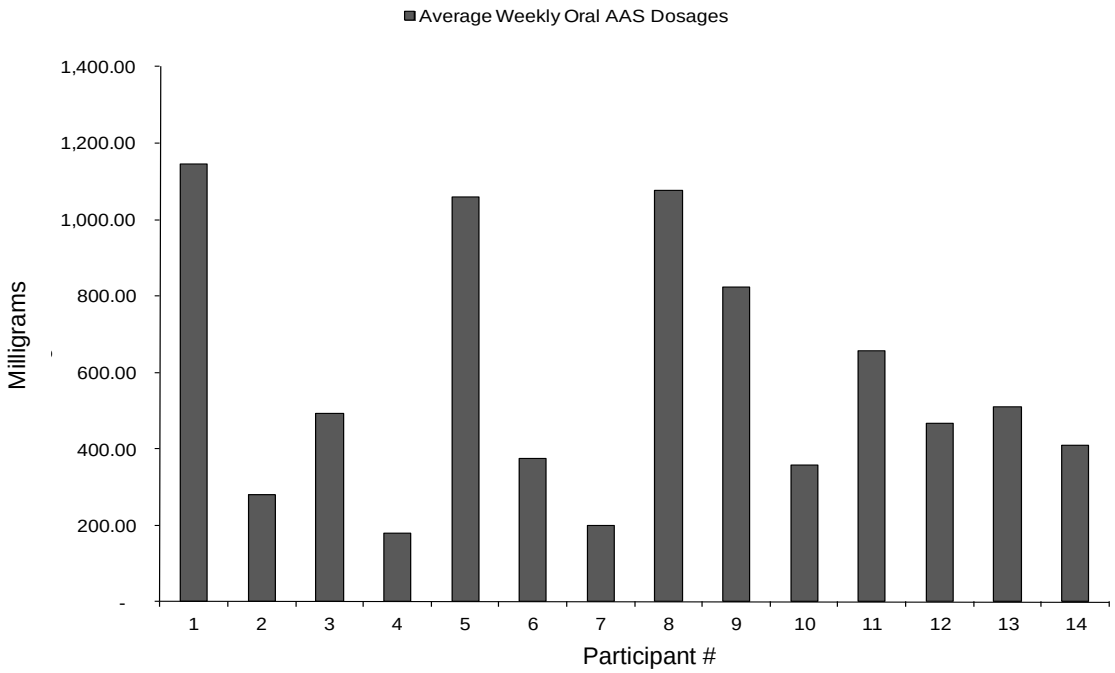


Figure A.29 Individual weekly average oral AAS dosages

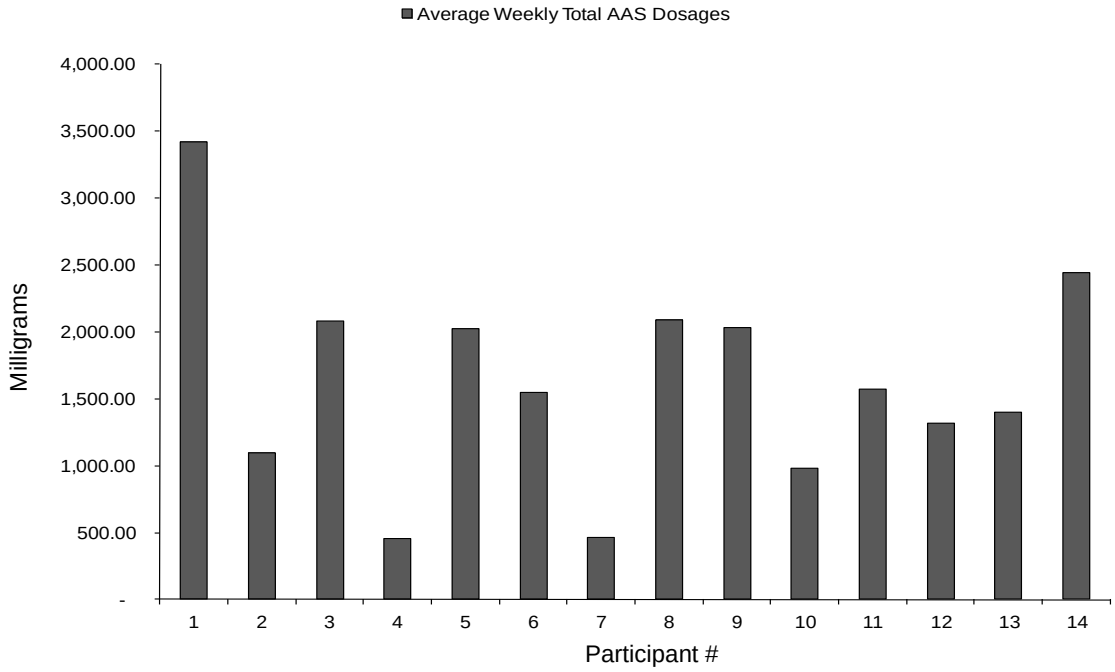


Figure A.30 Individual weekly average total AAS dosages

ANNEXURE B – AAS CYCLES

Participant 1 (Cycle length: 13 weeks)

Week Number	Testosterone Mixture (Supertest 350)	Testosterone Propionate (Testo-Prop)	Trenbolone Enanthate (Tren-E)	Trenbolone Acetate (Tren-Ace)	Drostanolone Enanthate (Masteron-Enan)	Drostanolone Propionate (Masteron-Prop)	Boldenone Undelcynate (Equipoise)	Methenolone Enanthate (Primobolan-Enan)	Stanozolol Aqua Suspension (Winstrol Injectable)	Nandrolone Decanoate (Deca-Durabolin)	Nandroione Phenylprorpionate (NPP)	Oxymetholone Oral (Anapolan-50)	Methandrostenolone Oral (Dianabol Tabs)	Oxandrolone (Anavar)	Mesterolone Oral (Proviron Tabs)	Stanozolol Oral (Winstrol Tabs)	Fluoxymesterone (Halotestan)	Human Growth Hormone (Normatropin / Kefei)	IGF1-LR3 (Igtropin)	Mechano Growth Factor (MGF)	Insulin (Apidra)	T3 (Tertroxin Drops)	Clenbuterol (Clen Drops)	Letrozole (Femara)	Tridenosene	Total Weekly Injectables	Total Weekly Orals	Total Weekly Average
	(mg)	(mg)	(mg)	(mg)	(mg)	(mg)	(mg)	(mg)		(mg)	(mg)	(mg/day)	(mg/wk)	(mg/day)	(mg/day)	(mg/day)	(mg/day)	IU/day	(mcg)	(mcg/day)	(IU)	(mcg/day)	(mcg/day) *2 Days on 2 Days off*	(mg/day)	(ml/day)	(mg)	(mg)	(mg)
13	1400		225		600		630			420		50								1000 (4 x wk)	14 (3 x d;4 x wk)					3275	350	3625
12	1400		225		450		630			420		50								1000 (4 x wk)	14 (3 x d;4 x wk)					3125	350	3475
11	1400		225		600		630			420		50	150							1000 (7 x wk)	14 (3 x d;7 x wk)					3275	500	3775
10	1050		225		450		315			420		50	50(/d)					2 (5 x wk)	100 (1 x wk)	1000 (7 x wk)	14 (3 x d;7 x wk)					2460	700	3160
9	1400		225		600		700	400		420		50(/wk)	90	320(/wk)	25	310(/wk)		6 IU	100 (1 x wk)	1000 (7 x wk)	12 (/d)	20	40	1		3745	1245	4990
8	1050		225		450		630	400		420				60	25	60		6 IU	100 (1 x wk)	1000 (7 x wk)	6 (/d)	20	40	1		3175	1015	4190
7	700	200		320	600		665	400						60	25	60		6 IU	100 (2 x wk)	1000 (7 x wk)		20	40	1		2885	1015	3900
6		300		240		300	350	400			300			60	50	60		6 IU	100 (3 x wk)			20		1	1	1890	1190	3080
5		400		320		400	700	400			150			60	50	60		6 IU	100 (2 x wk)			40		1	1	2370	1190	3560
4		300		240		300	700	400			150			60	50	60		6 IU	100 (1 x wk)			40		1	1	2090	1190	3280
3		100		496		600	525	400			150			60	100	60	10	6 IU				40		1	1	2271	1610	3881
2				360		300		1400						100	100	100	20	6 IU	100 (1 x wk)	1000 (7 x wk)				1.5	1	2060	2240	4300
1				320		400								100	100	150	40							1.5		720	2730	3450
Contest																									**Average**	**2564.7**	**1144.2**	**3421.2**

Participant 2 (Cycle length: 12 weeks)

Week Number	Testosterone Mixture (Sustanon)	Testosterone Enanthate (Testan 300)	Testosterone Propionate (Testo-Prop)	Testosterone Suspension	Drostanolone Propionate (Masteron-Prop)	Trenbolone Acetate (Tren-Ace)	Stanozolol Suspension (Winstrol Aqua Suspension)	Stanozolol Oral (Winstrol Tabs)	Methandrostenolone Oral (Dianabol Tabs)	Mesterolone Oral (Proviron Tabs)	Fluoxymesterone (Halotestin Tabs)	T3 (Tertroxin Tabs)	Total Weekly Injectables	Total Weekly Orals	Total Weekly AAS use
	(mg)	(mg)	(mg)	(mg)	(mg)	(mg)	(mg)	(mg/day)	(mg/day)	(mg/day)		(mcg/day)	(mg)	(mg)	(mg)
12	1000								40	25			1000	455	1455
11		600							40	25			600	455	1055
10		600							40	25			600	455	1055
9		600							40	25			600	455	1055
8			350				175			50			525	350	875
7			350				175			50			525	350	875
6				350	350	240	175	20				20	1115	140	1255
5				350	350	240	175	20				20	1115	140	1255
4				350	350	240	175	20				20	1115	140	1255
3				350	350	240	175	20				20	1115	140	1255
2					350	240	175	20				20	765	140	905
1					350	240	175	20				20	765	140	905
Contest												**Average**	820	280	1100

Participant 3 (Cycle length: 12 weeks)

Week Number	Testosterone Mixture (Sustanon) (mg)	Testosterone Enanthate (Test-Enan) (mg)	Testosterone Propionate (Test-Prop) (mg)	Drostanolone Propionate (Masteron-Prop) (mg)	Methenolone Enanthate (Primobolan-Depot) (mg)	Boldenone Undeclynate (Equipoise) (mg)	Trenbolone Acetate (Tren-Ace) (mg)	Testosterone Suspension (mg)	Stanozolol Suspension (Winstrol Aqua Suspension) (mg)	4-Clorodehydromethyltestosterone Oral (Oral Turinabol) (mg/day)	Methandrostenolone Oral (Dianabol Tabs) (mg/day)	Oxandrolone (Anavar Tabs) (mg/day)	Stanozolol Oral (Winstrol Tabs) (mg/day)	Mesterolone Oral (Proviron Tabs)	Clenbuterol (Clen Drops) (mcg/day)	IGF1-LR3 (mcg) 3 x wk	Human Growth Hormone (IU) Every 2nd day	T3 (Tertroxin Drops) (mcg/day)	Dyphenhydramine (Tylenol PM) (mg/day)	Letrozole (Femara) (mg/day)	Total Weekly Injectables (mg)	Total Weekly Orals (mg)	Total Weekly AAS Use (mg)
12	1000			250		400					50				20	60	8				1650	350	2000
11		600		250		400					50				20	60	8				1250	350	1600
10		600		250		400					50m				40	60	8		100		1250	350	1600
9		600		250		400				50					40	60	8				1250	350	1600
8			500	250		400				50					60	60	8				1150	350	1500
7			500	250	200	400	200		200	50			30		60	60	8	20	100	0.5	1750	560	2310
6			500	250	200	400	200		200			60	30		80	60	8	40		0.5	1750	630	2380
5			500	250	200	400	200		200			60	30		80	60	8	60		0.5	1750	630	2380
4			500	250	200	400	200		200			60	30		100	60	8	80	100	0.5	1750	630	2380
3			500	250	200	400	200		200			60	30		100	60	8	100		0.5	1750	630	2380
2				350	200		300	450	350			80	30		120			100		0.5	1650	770	2420
1				350			350	450	350			100	30					100		0.5	1500	910	2410
Contest																				**Average**	1537.5	492.5	2080

Participant 4 (Cycle length: 18 weeks)

Week Number	Trenbolone Acetate (Tren-Ace) (mg)	Stanozolol Suspension (Winstrol Aqua Suspension) (mg)	Mesterolone Oral (Proviron Tabs) (mg) (3 x d)	Oxandrolone (Anavar) (mg) (3 x d)	Clenbuterol (Clen Drops) (mcg) (3 x d; 2 d on, 2 d off)	T3 (Tertroxin Drops) (mcg) (3 x d)	Yohimbine HCl (Reverzine Injectable) (mg) (2 x d)	Human Growth Hormone (IU) (2 x d)	2,4-Dinitrophenol (DNP Capsules) (mg) (2 x d)	Total Weekly Injectables (mg)	Total Weekly Orals (mg)	Total Weekly AAS Use (mg)
12	300	200						6		500		500
11	300	200						6		500		500
10	300	200						6		500		500
9	300	200						6		500		500
8	300	200			40			6		500		500
7	300	200			40			6		500		500
6	300	200	40	20 mg 3 x p d	40	20	5	6		500	180	680
5	300	200	40	20 mg 3 x p d	40	20	5	6		500	180	680
4	300	200	40	20 mg 3 x p d	40	20	5	6		500	180	680
3	300	200	40	20 mg 3 x p d	40	20	5	6	150	500	180	680
2	300	200	40	20 mg 3 x p d	40	20	5	6	150	500	180	680
1	300	200	40	20 mg 3 x p d	40	20	5		150	500	180	680
Contest												
6	300	200	40	20 mg 3 x p d	40	20	5	6		500	180	680
5	300	200	40	20 mg 3 x p d	40	20	5	6		500	180	680
4	300	200	40	20 mg 3 x p d	40	20	5	6		500	180	680
3	300	200	40	20 mg 3 x p d	40	20	5	6	150	500	180	680
2	300	200	40	20 mg 3 x p d	40	20	5	6	150	500	180	680
1	300	200	40	20 mg 3 x p d	40	20	5		150	500	180	680
Grand Prix									**Weekly Average**	500	180	453,5

Participant 5 (Cycle length: 22 weeks)

Week Number	Testosterone Mixture (Sustanon) (mg)	Testosterone Propionate (Test-Prop) (mg)	Drostanolone Enanthate (Masteron Enan) (mg)	Drostanolone Propionate (Masteron-Prop) (mg)	Methenolone Enanthate (Primobolan-Depot) (mg)	Trenbolone Enanthate (Tren-E) (mg)	Trenbolone Acetate (Tren-Ace) (mg)	Methandrostenolone Oral (Dianabol Tabs) (mg/day)	Oxandrolone (Anavar) (mg/day)	Stanozolol Oral (Winstrol Tabs) (mg/day)	Mesterolone Oral (Proviron Tabs) (mg/day)	Fluoxymesterone (Halotestin Tabs) (mg/day)	Clenbuterol (Clen Drops) (mcg) (3 x d)	Human Growth Hormone (IU) (2 d on, 1 d off)	Letrozole (Femara) (mg/day)	Total Weekly Injectables (mg)	Total Weekly Orals (mg)	Total Weekly AAS Use (mg)
13	1000					200		40						5	0.5	1200	280	1480
12	1000					200		40						5	0.5	1200	280	1480
11	1000						150	40						5	0.5	1150	280	1430
10	1200						150	40						5	0.5	1350	280	1630
9	1200						150							5	0.5	1350		1350
8	1200		450				150							5	0.5	1800		1800
7	1000		450		200								40	5	0.5	1650		1650
6	1000		450		200								40	5	0.5	1650		1650
5	1000		450		300				40	50	40			5	1	1750	910	2660
4		500		350	300				40	50	40			5	1	1150	910	2060
3		500		350	400				40	60	60			5	1.25	1150	1120	2270
2		500		350	400				50	80	80	10	40		2.5	1150	1540	2690
1		500		350	400				50	80	100	20	40		2.5	1150	1750	2900
Contest																		
6		450		300			240							5	1	990		990
5		450		300			240		40	50	40			5	1	990	910	1900
4		450		300			240		40	50	40			5	1	990	910	1900
3		450		300			240		40	60	60			5	1.25	990	1120	2110
2		450		300			240		50	80	80	10		5	2.5	990	1540	2530
1		450		300			240		50	80	100	20			2.5	990	1750	2740
Grand Prix																		

Participant 5 (continued)

3	450	300	240	40	60	60		5	1.25	990	1120	2110
2	450	300	240	50	80	80	10	5	2.5	990	1540	2530
1	450	300	240	50	80	100	20		2.5	990	1750	2740
World Amateur Champs									**Average**	1209.5	1058.2	2027.3

Participant 6 (Cycle length: 28 weeks)

Week Number	Testosterone Mixture (Sustanon) (mg)	Testosterone Enanthate (Test-Enan) (mg)	Testosterone Propionate (Testo-Prop) (mg)	Testosterone Suspension (mg)	Trenbolone Acetate (Tren-Ace) (mg)	Drostanolone Enanthate (Masteron-Enan) (mg)	Drostanolone Propionate (Masteron-Prop) (mg)	Stanozolol Suspension (Winstrol Injectable) (mg)	Methenolone Enanthate (Primobolan-Depot) (mg)	Methandrostenolone Oral (Dianabol Tabs) (mg/day)	Oxandrolone (Anavar) (mg/day)	Fluoxymesterone (Halotestan) (mg/day)	Human Growth Hormone (Normatropin) (IU) (every 2nd day)	Insulin (Apidra) (IU/day)	Clenbuterol (Clen Drops) (mcg) (2 x d) (2 on, 2 off)	Letrozole (Femara) (mg/day)	Total Weekly Injectables (mg)	Total Weekly Orals (mg)	Total Weekly Average (mg)
16	1000									40			6-8	10	40		1000	280	1280
15		2000								40			6-8	10	40		2000	280	2280
14		2000								40			6-8	10	40		2000	280	2280
13		1000				300		175					6-8		40		1475		1475
12		1000				300		175					6-8		60		1475		1475
11		1000				300		175					6-8		60		1475		1475
10		1000				300		175					6-8		60		1475		1475
9		1000				300		175					6-8		60		1475		1475
8		1000				300		175					6-8		60	0.5	1475		1475
7		1000				300		175					6-8		60	0.5	1225		1225
6			750				400	175	300				6-8		60	0.5	1625		1625
5			750				400	175	300				6-8		60	0.5	1625		1625
4			750				400	175	300				6-8		60	0.5	1625		1625
3			750				400	175	300				6-8		60	0.5	1625		1625
2			750				400	175	300		60	10			60	1	1625	490	2115
1			750				400		300		60	20			60	2.5	1450	560	2010
Contest																			

Participant 6 (continued)

6	500	200	250	200	40	60		6-8	60	0.5	1150	700	1850
5	700	200	300	200	40	60		6-8	60	0.5	1400	700	2100
4	700		300		40	60		6-8	60	0.5	1000	700	1700
3	700		300			60		6-8	60	0.5	1000	420	1420
2	700		300				10		60	1	1000	70	1070
1		600					20		60	2.5	600	140	740
Grand Prix													
3	700		300			60		6-8	60	0.5	1000	420	1420
2	700		300			60	10		60	1	1000	70	1070
1		600					20		60	2.5	600	140	740
World Amateur Champs										**Average**	1336	375	1546

ANNEXURE C – SAMPLE DIETS

When it comes to different diet protocols followed by body builders during pre-contest preparation periods, these protocols become highly individualised. The researcher has documented many different diets over the years. Some athletes will have diets consisting of very high protein intake, moderate amounts of fat and low carbohydrate intake (also known as the ketogenic diet), while other athletes again will use very high carbohydrate intake with moderate amounts of protein intake. Some individuals will make use of a method called “carb-cycling” where the diet consists of 2 – 3 days of very high protein / moderate fat and extremely low carbohydrate intake, which is then followed by one day of low protein / low fat intake, combined with very high carbohydrate intake – similar to the levels used by endurance athletes when using carbohydrate-loading techniques.

The researcher has documented diets consisting of protein intake as high as 4.5-5 gr/kg body weight/day with carbohydrate intake as low as less than 1 gr/kg/day. Usually fat intake in general will be in the region of 0.8 - 1.2 gr/kg/day. Other diets again consisted of carbohydrate intake varying from 4 - 6 gr/kg/day, combined with protein intake of 2 gr/kg/day. Body builders rely heavily on whey liquid meal replacements as source of proteins and would opt for products that are in hydrolysed form, having high levels of available amino acids. Most elite level body builders, if not all, will weigh their food portions very accurately for each meal taken. Meal schedules are worked out for 6-8 meals per day and food may even be prepared the night before and placed in cooler bags for the following day. Body builders on pre-contest diets will consume food every 2 ½ to 3 hours and would even get up during the night to take a meal replacement drink. Calorie intake is calculated according to food portions so that the calorie intake per meal is usually close to the same for all the meals per day. Total daily calorie intake is carefully recorded and then titrated regularly (usually weekly changes are made) in accordance to the amount of training hours, type of training done and the measured skin fold fat % (the latter usually done on weekly basis).

The following diets are just generic samples that were followed by a few of the participants during the present study. These might have varied at different stages closer to competition day, but outline the diet generally followed during the pre-contest period.

Participant nr 5

This was for an athlete that participated in the over 100-kg+ weight category

Meal 1: 80 gr Whey Isolate
120gr Oats
Meal 2: 250-300 gr Chicken breast
1cup (raw) rice
Meal 3: 250-300 gr Fish
200 gr Potato
Meal 4: 80 gr Whey Isolate
60 gr Oats
Meal 5: Pre-workout drink
Meal 6: 80 gr Whey Isolate
30 gr Vitargo (complex Carbohydrate drink with very low GI)
Meal 7: 250-300 gr Fish
1-2 cups of mixed vegetables

Participant nr 8

This was for an athlete that participated in the 90-100 kg-weight category.

Meal 1: 60 gr Hydrolysed Whey
Meal 2: 100 gr Oats
2 whole eggs
Meal 3: 200 gr Chicken breast
30 gr mixed nuts
Meal 4: 300 gr Chicken breast
1 cup green vegetables
20ml Olive Oil
Meal 5: 200 gr Chicken breast
30 gr mixed nuts
Meal 6: 300 gr chicken breast or 250 gr lean beef or 400 gr salmon/hake
1 cup green vegetables

20ml Olive Oil

Participant nr 2

This was for an athlete that participated in the 80-85 kg-weight category.

Meal 1: 100 gr Oats
10 egg whites
Meal 2: 200 gr Hake
100 gr Cooked whole-wheat pasta
1 cup mixed vegetables
Meal 3: 300 gr Rump steak
1 cup mixed vegetables
Meal 4: 2 Tins of Tuna
1 cup chopped cucumber
1 Scoop (30 gr) Whey Isolate
Meal 5: 200 gr Hake
1 cup mixed vegetables
Meal 6: 20 gr Amino acids

Participant nr 4

This was for an athlete that participated in the 70-75 kg-weight category

Meal 1: 3 egg whites
1 cup dried oats
Meal 2: 1 Medium Grape Fruit
50 gr Cashew nuts
Meal 3: 100 gr Hake
½ cup boiled brown rice
Meal 4: 1 Medium Grape Fruit
50 gr Cashew nuts
Meal 5: 100 gr Hake
1 Bowl Green salad (lettuce, cucumber, lemon)

ANNEXURE D – DOCUMENTATION SENT TO PARTICIPANTS

Information Document regarding Research Study

You have been selected to participate in a research study, called ***"Body composition and blood measurements of elite Senior South African Body Builders during a competitive season."***

This proposed research study has specifically been designed to assess the physical (body) and biochemical (blood) changes over a competition preparation period of one year (2010 season).

You are participating in a very unique study, as these changes have never been followed over a pre-contest, competition and post-contest period. The duration of the study will be over a competitive season. Measurements will be taken at the beginning of your official pre-contest preparation period, irrespective of how long this period may be. Information will be gathered further during the course of your competition preparation, including the day of your final competition, as well as 6 weeks post-contest. If multiple competitions are planned, the measurements will start at the beginning of your pre-contest phase for the first planned competition and continue until the last competition of the season, including the 6 weeks post-contest period.

As the study has been designed to gather information and not to supply intervention, there will be no risk participating in the study. By gathering the information, risk profiling can be done in each participant's case and this will be relayed to you in person. This is the advantage to you for participating in the study – your health will be assessed over the course of the competitive season, at no cost to you as a participant. The only cost to you will be that of providing your own transportation to the nominated laboratories on the three occasions of blood sampling. If any assistance is needed regarding this, please feel free to contact the researcher.

The blood sampling will be free of cost. The results will be used for statistics only. You will receive your results in person after each occasion of sampling. These can then be taken either to your own physician of choice, or if assistance in interpretation of the results is needed, the researcher can be contacted in this regard. Blood samples will be only taken for the tests as indicated by the study protocol – no other tests will be performed. The blood samples will be discarded after the tests have been performed – they will not be kept for future use in any manner. The risk in providing the blood samples is minimal. The insertion and removal of the sampling needle will cause a slight burning sensation of about two seconds. The complication can be that of a small bruised (haematoma) area at the sample site – the latter can be largely minimalized by providing direct pressure to the sample site at the removal of the sample needle and continuing the pressure for 30-60 seconds. The AMPATH assistants will assist you in this procedure.

Participation in this study is voluntary, and you will not be penalized or lose benefits if you refuse to participate or decide to terminate your participation at any time during the study. Termination of participation on your behalf should be in writing,

addressed to the researcher, clearly stating the reason/s for termination. Participants will not be remunerated for participating in the study.

The information that will be obtained will be handled with the highest level of confidentiality. You will receive with this document, an accompanying consent form and a confidentiality agreement. Once these have been signed and returned to the researcher, the latter will allocate a case study number to you. This number will be the only thing used in further correspondence. Only the researcher will know your personal details. The confidentiality document is a legal agreement between you and the researcher alone. Please return all the original signed documents to the researcher by placing them in the accompanying envelope you have received with the information document. You will also receive signed copies of the consent form and the confidentiality agreement – this should be kept for your own records

The information obtained from the cohort (group of athletes) will be used for statistical analysis for answering the research questions. The results of the analysis of the cohort's information may be published and/or presented at a meeting/congress. It should be understood clearly that only the statistics, and not the individual results of any of the participants, would be published.

What will be measured and what will be needed from you as a participant.

The study has been designed in such a manner that it will take the minimum of your personal time to participate and will not interfere with your diet / training program. Once you have arrived at the AMPATH facility, the measurements on each occasion should not take more than 10 – 15 minutes of your time.

First measurements:

When starting your pre-contest phase

- Blood pressure
- Skin fold fat %
- Body weight

A registered, accredited personal trainer at any training facility should take the body weight and skin fold fat %, preferably at Virgin Active. It would help consistency, if you could have these measurements, if possible, taken by the same person on each occasion.

This will also be the first occasion for blood sampling. You will be supplied with numbered requisition forms (numbers one, two and three – each to be taken on each of the three occasions of blood sampling). These should be taken to the nominated laboratory. The latter can be selected once you have supplied the researcher with your address. On the requisition form, the planned tests will be indicated; your specific case number and the research study's grant number, as well as a space for your blood pressure reading. No other details should be filled in on the requisition forms. Your blood pressure will be taken at each occasion of blood sampling. Please note that this should be taken before the actual blood samples are collected – the blood pressure readings will be given to you on each occasion (for your own interest), but will be recorded by the venesectionist on the requisition forms that will be sent to the AMPATH main frame.

For the first occasion of sampling, you should be fasting from the previous night 22h00. No food / beverages should be taken until 08h00 the morning of the

sampling. The sampling should take place at 08h00. Once at the laboratory, this should not take more than 5 minutes of your time.
The results will be sent to the researcher, whom shall make them available to you as soon as possible.

The initial sampling is in order to establish base line values of the following:

- Glucose (sugar) levels
- Lipid (fat) profile
- Full blood count
- Cortisol levels
- Liver enzymes
- Kidney function test

You will also receive ***data collection sheets***. The first of these will need the skin fold fat % and the body weight measurements to be recorded on. Please record your highest off-season weight and skin fold fat % of the particular year's weight gaining phase.

You will also receive comprehensive data collection sheets on the different Androgenic-Anabolic Steroid (AAS) substances usually used by Body Building athletes. Please supply the information on the Excel spread sheets of every week's substance use. Only the total milligrams of each substance used per week should be supplied. Supplying this information is of utmost importance to the research study, as very little knowledge is available in the South African Medical Community on the types of AAS substances and dosing protocols used in general amongst South Africa's elite Body Builders.

These information sheets, where possible, should be sent to the researcher on a weekly basis. Only your case number should be quoted on the data collection sheets. These can be sent via fax, post or e-mail to the researcher – please liaise with the researcher on your preferred method.

Second measurement:

On the competition day, only your body weight and skin fold fat % need to be recorded on the data collection sheets and forwarded to the researcher in due course.

Third measurement:

These measurements will be taken one week after your last competition for the season.

Blood pressure, body weight and skin fold fat % will be recorded again in the same manner as previously.

The second blood sampling will take place in the same manner as on the first occasion – fasting for 10 hours and at 08h00. The same blood test will be repeated as on the first occasion, except the Cortisol levels. Again these results will be supplied to you as soon as possible.

Fourth measurement:

These will be taken at 4 – 6 weeks after the last competition for the year.

Blood pressure, body weight and skin fold fat % will be recorded again in the same manner as on previous occasions.

The last blood sampling will take place, but this occasion you do not need to be fasting. You should again be at the nominated laboratory at 08h00. The following tests will be done:

- Total Testosterone levels
- LH, FSH and Estradiol levels
- 08h00 Cortisol levels

These results, as on previous occasions, will be supplied to you as soon as possible.

Thank you for participating in the study and for the positive contribution you are making towards Sports Medicine in South Africa.

You are welcome to contact the researcher, Dr Riaan Barnard, if you require any further information or need assistance in any matters relating to the study. He can be reached at cell number 082 654 658 7 or e-mail docb@pemail.co.za.

Dr Riaan Barnard
MBChB (Pretoria)
BSc Hons (Stellenbosch)
Second year Masters Sports Medicine (Bloemfontein)

Consent to participate in research study

You have been selected to participate in a research study, called *"Body composition and blood measurements of elite Senior South African Body Builders during a competitive season."*

You have been informed about the study by means of an information document supplied to you by Dr Riaan Barnard, the researcher conducting the study.

You have been informed about available medical treatment / intervention if injury occurs as a result of study-related procedures.

You are welcome to contact the researcher, Dr Riaan Barnard, at 082 654 658 7 or via e-mail at docb@pemail.co.za any time if you have questions about the research study or if you are injured in any way as a result of the research.

You may contact the Secretariat of the Ethics Committee of the Faculty of Health Sciences, UFS at telephone number (051) 4052812 if you have any questions about your rights as a research subject.

Your participation in this research is voluntary, and you will not be penalized or lose benefits if you refuse to participate or decide to terminate participation at any time during the study. Termination of participation on your behalf should be in writing, addressed to the researcher, clearly stating the reason/s for termination.

You have also been informed that the only costs to you as a participant, is the expenses for transportation to the allocated laboratories on the three occasions of blood sampling. If any assistance regarding this is needed, you can contact the researcher.

You have been informed that the blood specimens taken will only be used for the indicated tests as stated in the information document. After the tests have been performed, the specimens will be discarded and not kept for future use. You will also be supplied with copies of the full results of the tests performed.

If you agree to participate, you will be given a signed copy of this document, as well as the participant information sheet, which is a written summary of the research project. You will also receive a signed copy of the confidentiality agreement between you and the researcher, providing you with your participant case number. Only this number will be used on the questionnaires provided to you in due course.

The research study, including the above information has been verbally described to me in full detail. I understand what my involvement in the study means and I voluntarily agree to participate.

Signed at _____ on the ____ of _____ 20___.

_____
Signature of participant

_____
Signature of witness

Memorandum of Confidentiality

Entered into between

Covenanter: **DR. RIAAN BARNARD**

Proprietor: _____

MEMORANDUM OF CONFIDENTIALITY

This entered into between:

Name:	DR RIAAN BARNARD
ID No.:	6906135243089
Designation:	Medical Practitioner / Sports Physician
Company:	
Registration Number:	MP 0434639
Postal Address:	PO Box 394, Kirkwood, 6120
Physical Address:	John Street 15, Kirkwood, 6120
Telephone Number:	042 2300 555
Facsimile Number:	042 2300 283
Mobile Number:	082 654 658 7
E-Mail Address:	docb@pemail.co.za

(Hereinafter referred to as "***The Covenanter***");

And

Name:	_____
ID No.:	_____
Designation:	_____
Company:	_____
Postal Address:	_____
	_____
Physical Address:	_____
Telephone Number:	_____
Facsimile Number:	_____
Mobile Number:	_____
E-Mail Address:	_____

(Hereinafter referred to as "***The Proprietor /Athlete***")

1. **INTERPRETATION AND DEFINITIONS**

a. In this ***Memorandum of Confidentiality***, unless inconsistent with, or otherwise indicated by the context:

i. "***the Covenanter"*** is ***Dr Riaan Barnard***;

ii. ***"the Covenanter's"*** address is ***PO Box 394, Kirkwood, 6120***;

iii. ***"the Proprietor"*** is _____ of _____;

iv. ***"Confidential Information"*** shall include, but shall not be limited in its interpretation to:- all personal information, medical samples taken; results of all laboratory testing done on the proprietor / athlete, all secret knowledge, technical information and specifications, training techniques, designs, circuit diagrams, instruction manuals, blueprints, samples, devices, demonstrations, formulae, know-how, information concerning materials, marketing and information generally in which ***The Proprietor*** has an interest in being kept confidential;

v. ***"Commencement Date"*** means the date of signature of this ***Memorandum of Confidentiality*** by ***the Covenanter.***

vi. words in the singular include the plural and vice versa;

vii. words importing any one gender include each of the other two genders; and

viii. A reference to a natural person includes a legal persona.

b. The headings of clauses are intended for convenience only and shall not affect the interpretation of this ***Memorandum of Confidentiality***.

2. **PREAMBLE**

a. ***The Proprietor*** has in its possession certain Confidential Information relating to, but in no way limited to, certain training supplements and other medicinal enhancement drugs and the use thereof.

b. It includes, but is in no way limited to, any and all information related to those products and their use.

c. The above mentioned confidential information is summarised, but in no way limited to, the above and as per Addendum ***A: DRUGS USED BY PROPRIETOR / ATHLETE.***

d. ***The Proprietor*** / ***Athlete*** has agreed to disclose certain of this Confidential Information to ***the Covenanter*** subject to ***the Covenanter*** agreeing to the terms of confidentiality set out herein.

3. **TITLE TO THE CONFIDENTIAL INFORMATION**

a. ***The Covenanter*** acknowledges that all right, title and interest in and to the Confidential Information vests in ***The Proprietor*** / ***Athlete*** and that they have no

claim of any nature in and to the Confidential Information.

4. **PERIOD OF CONFIDENTIALITY**

a. The provisions of this ***Memorandum of Confidentiality*** shall remain in force indefinitely.

5. **NON-DISCLOSURE**

a. ***The Covenanter*** undertakes to maintain the confidentiality of any Confidential Information to which ***the Covenanter*** should be allowed access to by ***The Proprietor***/ ***Athlete***, whether before or after the Commencement Date of this ***Memorandum of Confidentiality.***

b. ***The Covenanter*** is deemed to be bound by the whole agreement regardless of the time of disclosure .

c. ***The Covenanter*** will not divulge or permit to be divulged to any person any aspect of such Confidential Information otherwise than may be allowed in terms of this ***Memorandum of Confidentiality***.

d. ***The Covenanter*** shall take all such steps as may be reasonably necessary to prevent the Confidential Information falling into the hands of an unauthorised third party.

e. ***The Covenanter*** shall not make use, directly or indirectly, of any of the Confidential Information in the development, manufacture, marketing and/or sale of any goods / concepts / product / principle / strategy / program or any other endeavour, without the prior written consent of ***The Proprietor***/ ***Athlete***.

f. ***The Covenanter*** shall not use or disclose or attempt to use or disclose the Confidential Information for any purpose other than performing its contractual obligations to ***The Proprietor***/ ***Athlete***.

g. ***The Covenanter*** shall not use or attempt to use the Confidential Information in any manner which will cause or be likely to cause injury or loss to ***The Proprietor***/ ***Athlete***.

h. ***The Proprietor*** / **Athlete** may by written notice to ***the Covenanter*** specify which of ***the Covenanter***'s employees, officers or agents are required to sign a secrecy ***Memorandum of Confidentiality*** in a form specified by ***The Proprietor***/ ***Athlete*** from time to time and no such person may be employed in the conduct of the business of ***the Covenanter*** until such secrecy ***Memorandum of Confidentiality*** has been signed.

i. All documentation furnished to ***the Covenanter*** by ***The Proprietor*** / ***Athlete*** pursuant to this ***Memorandum of Confidentiality*** will remain the property of ***The Proprietor*** / ***Athlete*** and upon the request of ***The Proprietor***/ ***Athlete*** will be returned to ***The Proprietor***/ ***Athlete***. ***The Covenanter*** will not make copies of any

such documentation without the prior written consent of ***The Proprietor***/ ***Athlete***.

j. Any material of a confidential nature which comes into the possession of ***the Covenanter*** or one of its agents or employees, or which is generated by ***the Covenanter***, or one of its agents or employees, after the Commencement Date:

i. shall be deemed to form part of the Confidential Information of ***The Proprietor***/ ***Athlete***;

ii. shall be deemed to be the property of ***The Proprietor***/ ***Athlete***;

iii. shall not be copied, reproduced, published or circulated by ***the Covenanter*** and

iv. shall be surrendered to ***The Proprietor*** / ***Athlete*** on demand, unless ***The Proprietor***/ ***Athlete*** provides its prior written consent to the contrary.

6. **EXCEPTIONS**

a. The above ***Memorandum of Confidentiality*** by ***the Covenanter*** relating to the confidentiality shall not apply to information which:

i. is in fact lawfully in the public domain at the Commencement Date; or

ii. lawfully comes into the public domain after the Commencement Date otherwise than as a result of the conduct of ***the Covenanter*** or one of its employees or agents; or

iii. ***the Covenanter*** is compelled to disclose in terms of a court order.

b. The onus of proving the facts necessary to sustain any one of the exceptions listed in sub-paragraphs 6.a.i to 6.a.iii rests with ***the Covenanter***.

7. **JURISDICTION**

a. This ***Memorandum of Confidentiality*** shall be governed by South African law and ***the Covenanter*** hereby irrevocably agrees to the jurisdiction of the High Courts of South Africa in respect of any dispute flowing from this ***Memorandum of Confidentiality***.

8. **WHOLE AGREEMENT**

a. This document constitutes the whole of this ***Memorandum of Confidentiality*** to the exclusion of all else.

b. No amendment, alteration, addition, variation or consensual cancellation of this ***Memorandum of Confidentiality*** will be valid unless in writing and signed by ***the Covenanter*** and ***The Proprietor***/ ***Athlete***.

9. **WAIVER**

a. No waiver of any of the terms or conditions of this ***Memorandum of Confidentiality*** will be binding for any purpose unless expressed in writing and signed by ***The Proprietor*** / ***Athlete*** and any such waiver will be effective only in the specific instance and for the purpose given.

b. No failure or delay on the part of ***The Proprietor*** / ***Athlete*** in exercising any right, power or privilege will operate as a waiver, nor will any single or partial exercise by

The Proprietor / ***Athlete*** of any right, power or privilege preclude any other or further exercise thereof or the exercise of any other right, power or privilege.

10. **SEVERABILITY**

a. In the event that any of the provisions of this ***Memorandum of Confidentiality*** are found to be invalid, unlawful, or unenforceable such terms shall be severable from the remaining terms, which shall continue to be valid and enforceable.

11. **BREACH**

a. If ***The Covenanter*** , or any of the designated contacts or employees, or any other party to whom the ***Covenanter*** shall have alienated any information and / or revealed any documentation after receiving a written mandate by ***The Proprietor***/ ***Athlete***, and having signed a ***Memorandum of Confidentiality***, shall breach any provisions of this agreement, all of which shall be deemed to be material, then ***The Proprietor*** / ***Athlete*** shall be entitled, without prejudice to any other rights or remedies which he may have at law to:

i. forthwith cease his participation; and / or

ii. cancel any agreement of license or other transaction resulting from such a breach; and / or

iii. compel specific performance; and / or

iv. obtain an interdict or other similar relief, each without prejudice to his rights to claim damages, it being specifically agreed that damages shall include consequential and / or punitive damages.

12. **ACKNOWLEDGMENT**

a. ***The Covenanter*** hereby acknowledges that:

i. They understand the contents of this agreement;

ii. They have voluntarily agreed to enter into this agreement;

iii. They are bound by each and every provision hereof;

iv. Each and every provision hereof is reasonable and necessary to protect the rights of ***The Proprietor***/ ***Athlete*** in relation to the ***Memorandum of Confidentiality***.

SIGNATORIES

Thus done and signed on this the _____ day of

_____in the 2010.

Signed at:

_____

_____

_____

_____

_____

DR. RIAAN BARNARD / **The *Covenanter***

_____

The *Proprietor*/ *Athlete*

_____ Date: _____/_____/_____

Witness 1: .....

Identity No.: .....

_____ Date: _____/_____/_____

Witness 1: .....

Identity No.: .....

PATOLOË · PATHOLOGISTS
PRG5200005200431
Drs Du Buisson, Bruinette, Kramer Inc./Ing.
Drs Swart & Maré

AMPATH

Academic Study

PROJECT CODE: c 5 5 3 4 3
NUMBER

REQUISITION NUMBER

REFERRING DOCTOR: barnr00z
COPY DOCTOR: hougr00
COPY DOCTOR: ackze00

PATIENT NO:

DATE OF BIRTH: / (DD/MM/YYYY)

PATIENT BP:

DATE OF COLLECTION: 2 O
(DD/MM/YYYY)

SEX: M F

COLLECTION TIME: h

VISIT: FIRST VISIT

LABORATORY TESTS (Please tick appropriate blocks)

- □ Full Blood Count
- □ Fasting Glucose
- □ Fasting Lipogram
- □ U & E (Creat)
- □ 8H00 Cortisol
- □ Liver Enzymes

PATOLOË · PATHOLOGISTS
PRGS20005200431
Drs Du Buisson, Bruinette, Kramer Inc./Ing.
Drs Swart & Maré

AMPATH

Academic Study

PROJECT CODE: c 5 5 3 4 3
NUMBER

REQUISITION NUMBER

REFERRING DOCTOR: barnr00z
COPY DOCTOR: hougr00
COPY DOCTOR: ackze00

PATIENT NO:

DATE OF BIRTH: / (DD/MM/YYYY)

PATIENT BP:

DATE OF COLLECTION: 2 O
(DD/MM/YYYY)

SEX: M F

COLLECTION TIME: h

VISIT: SECOND VISIT

LABORATORY TESTS (Please tick appropriate blocks)

□ Full Blood Count
□ Fasting Glucose
□ Fasting Lipogram
□ U & E (Creat)
□ Liver Enzymes

PATOLOË · PATHOLOGISTS
PRGS20005200431
Drs Du Buisson, Bruinette, Kramer Inc./Ing.
Drs Swart & Maré

AMPATH

Academic Study

PROJECT CODE: c 5 5 3 4 3
NUMBER

REQUISITION NUMBER

REFERRING DOCTOR: barnr00z
COPY DOCTOR: hougr00
COPY DOCTOR: ackze00

PATIENT NO:

DATE OF BIRTH: / (DD/MM/YYYY)

PATIENT BP:

DATE OF COLLECTION: 2 O
(DD/MM/YYYY)

SEX: M F

COLLECTION TIME: h

VISIT: THIRD VISIT

LABORATORY TESTS (Please tick appropriate blocks)

□ **Total Testosterone**
□ **LH**
□ **FSH**
□ **Estradiol**
□ **8H00 Kortisol**

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