Cape Peninsula University of Technology

THE EFFECTS OF CONTRACEPTIVES ON THE ANTI-OXIDANT STATUS, SKIN PARAMETERS AND ANTHROPOMETRIC INDICATORS IN FEMALE STUDENTS: A PILOT STUDY

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DECLARATION

I, Martha Petronella Germishuys, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed **Date**

ABSTRACT

Introduction: The provision of access to safe and effective contraception is a critical element in the health of women that enables them to make choices about their fertility. This element of control empowers them and indirectly enables them to access better social and economic opportunities.

Hormonal contraceptives are a convenient, effective and relatively safe method of fertility control. Extensive research has been done on the effects of hormonal contraceptives on undesirable metabolic and haemostatic changes, but data on the relationship between oxidative stress and oral contraceptives is scarce and remains subject to debate. Aging of the skin due to oestrogen loss at menopause is thought to include atrophy, decreased collagen content, water content, and sebaceous secretions, loss of elasticity, wrinkling, poor wound healing and manifestations of hyperandrogenism. A number of studies have shown that oestrogens serve many important beneficial and protective functions in skin physiology.

Despite extensive clinical experience, many metabolic effects of oral contraceptive treatment remain to be explored. The effects of progesterone on body weight and composition are of interest from several standpoints. Since hormonal contraceptives are widely used, it is important to investigate the effect thereof on oxidative status, skin parameters and anthropometric indicators, to enable women make informed choices about the use of contraceptives, or to adapt their lifestyle if necessary. The aim of the present study was therefore, to assess certain effects of contraceptives in a student population at the Cape Peninsula University of Technology (CPUT).

Objectives of the study: To determine the differences in skin health, anthropometric \parameters and oxidative stress status in female university students using various hormonal contraceptives versus non-contraceptive users.

Research design: The study adopted a quantitative approach to examine a cross-sectional research sample in order to provide a snapshot of the population at a particular time. Concenting participants were selected through the use of questionnaires aimed at ascertaining the type of contraceptive used as well as general health and lifestyle patterns. Blood samples were collected and the antioxidant status was determined. Body composition and skin analysis was conducted on each of the participants in the selected groups and the results were

compared to determine the differences between contraceptive and non-contraceptive users.

Results: With regards to oxidative stress status, the results indicated a significant increase in superoxide dismutase (SOD) activities within the triphasic contraceptive group compared to the monophasic contraceptive group, suggesting higher levels of oxidative stress in monophasic contraceptive groups. There was also an increase in lipid peroxidation (TBARS) for the triphasic contraceptive group when compared to the control, monophasic contraceptive and injectable contraceptive groups respectively, indicative of increased oxidative stress levels in the triphasic contraceptive group. In this study, skin parameters evaluation revealed that there was a general increase in the presence of erythema in the monophasic contraceptive group compared to the control; injectable contraceptive; implant contraceptive and triphasic contraceptive groups, symptomatic of higher vascular activity in the monophasic group. Melanocyte activity measured in the forehead, cheek and chin areas were also significantly increased when the monophasic contraceptive group was compared to the control and other contraceptive groups, characterised by the pigmentation pattern of chloasma/melasma known to be caused by hormones. The hydration measurements were significantly increased in the implant contraceptive group compared to the control and monophasic contraceptive groups. Furthermore, a significant increase in hydration was evident in the injectable contraceptive group when compared to the control and monophasic contraceptive groups. Injectable contraceptives and implant contraceptives mainly contain progesterone which has been proven to combat signs of aging and increase collagen and elastin in the skin. With respect to anthropometric measurements, there was a significant increase in the measurement of waist to hip ratio in the implant contraceptive group compared to the control group (non-contraceptive). Progesterone influence on adipose tissue distribution indicated a more significant increase of adipose tissue in the abdominal region.

Conclusion:

In this study there was some evidence that the type of hormonal contraceptive used does have significant effects on the variables tested in the population sample. These effects are dependent on the composition of the contraceptive and the levels of progesterone and/or oestrogen.

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DEDICATION

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TABLE OF CONTENTS

CHAPTER THREE: RESEARCH DESIGN AND METHODOLOGY

LIST OF FIGURES

LIST OF TABLES

APPENDICES

GLOSSARY

Abbreviations	Explanation
AAE	Ascorbic acid equivalent
AGEs	Advanced glycation end products
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BCA	Bicinchoninic acid
BD vacutainers	Becton Dickinson
BIA	Bio-electrical impedance analysis
BMD	Bone mineral density
BMI	Body mass index
BP	Blood pressure
CAT	Catalase
CNS	Central Nervous System
COC	Combined oral contraceptives
CoQ10	Coenzyme Q10
COX	Cyclooxygenases
CPD	Cyclobutane pyrimidine dimers
CPG	Contraception policy guidelines
CPUT	Cape Peninsula University of Technology
CSM	Committee on Safety of Medicine
CVD	Cardiovascular disease
DETAPAC	Diethylenetriaminepentaacetic acid
DMPA	Depot medroxyprogesterone
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
ETG	Etonogestrel
FAD	Flavin adenine dinucleotide
Fe	Iron
$FeCl_3$	Iron trichloride
FR	Free radical
FRAP	Ferric reducing ability of plasma
FSH	Follicle stimulating hormone
GnRH	Gonadotropin-releasing hormone
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione

GSSG	Oxidised glutathione
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric acid
HDL	High density lipoprotein
HIV	Human immunodeficiency virus
HNO_2	Nitrous acid
HOCl	Hypochlorous acid
HPLC	High-performance liquid chromatography
HRT	Hormone replacement therapy
IHD	Ischaemic (or ischemic) heart disease
IUD	Intrauterine device
LARC	Long-acting reversal contraceptive
LDL	Low density lipoprotein
LH	Luteinizing hormone
LNG	Levonorgestrel
LOX	Lipoxygenases
LP	Lipid peroxidation
M_2VP	1-Methyl-2-vinylpyridiumtrifluoromethane sulphonate
MPO	Myeloperoxidase
N_2O_3	Sodium carbonate
NAD(P)H	Nicotinamide adenine dinucleotide phosphate
NADH	Nicotinamide adenine dinucleotide
NET-EN	Norethisterone enanthate
NMF	Natural moisturising factors
NO_2	Nitric oxide
NO_2Cl	Nitryl chloride
NOX	Oxidase isoforms
$O_2^{\bullet-}$	Superoxide radical
OC	Oral contraceptive
OCP	Oral contraceptive pill
OH	Hydroxide ion
OH•	Hydroxyl radical
ONOO	Peroxynitrite
OR	Oestrogen receptors
ORAC	Oxygen radical absorbance capacity
ORE	Oestrogen responsive element
OS	Oxidative stress
PMS	Premenstrual syndrome

POIC	Progesterone-only injectable contraceptive
POP	Progesterone-only pill
PR	Protein related
Prxs	Peroxiredoxins
PUFA	Polyunsaturated fatty acids
RNS	Reactive nitrogen species
RO_2	Peroxyl radicals
ROONO	Alkyl peroxynitrites
ROS	Reactive oxygen species
SA	South Africa
SADHS	South Africa Demographic and Health Survey
SD	Standard deviation
SHBG	Sex hormone binding globulin
SOD	Superoxide dismutase
TAC	Total antioxidant capacity
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid-reactive substance
TE	Trolox equivalent
TEWL	Transepidermal water loss
TPTZ	Tripyridyl triazine
UK	United Kingdom
UV	Ultra violet
WHO	World Health Organisation
WHR	Waist-to-hip ratio
XO	Xanthine oxidase

CHAPTER ONE
INTRODUCTION

1.1 Introduction

The provision of access to safe and effective contraceptives is a critical element in the health of women that enables them to make choices about their fertility. This element of control empowers them and indirectly enables them to access better social and economic opportunities. Birth spacing also improves the chances of children thriving physically and emotionally (National Contraception and Fertility Planning Policy and Service Delivery Guidelines, 2014).

Hormonal contraceptives are a convenient, effective and relatively safe method of fertility control. They have also been used to treat gynaecological disorders relating to ovulatory and menstrual dysfunction (Thane *et al.,* 2002). Extensive research has been done on the effects of hormonal contraceptives in terms of undesirable metabolic and haemostatic changes (Reubinoff *et al*., 1995b; Rivera *et al*., 1999; Borgelt-Hansen, 2001; Burkman *et al*., 2004; Lech & Ostrowska, 2005; Stevenson & Thornton, 2007; Shufelt & Merz, 2009; Sabatini *et al*., 2011; Shulman, 2011; Dahan-Farkas & Irhuma, 2016), but data on the relationship between oxidative stress and oral contraceptives is scarce (Akinloye *et al*., 2010). Nevertheless, among conditions known to affect oxidative stress, the use of contraceptives in women has been a matter of ongoing academic argument and discussion.

In recent decades, contraceptive use has risen markedly worldwide. In South Africa (SA), estimates of the proportion of women of reproductive age who are protected against unplanned pregnancies, using modern contraceptive methods, increased steadily from 26.3% in 2002/2003 to 37.3% in 2013/2014 (Chersich *et al.*, 2017). The South Africa Demographic and Health Survey (SADHS, 2018) provided information on both knowledge and patterns of contraceptive use. The data indicates that among married and sexually active unmarried women, 58% are using a modern contraceptive method (SADHS, 2018).

Modern methods of contraception include female and male sterilisation, oral hormonal pills, the copper intra-uterine device (Cu IUD), the male condom, injectables, the implant (including Norplant), vaginal barrier methods, the female condom and emergency contraception. The rate of usage varies according to ethnic group, age group and educational level. This accords with international trends, which show that the higher the person's level of education, the higher the likelihood of

contraceptive use (National Contraception and Fertility Planning Policy and Service Delivery Guidelines, 2014). Patterns of contraceptive methods used indicate that injectable progestogen contraception is predominant. Both the two-month and three-month hormonal injections are widely offered throughout the country (National Contraception and Fertility Planning Policy and Service Delivery Guidelines, 2014).

Oxidative stress and elevated levels of reactive oxygen species (ROS) are associated with the development of various diseases, ranging from cardiovascular-associated disorders such as heart failure and diabetes, to neurodegenerative diseases, cystic fibrosis, rheumatoid arthritis and cancer (Pincemail *et al.,* 2007; Akinloye *et al.*, 2010; Essick & Sam, 2010). Among factors known to influence oxidative stress, the use of oral contraceptives in women has been a matter of concern. Research studies have over the years investigated the influence of oral contraceptive usage on changes in carbohydrate, lipid, enzymes, mineral and vitamin metabolism (Akinloye *et al.*, 2010; Finco *et al.,* 2011; Sabatini *et al*., 2011).

It has been suggested (although not widely accepted) that oestrogens have an antioxidant effect that may contribute to the protection of the cardiovascular system through the inhibition of lipid oxidation. *In vitro* studies by Saha *et al*. (2000) and Chiang *et al.* (2004) report that oestrogens significantly reduce the oxidative damage to lipids exposed to free radical generating systems. However, only a few studies have examined the relationship between combined oral contraceptives containing oestrogens and progesterones and oxidative status (Akinloye *et al*., 2010).

Skin quality deteriorates with age due to the synergistic effects of chronologic aging, photo aging, environmental factors and hormonal influences. The hormonal aging of skin due to oestrogen loss at menopause is thought to cause atrophy, decreased collagen content, water content, and sebaceous secretions, loss of elasticity, wrinkling, poor wound healing and manifestations of hyperandrogenism. A number of studies have shown that oestrogens have many important beneficial and protective functions in skin physiology (Brincat *et al.,* 2005; Stevenson & Thornton, 2007; Raghunath *et al.,* 2015; Tobin, 2017).

Stevenson & Thornton (2007) and Raghunath *et al*. (2015) report a variation in skin thickness during the menstrual cycle, with skin thickness lowest at the start of the menstrual cycle, when oestrogen and progesterone levels are low, but increasing in thickness along with rising levels of oestrogen. When oestrogen levels are very low (menopause), hypo-oestrogenism accelerates age-related deterioration, which in turn results in thinner skin, an increase in number and depth of wrinkles, increased skin

dryness, and decreased skin firmness and elasticity (Stevenson & Thornton, 2007; Raghunath *et al.,* 2015; Tobin, 2017). Oestrogen therapy (hormone replacement therapy – HRT) may prevent collagen loss and can stimulate the synthesis of collagen in those who have lower initial collagen levels. There is also a relationship between oestrogen deprivation and degenerative changes in dermal elastic tissue (Stevenson & Thornton, 2007; Tobin, 2017). There is some evidence that HRT increases skin surface lipids and wound healing, increases epidermal hydration, skin elasticity, skin thickness, while also reducing skin wrinkles. Furthermore, the content and quality of collagen and the level of vascularisation have been found to be enhanced by HRT (Stevenson & Thornton, 2007; Tobin, 2017). Oestrogens also offer some degree of protection against skin photo-aging and epidemiological studies indicate that the mortality rates from both non-melanoma skin cancers and melanoma are significantly lower in women taking oestrogen (Brincat *et al*., 2005; Stevenson & Thornton, 2007).

Research into the effects of progesterone suggests that the presence of various dermatoses correlates with peak levels of progesterone. Raghunath *et al*. (2015) maintain that dermatoses that are exacerbated perimenstrually (at peak levels of progesterone) include acne, psoriasis, atopic eczema and irritant dermatitis, and possibly also erythema multiforme. Underlying mechanisms include reduced immune and barrier functions as a result of cyclical fluctuations in oestrogen and/or progesterone. Autoimmune progesterone and oestrogen dermatitis are the best-characterised examples of perimenstrual cutaneous reactions to hormones produced during the menstrual cycle (Raghunath *et al.,* 2015).

One of the most common dermatological conditions in older women is dry or dehydrated skin. Healthy skin varies during the menstrual cycle and decreases with age. However, it requires a substantial water content, which is determined by both the cutaneous evaporation rate and evaporation (Brincat *et al*., 2005). The loss of moisture within the skin is called transepidermal water loss (TEWL) and is the major factor responsible for dry skin (Lewis & Lewis, 2015).

Normal skin does not need topically applied water for moisturising. The water in skin cells is there as a result of normal physiological processes. What is needed is to keep this water within the cell, namely an improvement in barrier function. A critical skin component that is responsible for its barrier function comprises intercellular lipids. Lipid layers hold water and surround corneocytes to provide a barrier to permeability. The intercellular lipids and corneocytes containing proteins and natural moisturising factors work together to provide an efficient barrier against water loss,

promoting water retention to maintain the flexibility of the skin. The protective forces shield the skin from desiccation and environmental assaults (Lewis & Lewis, 2015). The activity of cutaneous sebaceous glands is regulated by the circulating levels of hormones. Oestrogen can reduce the size and number of sebaceous glands, as well as the production of sebum, while androgens oppose this action, thereby stimulating secretory activity (Brincat *et al.,* 2005).

Healthy skin requires integrity in both the structure and function of capillary blood vessels as well as core temperature homeostasis. The effect of oestrogen on cutaneous circulation is important in humans in maintaining core temperature equilibrium. However, the effect of oestrogen on the cutaneous circulation of women has not been extensively studied. Consistent with the formation of premenstruation oedema in women, cutaneous blood flow has been shown to vary over the course of the menstrual cycle. In addition, peripheral microcirculation at the level of the nail-fold capillaries has been shown to decrease significantly with menopause (Brincat *et al*., 2005; Lee *et al.*, 2014).

Progesterone is considered to be 'thermogenic', possibly because it inhibits cutaneous vasodilation (Charkoudian, 2003). All progestins have the unique effect of increasing core body temperature. Natural progesterone has no known influence on human skin other than having this effect at normal luteal phase levels. This progestin action results from raising the thermoregulatory set-point at which seating occurs. At the same time, evidence of the hormone's having a direct effect on cutaneous vasomotor tone is inconclusive (Charkoudian, 2003).

Androgenic progestins such as norethidrone and levonorgestrel have been important in cutaneous medicine, in combination with oestrogen, in the treatment of hirsutism and acne (Zouboulis *et al*., 2007; Schmidt & Shinkai, 2015). Progesterone plays a role in bone density and protects against osteoporosis (Leonetti *et al*., 1999; Zouboulis *et al*., 2007; Cartwright *et al*., 2016). It also helps burn fat for energy, acts as a natural diuretic, maintains thyroid hormone action for thermogenesis and normalises blood clotting (Zouboulis *et al*., 2007).

Chloasma, or melasma, is a pigmentary disorder that typically appears on the face, forehead, cheeks and chin. It occurs most frequently in women with Fitzpatrick skin type III or higher, especially those of Asian origin, and is associated with negative psychological and emotional effects. Chloasma can result from pregnancy or taking oral contraceptives, but it also occurs spontaneously. It can affect up to 50% - 70% of pregnant women, but genetic, ethnic (skin type), hormonal, and environmental

factors (i.e. ultraviolet [UVA and UVB] exposure) are also implicated (Stevenson & Thornton, 2007; Wang *et al*., 2014).

Young women may be especially concerned about weight gain. Despite extensive clinical experience, many metabolic effects of oral contraceptive treatment remain to be explored. Changes in appetite and weight are known to occur in some women, but the association with contraceptive treatment is unclear. There are only a few studies evaluating body composition during oral contraceptive treatments, and these indicate no significant change in body weight or body fat (Rickenlund *et al.,* 2004; Berenson & Rahman, 2009; Glintborg *et al*., 2014; Yancey & Raleigh, 2014; Bonny *et al*., 2015; Myllyaho, 2016; Batista *et al*., 2017; Dos Santos *et al*., 2017).

The effects of progesterone on body weight and composition are of interest from several standpoints. The body weight of animals treated with progesterone has been shown to increase; however, the composition of the weight gain remains unclear (Hervey & Hervey, 1967). The results were obtained from a number of experiments on rats of both sexes and predicate accurate definition of the effects of progesterone under the conditions of the experiments (Hervey & Hervey, 1967). In a study done by Lopez *et el.* (2017), there was little evidence of weight gain when using progesterone only contracpetives (POCs). Mean weight gain at 6 or 12 months was less than 2 kg for most studies reviewed by Lopez *et al.* (2017). The groups using other birth control methods had about the same weight gain (Laureen *et al.,* 2017). Oestrogen loss was shown to exert a prominent influence on women aging in a variety of body systems, including the cardiovascular system, brain, bones, joints, and skin (Piérard *et al.,* 2013a).

Sex steroids have been shown to interfere with appetite and metabolic functions. Estradiol inhibits feeding in animals, whereas high dose progestins are appetite stimulating. Oral contraceptives may also decrease insulin sensitivity, and a change in carbohydrate metabolism has been attributed to the progestin component (Rickenlund *et al*., 2004; Asarian & Geary, 2006). Furthermore, sex steroids may exert metabolic effects in adipose tissue (Pelkman *et al*., 2001; Rickenlund *et al*., 2004; Sitruk-Ware, 2006). The particular mechanisms responsible for the increase in body weight and body fat during oral contraceptive treatment remain unclear (Pelkman *et al*., 2001; Rickenlund *et al*., 2004; Sitruk-Ware, 2006).

Since hormonal contraceptives are widely used, it is important to investigate their effect on oxidative status, skin parameters and anthropometric indicators to enable women to make an informed choice or adapt their life style if necessary.

1.2 Aim

The aim of this study is to assess the effects of contraceptive use on the antioxidant status, skin parameters and anthropometric indicators in a student population of Cape Peninsula University of Technology (CPUT).

1.3 Research questions

1. Do hormonal contraceptives have any effect on skin health (level of inflammation, pigmentation, hydration, sebaceous activity and trans-epidermal water loss)?
2. Do hormonal contraceptives have any effect on body composition and body weight?
3. Do hormonal contraceptives have any effect on antioxidant status in human plasma?
4. Which hormonal contraceptive will have a more significant effect on skin health?
5. Which hormonal contraceptive will have a more significant effect on body composition?
6. Which hormonal contraceptive will have a more significant effect on antioxidant status?

1.4 Objectives of the study

1. To determine the differences in skin health (level of inflammation, pigmentation, hydration, sebaceous activity and transepidermal water loss) between females using various hormonal contraceptives and non-contraceptive users.
2. To determine the differences in body weight status (body mass index and hip-to-waist ratio) between females using various hormonal contraceptives and non-contraceptive users.
3. To determine the differences in body composition (moisture content, lean body mass and adipose tissue) between females using various hormonal contraceptives and non-contraceptive users.
4. To determine selected oxidative stress markers (lipid peroxidation, oxygen radical absorbance capacity and GSH:GSSG ratios) and levels of vitamin A and E in blood samples from females using various hormonal contraceptives, in comparison to those of non-contraceptive users.

1.5 Hypothesis

It was hypothesised that the various hormonal contraceptives would have different effects on the antioxidant status, skin parameters and anthropometric parameters of the sampled population, based on the composition of the contraceptives.

1.6 Research design

The study utilises a cross-sectional sampling design and a quantitative approach, in order to obtain a snapshot research view of the target population.

CHAPTER TWO

LITERATURE REVIEW

Over the past few years, the use of contraceptives has become increasingly popular in South Africa. The District Health Information System provides data to calculate the partners or couples' year protection rate. Data available for 2010 indicates this rate to be 31.6% nationally, but with considerable provincial variation (National Contraception and Fertility Planning Policy and Service Delivery Guidelines, 2014). The 2018 South Africa Demographic and Health Survey (SADHS) indicates that among currently married women and sexually active unmarried women, the use of modern contraceptive methods has increased to 58% (SADHS, 2018).

Due to their increasing popularity and rates of usage, it is essential to investigate the effects of hormonal contraceptives on the body, and specifically on the antioxidant status, skin and anthropometric parameters.

2.1 Contraceptive use

The practice of contraception was revolutionised by the advent of hormonal contraceptives, which provide an effective, convenient, safe and reversible solution. They enable couples to have almost complete control over the timing and number of children they choose to have. The array of modern contraceptive methods currently available is relatively free of major health risks (Rad *et al*., 2011; Sitruk-Ware & Nath, 2011). However, the most effective reversible methods have some troublesome side-effects, while the least effective methods have the fewest side-effects, and all have certain drawbacks. Although considerable progress has occurred in contraceptive technology, perfect contraceptives are not yet available and may never be (Sapire, 1986; Bahamondes *et al*., 2015).

Less than 1% of currently married and sexually active unmarried women use traditional methods of family planning (SADHS, 2018). Modern methods of contraception include female and male sterilisation, oral hormonal pills, the copper intrauterine device (Cu IUD), the male condom, injectables, the implant (including Norplant), vaginal barrier methods, the female condom and emergency contraception. The rate of use varies across ethnic groups, age group and educational levels. This agrees with international trends, which show that the higher the level of education, the higher the likelihood of contraceptive use (National

Contraception and Fertility Planning Policy and Service Delivery Guidelines, 2014; Fait *et al*., 2018).

The South Africa Demographic and Health Survey (SADHS, 2018) provides information on the population's knowledge and patterns of contraceptive use. As previously mentioned, the data indicates that 58% of married women and sexually active unmarried women are using a modern contraceptive method. For women in this combined group, the most commonly used methods are injectable contraceptives [(25%- 3-month injectables (18%) and 2-month injectables (7%)], male condom (15%), oral contraceptive pills (7%), and female sterilisation (6%).

Current contraception use as reflected in the results of SADHS 2016 can be compared with those from SADHS 1998. Contraceptive use has not changed significantly from 1998 to 2016. There was a slight increase in contraceptive use from 54.6 % to 56.3 % for married women and from 62.1 % to 64.2 % for unmarried women. Contraceptive use is highest among sexually active unmarried women, which is to be expected (National Contraception and Fertility Planning Policy and Service Delivery Guidelines, 2014).

2.2 Naturally occurring hormones in women

It is important to look at the function of natural hormones in the female body before reviewing the effects of introducing the synthetic hormones found in contraceptives.

Hormones are signalling molecules that regulate physiological and metabolic functions, such as growth, metabolism and reproduction. Hormones are produced by secretory cells all over the body and transported in the blood stream. By acting on receptors of target cells, hormones induce specific responses (Borer, 2003; Myllyaho, 2016). Hormones work both synergistically and independently to stimulate many metabolic functions in the body (Myllyaho, 2016).

The female body is characterised by changing patterns of hormones. Women must deal with fluctuations of endogenous hormones during development, the menstrual cycle, pregnancy, parturition and menopause (Lebrun, 2000; Myllyaho, 2016). The endogenous female hormones can be divided into gonadotropic hormones and sex hormones, which are secreted from the hypothalamus, anterior pituitary gland, ovaries, and adrenal glands (Guyton & Hall, 2006; Swiegers, 2015; Myllyaho, 2016).

2.2.1 Gonadotropic hormones

The gonadotropin-releasing hormone (GnRH) is released from the hypothalamus in short pulses once every 90 minutes and the concentration remains quite stable throughout the day. GnRH initiates secretion of the two anterior pituitary sex hormones: follicle stimulating hormone (FSH) and luteinizing hormone (LH). In the blood FSH and LH are bound to sex hormone binding globulin (SHBG) or to albumin. FSH and LH act synergistically, stimulating their ovarian target cells and activating the growth and proliferation of these cells. Concentrations of FSH and LH increase close to ovulation (Guyton & Hall, 2006; Myllyaho, 2016).

2.2.2 Sex hormones

Sex hormones can be divided into ovarian hormones and androgens. These steroid hormones are synthesised from cholesterol and acetyl coenzyme A. There are two types of ovarian hormones, the oestrogens and the progestins. Both oestrogens and progestins are secreted by the corpus luteum in the ovaries in response to FSH and LH. In the blood, oestrogens and progestins are transported bound with plasma albumin and with specific oestrogen- and progestin-binding globulins (Guyton & Hall, 2006; Myllyaho, 2016).

2.2.2.1 Oestrogens

The most important oestrogen is estradiol (β-estradiol), but oestrone and estriol are also present in small amounts in the plasma of the human female (Kraemer & Ratamess, 2005; Guyton & Hall, 2006; Verdier-Sévrain *et al.,* 2006; Myllyaho, 2016). In women, oestrogens are synthesised and secreted primarily by the ovarian follicle, the corpus luteum (LH), which is controlled by the follicle stimulating hormone (FSH) (Van Wysberghe *et al.,* 1995), and to a lesser extent by adrenals (Kraemer & Ratamess, 2005; Myllyaho, 2016). In addition, oestrogen can be synthesised locally in different tissues. Human skeletal muscle is able to synthesise oestrogen by aromatisation of androgens (Verdier-Sévrain *et al.,* 2006; Pöllänen *et al.*, 2011; Pöllänen *et al.,* 2015; Myllyaho, 2016).

Oestrogens have many functions in the human body, affecting the cardiovascular, musculoskeletal, immune, and central nervous systems (Van Wynsberghe *et al.,* 1995; Verdier-Sévrain *et al.,* 2006). The primary function of oestrogens is the development and maintenance of normal sexual and reproductive system function, including initiating the growth of the breasts. Oestrogen stimulates the thickening of the uterine wall, stimulates maturation of the oocyte, stimulates development of female sex characteristics, inhibits FSH secretion and increases LH secretion (Van Wynsberghe *et al.,* 1995). Oestrogens promote the proliferation and growth of sex

organs and specific cells that are related to reproduction (Verdier-Sévrain *et al.,* 2006; Myllyaho, 2016).

Oestrogens have a growth-promoting effect not only on the sex organs but also on bones and fat tissues. One important function of oestrogen is to stimulate bone growth by inhibiting osteoclastic activity in the bones. Oestrogens also increase the amount of fat accumulation in the subcutaneous tissues. This is why the percentage of body fat is generally greater in females than in males (Guyton & Hall, 2006; Myllyaho, 2016).

The secretion of oestrogen is increased significantly at puberty and is further increased during the first few years of reproductive life. The secretion progressively decreases toward the end of reproductive life, and beyond menopause there is almost no oestrogen or progesterone secretion (Guyton & Hall, 2006; Myllyaho, 2016).

In contraceptive formulations, the main effects of oestrogen are suppression of ovarian activity, in comparison with progestins, which have a high anti-gonadothropic activity (De Leo *et al.,* 2016).

2.2.2.2 Progestins

Naturally occurring progesterone is produced by the ovarian follicle, the corpus luteum (LH), which is controlled by FSH. The primary function of progestins is to prepare the uterus for pregnancy by promoting secretory changes in the uterine endometrium during the luteal phase of the monthly menstrual cycle (Van Wynsberghe *et al*., 1995; Verdier-Sévrain *et al.,* 2006). They also prepare the breasts for lactation by causing them to swell and inducing a proliferation of the alveolar cells into secretory cells (Guyton & Hall, 2006; Myllyaho, 2016).

Progesterone is the most important progestin. Although small amounts of another progestin, 17-α-hydroxyprogesterone, are also secreted, for practical purposes, progesterone is usually considered the only important progestin.

Progestins have some ‘anti-oestrogenic’ effects; for example, progesterone may play a secondary role in substrate metabolism as it has been reported to oppose the lipolytic effects of oestrogen (Ashley *et al.,* 2000; Verdier-Sévrain *et al.,* 2006; Myllyaho, 2016). In addition, progesterone seems to have a central thermogenic action, since high levels of progesterone have been associated with increased core temperature (Hessemer & Bruck, 1985; Grucza *et al*., 1993; Verdier-Sévrain *et al.,*

2006; Myllyaho, 2016). In contraceptive formulations, the progesterone and its derivatives bind to the progesterone receptor (PR) and strongly inhibit gonadotrophin secretion (De Leo *et al*., 2016).

2.2.2.3 Androgens

Androgens are generally considered male sex hormones because they have masculinising effects, but females also have small concentrations of androgens. In fact, androgens are necessary for the development of reproductive function and hormonal homeostasis in females. In addition, androgens affect bone density, muscle mass and strength, adipose tissue, mood and sexual desire. Testosterone is the most abundant androgen and it promotes muscle hypertrophy by enhancing protein synthesis. In females, testosterone is produced in the ovaries and in the outer layer of the adrenal glands (Bachmann *et al.,* 2002; Myllyaho, 2016).

2.3 Exogenous hormones and hormonal contraceptives

There are different types of synthetic sex steroids, also known as exogenous hormones. Synthetic androgens are usually referred to as anabolic steroids but combinations of synthetic female sex hormones are frequently used in hormonal contraceptives. These synthetic hormones reduce cyclical variability and provide a consistent menstrual cycle while also inhibiting ovulation and preventing pregnancy (Rickenlund *et al.,* 2004; Myllyaho, 2016).

The main mechanisms of hormonal contraceptives are ovulation inhibition and changes in the cervical mucus that inhibit sperm penetration (Rivera, 1999; Myllyaho, 2016). In normally-cycling women, follicle-stimulating hormone (FSH) promotes the growth of immature egg follicles in the ovary during the first phase of the cycle, termed the follicular phase (Salvaggio & Zaenglein, 2010; Welling, 2013). Luteinizing hormone (LH) surges instigate the release of a mature ovarian follicle at ovulation, causing progesterone levels to increase steeply in the luteal (second) phase of the menstrual cycle. If implantation of a fertilised egg does not occur, then progesterone and oestrogen levels decrease and menstruation occurs (Salvaggio & Zaenglein, 2010; Welling, 2013). Hormonal contraceptives work by preventing the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus, thereby blocking a signal to the pituitary gland to produce FSH and LH. This inhibits follicles from maturing/releasing and causes the ovaries to be relatively dormant. Therefore, the daily use of hormonal contraceptives mimics the hormonal state of pregnancy by increasing and flattening a woman's levels of both progesterone and oestrogen, resulting in the prevention of ovulation and a loss of normal fertility (Salvaggio & Zaenglein, 2010; Welling, 2013). Hormonal contraceptives even reduce the levels of

total and free testosterone, by inhibiting ovarian and adrenal androgen synthesis and by increasing the levels of sex hormone-binding globulin (Wiegratz *et al.,* 2003; Rickenlund *et al.,* 2004; Zimmermann *et al.,* 2013; Myllyaho, 2016).

There is a variety of different types and formulations of hormonal contraceptives in which the doses of synthetic oestrogens and progestins vary. Synthetic oestrogens are found as ethinylestradiol and synthetic progestins as levonorgestrel, norethindrone acetate, desogestrel, norgestimate, norgestrel or etynodiol (Burrows & Peters, 2007; Myllyaho, 2016).

Hormonal contraceptives are generally well tolerated, but some women experience a variety of negative physical side effects, such as headache, nausea, breast tenderness, increased risk of venous thromboembolism, myocardial infarction, ischemic stroke and weight gain (Sapire, 1986; Burkman *et al.,* 2004; Rickenlund *et al.,* 2004; Brynhildsen, 2014; Myllyaho, 2016). Hormonal contraceptive users also experience more sleep disruption than non-users and are at a higher risk of acquiring gallstones (Burkman *et al.,* 2004; Shulman, 2011). Additionally, there is some, albeit limited, evidence that these contraceptives may be associated with an increased risk of migraines, high blood pressure, cervical cancer, breast cancer, liver cancer and foetal abnormalities (Burkman *et al.,* 2004; Shulman, 2011).

Despite these slightly elevated health risks, it is generally agreed that hormonal contraceptives are a safe means of effectively preventing pregnancy (Burkman *et al.,* 2004; Shulman, 2011). However, smoking, hypertension, obesity and diabetes are risk factors that must be taken into account when prescribing OCs (Sapire, 1986; Burkman *et al*., 2004; Brynhildsen, 2014). In the present study these risk factors were accommodated as exclusion criteria.

Healthy non-smoking women using hormonal contraception do not appear to be at increased risk of myocardial infarction, embolic stroke, or venous thrombosis (Cedars, 2002; Myllyaho, 2016). The newer low dose oral contraceptives are safer in terms of cardiovascular morbidity and mortality (Sapire, 1986; Brynhildsen, 2014), but the clinical consequences of suppressed testosterone levels due to hormonal contraceptive use have so far gained little attention (Zimmermann *et al*., 2013; Myllyaho, 2016).

Hormonal contraceptives are also associated with several non-contraceptive health benefits. For example, women using hormonal contraceptives have a lower risk of both ovarian and endometrial cancer than those not using them (Milman *et al.,* 1998;

Kubba *et al.,* 2004). Other effects include a reduction in dysmenorrhea and premenstrual syndrome (PMS); and because hormonal contraceptives inhibit ovulation, susceptibility to functional ovarian cysts is almost eliminated (Mishell, 1982; Mishell, 1993; Bennell *et al.,* 1999; Davis & Westhoff, 2001; Lebrun *et al*., 2003; Burkman *et al*., 2004; Myllyaho, 2016). Iron-deficiency anaemia because of excessive monthly blood loss is reduced because hormonal contraceptives decrease menstrual blood flow and increase haemoglobin concentrations in anaemic women (Milman *et al.,* 1998; Bennell *et al.,* 1999; Kubba *et al.,* 2000; Davis & Westhoff, 2001; Lebrun *et al.*, 2003; Myllyaho, 2016).

Hormonal contraceptives may also help to maintain a predictable hormonal milieu (Cedars, 2002; Myllyaho, 2016). In women with low bone mineral density and menstrual disturbances, hormonal contraception treatment may help increase bone mineral density and prevent osteoporosis (Mishell, 1982; Mishell, 1993; Burkman *et al.,* 2004; Rickenlund *et al.,* 2004; Myllyaho, 2016). Oral contraceptives also protect women from developing rheumatoid arthritis and reduce menstrual-related symptoms; they reduce the risk of ectopic pregnancies and acne, and possibly offer protection against pelvic inflammation (Mishell, 1982; Mishell, 1993; Lucky *et al.,* 1997; Olson *et al*., 1998; Burkman *et al*., 2004; Rickenlund *et al.,* 2004; Shulman, 2011; Welling, 2013; Myllyaho, 2016).

The following types of contraceptive methods were investigated and will be discussed in more detail below: combined oral contraceptives, injectable contraceptives and subdermal implantable contraceptives.

2.3.1 Combined oral contraceptive

The oral contraceptive pill, commonly referred to as ‘the pill’, was introduced at the beginning of the 1960s and the significance of this development cannot be overestimated. For the first time in history, women themselves had the capacity to control their own fertility. The English magazine *The Economist* described the pill as ‘one of the seven wonders of the modern world’ and ‘the one invention that historians could possibly look back on and say: “That defined the 20th century”’ (Watkins, 2012; Welling, 2013).

The side effects of birth control pills kept some women from using them. Attempts to decrease these side effects led to the three-phase pill in the 1980s. Pills with three phases provide different amounts of hormones over three weeks. One-phase pills have the same amount of hormone for three weeks (Van Vliet *et al.,* 2011, Watkins, 2012).

Combined oral contraceptives (COCs) consist of elelments of both oestrogen and progestogen. In order to improve safety, tolerability and acceptability, COCs have undergone considerable development over the past 40 years (Steyn & Kluge, 2010; Watkin, 2012). The exogenous hormone dose is much lower in the newer forms, and the oestrogenic and progestrogenic content of pills in different brands can range from 0.02 to 0.5 mg and 0.1 to 1.0 mg, respectively (Elliott *et al*., 2005; Myllyaho, 2016). These pills are characterised by a reduction in the dose of oestrogen and the introduction of newer progestogens, which are less androgenic or anti-androgenic, giving them a more favourable clinical profile. Pills containing 35 µg or less oestrogen are now considered standard, and even lower dose pills, containing as little as 15 µg, are being marketed without compromising efficacy. The safest pills are likely to be those containing the lowest dose of hormones (Steyn & Kluge, 2010; Watkins, 2012). Hormonal contraceptives can also be divided into monophasic, biphasic and triphasic groups.

The monophasic type of contraceptive is a combination of oestrogen and progesterone that acts primarily through the mechanism of gonadotropin (luteinising hormone and follicle stimulation hormone) suppression, resulting in the prevention of ovulation (Sapire, 1986; Rivera *et al*., 1999). In monophasic hormonal contraceptives, the amounts of oestrogens and progestins remain constant over 21 days, followed by 7 days of placebo administration (Burrows & Peters 2007; Myllyaho, 2016).

Biphasic hormonal contraceptives contain a fixed amount of oestrogens and two different doses of progestins during the 21 days of consumption, followed by 7 days of placebo (Burrows & Peters 2007; Rechichi *et al*., 2009; Myllyaho, 2016).

Triphasic hormonal contraceptives contain three different doses of oestrogen and/or progestin during the pill cycle (Burrows & Peters 2007; Myllyaho, 2016) and can consequently mimic more closely the ovarian hormone variation that occurs during the normal menstrual cycle (Rechichi *et al*., 2009; Myllyaho, 2016). The oestrogen/ progestogen ratio varies during the cycle, the dosage ratios being administered as a six-day, five-day or ten-day regimen in order to ensure good cycle control and bring about distinct cyclical changes at the level of the vaginal epithelium and the endometrium, reducing the likelihood of implantation. Ovulation is inhibited by suppression of gonadotropin release, particularly at the mid-cycle peaks, and the viscosity of the cervical mucus is increased, impairing sperm penetration and making the endometrium less receptive to implantation (Figure 2.1). Sebaceous glands are androgen dependent and excessive androgen activity of the skin may exacerbate

acne. Oestrogens may exhibit androgen antagonism and suppress sebaceous gland activity (Upton & Corbin, 1989).

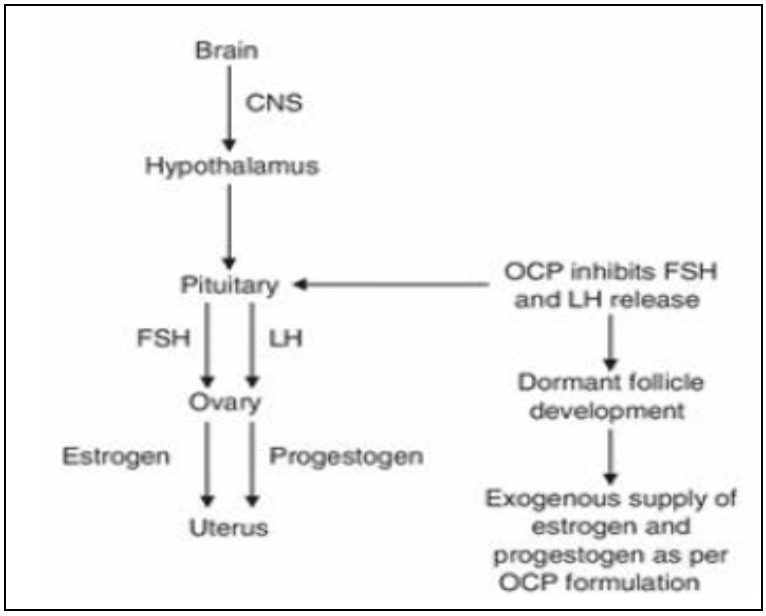

Figure 2.1: *The oral contraceptive pill and control of endogenous sex hormones (Myllyaho, 2016, p16). CNS- central nervous system, FSH- follicle stimulating hormone, LH- luteinizing hormone, OCP- oral contraceptive pill.*

Hormonal contraceptive pills systematically control the concentrations of endogenous female sex hormones by providing synthetic ovarian hormones for 21 out of 28 days. Actions on the hypothalamus and anterior pituitary gland via negative feedback lead to suppression of the Gonadotropin-releasing hormone (GnRH), follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Myllyaho, 2016). Therefore, the natural production of endogenous oestrogens and progestins are reduced to levels indicative of menopause, and, depending on the type of hormonal contraception administered, 3-5 times more exogenous oestrogen and 1-3 times more exogenous progesterone than endogenous levels can be provided (Lebrun, 2000; Elliott *et al.,* 2005; Burrows & Peters, 2007; Myllyaho, 2016).

There are several pills available in South Africa, making it possible to select the pill to suit each individual patient that will provide contraception at the lowest dose with the fewest side effects, and with added secondary benefits (Steyn & Kluge, 2010).

Low-dose combined oral contraceptive pills contain 35 µg or less of the synthetic oestrogen ethinyl estradiol and one of a range of synthetic progestogens like levonorgestrel. They are very effective in preventing pregnancy when taken daily as

prescribed, and are safe for most clients (National Contraception and Fertility Planning Policy and Service Delivery Guidelines, 2014). See Table 2.1 for key characteristics of low-dose combine oral contraceptives (COCs).

Table 2.1: Key characteristics of low-dose COCs (National Contraception and Fertility Planning Policy and Service Delivery Guidelines, 2014).

Effectiveness	92% during the first year as commonly used; when used correctly and consistently the effectiveness is as high as 99.7%
Age limitations	No restrictions on use from menarche to age 40. After age 40 generally can use, but more careful follow-up may be required
Parity limitations	No restrictions
Mode of action	Works primarily by preventing ovulation. Other mechanisms, such as thickening of the cervical mucus (which reduces sperm transport) and altering the endometrium are considered secondary
Common side effects	Nausea and inter-menstrual spotting/bleeding are not uncommon in the first 3 months. Mild headaches, dizziness, breast tenderness, light periods, break through bleeding or occasionally amenorrhoea may occur. Medical management is not usually necessary or recommended, but the side effects should be discussed
Non-contraceptive benefits	Non-contraceptive benefits associated with COCs use include: • Regular, lighter and less painful periods/menstruation • Prevention or improvement of iron-deficiency anaemia • Decrease in incidence of pelvic inflammatory disease (PID), ectopic pregnancy, ovarian and endometrial cancers and benign breast disease • Reduces symptoms of endometriosis and polycystic ovarian syndrome • Can improve or worsen acne (preparations containing cyproterone acetate or drosperinone as well as some other progestogens are particularly effective in management of acne) • Protection from risks associated with pregnancy
Effect on STI and HIV risk	Not protective
Duration of use	Can use COCs safely throughout the reproductive years; there is no value in periodic discontinuation
Return to fertility	Fertility returns without a delay

2.3.2 Progesterone-only injectable contraceptive

Injectable contraceptives are very popular, for a number of reasons. They are convenient, especially in that one cannot forget to take them, which is the case with oral contraceptives; they are effective, and they can be hidden from one's partner and others (Smit *et al*., 2002). In South Africa, POIC's are shown to be the most popular type of contraceptive and account for 49% of current contraceptive use nationally and up to 90% in some areas (National Contraception and Fertility Planning Policy and Service Delivery Guidelines, 2014). Of the two available injectable progestogens, depot medroxyprogesterone acetate (DMPA), given intramuscularly every 12 weeks, is more commonly used than norethisterone enanthate (NEN-EN), given intramuscularly every eight weeks (Sapire, 1986; World Health Orgaisation [WHO], 2003; Steyn & Kluge, 2010; National Contraception and Fertility Planning Policy and Service Delivery Guidelines: a Companion, 2012; Jacobstein & Polis, 2014; National Contraception and Fertility Planning Policy and Service Delivery Guidelines, 2014).

Depot medroxyprogesterone acetate (DMPA) is an aqueous suspension of 17-acetoxy 6-methyl progestin administered by intramuscular injection for long-term contraception. The principal mode of action is prevention of ovulation, while possible secondary mechanisms include thickening of the cervical mucus (preventing sperm penetration) and rendering the endometrium unfavourable for implantation (Sapire, 1986; Fleming, 2009; Steyn & Kluge, 2010; Jacobstein & Polis, 2014).

Most women experience changes in their bleeding pattern, such as infrequent or prolonged bleeding and spotting. However, up to 70% of women will be amenorrhoeic after one year. An average of 4.4 kg weight gain in the first 18 months of use is a side-effect of DMPA. Weight gain appears to be reversible to some extent when the DMPA is discontinued (Sapire, 1986; Fleming, 2009; Steyn & Kluge, 2010; Adams, 2015). A temporary reduction in bone mineral density (BMD) has been reported, as DMPA reduces the ovarian production of oestradiol. It appears that BMD that diminishes during use recovers after the discontinuation of DMPA (Mishell, 1996; Steyn & Kluge, 2010; Jacobstein & Polis, 2014). When compared to norethisterone enanthate (NET-EN), there is more incidence of breakthrough bleeding or menstrual chaos. Possible androgenic effects include loss of libido, dyspareunia, mood changes and hair loss. Patients who experience unacceptable side-effects when using DMPA are usually able to use NET-EN (Adams, 2015).

As previously mentioned, norethisterone enantate, a depot progestogen for hormonal contraception, is given every eight weeks (Fleming, 2009; Steyn & Kluge, 2010). A moderate suppression of FSH and LH occurs. Protection against conception is mainly effected by alterations in the cervical mucus which are present during the whole period of action. These impair sperm movement into the uterine cavity. Ovulation is suppressed by the antigonodotropic effect of norethisterone (Fleming, 2009; Steyn & Kluge, 2010; Bonny *et al.,* 2011; Adams, 2015).

The side-effects of NET-EN are not as pronounced as those experienced by users of DMPA. There is a lower incidence of breakthrough bleeding, headaches and androgenic effects. NET-EN does not appear to cause weight gain, and there is little evidence of BMD loss in adolescents, although a minimal effect is noted in older women (Bonny *et al*., 2011; Adams, 2015).

POICs are safer than combined oral contraceptives as there are no oestrogen-related side-effects, and thus no increased risk of deep vein thrombosis, pulmonary embolism, stroke or myocardial infarction. It is a method that mainly benefits women

who are unable to take oestrogen, and those aged 35 years and older who smoke (Fleming, 2009; Bonny *et al.,* 2011; Adams, 2015).

The Committee on Safety of Medicines (CSM) recommends that prescribers re-evaluate the suitability of treatment for those who continue to use the method for 2 years. The CSM recommends that POICs are used as a first-line treatment in adolescents after other methods have been discussed and declined (Fleming, 2009). Table 2.2 gives the key characteristics of progestogen-only injectables.

Table 2.2: Key characteristics of progestogen-only injectables (National Contraception and Fertility Planning Policy and Service Delivery Guidelines, 2014).

Effectiveness	As commonly used, injectables are 94% effective. If used correctly (for example woman comes for reinjection on time), the effectiveness is as high as 99.7%
Age limitations	Overall no restrictions, but some caution may be warranted in adolescents younger than 18 years and women older than 45 years due to concerns about reduced bone mineral density. There are no data to deny the choice of either injectable on the grounds of age alone
Parity limitations	No restrictions
Mode of action	Primarily inhibits ovulation but also thickens cervical mucus and thereby prevents sperm penetration
Common side effects	Changes in menstrual bleeding (irregular, prolonged or/and heavy bleeding, amenorrhoea) and weight gain, are important issues to cover during counselling. Other possible side effects include headaches, dizziness, mood changes and decrease in sex drive
Non-contraceptive benefits	The use of injectable contraceptives provides the following additional health benefits: • Prevention or improvement of iron deficiency anaemia • Decrease in occurrence of ectopic pregnancy, pelvic inflammatory disease, uterine fibroids and endometrial cancer • Reduction in the severity of sickle-cell crises among women with sickle-cell anaemia • Reduction in severity of symptoms of endometriosis
Effect on STI and HIV risk	Not protective. Recent observational studies suggest that use of hormonal contraception, in particular DMPA, might increase risk of HIV acquisition.[9,10] While there is need for further research, every effort must be made to emphasise the importance of consistent and proper condom use in conjunction with hormonal and other non-hormonal contraceptives for the prevention of HIV.[11] Alternatives, such as lower dose hormonal contraceptives, and non-hormonal options, such as Cu IUDs, need to be explored with the client. The benefits of the injectable to prevent pregnancy need to be weighed against the possible risk of HIV
Duration of use	Can be used throughout a woman's reproductive years but perimenopausal women may not have enough time until menopause to regain bone density. Switching to another method after reaching 45 years may be considered
Return to fertility	Average delay of about 4–6 months depending on type of injectable. No permanent damage to fertility has been associated with injectables. Cover this issue when counselling

2.3.3 Subdermal contraceptive implants

Various subdermal implants are available, which contain either levonorgestrel (LNG) or etonogestrel (ETG). Implanon® provides contraceptive effectiveness for approximately 3 years, while levonorgestrel provides contraceptive effectiveness for 4 years. Implanon and levonorgestrel are available and utilised in many countries, including many African countries (Pushpa *et al.,* 2011; Jacobstein & Polis, 2014; National Contraception and Fertility Planning Policy and Service Delivery Guidelines, 2014). Implanon® is the only subdermal implant registered in South Africa. It is a single, match-sized rod containing 68 mg ETG that is inserted subdermally on the

inner, upper arm and is licensed for three years' use (Sapire, 1986; WHO, 2003; Pushpa *et al.,* 2011; National Contraception and Fertility Planning Policy and Service Delivery Guidelines, 2014). The primary mode of action of the implant is to prevent ovulation by continuously releasing low amounts of progestin, which also thickens the cervical mucus and affects the endometrium (Steyn & Kluge, 2010; Pushpa *et al.,* 2011; Jacobstein & Polis, 2014; Ramdhan *et al*., 2018).

Implanon® is the most effective contraception available, with equal typical and perfect use failure rates. It is effective within 24 hours and has the highest effectiveness of any contraceptive method, with 0.05% of typical and perfect users expected to experience an unintended pregnancy in the first year of use (Pushpa *et al.,* 2011; Jacobstein & Polis, 2014; Ramdhan *et al*., 2018). It is more effective than female or male sterilisation (Steyn & Kluge, 2010; National Contraception and Fertility Planning Policy and Service Delivery Guidelines, 2014) and is effective for 3 years of use (Pushpa *et al.,* 2011; Jacobstein & Polis, 2014; Ramdhan *et al*., 2018).

Implants contain no oestrogen and are therefore suitable for most women, of any age (including adolescents), parity, marital status, or reproductive intentions (to delay, space, or limit); and for women who are post-abortal, breast feeding or living with HIV (Jacobstein & Polis, 2014), or cannot, or do not wish to, use oestrogen. Despite high initial costs they have proved to be cost-effective compared to pills and injections at one year (Steyn & Kluge, 2010; Pushpa *et al.,* 2011; Jacobstein & Polis, 2014; National Contraception and Fertility Planning Policy and Service Delivery Guidelines, 2014; Ramdhan *et al*., 2018). Table 2.3 shows the key characteristics of progesterone-only implants.

Table 2.3: Key characteristics of progesterone-only implants (National Contraception and Fertility Planning Policy and Service Delivery Guidelines, 2014).

Effectiveness	Implants are almost 100% effective. (With Implanon, one pregnancy occurs in 1000 women over a 3-year period)
Age limitations	No restrictions from menarche to menopause
Parity limitations	No restrictions
Mode of action	Primarily inhibits ovulation and thickens cervical mucus and thereby prevents sperm penetration
Common side effects	Changes in menstrual bleeding are common, including lighter bleeding, irregular bleeding, infrequent bleeding and amenorrhoea. Other side effects include headaches, nausea, dizziness, breast tenderness, mood changes and abdominal pain due to enlarged ovarian follicles
Non-contraceptive benefits	Prevention of symptomatic PID and iron-deficiency anemia
Effect on STI and HIV risk	Not protective
Duration of use	Can be used throughout the reproductive years
Return to fertility	Without a delay

2.4 The effects of sex hormones on the skin

The skin is the largest hormonally sensitive organ of the body (Brincat *et al.,* 2005; Owen *et al*., 2016). It consists of two main layers: the epidermis and the dermis. The epidermis forms the thin outer layer and is made up primarily of keratinocytes and melanocytes, while the dermis is the deeper layer that comprises the main bulk of skin (Brincat *et al.,* 2005). The dermis is predominantly made up of connective tissue and blood vessels. The fibres present in dermal connective tissue consist of two main types of fibrous proteins, collagen and elastin. Collagen fibres are arranged parallel to the skin surface, and are responsible for the main mass and tensile strength of skin. In contrast, elastin fibres are arranged as a thinly distributed sub epidermal network and provide the skin with elasticity and resilience (Brincat *et al.*, 2005).

Studies have shown that keratinocytes, Langerhans cells, melanocytes, sebaceous glands, and fibroblasts are under hormonal influence and that decreased oestrogen levels, which occur in menopause, have been associated with decreased capillary blood flow in the skin (Owen *et al.,* 2016). The aging of skin leads to decreased amounts of collagen, elastin, and hyaluronic acid (the three main components of the dermal tissue), with subsequent wrinkle formation and decreased skin rigidity (Owen, *et al.*, 2016). This loss of collagen has been shown to be accelerated in menopause (decrease in oestrogen), with an average decline of 2.1% in skin collagen per postmenopausal year (Brincat *et al.*, 2005; Owen, *et al.*, 2016).

Skin quality deteriorates with age due to the synergistic effects of chronologic aging, photo aging, environmental factors and hormonal deficiency. The hormonal aging of skin due to oestrogen loss at menopause is thought to include atrophy, decreased collagen content, water content, and sebaceous secretions, loss of elasticity, and manifestations of hyperandrogenism (Brincat *et al.*, 2005; Ramdhan *et al*., 2018).

Collagen is one of the main constituents of the skin and provides the major support for skin resistance. The most abundant type, collagen I, predominates in the reticular dermis, with type III collagen in the papillary dermis as well as at sites of new collagen deposition (Brincat *et al.*, 2005; Verdier-Sévrain *et al.,* 2006; Kanda & Watanabe, 2005; Owen *et al.,* 2016). Brincat *et al*. (2005) have demonstrated that there is a decrease in skin thickness and skin collagen content, corresponding to a reduction in bone mineral density, in the years following menopause, particularly in the initial postmenopausal years. Several controlled studies have also shown the effects of oestrogen on skin collagen and skin thickness (Sauerbronn *et al.,* 2000; Brincat *et al.*, 2005; Kanda & Watanabe, 2005; Verdier-Sévrain *et al.,* 2006; Owen *et al.,* 2016).

The epidermis is the outermost compartment of the skin and provides a barrier against what is outside the body. It can be divided into three lines of defence: the physical barrier against pathogens and mechanical injuries, the chemical/biochemical barrier with antimicrobial activity, and a barrier against the unregulated loss of water and solutes (Hänel *et al.,* 2013). The skin barrier is formed by differentiating keratinocytes and is continuously renewed (Hänel *et al.,* 2013). During cornification the keratinocytes develop a protein- and lipid-rich peripheral envelope, called the cell envelope (CE) (Bouwstra & Ponec, 2006; Hänel *et al.,* 2013). In the outermost layer of the CE, ceramides and other lipids are covalently bound and form the so-called lipid envelope. The main function of the lipid envelope is to prevent transepidermal water loss (TEWL) and the loss of solutes (Hänel *et al.*, 2013). The ability of the skin to hold water is related to the stratum corneum lipids that play a dominant role in maintaining the skin barrier (Verdier-Sévrain *et al.*, 2006).

One of the most common dermatological conditions in older women is dry skin. The health of the skin varies during the menstrual cycle and decreases with age. To be healthy it requires a substantial water content, which is determined by both the cutaneous evaporation rate and evaporation (Sauerbronn *et al.,* 2000; Brincat *et al.*, 2005; Kanda & Watanabe, 2005; Verdier-Sévrain *et al.,* 2006; Owen *et al.,* 2016). The positive effect of oestrogen on the water content of the skin may be related to oestrogen-stimulated increases in mucopolysaccharides and hyaluronic acid levels in skin, which correlate with an increase in dermal water content and skin thickness, which subsequently elevates natural moisturising factors (NMF) (Sauerbronn *et al.,* 2000; Kanda & Watanabe, 2005; Brincat *et al*., 2006; Verdier-Sévrain *et al.,* 2006; Owen *et al.,* 2016). An improvement in the water-holding capacity of the skin enhances the barrier function of the epidermis and results in less frequent development of dermatoses (Brincat *et al.*, 2005).

Ultraviolet light (UV) is an inducer of reactive oxygen species (ROS) as well as photoproducts and cyclobutane pyrimidine dimers (CPD) in the skin, which cause further damage to the skin cells (Slominski *et al.,* 2014; Janjetovic *et al*., 2017). Melanocytes, which are pigment-producing cells, are originally derived from neural crest cells in the embryonic skin (Hirobe, 2014). In human skin, the epidermal melanin unit, which comprises keratinocytes and melanocytes, has a key role in regulating pigmentation and homeostasis of the epidermis. The main function of melanocytes is to produce melanin which absorbs UV waves to prevent DNA damage to the keratinocytes (Hirobe, 2014; Slominski *et al.,* 2014).

Skin pigmentation is determined by genetic, environmental and endocrine factors that influence both melanin synthesis in melanocytes and the distribution of melanin throughout the epidermis (Stevenson & Thornton, 2007; Lee, 2015). Oestrogens regulate skin pigmentation. An increase in cutaneous pigmentation due to an increase in ovarian and/or pituitary hormones is common during pregnancy. With respect to female sex hormones, oestrogens and progesterones have been implicated in the development of melasma (Stevenson & Thornton, 2007; Lee, 2015). Melasma is a well-documented acquired pigmentation occurring exclusively in sun-exposed areas and can be exacerbated by pregnancy and oral contraceptive use (Stevenson & Thornton, 2007; Lee, 2015). Chloasma, on the other hand, is a common hyperpigmentation of the face seen in pregnant women, often accompanied by increased pigmentation in other areas including the areola, linea alba and perineal skin, all of which usually fade following parturition (Stevenson & Thornton, 2007). Variations of skin pigmentation with the menstrual cycle have also been reported and may result from the synergistic action of oestrogen and progesterone (Verdier-Sévrain *et al.,* 2006; Stevenson & Thornton, 2007; Lee, 2015).

Melasma has been reported as an adverse reaction to contraceptives containing the synthetic progestin levonorgestrel (Lee, 2015). The expression of pathogenesis-related **(**PR) proteins is increased in hyperpigmented skin in melasma (Jang *et al.,* 2010; Lee, 2015; Tamega *et al.,* 2015), suggesting that progesterone plays a role in the development of the condition. Conversely, it has been suggested that progesterone components in oral contraceptives may help prevent melasma, based on the finding that progesterone reduces proliferation without significant effects on tyrosinase activity, counteracting the stimulatory effects of oestrogen in cultured melanocytes (Wiedemann *et al.,* 2009; Lee, 2015).

Furthermore, the cumulative effect of oestrogen deficiency on skin is thought to contribute to the poor wound healing that accompanies aging (Calvin, 2000; Brincat *et al.*, 2005; Rieger *et al*., 2015; Crompton *et al.,* 2016). Oestrogen deprivation is associated with attenuated wound healing, while hormone replacement therapy positively improves acute wound healing and precludes the development of chronic wounds in ageing women (Rieger *et al.,* 2015). The positive effects of oestrogen during wound repair occur through its targeting of a collection of epithelial cells, fibroblasts and immune cells in the skin (Rieger *et al.,* 2015). Exogenous oestrogen can reverse the impaired wound healing observed in menopausal women, reducing local inflammation by down-regulating the macrophage migration inhibitory factor and improving matrix synthesis and deposition, leading to amelioration of scarring (Rieger *et al*., 2015; Crompton *et al.,* 2016).

Stevenson and Thornton (2007) report a variation in skin thickness during the menstrual cycle, with skin thickness lowest at the start of the menstrual cycle, when oestrogen and progesterone levels are low, but increasing with the rising levels of oestrogen. When oestrogen levels are very low (as in menopause), hypo-oestrogenism accelerates age-related deterioration, which in turn results in thinner skin, an increase in number and depth of wrinkles, increased skin dryness, and decreased skin firmness and elasticity (Stevenson & Thornton, 2007; Rieger *et al.,* 2015). The introduction of hormones into the body as in the case of hormone replacement therapy (HRT) has been shown to increase epidermal hydration, skin elasticity, skin thickness, and also reduce skin wrinkles (Stevenson & Thornton, 2007; Rieger *et al.,* 2015). Furthermore, the content and quality of collagen and the level of vascularisation is enhanced with the introduction of hormones into the body (Stevenson & Thornton, 2007; Rieger *et al.,* 2015). Oestrogen treatment is known to increase collagen content and deposition, thickness of the dermis (presumably via direct actions on fibroblasts and/or anagen hair follicles), elasticity and water content of skin, and reduce sebaceous secretion (Blume-Peytavi *et al.*, 2012; Rieger *et al.,* 2015).

The activity of cutaneous sebaceous glands is regulated by the circulating levels of hormones. Oestrogen can reduce the size and number of sebaceous glands, as well as the production of sebum, while androgens oppose this action, stimulating secretory activity (Brincat *et al.*, 2005; Sheng *et al.,* 2018). Oestrogens also suppress sebaceous gland size and function, both indirectly and directly, through the pituitary-gonadal suppression of androgen production. Although the efficacy of ethynylestradiol-containing oral contraceptives has been confirmed and approved in acne treatment, very little is known about the role of oestrogens in the pathogenesis of acne formation (Zouboulis *et al.*, 2007; Blume-Peytavi *et al.*, 2012; Akdoğan *et al.,* 2018).

Thus, while androgens contribute to the development of acne through an increase in sebum production, oestrogens, in sufficient amounts, have an inhibitory effect on acne through suppression of sebum production (Akdoğan *et al.,* 2018). The effects of oestrogen on acne are achieved through three different mechanisms, including opposition of androgens within the sebaceous glands, inhibition of gonadal androgen production via a negative feedback mechanism on gonadotrophin release, and effects on genes which play a role in sebaceous gland growth and lipid production (Akdoğan *et al*., 2018). Combined oral contraceptives containing oestrogen and progestin can be used for this purpose, to inhibit ovarian androgen production, decrease activation of androgen receptors on sebaceous glands and increase sex

hormone-binding globulin in the liver (Akdoğan *et al.,* 2018). This leads to a decrease in circulating levels of free testosterone (Zouboulis *et al.*, 2007; Blume-Peytavi *et al.*, 2012; Akdoğan *et al.,* 2018). Androgenic progestins such as norethindrone and levonorgestrel have been important in cutaneous medicine, in combination with oestrogen, in the treatment of hirsutism and acne (Zouboulis *et al.*, 2007; Blume-Peytavi *et al.*, 2012; Akdoğan *et al.,* 2018).

Healthy skin requires the integrity of both the structure and function of capillary blood vessels as well as the maintenance of core temperature homeostasis. The effect of oestrogen on cutaneous circulation in humans is important for maintaining core temperature homeostasis (Brincat *et al.,* 2005). Oestrogen may affect endothelial function by increasing sensitivity to vasodilatory factors, such as acetylcholine, reducing the concentrations required to evoke similar vasodilatory responses to those observed in oestrogen-deprived animals (Usselman *et al.,* 2016). Consistent with the formation of premenstruation oedema in women, cutaneous blood flow has been shown to vary over the course of the menstrual cycle (Brincat *et al.*, 2005; Blume-Peytavi *et al.,* 2012; Usselman *et al.,* 2016; De Melo & Campos, 2018).

2.5 Effect of contraceptives on body composition

Sex steroids have been shown to be associated with metabolic function and mechanisms of regulation (Rickenlund *et al.,* 2004; Myllyaho, 2016). Because of the regional distribution of receptors for sex steroid hormones, there is a gender difference in fat accumulation. In premenopausal women, for example, oestrogens increase the amount of fat accumulation in the subcutaneous tissues (Myllyaho, 2016; Borer, 2003). Therefore, the percentage of body fat is generally greater in females than in males (Guyton & Hall, 2006; Verdier-Sévrain *et al.*, 2006). In addition, progesterone may affect body weight because of water regulation and fluid retention via aldosterone (Burrows & Peters, 2007; Myllyaho, 2016).

There are potential changes in the distribution of body fluids throughout the menstrual cycle. Many women report changes in body weight and a bloated feeling (De Jonge, 2003; Myllyaho, 2016), usually observed with high doses of glucocorticoid-like activity, leading to salt and water retention (Sitruk-Ware, 2006). In addition, androgenic progestins stimulate insulin secretion, which may be responsible for true weight gain (Sitruk-Ware, 2006; Batista *et al*., 2017). However, most studies involving hormone verification have not found significant changes in body weight over the normal menstrual cycle (Lebrun *et al.,* 1995; Casazza *et al.*, 2002; De Jonge, 2003; Myllyaho, 2016).

Despite the worldwide use of hormonal contraceptives, their effects on body composition are not clear (Myllyaho, 2016). Individual responses to hormonal contraceptive use may involve some weight gain as a result of either fluid retention or appetite stimulation (Rosenberg & Waugh, 1998; Rickenlund *et al.,* 2004; Myllyaho, 2016). In addition, significant increases (1% to 5%) in total body fat percentage have been reported with triphasic hormonal contraceptive use (Casazza *et al.*, 2002; Lebrun *et al.*, 2003; Suh *et al.,* 2003; Myllyaho, 2016) and monophasic hormonal contraceptive use (Rickenlund *et al.,* 2004; Berenson & Rahman, 2009; Bonny *et al.,* 2015; Myllyaho, 2016).

Overall, it seems that the increases in body mass and body fat percentage occur within the first few months of hormonal contraceptive use (Lebrun *et al.,* 2003; Suh *et al.,* 2003; Rickenlund *et al.,* 2004; Myllyaho, 2016). It appears that the effect of hormonal contraceptives on body composition depends on the potency and androgenicity of the progesterone within the hormonal contraception pill (Casazza *et al.,* 2002; Suh *et al.,* 2003; Burrows & Peters, 2007; Myllyaho, 2016). Triphasic formulations with higher progestrogenic and androgenic activity may have more pronounced effects on body composition in the short term than formulations with lower potency and androgenicity (Casazza *et al.,* 2002; Suh *et al.,* 2003; Burrows & Peters, 2007; Myllyaho, 2016).

Sex steroids have been shown to interfere with appetite and metabolic functions (Rosenberg & Waugh, 1998; Rickenlund *et al.,* 2004; Myllyaho, 2016). Estradiol inhibits feeding in animals, whereas high-dose progestins are appetite stimulating. Oral contraceptives may also decrease insulin sensitivity, with the effect on carbohydrate metabolism being attributed to the progestin component. Furthermore, sex steroids may exert metabolic effects in adipose tissue. The mechanisms responsible for the increases in body weight and body fat during oral contraceptive treatment remain to be elucidated (Procter-Gray *et al.,* 2008; Rickenlund *et al.,* 2004; Myllyaho, 2016).

Hormonal contraceptive treatment significantly increases bone mineral density (BMD) in women with low BMD at baseline. Moreover, hormonal contraceptive treatment in female athletes has been shown to have beneficial effects on body composition without adverse effects on physical performance (Rickenlund *et al.,* 2004; Myllyaho, 2016).

2.6 Free radical species

A free radical (FR) may be defined as an atomic or molecular species with one or more unpaired electrons in its structure (Battino *et al.,* 1999; Swiegers, 2015). Free radicals can be positively (NAD^+) or negatively charged (O_2^-), or electrically neutral (OH). There are three possible means of free radical formation: a) by the homolytic cleavage of the covalent bond of a normal molecule, with each fragment retaining one of the paired electrons (i.e. homolytic fission), requiring a high energy input: A:B $\rightarrow A^{\cdot} + B^{\cdot}$ (electrically neutral FR); b) by the loss of a single electron from a normal molecule: A + B: $\rightarrow A^{\cdot -} + B^{\cdot -}$ ("-" and/or "+" charged FR); c) by the addition of a single electron to a normal molecule, otherwise called 'electron transfer', quite common in biological systems: A + e- $\rightarrow A^{\cdot -}$ (Battino *et al.,* 1999).

The presence of unpaired electrons makes free radicals highly reactive in terms of donating electrons to or extracting electrons from non-radicals, in an attempt to attain stability (Macharia *et al*., 2008). To achieve and sustain stability, the free radical finds a stable but vulnerable compound from which to collect an electron. With the loss of an electron, the formerly stable molecule becomes a free radical itself and collects an electron from some other nearby molecule, setting off an electron-snatching chain reaction, generating new radicals along the way (Sizer & Whitney, 2003). In a biological setup like the human body, vital macromolecules such as lipids, proteins and nucleic acids may be oxidatively modified, resulting in cell or tissue damage and the myriad pathologies linked to an excess of free radicals (Macharia *et al*., 2008). Not all oxidants in the body, however, are free radicals. 'Reactive oxygen species' (ROS) is a more inclusive term that describes both radical and non-radical oxidants, which may be oxygen, halide or nitrogen centred (Table 2.4).

In addition to normal body processes, environmental factors such as UV-radiation, pollution, tobacco smoke and others can act as oxidants and cause free-radical formation. Free radicals are like sparks, starting wildfires that lead to widespread damage by oxidative stress (Sizer & Whitney, 2003; Swiegers, 2015).

All the biological molecules present in the body are at risk of being attacked by free radicals. Such damaged molecules can impair cell functions and even lead to cell death, eventually resulting in various diseases (Devasagayam *et al.*, 2004). Membrane lipids present in subcellular organelles are highly susceptible to free radical damage. When reacted with free radicals, lipids can undergo the highly damaging changing reaction of lipid peroxidation (LP) producing both direct and indirect effects. During LP a large number of toxic by-products are formed that can affect a site away from the area of generation, behaving as 'second messengers'.

The damage caused by LP is highly detrimental to the functioning of the cell (Devasagayam *et al.,* 2004; Swiegers, 2015).

Table 2.4: Summary of *in vivo* biologically important free radicals (Swiegers, 2015).

Free radicals	Description
Superoxide anions ($O2^{-\bullet}$)	Produced as a result of incomplete reduction of oxygen during mitochondrial respiration, enzyme systems and auto-oxidation; superoxide dismutase (SOD) converts 2 $O_2^{-\bullet}$ to H_2O_2 and O_2.
Hydroxyl radicals ($OH^{\bullet}$)	Highly reactive radicals with very short half-life (10^{-9} s) that react with other molecules to form another radical; formed through the Fenton reaction especially in cases of altered homeostasis; attack proteins, DNA, polyunsaturated fatty acid and almost all other biomolecules.
Hydrogen peroxide (H_2O_2)	Not a free radical, but a reactive oxygen species formed when $O_2^{-\bullet}$ is converted by superoxide dismutase and other oxidase enzymes; forms hydroxyl radicals in the presence of transition metals.
Hypochlorous acid (HOCl)	Produced by the neutrophil-derived enzyme myeloperoxidase during inflammation when chloride ions are oxidized in the presence of H_2O_2.
Nitric oxide ($NO_2^{\bullet}$)	Free radical produced by damaged vascular endothelium; promotes vasodilation and oxidation of low-density lipoproteins.
Peroxyl Radicals ($RO_2^{\bullet}$)	Intermediate species formed during lipid peroxidation chain reactions; increased production during oxidative stress as a result of smoking, xenobiotics, and inflammation.

Free radicals such as hydroxide (OH) react with carbohydrates by randomly attracting a hydrogen atom from one of the carbon atoms, producing a carbon-centred radical leading to chain breaks in important molecules like hyaluronic acid (Devasagayam *et al.*, 2004).

2.6.1 Reactive oxygen species

Reactive oxygen species (ROS) can be defined literally as entities containing one or more oxygen atoms that meet the defining criteria for being chemically reactive (Frei, 1994; Frei, 2012). The defining criteria require identification of the molecular environment, but ROS is generally a more appropriate and useful term than oxyradicals, oxygen free radicals, toxic oxygen radical, and related terms, unless the more limited meaning of one of the latter terms is intended. Oxygen-free radicals, for example, would not include peroxide or bound forms of oxygen that might nevertheless be chemically active (Frei, 1994).

Reactive oxygen species / reactive nitrogen species (ROS/RNS) are constantly being formed in living organisms. In the course of oxygen metabolism, 1 - 5% of all inhaled oxygen becomes ROS. Endogenously, ROS are produced from various sources such

as mitochondria, activated macrophages and leucocytes, oxidase enzymes (NADPH), cyclo-oxygenase and lipoxygenase (Frei, 2012). Reactive oxygen species have oxidative ability and are classified either as free radicals (superoxide anion $O_2^{\bullet}$ hydroxyl radical OH•, nitric oxide NO) or as non-free radicals (hydrogen peroxide H_2O_2, peroxynitrite ONOO-) (Oguntibeju *et al.,* 2010).

Oxidation and production of ROS is an integral part of human living and all cells in the body are constantly exposed to oxidants from both endogenous and exogenous sources (Table 2.5). Reactive oxygen species (ROS), including oxygen free radicals and non-radicals, are generated endogenously and exogenously from enzymatic and non-enzymatic systems (Wiseman & Halliwell, 1996). Endogenously, ROS are mostly derived from the incomplete reactions of oxygen during aerobic metabolism *in vivo*. Reactive oxygen species are produced from various sources such as mitochondrial electron transport chain, nicotine adenine dinucleotide phosphate NADPH oxidases, arachidonic acid pathway enzymes (namely cyclooxygenase and lipoxygenase), NO synthase, peroxidases, xanthine oxidases and phagocytes-derived myeloperoxidase (Cai & Harrison, 2000; Alinde *et al.*, 2012). Exogenously, ROS are derived from exposure to environmental agents such as UV radiation and redox cycle agents (Alinde *et al.*, 2012).

Table 2.5: Sources of reactive oxygen species (Sullivan & Chandel, 2014).

Origin	Source
Internally generated sources	Mitochondria Activated phagocytes Xanthine oxidase Transition metals-mediated reactions Arachidonate pathways Inflammation Ischaemia & reperfusion
Externally induced sources	Diet Cigarette smoke Radiation and ultra violet light Ozone Pesticides Drugs e.g. cyclosporine A

Nicotine adenine dinucleotide phosphate NAD(P)H oxidases have been identified as a major source of production of superoxide in the vasculature of living organisms. The superoxide anion is produced *in vivo* because of most aerobic mechanisms. There is strong evidence showing that the principal source of superoxide anions in

the vascular system prevails in the NAD(P)H oxidase metabolism (Alinde *et al.*, 2012).

The generation of ROS is not however to be avoided at all costs. In fact, production of super oxide and hypochlorous acid by activated phagocytic cells and leukocytes, respectively, is deliberate and directed at eliminating potentially pathogenic microorganisms (Macharia *et al*., 2008). Oxygen radicals are involved in signal transduction, gene transcription and regulation of soluble guanylate cyclase activity in cells, while nitric oxide (NO) is essential in the regulation of vascular tone, leukocyte adhesion, platelet aggregation and thrombosis, as well as being a potent synaptic neurotransmitter (Macharia *et al*., 2008). In addition, the involvement of ROS in cell differentiation and apoptosis has been suggested (Macharia *et al*., 2008).

Reactive oxygen species are implicated in the pathogenesis of certain human diseases, including cancer, ageing, neurodegenerative diseases, type-II diabetes, cardiovascular diseases, muscular disorders, hepatic encephalopathy, immunity diseases, atherosclerosis, rheumatoid arthritis, drug-associated toxicity, and postischemic reoxygenation injury (Frei, 1994; Wen *et al.,* 2013; Swiegers, 2015). They are generated in biological systems through endogenous physiological or pathological processes, as well as by exogenous factors such as food components, drugs, ultraviolet light, ionizing radiation, and pollution (Frei, 1994).

The different types of pro-oxidants require antioxidant defence systems employing various strategies, and a disturbance in the prooxidant/antioxidant balance has been termed ‘oxidative stress’. Antioxidants may act at different levels in the oxidative process, including (and among others) by (1) scavenging initiating radicals, (2) binding metal ions, (3) scavenging peroxyl radicals, or (4) removing oxidatively damaged biomolecules. Some of the antioxidant molecules are synthesised in the body, for example, glutathione or uniquinol, whereas others have to be provided as micronutrients, for example, in antioxidant vitamins and trace metals (Frei, 1994; Pisoschi & Pop, 2015).

Uncontrolled free radical production in the aging process is considered to be the result of increased production of ROS – mainly generated in the organism through by-products of normal cellular metabolism, especially through the mitochondria pathway and lowered antioxidant defences (Mariani *et al.*, 2008). This will be further discussed later.

2.6.2 Activation of oxygen

Oxygen is essential for energy metabolism and respiration but it has been implicated in many diseases and degenerative conditions. Activation of oxygen may occur through two different mechanisms: absorption of sufficient energy to reverse the spin on one of the unpaired electrons, and monovalent reduction (Oguntibeju *et al*., 2010; Fang *et al.,* 2015).

Non-activated oxygen is bi-radical, and can be activated either by reversing the spin on one of the unpaired electrons to form the singlet state, or by reduction. In the monovalent reduction of oxygen, superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH) and finally, water (H_2O), are formed. Superoxide forms the hydroxyl radical (OOH) which is a powerful oxidant in its protonated form. Numerous enzymes (peroxidases) use hydrogen peroxide as a substrate in oxidation reactions involving the synthesis of complex organic molecules (Oguntibeju *et al*., 2010).

Most oxygen is consumed by the cytochrome oxidase enzyme in the mitochondrial electron transport system. Isolated mitochondria produce H_2O_2 and O^{2-} in the presence of NADH (Boveris *et al.,* 1976). Iron–sulfur proteins (Fe-S-proteins) and NADH dehydrogenase have also been implicated as possible sites of super-oxide and hydrogen peroxide formation (Oguntibeju *et al.,* 2010).

2.6.3 Reactive oxygen species: functions and dysfunctions

Reactive oxygen species can affect tissues positively and negatively, depending on whether or not they are well eliminated by their counteracting antioxidants. Reactive oxygen species, as an unavoidable product of aerobic respiration, are reported at low levels to be excellent second messengers in cellular signalling (Valko *et al.,* 2007; Alinde *et al.*, 2012). Cellular signalling or signal transduction can be defined as biological mechanisms by which ‘cells communicate with each other’ (Valko *et al*., 2007).

Reported functions of ROS include: signalling molecules that regulate cellular processes such as proliferation, differentiation, migration and death; regulators of signalling molecules; regulators of developmental process such as embryogenesis, haematopoiesis, spermatogenesis, oogenesis and growth (Alinde *et al.*, 2012). In the event of oxidative damage to DNA, lipids and proteins at high concentrations, ROS can be important mediators of damage to cell structures, nucleic acids, lipids and proteins (Valko *et al*., 2007). Permanent modification of genetic material resulting from these “oxidative damage” incidents represents the first step involved in mutagenesis, carcinogenesis, and ageing. It is known that metal-induced generation

of ROS results in an attack not only on DNA, but also on other cellular components involving polyunsaturated fatty acid residues of phospholipids, which are extremely sensitive to oxidation (Valko *et al.,* 2007).

Advanced glycation end products (AGEs) constitute a class of complex products. Most of the AGEs are very unstable, reactive compounds, and the end products are difficult to analyse thoroughly. The involvement of reactive oxygen species (ROS) in the aging process has been well documented, and enhanced oxidative stress in the elderly has been reported as being related to several pathologies, such as neurodegenerative and vascular diseases (Mariani *et al.,* 2008).

Previous studies have shown the involvement of ROS in physiological and pathophysiological conditions. At low concentrations, ROS are involved in normal cell signalling pathways (smooth muscle and endothelial cell growth, apoptosis and survival) and in the remodelling of vessel walls. At high concentrations, ROS are identified as harmful compounds and constitute an important risk factor for the development of many diseases, including cardiovascular problems. The pathophysiology of cardiovascular disease (CVD) and ischaemic (or ischemic) heart disease (IHD) is multifactorial, but it has been shown that the underlying pathogenesis is the deposition of fatty material, mainly low-density lipoprotein cholesterol (LDL-C), on the inner vascular wall of the blood vessels of the heart (Oguntibeju *et al.,* 2010).

2.7 Oxidative stress and oxidative damage

With the wide variety of functions and distribution of ROS throughout the body, any dysfunction in the balance between ROS and antioxidants can easily threaten the integrity of the whole health system. In normal cellular signalling, ROS production plays a very important physiological role as a secondary messenger (Valko *et al*., 2007), but with loss of regulation in ROS production, the normal cell transduction is shifted to generate pathological conditions through oxidative stress.

Oxidative stress is a metabolic state that occurs when there is a dysfunction in favour of ROS production in the overall balance between the production of reactive oxygen and nitrogen species and the antioxidant defence mechanisms (Willcox *et al.,* 2004; Kuhnt *et al*., 2006; Awoniyi *et al.,* 2010; Alinde *et al.*, 2012; Swiegers, 2015). A physiological metabolism in the human body maintains a state of equilibrium between the levels of oxidants and antioxidants (Awoniyi *et al.,* 2010; Swiegers, 2015).

An imbalance can result from a lack of antioxidant capacity caused by a disturbance in the production or distribution of ROS, or by an overabundance of ROS from endogenous sources or environmental stressors. If not properly regulated, excess ROS can damage cellular lipids and proteins of DNA, thus inhibiting signal transduction pathways, and, in general, normal cellular function (Brenneisen *et al.,* 2005).

Because of this, oxidative stress has been implicated in a growing list of human ailments such as cardiovascular and neurodegenerative diseases, cancer, lung diseases, and UV-mediated skin diseases, as well as the aging process (Langseth, 1995; Young & Woodside, 2001; Brenneisen *et al.,* 2005; Alinde *et al.*, 2012). Oxidative stress is involved in apoptosis, genotoxicity, mitochondrial damage and carcinogenesis (Alinde *et al.*, 2012).

Antioxidant defences that have been identified include superoxide dismustases (SODs), peroxiredoxins (Prxs), heme oxygenases (HOs), glutathione peroxidases (GPxs), catalase (CAT), NAD(P)H:quinone oxidoreductase 1 (NQO1) and NHR:quinone Oxidoreductase 2 (NQO2) (Schreibelt *et al.,* 2007; Nagaraju & Belur, 2008). Endogenous antioxidant enzymes such as SOD, catalase (CAT) and GPx defend the host against the damaging effects of free radical species (Awoniyi *et al*., 2010).

The intake of oxygen (O_2) is fundamental to sustain aerobic life. During cellular respiration in most mitochondria, oxygen is reduced to water to stimulate adenosine triphosphate (ATP) generation, the core energy source of cellular metabolism (Figure 2.2). However, it is believed that in the course of oxygen metabolism, 1-5% of all inhaled molecular oxygen reacts strongly with other molecules to generate downstream oxygen derivatives called reactive oxygen species / reactive nitrogen species (ROS/RNS) (Alinde *et al.*, 2012).

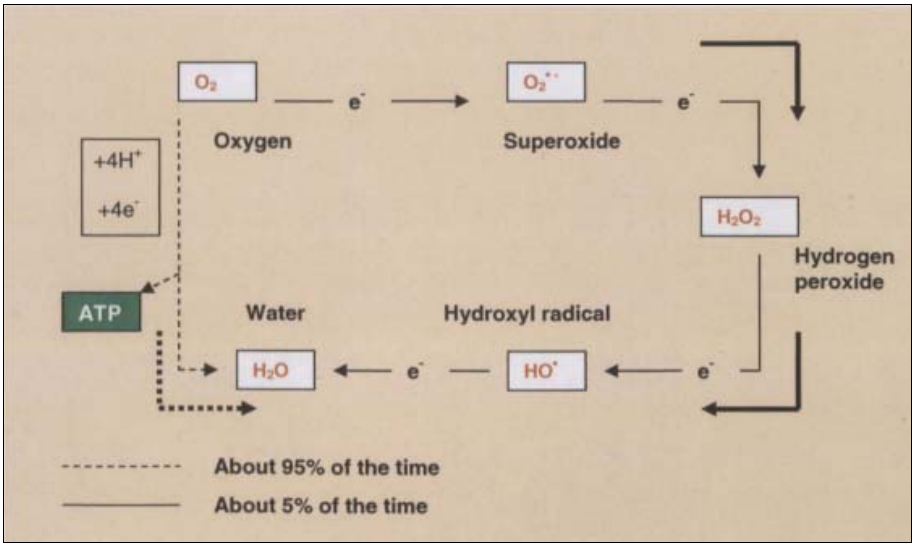

Figure 2.2: Production of ROS during normal cellular respiration (Alinde *et al.*, 2012, p3).

Oxidising agents can be produced by both endogenous sources (inflammatory cells, fibroblast, epithelial cells, endothelial cells, respiratory chain, xanthine and NADPH oxidase) and exogenous sources (cigarette smoke, exogenous toxins, pollution, radiation, carcinogens and drugs) (Palipoch & Koomhin, 2015). Risk factors related to oxidative stress-induced pathologies include alcohol consumption, cigarette smoking, diet, gender, geographic location (specifically at high altitude) and occupation, as indicated in Figure 2.3. Alcohol metabolism is linked to ROS/RNS generation, leading to increased oxidative stress biomarkers such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE), and decreased antioxidative defence systems (Palipoch & Koomhin, 2015).

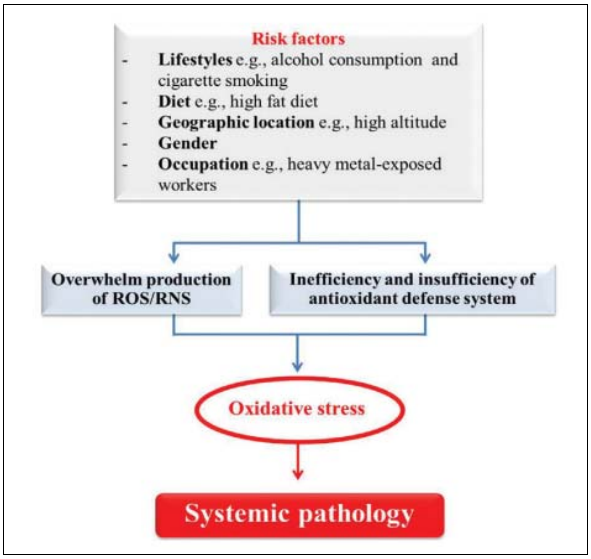

Figure 2.3: Risk factors associated with OS-induced pathologies (Palipoch & Koomhin, 2015, p1442).

2.8 Antioxidant defence systems of the body

Oxidation reactions are crucial for aerobic life, but uncontrolled ROS generation is damaging (Figure 2.4). Although free radicals are continuously generated, the body is equipped to defend itself against the harmful effects of ROS with the help of 2antioxidants, collectively called the antioxidant defence system (comprising both enzymatic and nonenzymatic mechanisms). Antioxidants remove free radicals from the system and inhibit oxidation by being oxidised themselves (Devasagayam *et al.*, 2004; Bhattacharyya *et al.*, 2014). An antioxidant is defined as "any substance that when present in low concentrations compared to that of an oxidisable substrate significantly delays or inhibits the oxidation of that substrate" (Macharia *et al*., 2008).

Antioxidants are compounds that dispose of, scavenge or suppress the formation of ROS, or oppose their actions (Awoniyi *et al*., 2010). As previously mentioned, oxidisable substrates might be proteins, lipids, DNA or any other susceptible biological molecule. Antioxidants can proceed from either exogenous or endogenous sources (Macharia *et al*., 2008). In physiological conditions, antioxidants help to equilibrate the oxidative stress-status balance and protect against the onset of CVD, carcinogenesis and other oxidative stress-related health disorders (Alinde *et al.,* 2012).

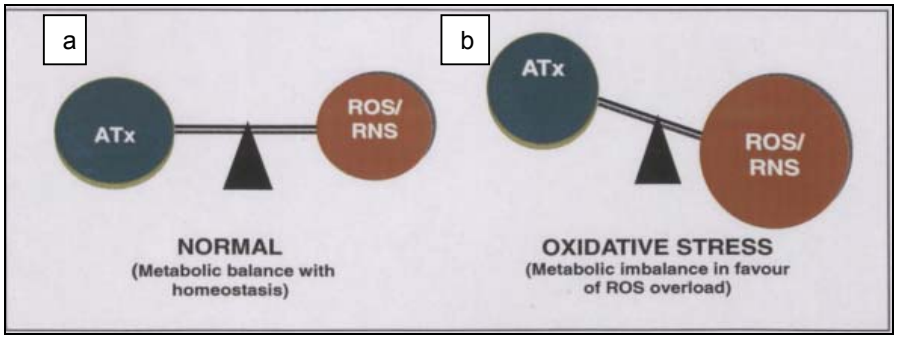

Figure 2.4: General concept of oxidative stress (a) Normal condition is indicated the balance between oxidant production and antioxidant defence system and (b) OS is demonstrated by the imbalance between the generation and clearance of oxidants (Alinde *et al.*, 2012, p1442).
ATx- antioxidant production, ROS/RNS- reactive oxygen species / reactive nitrogen species

Dietary intake is a very important source of antioxidants and points to the potential effects of malnutrition or malabsorption of nutrients on the regulation of these mediators (Bhattacharyya *et al.*, 2014). Antioxidant components are most important in food because of their ability to reduce the free radical-mediated degradation of

cells and tissues in an organism (Awoniyi *et al.,* 2010). Among antioxidants derived from dietary sources (e.g. fruits and vegetables, including red palm oil), many come from the phenol family (Fusco *et al.*, 2007; Oguntibeju *et al*., 2010; Alinde *et al.,* 2012).

The body's antioxidant defences can be approximated by measuring plasma levels of antioxidants (micronutrients, enzymes, and other antioxidants), keeping in mind that the circulating compartment only reflects the flow between organs and tissues. The tissue levels of the various antioxidants are only available when research protocols such as tissue biopsies are required (Fusco *et al.*, 2007).

Although specific antioxidants have different mechanisms of action (as made clear in Table 2.6), the functional hierarchy in terms of which any antioxidant protects against ROS will fall within one of the following three broad categories (Macharia *et al.,* 2008; Alinde *et al.,* 2012): 1) primary/preventive is the first line of defence and suppresses the formation of ROS; examples include enzymes like glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase; 2) Radical scavengers/chain breakers are the second line of defence, trapping ROS to inhibit oxidation chain initiation and thus breaking the reaction; e.g. polyphenols, carotenoids and vitamins C and E; 3) Repair enzymes act as third line of defence by repairing damaged macromolecules. These include lipases, proteases and transferases (Devasagayam *et al.*, 2004; Fusco *et al.*, 2007; Macharia *et al.,* 2008; Oguntibeju *et al*., 2010; Alinde *et al.,* 2012).

Table 2.6: The role of antioxidants in protecting against free radical damage (Ayepola, 2014).

Antioxidants	Cellular location	Role
Enzymatic antioxidants		
(A) Catalase	Peroxisomes	Decomposes H_2O_2 to water and oxygen
(B) Glutathione peroxidase	Cytoplasm, mitochondria, and nucleus	Detoxifies H_2O_2 and lipid peroxides with simultaneous oxidation of GSH and generation of GSSG
(C) Glutathione reductase	Cytoplasm, mitochondria, and nucleus	Recycles Glutathione disulphide back to glutathione using the cofactor NADPH
(D) Superoxide dismutase	Cytoplasm, nucleus lysosomes, mitochondria	Converts superoxide radicals to H_2O_2
Non enzymatic antioxidants		
(A) GSH	Cytoplasm, mitochondria and nucleus	Acts as a cofactor for antioxidant enzymes (GPx, GST), regenerate other antioxidants such as Vitamins C and E to their active form
(B) Vitamin E	Membrane	Directly scavenges singlet oxygen, peroxyl and superoxide radicals, protect against peroxidation of membrane lipids
(C) Vitamin C	Cytosol	Acts synergistically with vitamin E to terminate radical induced lipid peroxidation
(D) α-Lipoic acid	Cell membrane and cytoplasm	Increases glutathione and vitamin C levels

Sources of antioxidants include teas, fruits, vegetables, legumes and whole-grain cereals. Herbal infusions are also important sources of antioxidants (Marongiu *et al.,* 2004). Humans have a highly sophisticated and complex antioxidant protection system to defend the cells and organ systems. This includes prevention antioxidants that block the formation of new ROS, and scavenger antioxidants that remove already formed ROS (Jacob, 1995; Agarwal *et al*., 2005b; Awoniyi *et al.,* 2010). Table 2.7 lists examples of antioxidants and their mechanism of action.

Table 2.7: Examples of antioxidants and their mechanisms of action (Alinde *et al.*, 2012).

Antioxidant/source	Mechanism of action
Endogenous	
Superoxide dismutase (SOD)	Dismutate $O_2^{\bullet-}$ to H_2O_2
Catalase (CAT)	Decompose H_2O_2 to molecular oxygen and water
Glutathione (GSH)	intracellular reducing agent
CoQ10	Inhibit lipid peroxidation; reduce mitochondrial
Uric acid	oxidative stress
	Scavenge peroxyl and $OH^{\cdot}$ radicals; chelate transition metal ions
Dietary antioxidants	
Vitamin E	Scavenge $O_2^{\bullet-}$; up-regulate antioxidant enzymes; inhibit LPO
Vitamin C	Scavenge $O_2^{\bullet-}$, tocopherol regeneration
Lycopene	
Ellagic acid	Trap $^1O_2$
Genistein, quercetin	Scavenge H_2O_2; stimulate glutathione-S-transferase
Catechins	Metal chelation; scavenge O_2 •-, H_2O_2, $OH^{\cdot}$ and $^1O_2$ tocopherol regeneration.

CoQ10= coenzymeQ10, LPO= lipid peroxidation

2.8.1 Endogenous antioxidant defence system

Antioxidants that are synthesised in the human body include antioxidant enzymes, metal binding proteins and other small molecule antioxidants (Evans & Halliwell, 2001). The major enzymatic antioxidants are catalase (CAT), superoxide dismutases (SOD), glutathione peroxidase (GPx), and glutathione-reductase (Bhattacharyya *et al.*, 2014; Nagaraju & Belur, 2008; Young & Woodside, 2001). SOD and catalase provide major antioxidant defences against ROS (Bhattacharyya *et al.,* 2014).

Intracellular compartments, including mitochondria, the endoplasmic reticulum, peroxisomes, nuclei, the cytosol, plasma membranes, and even extracellular spaces, are capable of ROS generation (Figure 2.5) (Bhattacharyya *et al.,* 2014).

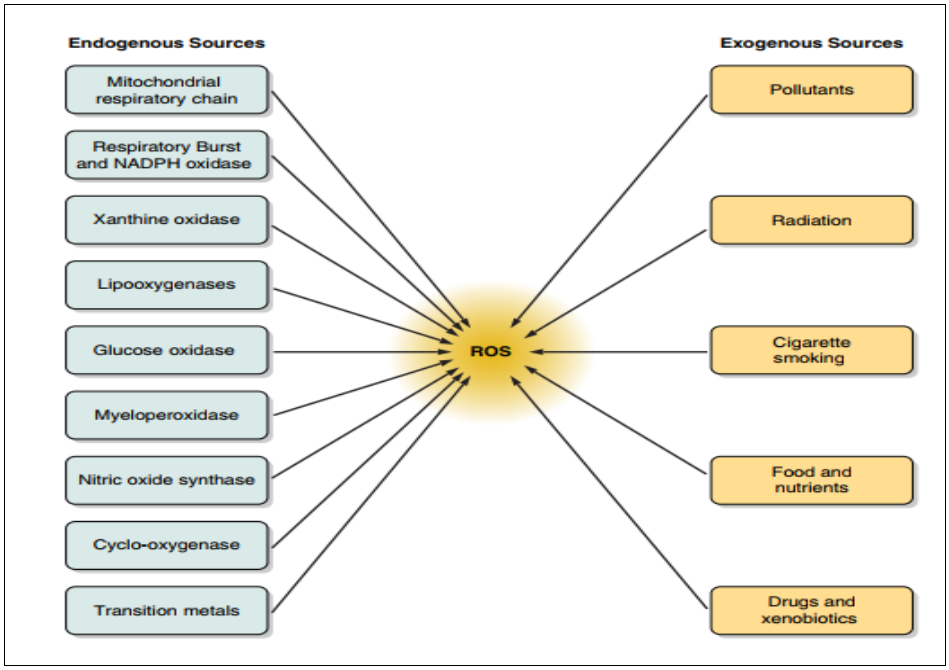

Figure 2.5: Endogenous and exogenous factors leading to ROS generation (Bhattacharyya *et al.*, 2014, p334).

2.8.2 Exogenous antioxidants defence system

Endogenous antioxidants are insufficient to prevent every kind of oxidative damage *in vivo*. Medical experts and nutritionists therefore agree on the necessity for an adequate supplementation of antioxidant-rich products to reinforce the body's overall antioxidant defence system. Exogenous antioxidants occur in diverse forms, including vitamins, enzymes, trace elements and proteins. They derive from both natural and synthetic sources, but natural antioxidants are preferred as they are readily available, safer and more efficient (Pokorny *et al.,* 2001).

Natural supplementation of antioxidants is achieved through the dietary intake of animal products, fruits and vegetables (cereals, oil seeds, legumes, herbs, spices, tomato, spinach and carrots), vegetable oils (red palm oil, olive oil and sunflower oil) and natural beverages (rooibos and honey bush teas). These antioxidants may inhibit the formation of reactive oxygen species through the sequestration of metal ions (Hu *et al*., 2007), the reduction of hydroperoxides and hydrogen peroxide, direct combination with superoxide and singlet oxygen, the scavenging of radicals and the inhibition of lipid chain breaking reaction, initiation and propagation. Antioxidants may also repair damaged molecules (DNA, proteins and lipids) and reconstitute enzymes (Pokorny *et al*., 2001).

2.8.3 Antioxidant non-enzymatic defences of the body

Other small-molecular-weight antioxidants are found in food, the best known being vitamin E, vitamin C and carotenoids. Some foods contain other antioxidant substances, mostly in the form of phenolic or polyphenolic compounds. Although these substances have unknown nutritional function, they may be important to human health because of their antioxidant potency (Langseth, 1995).

Glutathione is found in all eukaryotic cells and is one of the key non-enzyme antioxidants in the body. It is generally present in its reduced form, GSH. This is ubiquitously expressed, and together with three enzymes, glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione S-transferases (GST), forms the glutathione system (Bhattacharyya *et al.*, 2014).

2.8.3.1 Glutathione

Reduced glutathione (GSH) is a major antioxidant in human tissues that provides reducing equivalents to the GPx-catalysed reduction of hydrogen peroxide and lipid hydroperoxides to water and the respective alcohol (Young & Woodside, 2001; Awoniyi *et al.*, 2010; Alinde *et al*., 2012). It directly quenches ROS such as lipid peroxides and also plays a prominent role in xenobiotic metabolism (Awoniyi *et al.,* 2010; Kwiecien *et al.,* 2014). In the process, GSH becomes oxidised to GSSG (oxidised glutathione) which is then recycled back to GSH in the presence of NADPH (Young & Woodside, 2001; Swiegers, 2015). The oxidised glutathione disulphide is reverted to reduced glutathione by the action of glutathione reductase (Szasz *et al.,* 2007). The ratio between oxidised and reduced glutathione is important to evaluate toxicity in the cells.

Exposure of mammalian cells to increased oxidative stress leads to a decrease in the ratio of GSH/GSSG due to GSSG accumulation or reduction in GSH levels (Awoniyi *et al.*, 2010). GSH represents reduced monomeric glutathione and GSSG represents the oxidized glutathione. GSH is recycled to its initial reduced form by the enzyme glutathione reductase (GR). Glutathione plays an important role in the detoxification of peroxide, hydrogen peroxide and other free radicals (Alinde *et al.*, 2012). Research suggests that glutathione and vitamin C work interactively to neutralise free radicals. These two also have a sparing effect upon each other (Yadav *et al.,* 2016).

2.8.3.2 Vitamins

Vitamins are essential organic compounds for humans, who have lost the ability to synthesize them *de novo*. Most vitamins synthesized by plants present amino acids as precursors (B_1, B_2, B_3, B_5, B_7, B_9 and E) and are therefore linked to plant

nitrogen metabolism. Amino acids play different roles in their biosynthesis and metabolism, either incorporated into the backbone of the vitamin or as amino, sulfur or one-carbon group donors. There is a high natural variation in the vitamin content of crops and its exploitation through breeding, metabolic engineering and agronomic practices can enhance their nutritional quality. While the underlying biochemical roles of vitamins as co-substrates or co-factors are usually common for most eukaryotes, the impact of vitamins B and E in metabolism and physiology can be quite different in plants and animals (Wintergerst *et al.,* 2007; Miret & Munné-Bosch, 2014).

2.8.3.2.1 Vitamin C (Ascorbic acid)

Vitamin C is the major water-soluble antioxidant and acts as first defence against free radicals in whole blood and plasma. It is a powerful inhibitor of lipid peroxidation and regenerates vitamin E in lipoproteins and membranes (Devasagayam *et al.,* 2004). It is considered the most important antioxidant in extracellular fluids and performs many cellular activities of an antioxidant nature as well. It has been shown to efficiently scavenge superoxide, hydrogen peroxide, hypochlorite, the hydroxyl radical, peroxyl radicals, and oxygen. It also protects membranes against pro-oxidants by enhancing the activity of tocopherol, the chief lipid-soluble, chain-breaking antioxidant (Frei, 1994; Sies & Stahl, 1995; Agarwal *et al*., 2005). Furthermore, it acts indirectly as an antioxidant by regenerating the lipophilic vitamin E at the aqueous-lipid interphase (Tanaka *et al*., 1997).

Vegetables and fruits contain considerable amounts of ascorbate – e.g. broccoli, spinach, tomato, garlic, potato, and citrus fruits – and as part of a balanced diet provide sufficient vitamin C (Frei, 1994).

Reduced levels of ascorbic acid have been observed in the plasma, leukocytes, thrombocytes, platelets and urine of oral contraceptive users compared to non-users. OCs may increase the breakdown of ascorbic acid by stimulating the release of ceruloplasmin (a copper-containing protein with ascorbate oxidase activity) from the liver. Alternatively, the reduction may be due to decreased absorption of the vitamin or changes in tissue distribution. A mega dose of vitamin C (1000 mg ascorbic acid) converts a low dose oestrogen pill into a high dose pill. This enhances the effect of OC on plasma proteins and may theoretically increase the risk of cardiovascular disease in the long term (Palmery *et al.,* 2013).

2.8.3.2.2 Vitamin E

Vitamin E is one of the most important lipid-soluble primary defence antioxidants. It is a generic term for several naturally occurring tocopherols and tocotrienols. In its function as a chain-breaking antioxidant, vitamin E rapidly transfers its phenolic H-atom to a lipid peroxyl radical, converting it into a lipid hydroperoxide and a vitamin E radical (Frei, 1994; Yadav *et al.,* 2016). Vitamin E scavenges peroxyl radical intermediates in lipid peroxidation and is responsible for protecting polyunsaturated fatty acid (PUFA) present in cell membranes and low density lipoprotein (LDL), against lipid peroxidation. A fat-soluble vitamin that can be stored with fat in the liver and other tissues, vitamin E (tocopherols, tocotrienols) is promoted for a range of actions from delaying aging, to healing sun burn. In plasma and red blood cells, vitamin E is the major lipid-soluble antioxidant protecting lipids against peroxidative damage. It is essential for inhibiting the oxidation and breakdown of body tissues, and for forming red blood cells. It efficiently scavenges peroxyl radicals in cell membranes to inhibit lipid peroxidation (Frei, 1994; Ibrahim *et al*., 2000; Agarwal *et al*., 2004; Yadav *et al.,* 2016).

The vitamin serves variously to maintain the normal condition of cells and healthy skin and tissues, protect red blood cells, promote antioxidation and enhance immunity. The important sources of vitamin E include wheat germ, nuts, seeds, whole grains, green leafy vegetables, vegetable oil and fish-liver oil (Frei, 1994; Sies & Stahl, 1995; Brigelius-Flohé & Traber, 1999; Yadav *et al.,* 2016).

2.8.3.2.3 Vitamin A (Carotenes)

In plants, vitamin A exists only in its precursor form carotene, the most abundant of the carotenoid precursors with the highest vitamin A activity. It takes about 12 micrograms of beta-carotene from food to supply the equivalent of one microgram of retinol to the body. Scientists also recognise beta-carotene and its carotene relatives for their antioxidant actions in the body. In general, carotenoids possess the ability to quench $^1O_2$ and are useful for protection against UV-induced damage (Masaki, 2010). The plant-made orange pigment with antioxidant activity and a vitamin A precursor is stored in human fat tissue (Sizer & Whitney, 2003). Natural and synthetic analogues of vitamin A have been used successfully in the treatment of skin disorders, including acne *vulgaris* and psoriasis (Masaki, 2010; Cunliffe, 2017).

The major sources of carotenoids are vegetables and fruits, e.g., carrot, tomato, grapefruit, bean, broccoli, orange, and mango. Dietary vitamin A is available in the form of provitamin A precursor compounds or directly from animal food: liver, milk, egg, and fish (Frei, 1994). There are three known mechanisms by which carotenoids

protect cells from oxidative stress: by quenching (1) triplet-state sensitizers and (2) singlet oxygen, and by (3) scavenging peroxyl radicals (Frei, 1994; Palace *et al*., 1999).

Wild *et al*. (1974) have confirmed a significant increase in vitamin A levels in OC users, but show that during early pregnancy there is no significant difference between recent OC users and non-users. The use of OCs had neither detrimental effect on the outcome of pregnancy nor any teratogenic risk due to increased vitamin A levels.

2.8.4 Antioxidant enzymatic defences of the body

While antioxidant defences of the body are composed of molecular and enzymatic players, the composition of this network differs markedly, in terms of concentration and components, between body compartments. The multifunctional properties of the antioxidant network highlight the crucial importance of dynamic interactions among the components of the network in protecting body fluids from oxidative stress (Bhattacharyya, 2014).

The human body has several mechanisms for defence against free radicals and other reactive oxygen species. One important line of defence is a system of enzymes, including glutathione peroxidases, superoxide dismutases and catalase, which decrease the concentration of the most harmful oxidants. Superoxide dismutases are a family of antioxidant enzymes that are important in the catalytic decomposition of the superoxide radical to hydrogen peroxide and oxygen. Catalase specifically catalyses the decomposition of hydrogen peroxide. Glutathione peroxidases are a family of antioxidant enzymes containing selenium and are important in the reduction of hydroperoxides, for example, those resulting from lipid oxidation (Langseth, 1995; Sies, 1997; Swiegers, 2015). These antioxidant enzymes operate in concert together with several non-enzymatic molecules to contest the action of ROS and avoid oxidative damage (Sies, 1997; Swiegers, 2015).

2.8.4.1 Catalase

Catalase (CAT) is an intracellular antioxidant enzyme that is produced naturally in the body and found in peroxisomes in eukaryotic cells (Alinde *et al*., 2012). Catalases are enzymes that catalyse the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor. Subcellularly, catalases are found in mitochondria and peroxisomes (Devasagayam *et al.,* 2014).

Catalase (CAT) has two enzymatic activities, namely catalytic and peroxodic, depending on the concentration of H_2O_2, a powerful oxidising agent. If the concentration of H_2O_2 is high, CAT catalyses the conversion of hydrogen peroxide into water and molecular oxygen, also favouring the oxidation of hydrogen donors such as alcohols and phenol formic acids. Catalase is particularly important in the event of limited glutathione availability and plays a significant role in the development of tolerance to cellular oxidative stress (Awoniyi *et al*., 2010).

CAT degrades hydrogen peroxide (H_2O_2) to water and oxygen and hence finishes the detoxification reaction started by superoxide dismutase (SOD). A catalase molecule can convert millions of H_2O_2 molecules per second, preventing excessive build-up of hydrogen peroxide and protecting against hydrogen peroxide-mediated oxidative damage (Awoniyi *et al*., 2010).

2.8.4.2 Superoxide dismutase

Superoxide dismutase (SOD) is an antioxidant metal ion cofactor-requiring enzyme that catalyses the dismutation of two superoxides into H_2O_2 and oxygen (Johnson & Giulivi, 2005; Bhattacharyya *et al.*, 2014). It acts as a major defence system against the cytotoxic effects of superoxide radicals (Caldwell *et al.*, 2008). Three isoforms of SOD exist in humans: cytosolic copper and zinc-containing enzyme (Cu-Zn-SOD), manganese-requiring mitochondrial enzyme (Mn-SOD), and an extracellular Cu-Zn containing SOD (EC-SOD). Each type of SOD plays a different role in keeping cells healthy (Caldwell *et al*., 2008; Bhattacharyya *et al.*, 2014). Superoxide dismutase families are characterised according to their metal ion content and their location in organisms. SOD is a metal-containing enzyme that depends on bound trace metals for antioxidant activity. These enzymes are present in almost all aerobic cells as well as in extracellular fluids (Bhattacharyya *et al.*, 2014).

Oxygen formed in the mitochondria is dismuted to H_2O_2 by Cu-Zn-SOD present in the mitochondrial inter-membranous space and Mn-SOD present in the mitochondrial matrix. GPx present in the mitochondrial matrix can scavenge H_2O_2. Uncharged H_2O_2 crosses the mitochondrial membranes and in the cytosol can be scavenged by either cytosolic Cu-Zn-SOD or catalase (Chan, 1996; Miller, 2004; Brand, 2010; Bhattacharyya *et al.*, 2014).

2.8.4.3 Glutathione peroxidase

Glutathione peroxidase (GPx) converts glutathione (GSH), a tripeptide consisting of glutamate, cysteine, and glycine, into oxidised glutathione (also called glutathione disulfide, GSSG) and, during this process, reduces H_2O_2 to H_2O and lipid

hydroperoxides (ROOH) to corresponding stable alcohols (Maritim *et al.,* 2003; Bhattacharyya *et al.*, 2014). This enzyme requires selenium as a cofactor and contains a seleno-cysteine amino acid residue in the active site of each monomer (Herbette *et al.,* 2007; Bhattacharyya *et al.*, 2014).

Glutathione peroxidase is an enzyme that protects erythrocytes against oxidative damage (Mills, 1957; Herbette *et al.,* 2007). Together with SOD and CAT, GPx constitutes the enzymatic antioxidant system that recycles active oxygen species (AOS) and limits their toxicity in mammals (Herbette *et al.,* 2007).

Rikans and Hornbook (1997) have shown that the glutathione metabolism is one of the most essential antioxidant defence mechanisms. Glutathione peroxides in particular play an important role in the detoxification of peroxides in the cell. They prevent the destruction of cell membranes since peroxides decompose in high reactive free radicals. Therefore, they are generally helpful in preventing lipid peroxidation of cell membranes (Covarrubias *et al.*, 2008).

2.8.4.4 Glutathione reductase

Glutathione reductase (GR or GSR) reduces oxidised glutathione disulfide (GSSG) to GSH. GR protects red blood cells, hemoglobin, and cell membranes from oxidative stress by generating GSH. Riboflavin deficiency leads to reduced GR activity. An increased level of GSH is often associated with the resistance to drugs of various cancers, including colon cancer (Bhattacharyya *et al.*, 2014; Anjum *et al.,* 2015).
Extensive research has been done on contraceptives, antioxidant status, and skin and body composition, but these studies has often focused on individual elements in isolation from each other. There remain several unanswered questions regarding the effects of contraceptives on essential human biological functions, questions whose answers prospective contraceptive users should know.

CHAPTER THREE

RESEARCH DESIGN AND METHODOLOGY

To address the research problem effectively, an overall strategy was devised to integrate the various components of the study in a coherent and logical way. Chapter Three describes the strategy for the collection, measurement, and analysis of samples and data.

3.1 Research design

This study employed a quantitative approach to achieve a cross-sectional research sampling that provided a snapshot of the target population at a particular time.

3.2 Study site and experimental design

The study was conducted at Cape Peninsula University of Technology, South Africa. Participants were selected through quantitative sampling, using questionnaires to ascertain the type of contraceptive used as well as general health and lifestyle patterns (Figure 3.1). Blood samples were collected from participants and their antioxidant status determined. Body composition and skin analysis was conducted on each of the participants in the selected groups, and the results were compared to determine the differences between contraceptive and non-contraceptive users.

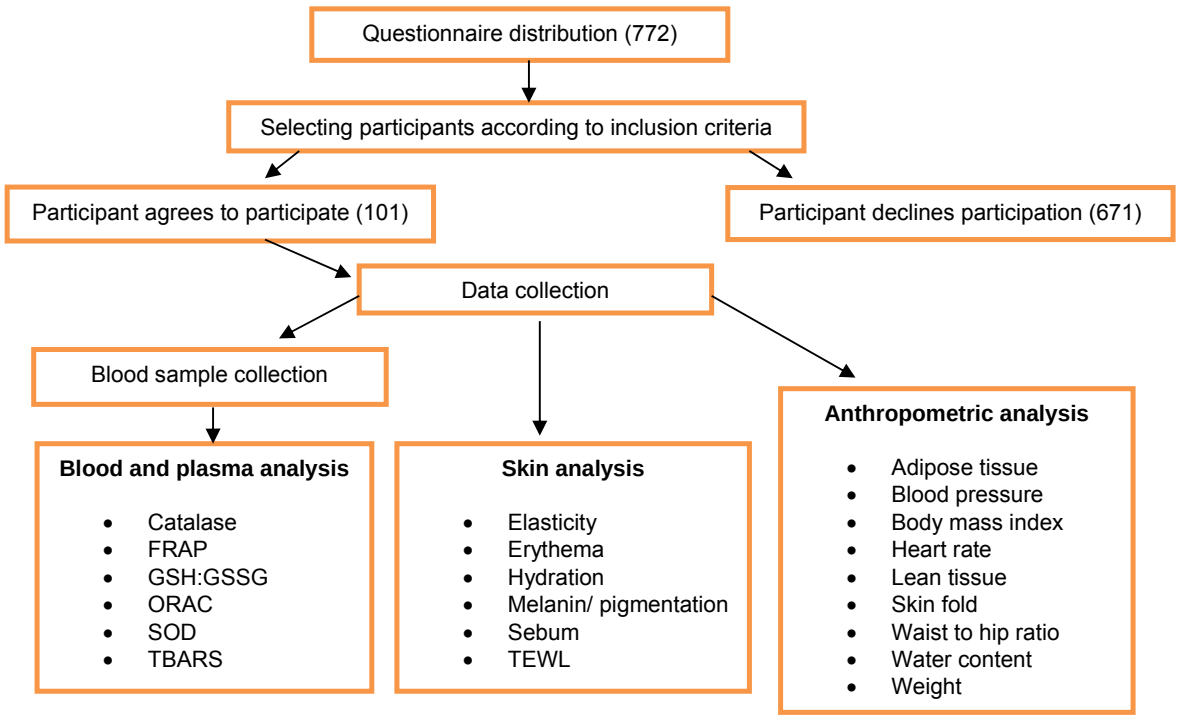

Figure 3.1: Diagram outlining the research design.

3.3 Participant selection

Seven hundred and seventy-two questionnaires were distributed to female students at Cape Peninsula University of Technology's (CPUT) Cape Town (District Six) and Bellville campuses through the Health Clinics and Health Sciences departments after ethical approval and informed consent form had been obtained. The aim was to collect information pertaining to the participants' socio-demographic profile, dietary and lifestyle habits, medication and supplements usage, as well as contraceptive use (Appendix A). The aim of the study was explained in the introduction to the questionnaire. After completion the questionnaires were analysed and participants were selected according to their age, contraceptive use and current health status (taking into account the exclusion criteria). The following contraceptive use data was gathered from the questionnaire feedback: monophasic – 55; triphasic – 21; injectable – 124; implant – 27; barrier method – 8; IUD – 7; patch – 2; periodic abstinence – 11; sterilisation – 2; none – 496; un known – 19. After consulting with a statistician, it was decided that one hundred participants would be selected: 20 progesterone injection users, 20 progestogen implant users, 20 monophasic oral contraceptive users, 20 triphasic oral contraceptive users, and 20 non-contraceptive users as a control group. The group selection size was based on 'A Power Primer' (Cohen, 1992). Participants completed an informed consent form that outlined the purpose and requirements of the study, and highlighted the guarantee of anonymity.

3.4 Inclusion criteria

Apparently healthy, non-lactating, non-pregnant females aged between 18 and 30 at a tertiary educational institution were recruited for the study. Blood samples were collected when participants were not menstruating, as this might have influenced the body composition, skin parameters and antioxidant status.

3.5 Exclusion criteria

There are several contraindications (possible side-effects and special precautions) to be considered before women are prescribed a specific hormonal contraceptive. The following exclusion criteria were invoked in this study: hypertension, fluid retention, cloasma or melasma, carbohydrate and lipid metabolic effects, increase or decrease in mass, change in appetite (Chernev *et al.*, 1998).

3.6 Informed consent from participants

Each participant completed an informed consent form that outlined the purpose and methodology of the study. Participants remained anonymous and participation was voluntary (Appendix C).

3.7 Ethical approval

Ethical approval was sought for this study as it involved the participation of human subjects and the collection of blood samples. This was granted by the Faculty of Health and Wellness Sciences Research Ethics Committee of the Cape Peninsula University of Technology (CPUT/HW-REC 2014/H13) (Appendix B).

3.8 Materials and methods

The quantitative data was obtained by utilises a cross-sectional sampling design, in order to obtain a snapshot research view of the target population and measuring selected oxidative stress markers as well as skin and anthropometric parameters.

3.8.1 Blood sampling

A qualified, registered nurse (phlebotomist) drew intravenous blood samples from participants. The blood samples were collected into two EDTA tubes (BD vacutainers, Plymouth, UK) using a Vacutainer® with a 21-gauge needle. Samples were protected from light and transported on ice to the Oxidative Research Centre at the Cape Peninsula University of Technology (CPUT) Bellville campus for processing the same day. Blood samples were centrifuged (3500 rpm / 1000 x g, 10 minutes, 4˚C) to obtain plasma. Both the plasma and whole blood was stored at -80˚C until analysed. Whole blood samples of oxidised glutathione (GSGG) analysis were treated with 30 mM of 1-methyl-2-vinylpyridinium trifluoromentanesulphonate (M2VP) obtained from Merck, SA, before storage at -80˚C. All biological waste was disposed of into a medical waste box and collected by a reputable company that incinerates biological waste ethically.

The plasma samples were collected from blood samples by centrifugation at 4000 rpm for 3 minutes and then transferred to new Eppendorf tubes. These were then stored at -80°C and thawed on the day of the analysis. These plasma samples were used for the FRAP, ORAC and TBARS assays.

3.8.2 Oxidative stress assays

Blood samples were analysed at the Oxidative Stress Research Unit at Cape Peninsula University of Technology (CPUT), Bellville campus, for the following: 1) Catalase (Young & Woodside, 2001); 2) The index of antioxidant potential by ferric reducing ability of plasma (FRAP) (Benzie & Strain, 1996); 3) The ratio of reduced glutathione (GSH) and oxidised glutathione (GSSG) levels in the blood (Gutteridge, 1995; Young & Woodside, 2001); 4) Total antioxidant capacity using the oxygen radical absorbance capacity (ORAC) assay (Cao *et al*., 1998; Ou *et al.*, 2001); 5) Superoxide dismutase (SOD) (Devasagayam *et al.*, 2004); and 6) Lipid

peroxidation determined using the thiobarbituric acid-reactive substance (TBARS) assay (Cao *et al.*, 2003). After the analyses all samples were disposed of into a medical waste box which was collected by a reputable company for incineration.

3.8.2.1 Catalase activity

Catalase activity (CAT) was determined by a method modified from Aebi (1974) and Ellerby and Bredesen (2000). The assay is based on the principle of measuring (at 240nm) the decomposition of hydrogen peroxide (H_2O_2) by catalase.

A phosphate buffer (50 mM potassium phosphate buffer 0,5% (v/v), pH 7.5) was freshly prepared and 170 µl of the phosphate buffer agent (P-buffer) was put into a 96-well microplate. Ten microliters of diluted blood sample were then added to each well except for the first 3 wells, which was filled with water as a control. Lastly, 75 µl of H_2O_2 was added to each well. Using a thermostatted spectrophotometer, the rate of decomposition of H_2O_2 was measured at 240 nm for 2 minutes at 15 second intervals. Catalase activity was expressed as U/mg total.

3.8.2.2 Ferric ion-reducing ability

The index of antioxidant potential by the ferric ion-reducing ability of plasma (FRAP) (Benzie & Strain, 1996; Benzie & Strain, 1999) is a colorimetric spectrophotometric assay to assess the antioxidant power of biological fluids. The principle of this assay is the reduction ability of antioxidants to convert the ferric ion (Fe^{3+}) into its oxidised counterpart (Fe^{2+}) in acidic media. The electron transfer redox (oxidation/reduction) reactions occurring in the assay are signalled by the development of a characteristic blue coloration. At low pH, a ferric salt, ferric chloride hexahydrate $Fe^3(TPTZ)_2Cl_3$ (TPTZ=2,4,6, Tripyridyl-s-triazine) used as an oxidant is reduced by biological antioxidants in a sample to give the blue-coloured ferrous tripyridyltriazine complex. The colour development occurs only in the presence of electron-donating antioxidants in the sample and is monitored using a spectrophotometer that measures the change in absorption maximum at 593 nm (Benzie & Strain, 1996; Alinde *et al*., 2012).

The standard solution was made by dissolving 0.0085g ascorbic acid in 50 ml distilled water. This solution was used as stock to prepare the standard series (0, 50, 75, 125, 250, 500 µM) using distilled water as the diluent. The FRAP reagent was prepared in a 50 mL conical tube by adding together, 30 mL acetate buffer, 3 mL iron chloride hexahydrate and 3 mL 2,4,6-tri[2-pyridyl]-s-triazine (TPTZ) solution, 3 mL $FeCl_3$ and 6.6 mL distilled water. TPTZ was prepared in 0.1 M hydrochloric acid (HCl).

Ten microliters of the sample were then added to the 96 clear well microplate with 300 µL of the FRAP reagent. The microplates were incubated in the oven at 37ºC for 30 minutes before the absorbance was read using the Multiskan spectropohotometer (Thermo Electron Corporation, Finland) set at 25ºC and 593 nm. Each sample was run in triplicate and final results were obtained by comparison to the calibration curve standard, using a regression equation (y = a + bx) and expressed as µmolAAE/L (Alinde *et al*., 2012).

3.8.2.3 Total glutathione and glutathione disulphide levels

The ratio between reduced glutathione (GSH) and oxidised glutathione (GSSG) levels in the blood was determined by first analysing total glutathione (GSH_t) and glutathione disulphide (GSSG), and then using the following calculation: (GSH_t – 2GSSG) / GSSG. GSH is a tripeptide present at high levels in all living cells and participates in numerous cellular functions, including protection against oxidative damage caused by free radicals. Thus, the glutathione status (GSH/GSSG ratio) is a good indicator of oxidative stress (Asensi *et al*., 1999).

Glutathione concentration was determined according to the method of Asensi *et al*. (1999). GSSG whole blood samples were prepared by adding 10 µl 1-methyl-2-vinyl-pyridinium trifluoromethane sulfonate (M2VP) to the microcentrifuge tube. Both the GSSG and GSH samples where frozen at -70°C before thawing, and both samples were prepared in exactly the same manner. Seven hundred microliters of metaphosphoric acid (MPA) (v, 5%) were added to 100µl blood and centrifuged at 10 000 x g for 10 minutes to obtain the supernatant. Six hundred microliters of buffer (500 mM $NaPO_4$, 1 mM EDTA, pH 7.5) was then added to the supernatant, and this was used in the assay.

The sample volume was measured into a 96 microliter plate and 50 µl of 5,5’ Dithiobis-(nitrobenzoic acid (DTNB) (v), 0.3 mM in buffer with 50 µl glutathione reductase (GR), and left for 5 minutes. To start the reaction, 50 µl of nicotinamide adenine dinucleotide phosphate (NADPH) (v) was added, and absorbance was measured at 412 nm for 5 minutes. GSH / GSSG standards were used for comparison and all samples and standards were done in triplicate. A linear slope of standards was used to calculate the concentrations as follows: GSHt = µM x dilution factor (GSH = GSHt - 2GSSG); GSSG = µM x dilution factor; Ratio = (GSHt – 2GSSG) / GSSG (Macharia *et al*., 2008).

3.8.2.4 Oxygen radical absorbance capacity

Total antioxidant capacity was measured using the oxygen radical absorbance capacity (ORAC) assay (Cao *et al*., 1998; Ou *et al.*, 2001). The ORAC assay is a method used to measure the antioxidant scavenging activity of a substance (e.g. lipophilic and hydrophilic), and is based upon measurement of the inhibition of free radical damage to a fluorescent probe by antioxidants (Prior & Cao, 2001). The loss of the fluorescent intensity reflects the intensity of free radical damage and the extent of their concentrations (Alinde *et al*., 2012). The extent of pre-existing antioxidant scavenging of free radicals' activity is then indicated by the delay in the degradation of the fluorescent probe. In this study the ORAC method was performed using a fluorescence spectrophotometer until zero fluorescence occurred. The results were reported as the ORAC value, which refers to the net protection area under the quenching curve of β-PE (fluorescein) in the presence of an antioxidant. The ORAC value was calculated by dividing the area under the sample curve (fluorescence decay curve or AUC) by the area under the control mixtures, which were prepared using a 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic Acid (Trolox) solution with both areas being corrected by subtracting the area under the blank curve. Trolox is a synthetic water solution antioxidant derivative of vitamin E. One ORAC unit was assigned as being the net protection area provided by 1 μM Trolox in final concentration. When the curve for the sample is compared to the area under the curve for Trolox, the result generated is given in Trolox equivalents (Alinde *et al.,* 2012).

For analysis, the samples were prepared by mixing 50 μl of plasma with 50 μl of 5% Perchloric acid (PCA), which was vortexed to precipitate the proteins. The protein precipitate was then centrifuged at 10 000 rpm for 1 minute. Thereafter, 400 μl ORAC buffer was added to prepare the sample for use. This procedure was a modified method of Rautenbach *et al*. (2010). All reagents and standards (AAPH, FL and Trolox) were prepared in a phosphate buffer (75 mM, pH 7.4, ORAC buffer).

Twelve microliters of the sample were then added to a black 96-microwell plate in triplicate to ensure accurate readings. One hundred and thirty-eight microliters of fluorescein were added, a stock solution of AAPH (500 μM) was prepared, and 50 μl of this was added to the plate just before the readings. Control mixtures were prepared within a range of 0-417 μM Trolox.

Fluorescence readings were carried out on a fluoroskan ascent plate reader (Thermo Fisher Scientific, Waltham, Mass., U.S.A.). The fluoroskan should be switched on at least 30 minutes before starting the assay to allow the machine to reach a

temperature of 37°C. The excitation wavelength was set at 485 nm and the emission wavelength at 530 nm (Cao & Prior, 1998; Prior *et al*., 2003). Each reading was taken after shaking at the end of every cycle (1 min) over two hours. Antioxidant activity was expressed in Trolox equivalents (µmole TE/L) (Alinde *et al*., 2012).

3.8.2.5 Superoxide dismutase activity

Superoxide dismutase (SOD) activity was determined by a modified method from Ellerby and Bredesen (2000). The Thermo Scientific™ Pierce™ BCA Protein Assay kit was used. This is a detergent-compatible formulation based on bicinchoninic acid (BCA) for the colorimetric detection and quantitation of total protein. The method combines the well-known reduction of Cu^{+2} to Cu^{+1} by protein in an alkaline medium (biuret reaction) with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu^{+1}) using a unique reagent containing BCA (Smith *et al.,* 1985; Weydert & Cullen, 2010).

Twelve microliters of each blood sample were put into a 96-well microplate, then 15 µL of 6HD (6-hydroxydopamine) was added, and just before reading, 170 µl Diethylenetriaminepentaacetic acid (DETAPAC) was added. Twelve microliters of an SOD assay buffer ($NaPO_4$-buffer, 50 mM, pH 7.4) was used in each plate as a comparison. The auto oxidation was then recorded at 490 nm for 4 minutes at 1 minute intervals. Each sample was run in triplicate. The activity of SOD was calculated from a linear calibration curve, in the range of 2-20 U/mg.

3.8.2.6 Thiobarbituric acid reactive substances

Plasma concentrations of thiobarbituric acid reactive substances (TBARS) are an index of lipid peroxidation and oxidative stress. Plasma MDA (malondialdehyde), an end product of lipid peroxidation, was determined through a modern HPLC-based thiobarbituric acid (TBA) assay. The quantitative analysis of the plasma content of MDA was performed via a modified version of Cuny *et al*.'s (2004) method, using the Spectra HPLC system (Thermo Fischer Scientific, South Africa).

The sample was prepared by adding 100 µl of plasma, 12.5 µl ETOH, 100 µl OPA and 12.5 µl TBA. The microcentrifuge tubes were then punctured and heated to 90°C for 45 minutes. Thereafter, the tubes were placed on ice for 2 minutes and then left at room temperature for 5 minutes. One hundred µl of saturated NaCl solution and 1000 µl butanol were added and microfuged at 10000 rpm for 2 minutes. Two hundred-and-fifty microliters of the top butanol phase were then added into the microliter plate wells in triplicate. Butanol was put into wells A1-A3 as a comparison. Spectro photometric detection was performed at 532 nm.

3.8.3 Skin parameters analysis

Participants had a skin analysis test performed using the Multi Skin Test Centre® Model MC 750 and Visioscope® Imager Dual (Mahler *et al.*, 2010). These tests were conducted at the Department of Wellness Sciences after the initial blood collection. The skin's elasticity, level of inflammation, pigmentation, stratum corneum moisture content, trans-epidermal water loss (TEWL) and sebaceous activity were determined using skin biophysical techniques. These comprised Cutometer® (MPA 580; Courage & Khazaka Electronic GmbH, Cologne, Germany), Mexameter MX 18 (Courage & Khazaka electronic GmbH, Cologne, Germany), Corneometers CM 825, Tewameters CM 210 (from the Multi Skin Test Centre®, Model MC 750) (Campos *et al*., 2008). All biophysical measurements were taken on the three facial regions of the forehead, right cheek and chin. Digital photos were taken with the Visioscope® Imager Dual and digital camera, with the participants' consent. The participants' identities were protected by blocking out the eyes on the images. The skin age of each participant was rated using the Rao-Goldman 5-point Facial Wrinkle Scale (Khoury *et al.*, 2008).

3.8.3.1 Skin elasticity

The Cutometer® (MPA 580; Courage & Khazaka Electronic GmbH, Cologne, Germany) is a standard device for measuring the elasticity and other biomechanical parameters of the skin. The measurement is based on computer assisted suction devices (Piérard *et al*., 2013b). The handheld probe is maintained on the skin surface under constant pressure guaranteed by a built-in spring. Upon suction, the skin surface is pulled upwards inside the aperture of the probe by the applied negative pressure. After three seconds the negative pressure of 400 mbar stops and the skin relaxes and returns from the probe opening within the next 3 seconds. The penetration depth of the skin is determined optically during suction and relaxation at 0.01 mm accuracy. The measurement assesses the skin's ability to resist the suction (firmness) and its ability to return to the original position (elasticity) (Ryu *et al*., 2008; Piérard *et al*., 2013).

The elasticity is displayed as a percentage. It is measured by how the skin can resist the pressure (a) in relation to its ability to return into the original position (b) and calculated as: a - b x 100 = E (elasticity in %) (Piérard *et al*., 2013b; Ryu *et al*., 2008; Addor *et al*., 2018; Mota *et al*., 2018).

3.8.3.2 Erythema

The erythema (haemoglobin) was measured using the Mexameter MX 18 probe (Courage & Khazaka Electronic GmbH, Cologne, Germany). Skin colour as

perceived by the human eye consists mainly of melanin and erythema. The skin redness (erythema) is induced by the haemoglobin in the skin (red blood cells), a complex molecule responsible for oxygen transport through our body. The human eye cannot detect small colour changes, especially when viewed at different points in time. A precise and objective skin colour measurement was therefore very important.

The measurement of erythema is based on the absorption/reflection principle. The probe emits light of three defined wavelengths (568, 660 and 880 nm), and measurements are displayed in arbitrary units (0–99 AU) (Mahler *et al*., 2009). A receiver measures the light reflected by the skin. These specific wavelengths were chosen because they are known to be absorbed by melanin and haemoglobin (the colouring of the red blood particles). The probe was pressed on the skin surface and held lightly according to the pressure of the spring in the probe. The positions of emitter and receiver guarantee that only diffuse and scattered light is measured. As the quantity of emitted light is defined, the quantity of light absorbed by the skin can be calculated. The erythema was measured by two specific wavelengths (green: 568 nm and red: 660 nm), corresponding to the spectral absorption peak of haemoglobin and to avoid other colour influences (e. g. bilirubin) (Matias *et al*., 2015; Majid *et al*., 2016; Mazurek & Pierzchala, 2016; Zasada *et al*., 2016; Duman *et al*., 2017; Khosrowpour *et al*., 2018).

3.8.3.3 Skin hydration

The moisture content of stratum corneum can vary greatly depending on its storage capacity. It is a critical parameter for the hydrolipidic film of the skin, and plays an important role in our daily life as our skin is dried out by the hazardous effects of the sun, air-conditioned rooms, pollution etc. Dry skin tends to wrinkle. The measurement of skin moisture was based on the capacitive Corneometer® method (Morganti *et al*., 1986; Berardesca & EEMCO, 1997; Clarys *et al.,* 1999; Campos *et al*., 2008; Darlenski *et al.,* 2018). The device determines the water content of the superficial epidermal layers down to a depth of about 0.1 mm, and water content values were expressed in arbitrary units on a scale from 0-99. The probe head was placed vertically on the skin area to be measured according to the pressure of the spring in the probe (Morganti *et al*., 1986; Berardesca & EEMCO, 1997; Clarys *et al.,* 1999; Campos *et al*., 2008; Darlenski *et al.,* 2018).

3.8.3.4 Pigmentation

Pigmentation (melanin production) was also measured using the Mexameter MX 18 (Courage & Khazaka Electronic GmbH, Cologne, Germany) probe. Skin colour perceived with the human eye consists mainly of melanin and erythema. The melanin

pigment is produced and distributed by the melanocytes in the skin, creating the pigmentation which provides protection against UV radiation. It comprises eumelanin, occurring especially in dark skin, and pheomelanin, providing pigmentation in fair skin types.

The measurement of pigmentation is based on the absorption principle. The probe emits light of three defined wavelengths (568, 660 and 880 nm), and measurements are displayed in arbitrary units (0–99 AU) (Mahler *et al*., 2009). A receiver measures the light reflected by the skin. These specific wavelengths have been chosen because it is known how they are absorbed by melanin and haemoglobin (the colouring of the red blood particles). The positions of emitter and receiver guarantee that only diffuse and scattered light is measured. As the quantity of emitted light is defined, the quantity of light absorbed by the skin can be calculated (Matias *et al*., 2015; Majid *et al*., 2016; Mazurek & Pierzchala, 2016; Zasada *et al*., 2016; Duman *et al*., 2017; Xu *et al.*, 2017; Khosrowpour *et al.,* 2018). The probe is pressed on the skin surface and held lightly according to the pressure of the spring in the probe.

3.8.3.5 Sebaceous activity

Sebaceous activity was measured using the Sebumeter SM815 (Courage & Khazaka), which enables the direct measurement of the sebum secretion on skin, hair and scalp. The measurement principle is the photometric method, the grease spot photometer. This method is independent of moisture. The supplied sebum measurement cartridge contains a mat synthetic tape which is 0.1 mm thick. The measuring head of the cartridge exposes a 64 mm^2 measuring section of the tape. For the next measurement the tape has to be transported forward by a trigger at the side of the cartridge so that a new measuring section is exposed. The used tape is rewound inside the cartridge. One cartridge can be used for approx. 400 measurements. The complete cassette is exchanged for hygienic reasons. A mirror under the measuring section of the tape protrudes approximately 1 mm from the measuring head. The mirror is linked with the cartridge by a 4 N spring. This ensures that the tape is pressed onto the measuring area with constant pressure by the mirror (Courage*,* 1994; Pouradier *et al*., 2017; Yonezawa *et al*., 2018).

The Sebumeter was first calibrated to zero with an unused tape section prior to each measurement. Sebum was collected from each site on a plastic strip using a constant pressure of 10 N for 10 seconds. The values were displayed as arbitrary units on a scale from 0-99 (Courage*,* 1994; Pouradier *et al*., 2017; Yonezawa *et al*., 2018).

3.8.3.6 Transepidermal water loss

Transepidermal water loss (TEWL) was measured with an evaporimeter, TEWAmeter TM 300 (Courage & Khazaka, Koln, Germany). Water is constantly evaporating through the skin due to regular metabolism (TEWL). Skin is the barrier between the inside of the body and the outside environment. Even the slightest damage to the skin not visible to the human eye manifests itself immediately in increased TEWL. The measurement is based on the open chamber method. Water evaporates through the probe's hollow cylinder. The resulting density gradient is measured indirectly by two pairs of sensors (temperature and relative humidity) and is analysed by a microprocessor. The microprocessor analyses the values and expresses the evaporation rate in g/m^2h. The probe was held flat on the skin with a constant but low pressure with the short end to the skin and the measurement started by pressing the button on the handle of the probe. The measurement time of 15 seconds was counted backwards. After this time the TEWL-Index-value (1-20) was displayed, together with the interpretation (Dal'Belo *et al*., 2006; Campos *et al*., 2008; Mahler *et al*., 2009; Berardesca *et al*., 2018; Khosrowpour *et al*., 2018;).

3.8.4 Anthropometric indicator analysis

Body composition and bioelectrical impedance was measured using a hand-to-foot multifrequency tetrapolar device (BodyStat 1500 MD; BodyStat, Isle of Man, United Kingdom), adhering to standard operating procedures with the subject's sex, age, height, and weight entered into the device. This enables fat-free mass (FFM) to be directly calculated from the internal algorithm (the default equation being Houtkooper *et al*., 1985 - Fat-Free Mass (FFM) = a * HEIGHT2 + b * WEIGHT + c * AGE + d * R(resistance) + e. Variables a, b, c, d, and e represent constant coefficients calculated by regression analysis in each instance) (Kyle *et al*., 2004; Cleary *et al.,* 2008; Sánchez-Rodríguez *et al*., 2014; Maddocks *et al*., 2015).

The Bodystat 1500 analyser includes among its functions a range of normality adjusted for age and sex in geriatric population: fat-free mass (FFM) (kg) ¼ (0.360 x $l0^4$ x H^2 /R) + 0.359BW + 4.5S - 20T + 7.0 where; H is height (m), R is resistance (Ω), BW is body weight (kg), S is sex (females = 0; males = 1), and T is thigh circumference (m) (Deurenberg *et al*., 1990; Sánchez-Rodríguez *et al*., 2014).

Bioelectrical impedance measures the resistance and conductance of a mild electrical current, ranging from 5 to 500 kHz, delivered through the participant's body via electrodes placed on the right hand and foot (Visscher *et al.*, 2001; Rochette, 2004; Rinninella *et al*., 2018).

Lean and fat body mass were measured by bio-impedance (Bodystat 1500, Bodystat Ltd, Isle of Man, British Isles) in kilograms, and expressed as normal, low or high values, according to normal values for the population by age, sex, height and weight (Deurenberg *et al*., 1990; Sánchez-Rodríguez *et al*., 2014).

To assess each participant's level of hydration, the Multiscan 5000 multi-frequency bioelectrical impedance instrument (Bodystat Ltd, Isle of Man, UK) was used to measure total body water (TBW) and the distribution of extracellular water (ECW) and intracellular water (ICW). The 5 kHz signal has been found to accurately assess ECW whereas the 100 kHz signal is adequate for assessing TBW (Deurenberg *et al.,* 1993; Deurenberg *et al.,* 1995). Multi-frequency bioelectrical impedance analysis, which ranges from low to high frequencies, has been found to accurately track changes in ICW, ECW, and TBW (Rochette, 2004; Bahadori *et al.,* 2005). TBW, ECW, ICW are expressed in liters of water (Shanholtzer & Patterson, 2002). Body mass index (BMI) was also calculated using the Bodystats ® 1500 Body Composition Monitor unit, as all the measurements were entered into the device, i.e. weight and height (Schutz *et al*., 2002; Rider *et al.,* 2009; Salaun & Berthouze-Aranda, 2011; Darokar *et al.,* 2015; Andreoli *et al.,* 2016; Kammar-García *et al.,* 2018).

Hip and waist circumference measurements were obtained at a) the level of the umbilicus, and b) the level of the greater trochanter of the femur, to determine the hip-to-waist ratio (Visscher *et al.*, 2001; Rider *et al*., 2009).

Skinfold test was administered on the upper left arm muscle, the triceps, using a skinfold test calliper to determine the percentage of subcutaneous adipose tissue (Clasey *et al.*, 1999).

Blood pressure was taken with a sphygmomanometer by a registered qualified nurse. Blood pressure measurements are a noninvasive method for collecting real-time data that provide insight into the physiologic function and status of individuals and can be collected in virtually every physical therapy practice setting. Blood pressure measurements provide data essential for making crucial clinical decisions (Agabiti-Rosei *et al.,* 2007; Morris, 2018).

The heart rate (HR) of each participant was measured together with their blood pressure (BP) by a registered qualified nurse before the blood samples were collected. HR is the speed of the heartbeat measured by the number of contractions (beats) of the heart per minute (bpm). The heart rate can vary according to the

body's physical needs, including the need to absorb oxygen and excrete carbon dioxide. It is usually equal or close to the pulse measured at any peripheral point. Activities that can provoke change include physical exercise, sleep, anxiety, stress, illness, and the ingestion of drugs. The normal resting adult human heart rate is 60–100 bpm (Fox *et al*., 2007).

3.9 Statistical analysis

Differences between group means were estimated using the one-way analysis of variance (ANOVA). ANOVA is a hypothesis testing procedure used to determine if mean differences exist for two or more samples or treatments (Burns & Burns, 2008). The data were expressed as means ± standard deviation (SD), and results were considered significantly different at $P<0.05$.

CHAPTER FOUR

RESULTS

Data was collected for the anti-oxidant status of blood and skin parameters using the Multi Skin Test Centre®, and for anthropometric indicators using Bodystats® 1500 Body Composition Monitor unit (Appendix E). All the results were analysed by a professional and experienced statistician and are displayed in tables and graphs. The n-value for the implant contraceptive group was 21, all other groups n-value was 20.

4.1 Oxidative stress parameters

Table 4.1 shows the oxidative stress parameters for each of the four contraceptive groups compared to the control group of non-contraceptive users. The only significant difference ($p<0.05$) for SOD was evident between the monophasic contraceptive group and the triphasic contraceptive group. There was also a significant difference revealed by TBARS analysis of the blood samples between the control group, the monophasic contraceptive group and the injectable contraceptive group as compared to the triphasic contraceptive group. No other significant differences were found between any other groups for all the other oxidative stress parameters measured.

Table 4.1: Comparison of the oxidative stress parameters for non-contraceptive and contraceptive user groups.

Groups	Catalase	FRAP	GSH	GSSG	GSH/GSSG	ORAC	SOD	TBARS
Non contraceptive (n=20)	8.17 ± 1.92	389.67 ± 61.43	988.53 ± 162.25	17.88 ± 11.24	82.56 ± 61.92	1872.10 ± 576.81	46.79 ± 19.12	0.29 ± 0.02*
Monophasic contraceptive (n=20)	8.24 ± 1.93	412.50 ± 57.77	1086.86 ± 281.75	17.22 ± 9.37	88.14 ± 58.53	2156.71 ± 576.81	36.55 ± 17.10*	0.30 ± 0.01*
Injectable contraceptive (n=20)	6.99 ± 2.46	424.50 ± 96.38	1007.14 ± 169.99	12.58 ± 9.20	110.67 ± 60.23	2019.47 ± 662.46	50.23 ± 22.93	0.29 ± 0.01*
Implant contraceptive (n=21)	7.53 ± 2.03	431.84 ± 83.19	1088.72 ± 177.83	13.15 ± 5.51	101.16 ± 59.22	2031.08 ± 506.18	52.27 ± 25.66	0.32 ± 0.07
Triphasic contraceptive (n=20)	6.96 ± 2.25	372.36 ± 62.71	1047.79 ± 213.86	13.56 ± 3.60	80.52 ± 19.27	1900.73 ± 298.77	61.56 ± 16.44	0.34 ± 0.06

Data are presented as means ± SD. * significantly different when compared to the triphasic contraceptive group ($p<0.05$).

Figures 4.1.1-4.1.6 graphically indicate the activities of CAT, FRAP, GSH/GSSG, ORAC, SOD and TBARS in the blood of participants for the non-contraceptive and contraceptive user groups. No significant differences were evident for catalase when all the contraceptive groups (monophasic, injectable, implant and triphasic) were compared to the control group and each other (Figure 4.1.1). No significant differences ($p>0.05$) were evident for FRAP and GSH/GSSH for any of the contraceptive groups when they were compared to each other and to the non-contraceptive group (Figures 4.1.2 and 4.1.3). No significant differences were evident for ORAC when all the contraceptive groups (monophasic, injectable, implant and triphasic) and the control group were compared with each other (Figure 4.1.4). However, there was a significant increase in the SOD activities of the triphasic contraceptive group when compared to the monophasic contraceptive group (Figure 4.1.5). No significant ($p>0.05$) increase or decrease in SOD activities was observed for all the other contraceptive groups when they were compared to each other and to the control group. There was a significant difference in TBARS for the triphasic contraceptive group when compared to the control group, monophasic contraceptive group and injectable contraceptive group, as shown in Figure 4.1.6.

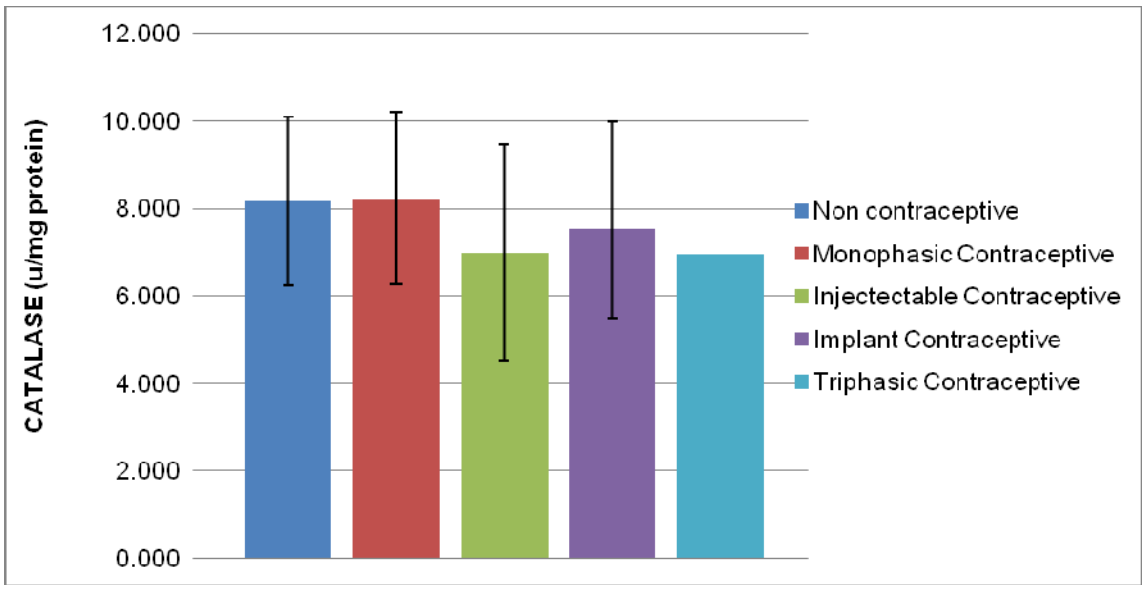

Figure 4.1.1: Catalase levels (u/mg protein) in the blood.
Data are presented as means ± SD

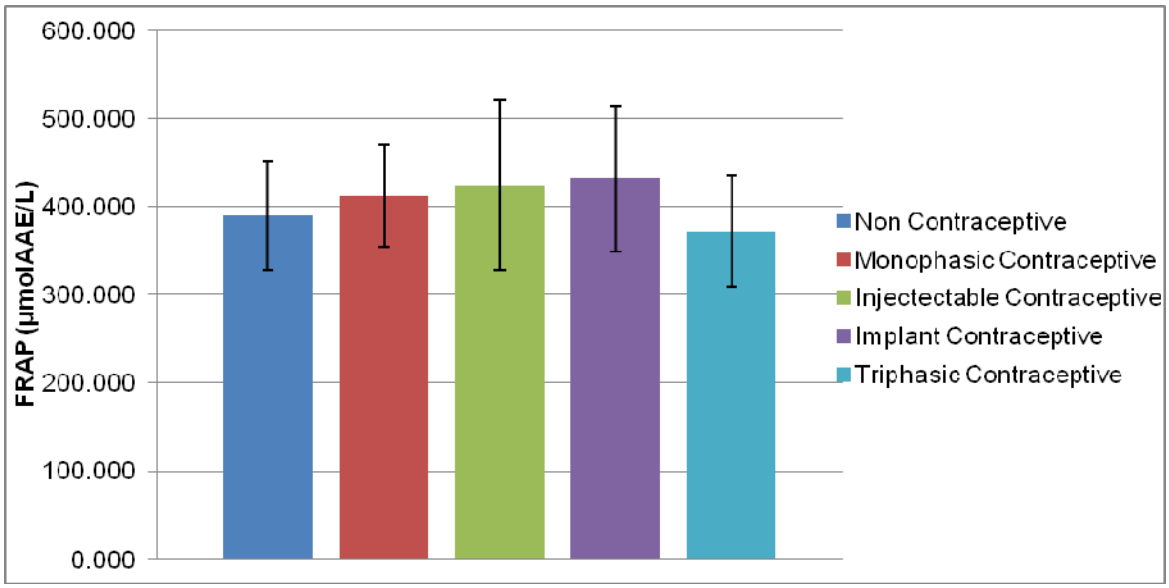

Figure 4.1.2: Index of antioxidant potential by ferric reducing ability of plasma (µmolAAE/L).

Data are presented as means ± SD.

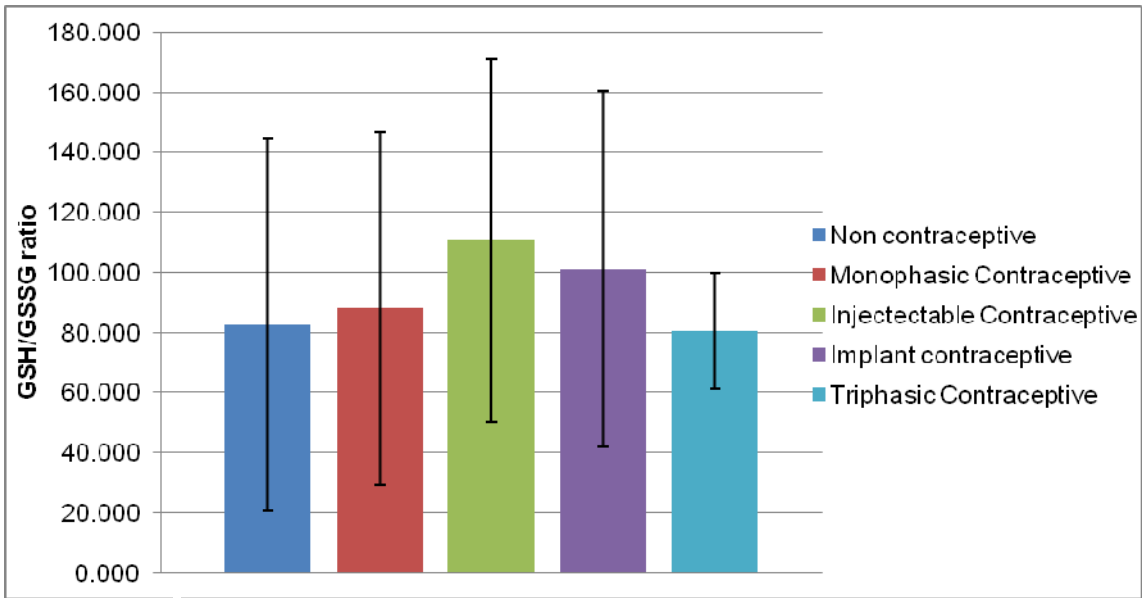

Figure 4.1.3: Ratio of reduced glutathione (GSH) and oxidised glutathione (GSSG) levels in the blood.

Data are presented as means ± SD

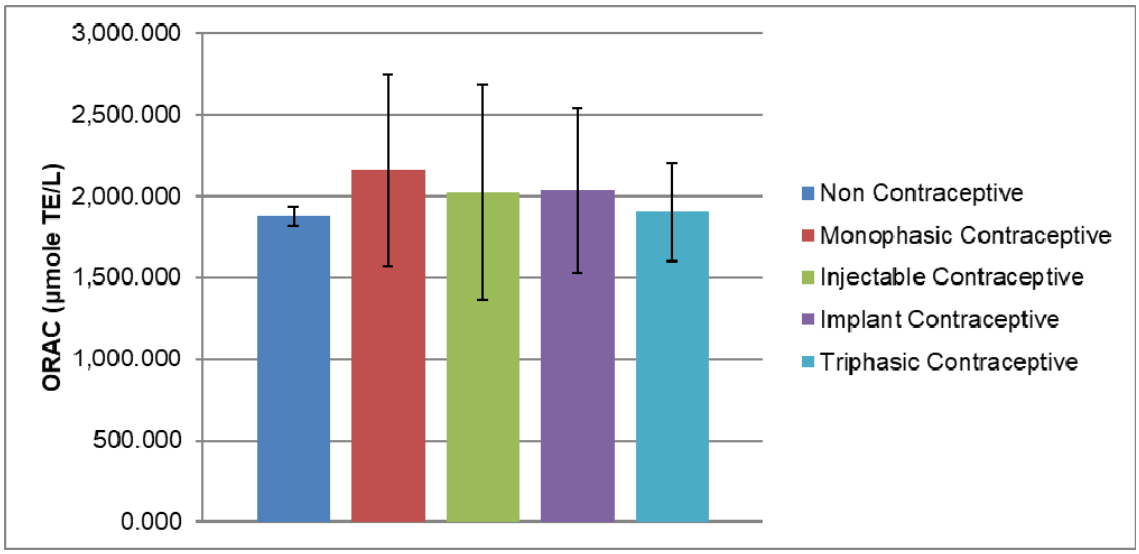

Figure 4.1.4: Total antioxidant capacities (µmole TE/L) in the blood.

Data are presented as means ± SD.

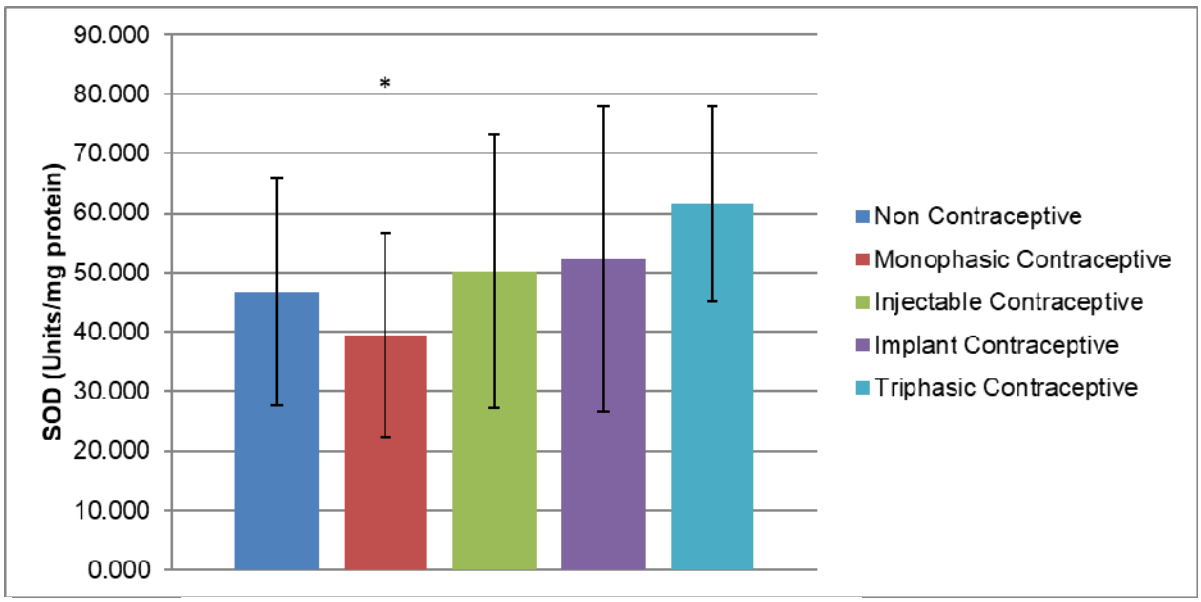

Figure 4.1.5: Plasma superoxide dismutase (SOD, units/mg protein) levels in the blood.

Data are presented as means ± SD.

*Significant difference when compared to the triphasic contraceptive group ($p<0.05$).

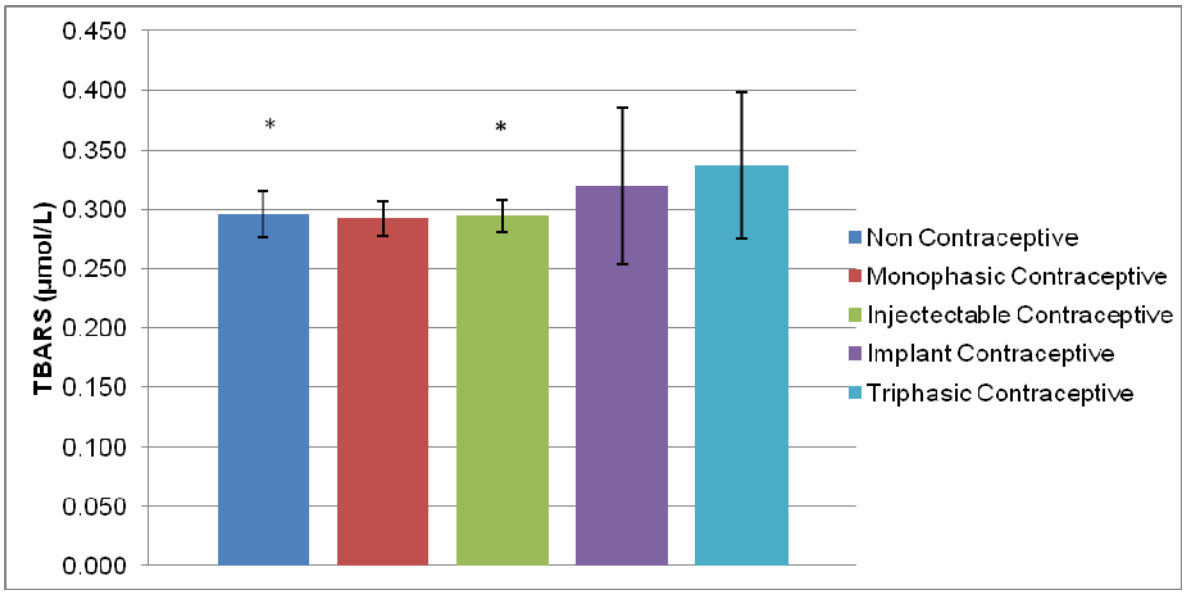

Figure 4.1.6: Lipid peroxidation levels (µmol/L) in the blood.

Data are presented as means ± SD

*Significant difference when compared to the triphasic contraceptive group ($p<0.05$).

4.2 Skin parameters

Table 4.2 shows the facial parameters for the four different contraceptive groups and the control (non-contraceptive) group. According to the results of the erythema measurements for each group, there were significant differences ($p<0.05$) evident between the monophasic contraceptive group and the control group; the monophasic contraceptive group and the injectable contraceptive group; the monophasic contraceptive group and the implant contraceptive group, and the monophasic contraceptive group and the triphasic contraceptive group.

The average hydration measurements indicated a significant difference ($p<0.05$) between the control and injectable contraceptive groups; the control and implant contraceptive groups; the monophasic contraceptive and the injectable contraceptive groups, as well as the monophasic contraceptive and the implant contraceptive groups.

The results of the melanin/hyperpigmentation measurements on the forehead, cheeks and chin showed significant ($p<0.05$) differences between the monophasic contraceptive group and the control group; the monophasic contraceptive group and the injectable contraceptive group; the monophasic contraceptive group and the implant contraceptive group and the monophasic contraceptive group and the triphasic contraceptive group. No significant differences were evident for elasticity, sebum and TEWL between groups.

Table 4.2: Comparison of the skin parameters for non-contraceptive and contraceptive groups

Groups	Elasticity	Erythema	Skin Hydration	Melanin on forehead	Melanin on cheek	Melanin on chin	Sebum	TEWL
Non contraceptive (n=20)	81.32 ± 5.91	43.43 ± 4.62[b]	33.61 ± 3.78[cd]	54.10 ± 23.60[b]	46.35 ± 21.70[b]	64.30 ± 25.48[b]	32.12 ± 16.57	6.73 ± 1.58
Monophasic contraceptive (n=20)	77.87 ± 7.56	37.32 ± 6.05 [acde]	33.12 ± 3.26[cd]	27.80 ± 22.29[acde]	23.45 ± 15.62[acde]	33.10 ± 20.91[acde]	24.97 ± 10.82	6.73 ± 1.95
Injectable contraceptive (n=20)	80.07 ± 5.99	47.87 ± 4.73[b]	37.83 ± 5.06[ab]	64.70 ± 23.62[b]	57.90 ± 21.47[b]	75.35 ± 21.26[b]	33.70 ± 14.53	6.55 ± 1.86
Implant contraceptive (n=21)	81.49 ± 9.07	46.19 ± 6.17[b]	39.17 ± 5.02[ab]	60.14 ± 25.83[b]	54.43 ± 25.75[b]	72.33 ± 27.73[b]	28.37 ± 12.18	6.67 ± 2.95
Triphasic contraceptive (n=20)	80.40 ± 6.80	44.82 ± 4.48[b]	35.74 ± 4.29	60.15 ± 27.87[b]	57.40 ± 25.97[b]	68.35 ± 24.74[b]	27.48 ± 13.09	7.07 ± 2.18

Data are presented as means ± SD

[a] significant difference when compared to the control (non-contraceptive) group ($p<0.05$).

[b] significant difference when compared to the monophasic contraceptive group ($p<0.05$).

[c] significant difference when compared to the injectable contraceptive group ($p<0.05$).

[d] significant difference when compared to the implant contraceptive group ($p<0.05$).

[e] significant difference when compared to the triphasic contraceptive group ($p<0.05$).

Figures 4.2.1-4.2.6 display the results of the facial parameters measurements, which include elasticity, erythema, hydration, melanin/hyperpigmentation, sebum and transepidermal water loss (TEWL). There was a significant ($p<0.05$) increase in the presence of erythema in the monophasic contraceptive group in comparison to the control group, the injectable contraceptive group, the implant contraceptive group and triphasic contraceptive group (Figure 4.2.2).

The hydration measurement (Figure 4.2.3) was significantly higher ($p<0.05$) in the implant contraceptive group than in the control and monophasic contraceptive groups. Furthermore, a significantly ($p>0.05$) higher level of hydration was evident in the injectable contraceptive group as compared to the control and monophasic contraceptive groups.

Melanocyte activity measured in the forehead, cheek and chin areas (Figures 4.2.4.1, 4.2.4.2 and 4.2.4.3 respectively) were also significantly ($p<0.05$) higher in the monophasic contraceptive group than in the control group, the injectable contraceptive group, the implant contraceptive group and the triphasic contraceptive group. All other facial parameters measurements showed no significant differences ($p>0.05$) when the contraceptive groups (monophasic, injectable, implant and triphasic) were compared to each other and to the control or non-contraceptive group.

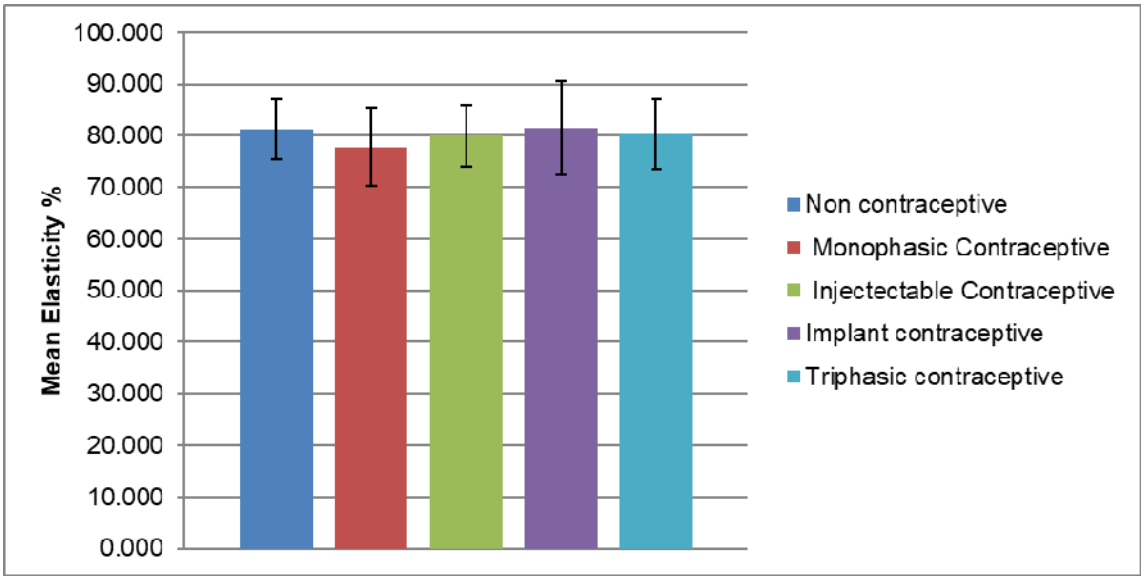

Figure 4.2.1: Average percentage of skin elasticity present on the facial area.
Data are presented as means ± SD

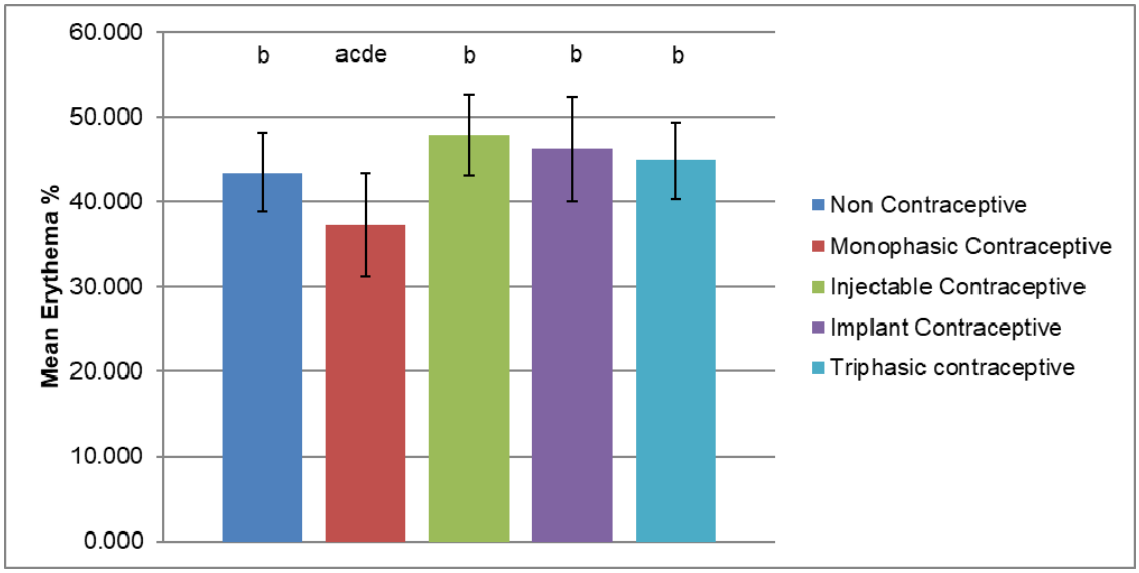

Figure 4.2.2: Average percentage erythema presented on the facial area.

Data are presented as means ± SD

[a] significant difference when compared to the control (non-contraceptive) group ($p<0.05$).
[b] significant difference when compared to the monophasic contraceptive group ($p<0.05$).
[c] significant difference when compared to the injectable contraceptive group ($p<0.05$).
[d] significant difference when compared to the implant contraceptive group ($p<0.05$).
[e] significant difference when compared to the triphasic contraceptive group ($p<0.05$).

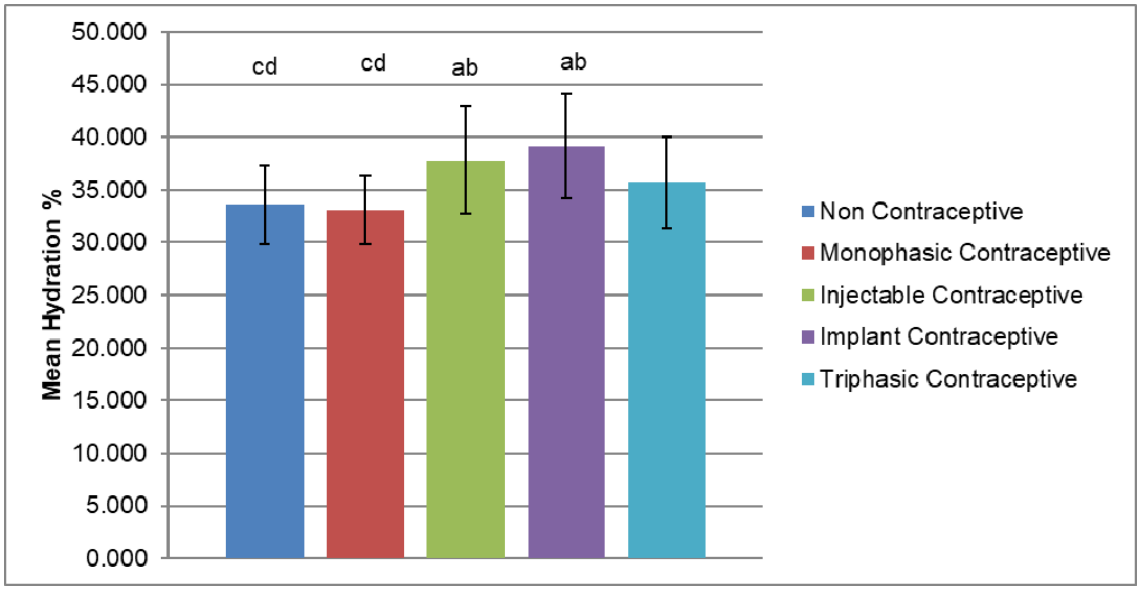

Figure 4.2.3: Average percentage hydration presented on the facial area.

Data are presented as means ± SD.

[a] significant difference when compared to the control (non-contraceptive) group ($p<0.05$).
[b] significant difference when compared to the monophasic contraceptive group ($p<0.05$).
[c] significant difference when compared to the injectable contraceptive group ($p<0.05$).
[d] significant difference when compared to the implant contraceptive group ($p<0.05$).

Pigmentation was measured in 3 areas: forehead, right cheek and chin (a good indicator for chloasma), in order to determine the pigmentation caused by hormonal influences in non-pregnant women.

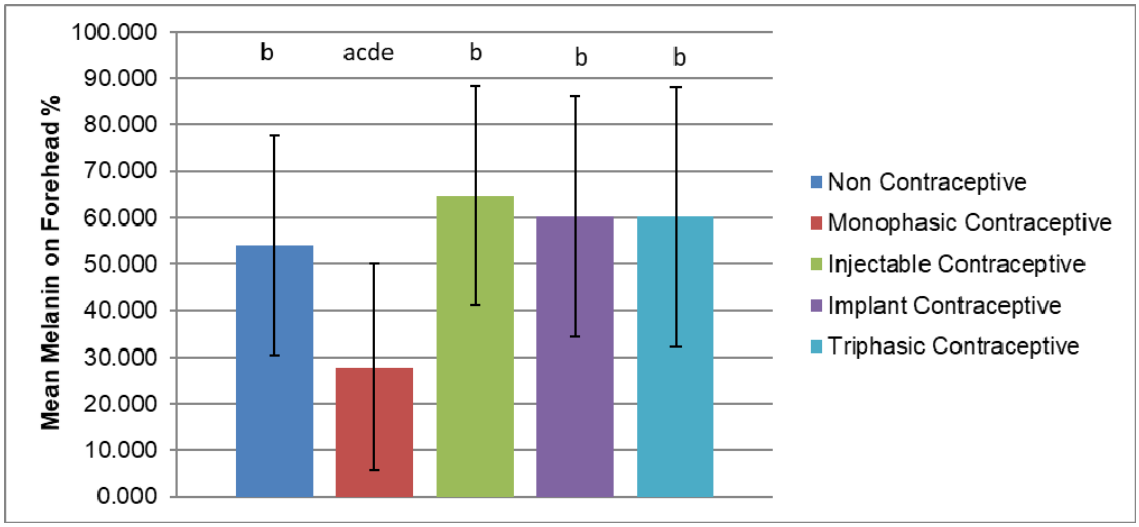

Figure 4.2.4.1: Percentage melanin/hyperpigmentation presented on the forehead area.

Data are presented as means ± SD.

[a] significant difference when compared to the control (non- contraceptive) group ($p<0.05$).
[b] significant difference when compared to the monophasic contraceptive group ($p<0.05$).
[c] significant difference when compared to the injectable contraceptive group ($p<0.05$).
[d] significant difference when compared to the implant contraceptive group ($p<0.05$).
[e] significant difference when compared to the triphasic contraceptive group ($p<0.05$).

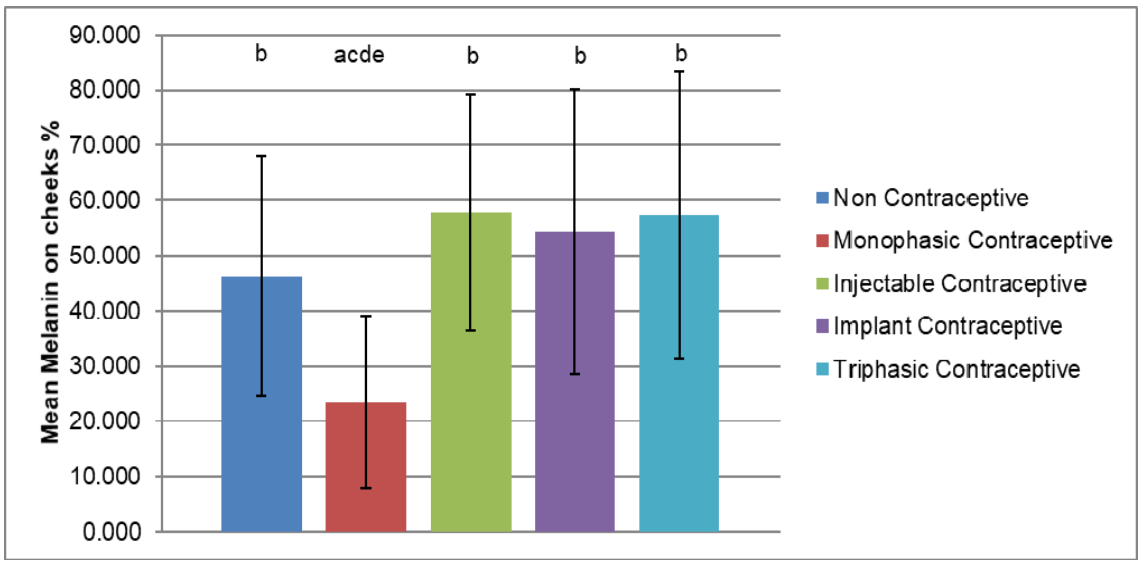

Figure 4.2.4.2: Percentage melanin/hyperpigmentation presented on the cheek area.

Data are presented as means ± SD.

[a] significant difference when compared to the control (non-contraceptive) group ($p<0.05$).
[b] significant difference when compared to the monophasic contraceptive group ($p<0.05$).
[c] significant difference when compared to the injectable contraceptive group ($p<0.05$).
[d] significant difference when compared to the implant contraceptive group ($p<0.05$).
[e] significant difference when compared to the triphasic contraceptive group ($p<0.05$).

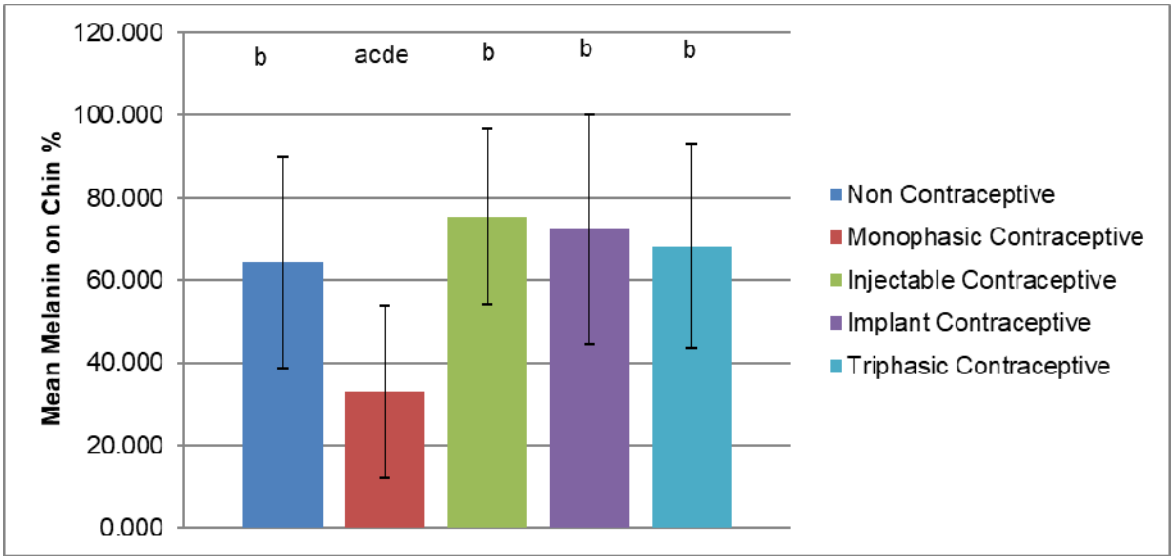

Figure 4.2.4.3: Percentage melanin/hyperpigmentation presented on the chin area.

Data are presented as means ± SD.

[a] significant difference when compared to the control (non- contraceptive) group ($p<0.05$).
[b] significant difference when compared to the monophasic contraceptive group ($p<0.05$).
[c] significant difference when compared to the injectable contraceptive group ($p<0.05$).
[d] significant difference when compared to the implant contraceptive group ($p<0.05$).
[e] significant difference when compared to the triphasic contraceptive group ($p<0.05$).

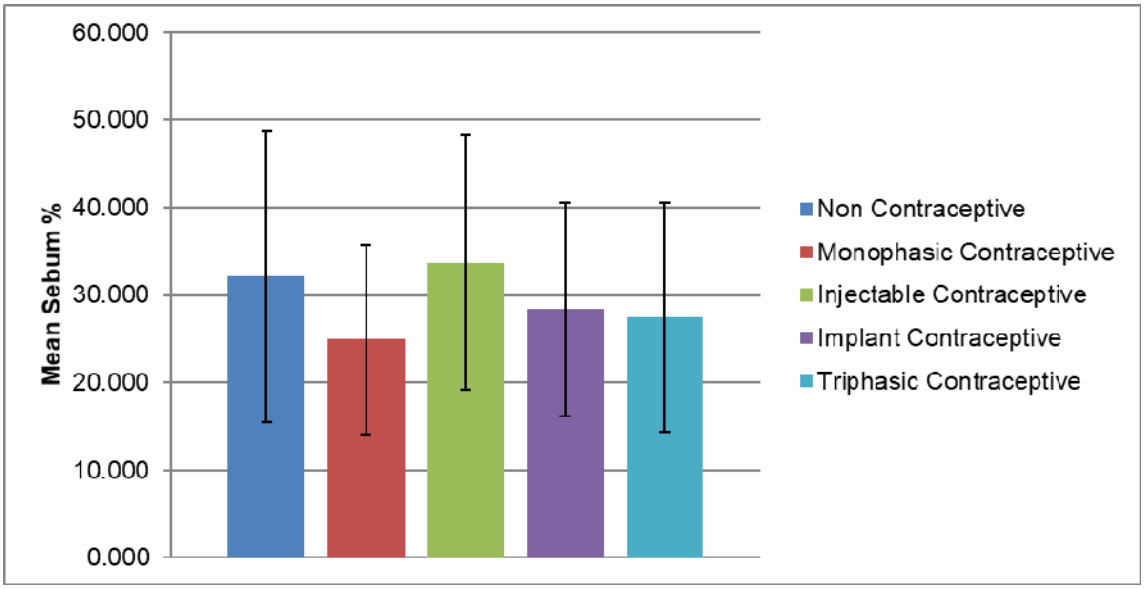

Figure 4.2.5: Average percentage sebum present on the facial area.

Data are presented as means ± SD.

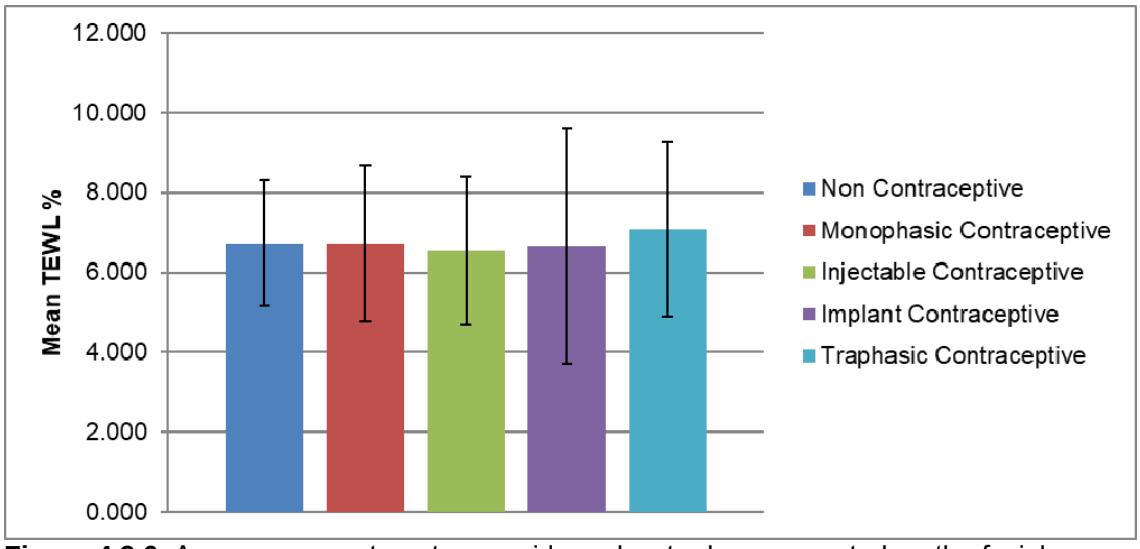

Figure 4.2.6: Average percentage trans-epidermal water loss presented on the facial area.

Data are presented as means ± SD.

4.3 Anthropometric indicators

Table 4.3 indicates the anthropometric parameters, comparing these for the four different contraceptive groups and the control or non-contraceptive group. The only significant ($p>0.05$) difference was the discrepancy in the waist to hip ratio between the control group and the implant contraceptive group. No other significant differences were found in any of the anthropometric measurements.

Table 4.3: Comparison of anthropometric indicators for non-contraceptive and contraceptive groups

Groups	Adipose tissue	Systolic blood pressure (BP)	Diastolic BP	BMI	Heart rate	Lean tissue	Skin fold	Waist to hip ratio	Water content	Average weight
Non contraceptive (n=20)	33.21± 6.34	125.05 ± 13.63	80.65 ± 7.75	24.15 ± 5.59	81.10 ± 13.70	65.13 ± 9.72	20.35 ± 8.50	0.71 ± 0.05	46.20 ± 7.10	60.53 ± 13.65
Monophasic contraceptive (n=20)	32.34 ± 6.54	128.20 ± 17.39	89.50 ± 16.63	24.11± 5.21	80.00 ± 9.13	67.63 ± 6.55	22.15± 10.95	0.72 ± 0.05	47.63± 5.25	60.51 ± 13.42
Injectable contraceptive (n=20)	36.86 ± 9.40	132.75 ± 15.53	84.45 ± 10.23	27.73 ± 7.03	86.65 ± 14.93	63.11± 9.40	26.45 ± 9.80	0.75 ± 0.07	44.27 ± 6.97	69.30 ± 17.08
Implant contraceptive (n=21)	35.29± 11.60	131.05 ± 14.33	85.43 ± 9.61	28.71± 6.98	83.76 ± 15.70	64.71 ± 11.61	27.48 ± 11.94	0.77 ± 0.08*	45.62± 9.21	73.60 ± 21.76
Triphasic contraceptive (n=20)	35.42 ± 8.80	126.00 ± 9.89	84.50 ± 9.43	27.58 ± 7.86	85.75 ± 14.66	64.57 ± 8.79	25.65 ± 11.20	0.74 ± 0.07	45.57 ± 7.02	69.80 ± 21.34

Data are presented as means ± SD.

* significant difference when compared to the control (non-contraceptive) group ($p<0.05$).

Figures 4.3.1-4.3.10 graphically represent the results of each anthropometric parameter including adipose tissue, blood pressure, body mass index (BMI), heart rate, lean tissue, skin fold measurement (sub cutaneous adipose tissue), waist to hip ratio, water content/hydration and weight. The mean waist to hip ratio in the implant contraceptive group was significantly higher ($p>0.05$) than that of the control group (Figure 4.3.8). All other anthropometric parameters showed no significant ($p>0.05$) difference when the various contraceptive groups (monophasic, injectable, implant and triphasic) and the control group were compared.

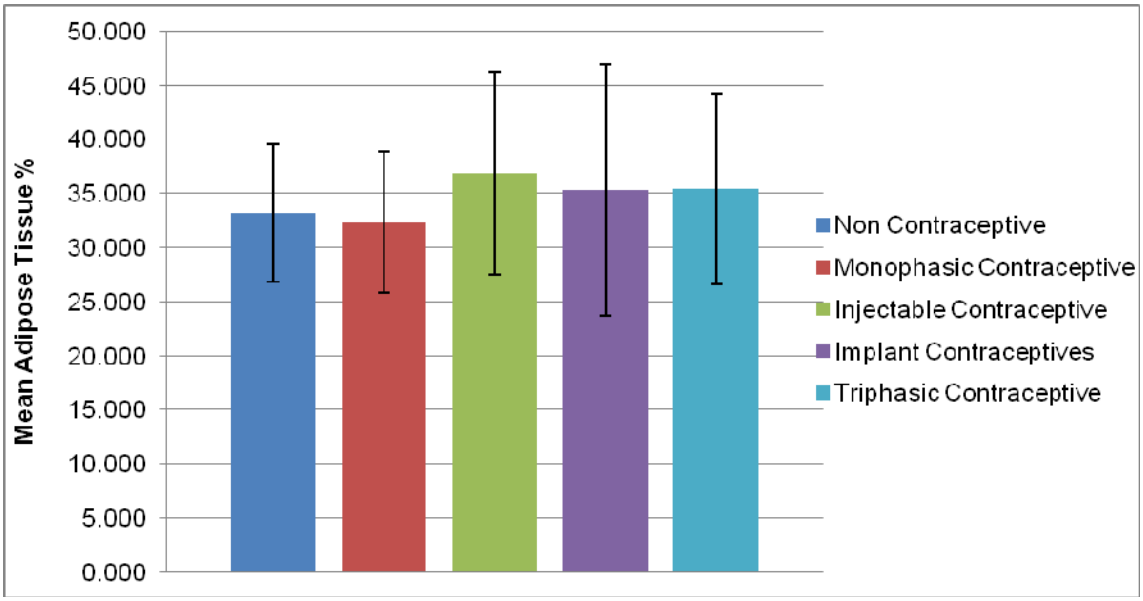

Figure 4.3.1: Average percentage adipose tissue per group.
Data are presented as means ± SD.

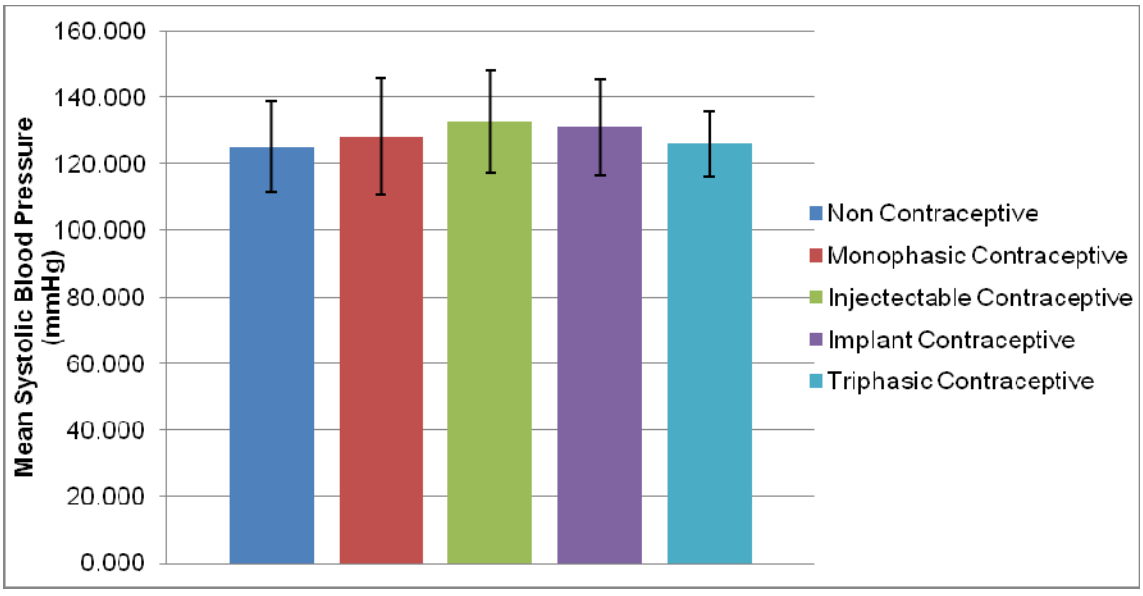

Figure 4.3.2: Average systolic blood pressure (mmHg) measurement per group.
Data are presented as means ± SD.

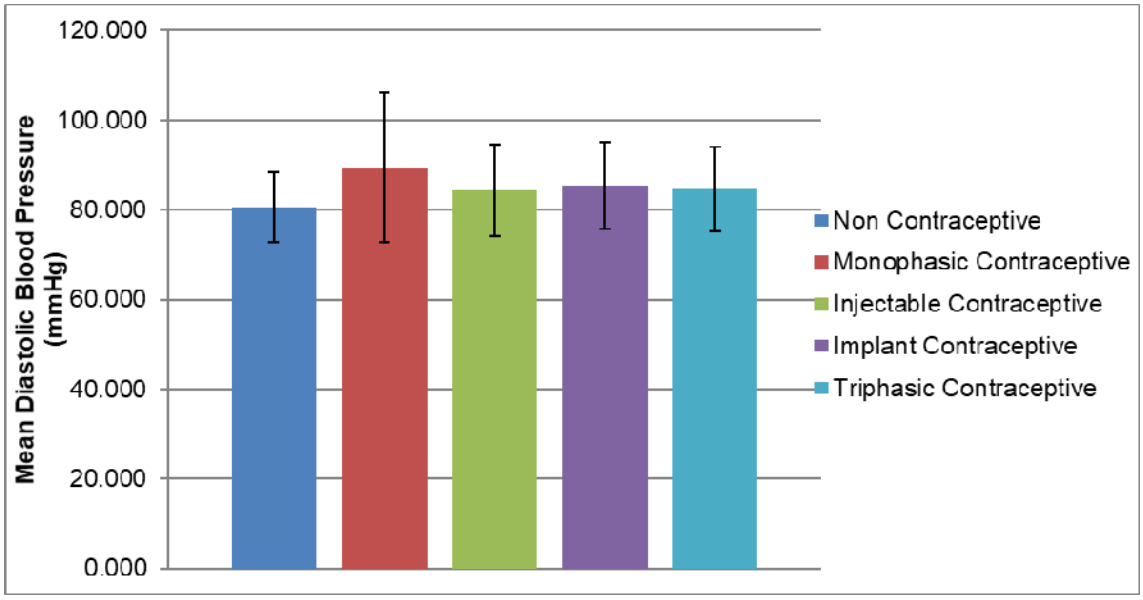

Figure 4.3.3: Average diastolic blood pressure (mmHG) measurement per group.

Data are presented as means ± SD

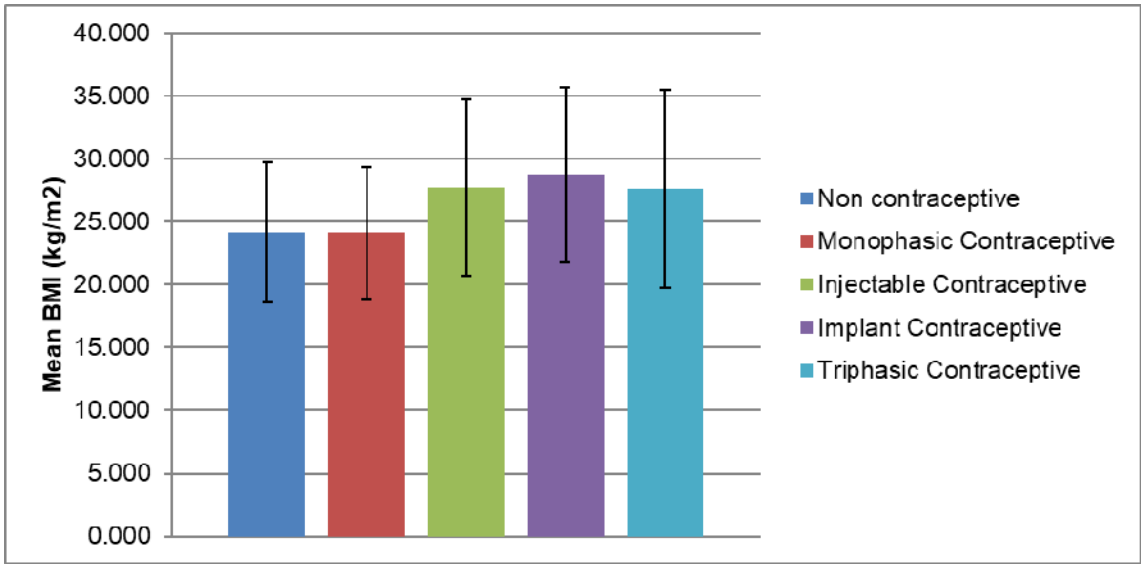

Figure 4.3.4: BMI values (kg/m^2) per group.

Data are presented as means ± SD.

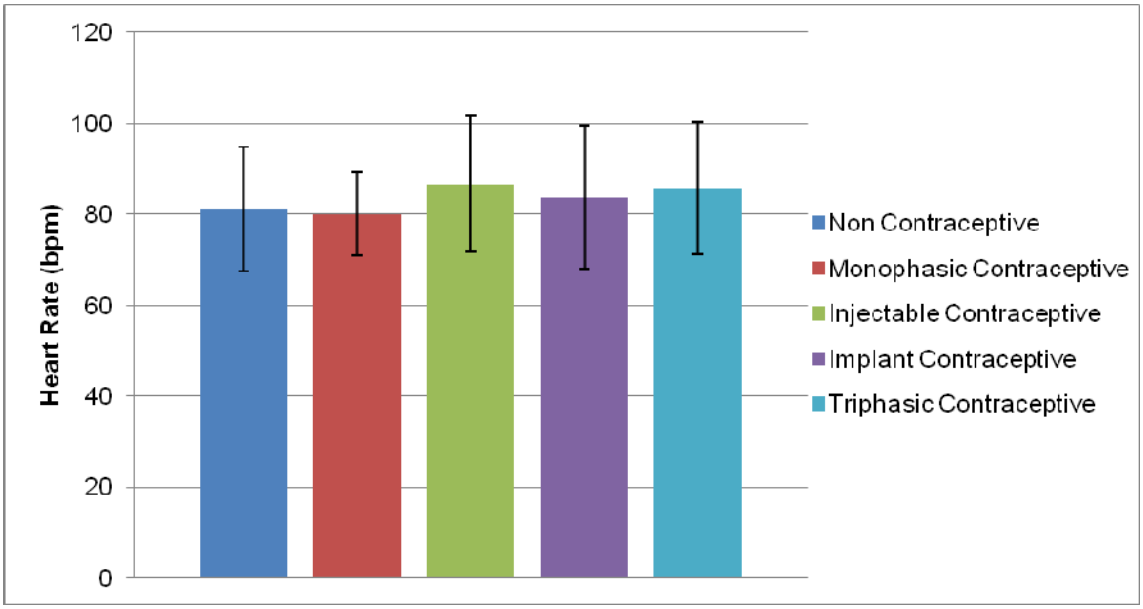

Figure 4.3.5: Average heart rate (bpm) per group.

Data are presented as means ± SD.

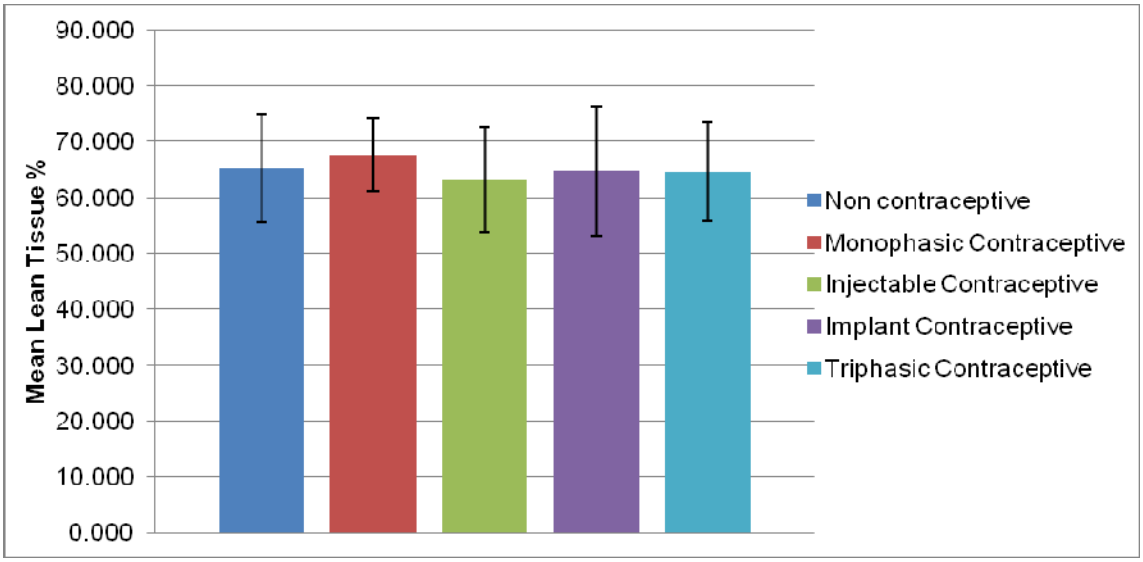

Figure 4.3.6: Average percentage lean tissue per group.

Data are presented as means ± SD.

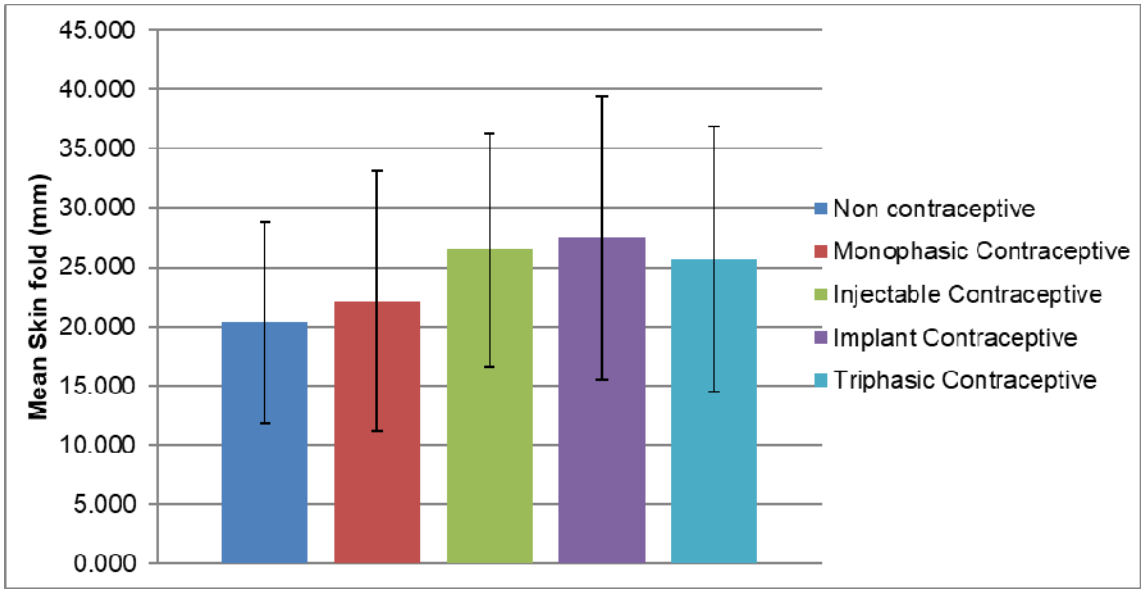

Figure 4.3.7: Average skin fold (mm) measurement.

Data are presented as means ± SD.

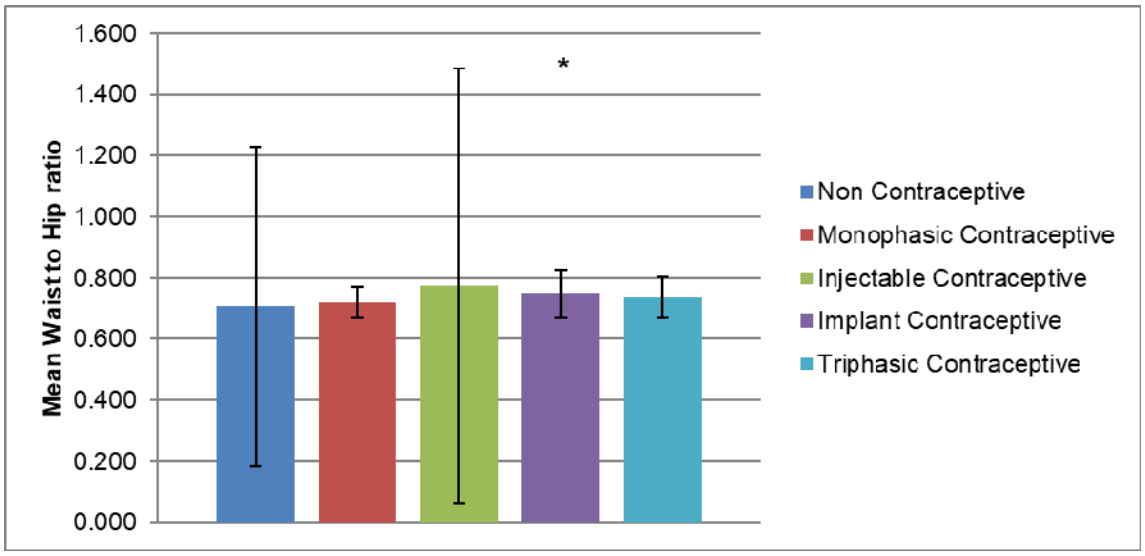

Figure 4.3.8: Average waist to hip ratio per group.

Data are presented as means ± SD

* Significant difference when compared to the control (non-contraceptive) group ($p<0.05$).

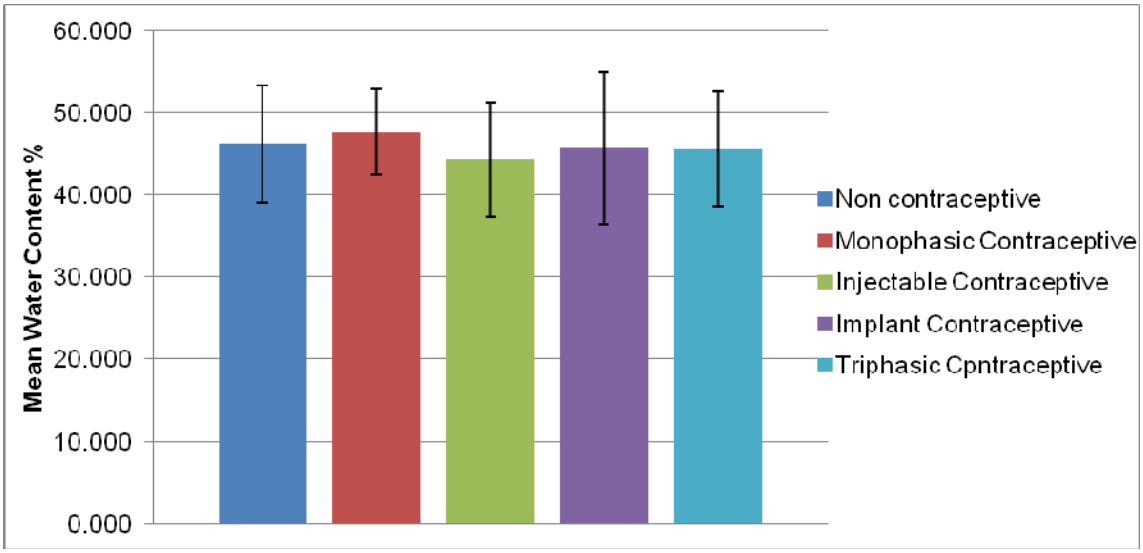

Figure 4.3.9: Average percentage hydration levels in the body per group.

Data are presented as means ± SD

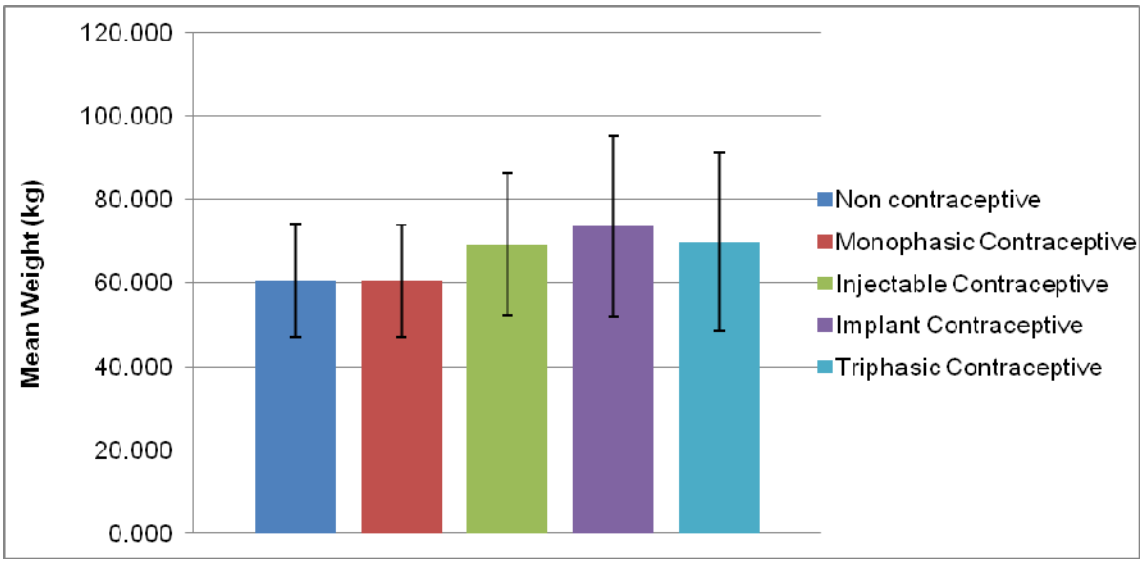

Figure 4.3.10: Average weight (kg) of participants per group.

Data are presented as means ± SD

CHAPTER FIVE

DISCUSSION

With high rates of unintended pregnancy worldwide, there is a need for improvement of hormonal contraceptive acceptability, compliance and continuation (Sabatini *et al.,* 2011; Adeniyi *et al.*, 2018). Alternative delivery systems have recently been introduced to improve tolerability, continuance and convenience in the use of contraceptives. These include new progestins that decrease androgenic side-effects (Shulman, 2011).

Currently, pharmacological methods of contraception consist of reversible contraceptive steroids formulated in pills, patches, intravaginal rings, subdermal implants and injections. Despite the safety profile of current combined oral contraceptives, fears of adverse metabolic and vascular effects caused by the oestrogen component, and of the possible neoplastic effects of these formulations remain. Misperceptions and concerns about side-effects, especially those affecting the menstrual cycle and body weight, are often cited, and although these disorders are not clinically significant they can lead to erratic method use or even discontinuation (Reubinoff *et al*., 1995; Rivera *et al*., 1999; Borgelt-Hansen, 2001; Burkman *et al*., 2004; Lech & Ostrowska, 2005; Stevenson & Thornton, 2007; Shufelt & Merz, 2009; Sabatini *et al*., 2011; Shulman, 2011; Dahan-Farkas & Irhuma, 2016).

Because of the popularity of contraceptive use, it is important to evaluate and compare the possible side effects of each hormonal contraceptive on antioxidant status, skin and anthropometric parameters, as such information could contribute to informed decision making among potential users.

5.1 Oxidative stress parameters

Since the discovery that oral progestational 19-nor steroids could inhibit ovulation (Pincemail *et al*., 2007), millions of women have used various types of synthetic oestrogens and progestins to prevent conception. Data is scarce on the relationship between the use of contraceptives and oxidative stress, and the topic remains a subject for debate. It has been suggested, but not generally admitted, that oestrogens have an antioxidant effect that may contribute to protective effects on the cardiovascular system through inhibition of lipid oxidation (Ling *et al*., 2006; Pincemail *et al*., 2007; Adejumo *et al*., 2015). Studies conducted *in vitro* have shown that oestrogens, and more particularly estradiol, were able to reduce significantly the

oxidative damage to lipids exposed to several free radical-generating systems (Sugioka *et al*., 1987; Hwang *et al*., 2000; Saha *et al.,* 2000; Pincemail *et al*., 2007; Adejumo *et al*., 2015).

In a study conducted by Chen and Kotani (2012), it was demonstrated that the use of oral contraceptive therapy among pre-menopausal women, most especially the triphasic preparations, resulted in significantly higher reactive oxygen metabolite levels than those present in non-contraceptive users (Chen & Kotani, 2012 and 2018). In a study that involved female athletes, oxidative stress in relation to combined oral contraceptive use and lifestyle habits was investigated (Cauci *et al*., 2016). The study found that elevated oxidative stress levels were evident and varied considerably according to the oral contraceptive used (Chen & Kotani, 2012 and 2018; Adejumo *et al*., 2015; Cauci *et al*., 2016).

A study by Adejumo *et al.* (2015) revealed a significant decrease in the serum levels of total antioxidant status among hormonal contraceptive users. This suggests that women taking hormonal contraceptives are at higher risk of oxidative stress-related diseases. Thibodeau *et al*. (2002) argue that this divergence in reports could be attributed to the chemical heterogeneity of the oestrogen family and the varying concentrations of the hormone in the contraceptives (Adejumo *et al*., 2015). In the present study, there was evidence of increased oxidative status among participants using triphasic contraceptives when they were compared to those using other hormonal contraceptives.

5.1.1 Catalase activity

Catalase (CAT) is a common enzyme found in nearly all living organisms that are exposed to oxygen. It functions to catalyse the decomposition of hydrogen peroxide to water and oxygen. Hydrogen peroxide is a harmful by-product of many normal metabolic processes and must be quickly converted into other less dangerous substances to prevent damage, and catalase is frequently used by cells for this purpose (Türsen, 2016).

Studies conducted by Capel *et al*. (1981) and Massafra *et al.* (1993), found that a 9-cycle course of a combined oral contraceptive (ethinylestradiol 20 mg and desogestrel 150 mg) in young women led to significant increased activity of antioxidative enzymes, namely catalase and glutathione peroxidase (GPx) (Pincemail *et al.,* 2007). In the present study, there were no significant differences between the

contraceptive groups, although the highest level of CAT was evident in the monophasic contraceptive group.

The mechanism by which hormonal contraceptives alter antioxidant enzyme activity is not clearly understood (Massafra *et al*., 1993; Fallah *et al*., 2011). It has been shown that in women using low-dose oral contraceptives for a prolonged period there is a significant increase in GPx activity (six cycles), and later in CAT activity (nine cycles). It is therefore likely that even a small amount of the steroids in oral contraceptive pills could be converted into peroxides to a sufficient extent to induce synthesis of the new molecules of GPx enzymes (Massafra *et al*., 1993; Fallah *et al*., 2011). In the present study there was no evidence to support these findings.

5.1.2 Index of antioxidant potential using the ferric reducing ability

The index of antioxidant potential resulting from the ferric ion reducing ability of plasma (FRAP) is a colorimetric spectrophotometric assay used to assess the antioxidant power of biological fluids (Benzie & Strain, 1996).

In a study conducted by Swiegers (2015) there was an indication that, on average, the contraceptive groups in the study had a lower concentration of antioxidants in the blood than the control group (non-contraceptive group). The chronic high reactive oxidative stress (ROS) conditions found in the contraceptive groups suggest that these groups are subjected to systemic depletion of antioxidants and therefore indicate a lower antioxidant potential (Swiegers, 2015). This is consistent with the results reported by Finco *et al*. (2011; 2012). Both these studies focused on the oxidative stress caused by the use of combined oral contraceptives, and the fact that the users seemingly possess the necessary antioxidant potential to bind and clear ROS (Swiegers, 2015). There was no evidence supporting these findings in the present study. Even though the non-contraceptive group had low FRAP levels, the triphasic contraceptive group exhibited the lowest levels, which contradicts the findings described above.

5.1.3 Ratio of reduced glutathione and oxidised glutathione

Glutathione plays an important role in the detoxification of peroxide, hydrogen peroxide and other free radicals (Alinde *et al*., 2012). Therefore, the ratio between oxidised and reduced glutathione is important in evaluating levels of toxicity in the cells. According to Awoniyi *et al*. (2010), exposure of mammalian cells to increased oxidative stress leads to a decrease in the ratio of GSH/GSSG due to accumulation of GSSG or reduction in GSH levels.

In the present study, the lowest ratio of GSH/GSSG was found in participants using triphasic contraceptives and the highest ratio of GSH/GSSG in participants using injectable contraceptives. As injectable contraceptives only contain progestins, this may indicate that progestins have a more significant impact on GSH/GSSG ratios.

As previously mentioned, studies by Capel *et al*. (1981) and by Massafra *et al.* (1993) found that a 9-cycle course of a combined oral contraceptive (ethinylestradiol 20 mg and desogestrel 150 mg) in young women led to significant increased activity of antioxidative enzymes, namely catalase and glutathione peroxidase (GPx) (Pincemail *et al.,* 2007). This is supported by studies in which it was demonstrated that hormonal contraceptive intake resulted in a significant increase in GPx activity and a decrease (insignificant) in erythrocyte SOD activity (Massafra *et al*., 1993; Fallah *et al*., 2011). The mechanism by which hormonal contraceptives alter antioxidant enzyme activity is not clearly understood (Massafra *et al*., 1993; Fallah *et al*., 2011). It is suggested that hormonal contraceptives may induce GPx activity. It has been shown that in women using low-dose oral contraceptives for a prolonged period there is a significant increase in GPx activity (six cycles) and later in catalase (CAT) activity (nine cycles). Therefore, it is likely that even a small amount of the steroids in OCPs could be converted to peroxides in sufficient measure to induce synthesis of the new molecules of GPx enzymes (Massafra *et al*., 1993; Fallah *et al*., 2011).

5.1.4 Oxygen radical absorbance capacity

Oxygen radical absorbance capacity (ORAC) is a method of measuring antioxidant capacities in biological samples (Prior *et al.*, 2001). In this study, the highest levels of ORAC were found in the monophasic contraceptive group and lowest levels of ORAC in participants using no contraceptives.

Higher levels of ORAC indicate higher resistance to oxidation and free radical attack (Prior *et al.*, 2001). Adejumo *et al.* (2015) investigated the effect of hormonal contraceptives on the Total Antioxidants Status (TAS) of Women from Isolo, Lagos State, Nigeria. Their study revealed significantly lower levels of serum TAS in users of both oral and injectable hormonal contraceptives than in non-contraceptive users. Palan *et al.* (2010) also reported that hormonal contraceptives deplete antioxidant vitamins and trace elements, as measured by the activity of coenzyme Q10, vitamin E and total antioxidant activity. Akinloye *et al.* (2011) attribute a decrease in the serum levels of antioxidant trace elements more specifically to the increased use of oral contraceptives. There was no definitive evidence to support these findings in the current study.

5.1.5 Superoxide dismutase activity

Superoxide dismutases (SODs) are a class of closely related enzymes that catalyse the breakdown of the superoxide anion into oxygen and hydrogen peroxide. SOD is defined as the body's first line of antioxidant defence and characterised as a primary antioxidant. As an enzyme, SOD exhibits a very high catalytic rate of reaction and is constantly renewing itself (Türsen, 2016).

In the present study, the highest levels of plasma superoxide dismutase (SOD) were found in the triphasic contraceptive group and the lowest levels in the monophasic contraceptive group, with a significant difference ($p<0.05$) between the groups. As SOD plays a major role in the defence system against the cytotoxic effects of superoxide radicals (Caldwell *et al.*, 2008), this finding may indicate that monophasic contraceptives have a negative effect on the antioxidant status of the body.

A study conducted by Cauci *et al.* (2016) found that in oral contraceptive users, there was an inverse relationship between hydroperoxides and the total defence capacity against free oxygen radicals. However, the study could not assess whether oral contraceptives directly increased reactive oxygen species production that provoked the formation of hydroperoxides and consumed antioxidant defences, and/or whether oral contraceptive use directly reduced antioxidant defences, which became insufficient to neutralise free radicals, in turn provoking hydroperoxidation (Cauci *et al.,* 2016).

Some evidence suggests that oestrogens are inversely related to antioxidant defence; in particular, high oestrogen levels were correlated with decreased blood superoxide dismutase (SOD) levels (Joo *et al*., 2004; Cauci *et al.,* 2016). A study on female rats reported no relationship between administered oestrogen and SOD, but a positive relationship became evident with increased lipid peroxidation (Gómez-Zubeldia *et al*., 2001; Adejumo *et al.,* 2015; Cauci *et al.,* 2016).

The effects of oestrogens and progestin on oxidative stress are controversial. Oestrogens display an antioxidant capacity by stimulating the expression and activity of the manganese SOD (MnSOD) and extracellular SOD (ecSOD). This antioxidant activity is counteracted by progestins via the activation of the NADPH oxidase and the inhibition of the expression and activity of MnSOD and ecSOD (De Groote *et al*., 2009).

The significant decrease in β-carotene levels in the oral contraceptive group compared to non-contraceptive group is supported by studies conducted by Pincemail *et al*. (2007), De Groote *et al.* (2009), Fallah *et al.* (2011) and Palan *et al*. (2010) and can be attributed to the oestrogen induction and activation of the retinol-binding protein, increasing the conversion of β-carotene into retinol (De Groote *et al*., 2009). De Groote *et al*. (2009) note that these results were based on a cross-sectional comparison, and recommend confirmation of them in longitudinal studies in which women act as their own control, or with randomisation to nonsteroidal contraception or active treatment.

5.1.6 Thiobarbituric acid reactive substances

There were significant differences in thiobarbituric acid reactive substances (TBARS) levels in the present study between groups.

Higher levels of TBARS might indicate higher oxidative stress status, because they signal higher levels of lipid peroxidation, the oxidative degradation of lipids. This process results in free radical damage to cell membranes. Pincemail *et al.* (2007) reported a dramatic and significant increase in lipid peroxides in a group of women taking a steroid contraceptive. In this study it was not clear what difference to the intensity of lipid peroxide increase was made by which type of OC used (mono, bi and tri-phasic pills) (Pincemail *et al.,* 2007). These findings were supported by Adejumo *et al.* (2015), who concluded that there were significantly lower levels of serum TAS in users of both oral and injectable hormonal contraceptives when compared with non-contraceptive users. In the present study, the non-contraceptive group had the lowest levels of TBARS, which is similar to the findings of Adejumo *et al*. (2015). Palan *et al.* (2010) also reported that hormonal contraceptives deplete antioxidant vitamins and trace elements, as measured by the activity of coenzyme Q10, vitamin E and total antioxidant activity.

Akinloye *et al.* (2011) attributed a decrease in the serum levels of antioxidant trace elements specifically to the use of oral contraceptives. In the present study, the highest level of TBARS was found in the triphasic contraceptive group. Because of the oestrogen-associated increase in fatty acids and also the high levels of ROS in the contraceptive group, the assumption can be made that the increased concentrations of TBARS in this group are the result of lipid peroxidation brought on by higher amounts of lipids that are readily oxidised by the high levels of ROS present. Since long-term use of Combined oral contraceptives (COCs) can increase lipids in the blood of users (Pincemail *et al*., 2007; De Groote *et al*., 2009; Swiegers,

2015), it can be assumed that the increase in lipid peroxidation will occur chronically as a result of the chronically high ROS state (Swiegers, 2015).

5.2 Facial parameters

The skin is considered a kind of protective armour in everyday life, the primary interface with the environment. It has an area of some 2 m^2, and is thus the largest single organ in the human body. One of the main functions of the skin is to protect the body from external factors, such as mechanical injuries, extremes of temperature and radiation, as well as to transport various substances (Dąbrowska *et al*., 2016).

Several functions of the human skin appear strongly dependent on biologically active sexual hormones, namely androgens, oestrogens, and progestins (Zouboulis *et al*., 2007). In the present study, there was a significant difference between the contraceptive groups and control group, which is a strong indication of the influence of hormones on the skin.

5.2.1 Skin Elasticity

Wrinkles are modifications of the skin associated with cutaneous aging appearing preferentially on sun-exposed areas (actinic aging). Their prevalence can be increased by various intrinsic (heredity, ethnic, hormonal, and pathological) or extrinsic factors (irradiation, pollution, temperature, and humidity). Histological studies of wrinkles or rhytides have shown changes in dermal components with atrophy of dermal collagen, alterations of elastic fibres and a marked decrease in glycosaminoglycans. Oestrogens cause an increase in collagen and glycosaminoglycans in the dermis, which may explain the decrease in skin wrinkling with oestrogen treatment (Verdier-Sévrain *et al*., 2006).

Skin quality deteriorates with age due to the synergistic effects of chronologic aging, photo aging, environmental factors and hormonal deficiency. The hormonal aging of skin due to oestrogen loss at menopause is thought to include atrophy, decrease in collagen content, water content and sebaceous secretions, as well as loss of elasticity, and manifestations of hyperandrogenism (Brincat *et al.*, 2005; Stevenson & Thornton, 2007; Owen, *et al.*, 2016; Ramdhan *et al*., 2018).

Oestrogen treatment is known to increase collagen content/deposition, the thickness of the dermis (presumably via direct actions on fibroblasts and/or anagen hair follicles), and the elasticity and water content of the skin, while reducing sebaceous secretion (Blume-Peytavi *et al.*, 2012; Rieger *et al.,* 2015). Although there is scientific

evidence of the influence of hormonal contraceptives on skin health and the skin's elasticity, the present study did not have conclusive data to support this.

5.2.2 Erythema

Erythema is defined as redness due to vasodilation and increased blood volume in the skin (Parrish *et al*., 1982). According to a study conducted by Sobrino *et al*. (2009), it has been assumed that the vasodilatory effects of estradiol drive these changes in facial coloration. Estradiol may increase blood flow to vessels close to the surface of the skin, increasing skin redness (Jones *et al*., 2015).

In the present study, participants using injectable contraceptives showed the highest levels of erythema, and the monophasic contraceptive group had the lowest levels of erythema. There were significantly lower levels of erythema in the monophasic contraceptive group compared to the other groups.

Studies have shown that keratinocytes, Langerhans cells, melanocytes, sebaceous glands, and fibroblasts are subject to hormonal influence and that a decrease in oestrogen levels, which occurs in menopause, has been associated with decreased capillary blood flow in the skin (Brincat *et al.*, 2005; Owen *et al.*, 2016).

Healthy skin requires integrity of both the structure and function of capillary blood vessels as well as the maintenance of core temperature homeostasis. The effect of oestrogen on cutaneous circulation in humans is important in maintaining core temperature homeostasis. However, the effect of oestrogen on the cutaneous circulation of women has not been well studied (Brincat *et al.,* 2005). Oestrogen may affect endothelial function by increasing sensitivity to vasodilatory factors, such as acetylcholine, reducing the concentrations required to evoke similar vasodilatory responses to those observed in oestrogen-deprived animals (Usselman *et al.,* 2016). Consistent with the formation of premenstruation oedema in women, cutaneous blood flow has been shown to vary over the course of the menstrual cycle (Brincat *et al.*, 2005; Blume-Peytavi *et al.,* 2012; Usselman *et al.,* 2016; De Melo & Maia Campos, 2018).

5.2.3 Hydration

The hydration level of the stratum corneum can vary depending on environmental conditions, as corneocytes can take up water until the hydration level of the stratum corneum is in equilibrium with the environment. The hydration level of the stratum corneum is responsible for the physiology and homeostasis of the skin. Hydration is

important for the functions and properties of the skin because of its influence on the mechanical toughness of skin, its barrier functions, and its regulation of enzyme activity (Dąbrowska *et al*., 2016).

In this study, the implant contraceptive group showed the highest levels of hydration, with a significant difference from the monophasic contraceptive group, which evinced the lowest levels of hydration. A significant difference between the non-contraceptive group and the injectable contraceptive group was also noticed, with the injectable contraceptive group showing higher levels of hydration. Again significant was the difference between the non-contraceptive group and the implant contraceptive group, with the latter showing higher levels of hydration (p-value 0.001).

The ability of the skin to hold water is related to the stratum corneum lipids, which play a predominant role in maintaining the skin function, and also to the dermal glycosaminoglycans, which have a high water-holding capacity. Oestrogens also affect dermal water-holding capacity, producing marked increases in glycosaminoglycans and an increase in dermal hydroscopic qualities (Verdier-Sévrain *et al*., 2006).

Research into the effects of oestrogen on the skin has provided evidence to suggest that oestrogen is associated with increases in skin thickness and dermal water content, improved barrier function, and enhanced wound healing (Raghunath *et al*., 2015). The positive effect of oestrogen on the water content of skin may be related to oestrogen-stimulated increases in mucopolysaccharides and hyaluronic acid levels in skin, which correlate with an increase in dermal water content and skin thickness, subsequently elevating natural moisturising factors (NMF). An improvement in the water-holding capacity of the skin enhances the barrier function of the epidermis and inhibits the development of dermatoses (Brincat *et al*., 2005). This was also evident in the present study, with the implant contraceptive group demonstrating a greater capacity for water retention.

5.2.4 Pigmentation

Melasma (chloasma) is an acquired hypermelanosis occurring symmetrically on sun-exposed areas of the body. Lesions are irregular light-to-dark brown macules and patches, usually involving the forehead, temples, upper lip, and cheek. Melasma can affect any race, though Asian and Hispanic women are most commonly affected. Adult women are affected in 90% of cases, as melasma is rare before puberty and most commonly occurs during the reproductive years. Three patterns of melasma are

recognised clinically: a centrofacial pattern, a malar pattern, and a mandibular pattern (Mohamed Ali *et al.,* 2017).

In the present study, pigmentation was measured in 3 areas: forehead, right cheek, and the chin, which acts as a good indicator of melasma or chloasma. Natural and synthetic oestrogen and progesterone have been blamed for the pathogenesis of melasma because of its frequent association with pregnancy, the use of contraceptive drugs, the use of oestrogens in postmenopausal women, and diethylbestrol treatment of prostate cancer (Mohamed Ali *et al.,* 2017).

Te observed significant differences in pigmentation in the forehead, cheek and chin of participants as reported earlier in this study indicate that the monophasic contraceptive group had lower values in pigmentation. Skin pigmentation is determined by genetic, environmental, and endocrine factors, which influence both melanin syntheses in melanocytes and the distribution of melanin throughout the epidermis. Oestrogens regulate skin pigmentation. An increase in cutaneous pigmentation due to an increase in ovarian and/or pituitary hormones is common during pregnancy. Melasma, a well characterised acquired pigmentation occurring exclusively in sun-exposed areas, is exacerbated by pregnancy and oral contraceptives (Verdier-Sévrain *et al*., 2006).

5.2.5 Sebaceous activity

In the present study, the injection contraceptive group showed the highest levels of sebaceous gland activity, while the monophasic contraceptive group showed the lowest levels. However, there was no significant deference between the groups.

The amount of sebum a person produces varies throughout the course of his or her life. Sebaceous glands are present at birth and display relatively high production of sebum at this time. Shortly after birth, sebum production decreases until puberty, at which time it increases dramatically (Endly & Miller, 2017).

Acne is a common condition that affects men and women and is thought to be controlled to a large extent by androgenic sex hormones. Combined oral contraceptives can reduce acne in women primarily by reducing the production of testosterone. All combined oral contraceptives increase the production of sex hormone-binding globulin (SHBG), which binds free circulating androgens (Bitzer & Simon, 2011). Androgens and oestrogens significantly impact the pathogenesis of acne, and oestrogens have inhibitory effects on acne (Graber, 2017).

Oral contraceptives are beneficial for oily skin in that they result in a decrease in ovarian and adrenal androgens and an increase in sex hormone-binding globulin, which limits free testosterone. Oestrogens have been found to exhibit an inhibitory effect on excessive sebaceous gland activity *in vivo* (Endly & Miller, 2017). Even though there are previous studies indicating a strong correlation between hormonal contraceptives and sebum production, there was no conclusive evidence in this study to support these findings.

5.2.6 Transepidermal water loss

In the present study, no significant differences were observed among the groups with regards to TEWL. Triphasic contraceptive users showed the highest levels of TEWL and injection contraceptive users the lowest levels of TEWL.

Research credits adequate hydration with improved skin appearance and health. This general concept has been researched by many food and beverage industries to ascertain whether increased water intake may have anti-aging effects. Research attributes dermal water to decreasing the friction between fibres, thus acting as a lubricant. The association between skin health and improved skin appearance has therefore been widely accepted. Water is an essential component of the skin, an organ comprising cells that consist of 80% water. Without proper hydration, this organ will not have the ability to carry out its intended functions, becoming dry, tight, flaky and less resilient. Further research explains that if the epidermal layer of the skin lacks water, the skin becomes rough and loses elasticity. These symptoms may be due to the fact that skin cells undergo crenation in the absence of a sufficient amount of water (Castillo, 2017).

5.3 Anthropometric indicators

The assessment of body composition has become imperative because of the important role of body components in human health, especially the influence of excess body fat on the onset of non-communicable chronic diseases (Mialich *et al*., 2014).

Despite extensive clinical experience, many of the metabolic effects of oral contraceptive treatment remain to be explored. Changes in appetite and weight are known to occur in some women, but the association with treatment is unclear. There are only a few studies evaluating body composition during oral contraceptive treatment, and these indicate no significant change in body weight or body fat (Franchini *et al*., 1995; Reubinoff, *et al*., 1995; Lloyd *et al*., 2000). In this study, there

were some indications that contraceptives affected anthropometric parameters, but the only significant difference was in the waist-to-hip ratio measurement among the implant contraceptive group.

5.3.1 Adipose tissue

A study by Berenson and Rahman (2009) has demonstrated that increase in weight associated with injection contraceptive, Depomedroxyprogesterone acetate (DMPA), was due to an increase in fat mass and not lean mass although the mechanism by which DMPA causes an increase in fat mass is not known (Berenson & Rahman, 2009). In the present study the influence of contraceptives adipose tissue was also evident, with the injectable contraceptive group having the highest percentage of adipose tissue and the monophasic contraceptive group the lowest percentage of adipose tissue. No significant differences were found among any of the groups. An increase in total body fat percentage has been reported with triphasic hormonal contraceptive use (Casazza *et al.*, 2002; Lebrun *et al.*, 2003; Suh *et al.,* 2003; Myllyaho, 2016) and monophasic hormonal contraceptive use (Rickenlund *et al.,* 2004; Berenson & Rahman, 2009; Bonny *et al.,* 2015; Myllyaho, 2016).

In another study, Rickenlund *et al.* (2004) investigated the hormonal effect of oral contraceptive treatment among female athletes and reported relatively similar results for the athlete groups and control groups. Marked changes in body composition were recorded only among the oligo-/amenorrheic athletes. The increase in body weight was mainly caused by an increase in body fat, and there was no change in lean body mass. Within the groups of athletes, the largest increase in weight and body fat was found in women with menstrual disturbances. There was also an association between low fat mass at baseline and a larger increase in body fat during oral contraceptive use (Rickenlund *et al*., 2004). In this study there was no significant evidence to support these findings.

Overall, it seems that increase in body mass and body fat percentage occur within the first few months of hormonal contraceptive use (Suh *et al.,* 2002; Lebrun *et al.,* 2003; Rickenlund *et al.,* 2004; Myllyaho, 2016). In addition, the effect of hormonal contraceptives on body composition depends on the potency and androgenicity of the progesterone within the hormonal contraceptive pill (Casazza *et al.,* 2002; Suh *et al.,* 2003; Burrows & Peters, 2007; Myllyaho, 2016). Triphasic formulations with higher progestrogenic and androgenic activity may have more pronounced effects on body composition in the short term compared with formulations with lower potency and

androgenicity (Casazza *et al.,* 2002; Suh *et al.,* 2003; Burrows & Peters, 2007; Myllyaho, 2016).

Estradiol inhibits feeding in animals, whereas high dose progestins are appetite stimulating (Rickenlund *et al.,* 2004; Procter-Gray *et al.,* 2008; Myllyaho, 2016). Oral contraceptives may also decrease insulin sensitivity, and the effect on carbohydrate metabolism has been attributed to the progestin component. Furthermore, sex steroids may exert metabolic effects in adipose tissue. The mechanisms responsible for the increased body weight and body fat during oral contraceptive treatment remain to be elucidated (Rickenlund *et al.,* 2004; Procter-Gray *et al.,* 2008; Myllyaho, 2016).

5.3.2 Blood pressure

A normal young adult's blood pressure is 120/80 mmHg or lower. Blood pressure varies with age. Systolic pressure may progress from 100 to 120 during adolescence, and continues to rise slightly throughout adulthood (Van Wynsberghe *et al*., 1995).

Sexual dimorphism in arterial blood pressure appears in adolescence and persists throughout adulthood. Average systolic and diastolic blood pressures in men under 60 years of age are higher than in age-matched women by 6–7 and 3–5 mmHg, respectively. After that time, blood pressure (particularly systolic blood pressure) increases in women so that hypertension becomes at least as prevalent in women as men. While gender-associated differences in hypertension prevalence either disappear or cross over after women enter menopause, ovarian hormones may be responsible in part for lower blood pressure in premenopausal women and for the increase in blood pressure in postmenopausal women (Dubey *et al*., 2002).

In the present study, the injectable contraceptive group recorded the highest average systolic blood pressure and the non-contraceptive group the lowest. No significant differences were found among any of the groups. With regard to diastolic blood pressure, the monophasic contraceptive group exhibited the highest measurement and the non-contraceptive group the lowest. Again, no statistically significant differences were found among any of the groups.

This is similar to the study conducted by Kharbanda *et al.* (2014), where no statistically significant changes in systolic or diastolic blood pressure were observed between adolescents treated with combined oral contraceptives (COC) and a control group at 3, 6 and 12 months following COC initiation/index date. At all stages, progression to having systolic blood pressure or diastolic blood pressure in the

hypertensive range was rare and did not differ between COC-users and non-users (Kharbanda *et al*., 2014).

In another study by Shufelt and Merz (2009) on blood pressure in normotensive women, an increase in blood pressure associated with oral contraceptive use was reported. It has also been suggested that the newer progestins such as drospirenone, with anti-mineralocorticoid diuretic effects, produce lower blood pressure (Shufelt & Merz, 2009).

5.3.3 Body mass index and weight

In the current study, the subdermal implant contraceptive group displayed the highest body mass index (BMI) value and the monophasic contraceptive group the lowest BMI value. No significant differences were found among any of the groups. With regards to weight, the implant contraceptive group displayed the highest average weight and the monophasic contraceptive group the lowest average weight. No significant differences were found in any of the other groups. These observations could be an indication that contraceptive use does not necessarily result in weight gain, but could be a pre-disposing factor.

Both generalised and abdominal obesity are associated with increased risk of morbidity and mortality. The main cause of obesity-related deaths is cardiovascular disease (CVD), for which abdominal obesity is a predisposing factor. BMI has traditionally been the chosen indicator by which to measure body size and composition, and to diagnose underweight and overweight conditions (Huxley *et al*., 2010; Seidell, 2010; World Health Organisation, 2011). No statistically significant changes in BMI were observed between combined oral contraceptives (COC) users and control adolescents at 3, 6 and 12 months following COC initiation/index date. Similarly, at 3, 6 and 12-months, COC-use was not associated with a statistically significant weight gain (Kharbanda *et al*., 2014).

Dos Santos *et al*. (2017) found that measurements of weight, BMI, and body composition among those with 12 months of continuous contraceptive use did not differ between users of the levonorgestrel intrauterine system (LNG-IUS), the etonogestrel implant (ENG), and the copper IUD (intrauterine device). There was also no statistically significant difference in weight or body fat percentage when they compared participants who continued use for 12 months with those who discontinued use. Additionally, there was no significant difference in the BMI at 6 months between the continued and discontinued groups. Moreover, changes in weight, BMI, eating

behaviour, and body composition over 12 months of continuous use revealed no differences between the three groups (Dos Santos *et al*., 2017).

One of the most common reasons that young women are reluctant to use oral contraceptives (OC) is their concern about possible weight gain (Reubinoff *et al.,* 1995; Berenson & Rahman, 2009; Nault *et al.,* 2013). It is a common clinical experience that some women who start to use contraceptives report a subjective sensation of weight gain and bloating. However, studies have differed in their findings as to whether this birth control method actually does cause an increase in weight. Many of the studies were retrospective in design or did not include a comparison group using non-hormonal contraception. In contrast, studies on low-dose oral contraceptives have not reported any effect on weight or body composition. However, many of these studies were limited by small sample sizes, or merged different formulations of oral contraceptives (Berenson & Rahman, 2009).

5.3.4 Heart rate

The current study found that the injectable contraceptive group evinced the highest heart rate and the monophasic contraceptive group the lowest. No significant differences were found among any of the groups examined in this study.

In the resting homeostatic state, the heart rate (inherent rate) is set by the tone of the parasympathetic system, in the range of 50 to 100 beats per minute (Van Wynsberghe *et al*., 1995). There have been reports of significantly greater heart rate variations and vagal activity in the follicular phase (Sato *et al*., 1995; Leicht *et al*., 2003) and greater sympathetic activity during the luteal phase (Leicht *et al*., 2003; Yazar, 2016), compared with other phases of the menstrual cycle. The enhanced vagal activity at ovulation has been attributed to higher endogenous oestrogen levels, while the reduced heart rate variations and greater sympathetic activity during the luteal phase have been attributed to greater endogenous progesterone levels (Sato *et al.*, 1995; Leicht *et al.,* 2003). This indicates significant correlations between oestrogen levels and all absolute measures of heart rate variations at ovulation. This in turn suggests a positive relationship between oestrogen and vagal activity, as only the parasympathetic nervous system regulates heart rate control (Leicht *et al.,* 2003).

5.3.5 Lean tissue

In this study, the monophasic contraceptive group had the highest percentage of lean tissue and the injectable contraceptive group the lowest percentage of lean tissue. No significant differences were found among any of the groups.

Berenson and Rahma (2009) and Rickenlund *et al*. (2004) have proposed that with the use of contraceptives an increase in weight was due to an increase in fat mass and not lean mass. In the current study, there were no significant differences between the contraceptive groups and the control group (non-contraceptive group).

Despite worldwide use of hormonal contraceptives, their effects on body composition remain unclear (Myllyaho, 2016). Individual responses to hormonal contraceptive use may involve some weight gain as a result of either fluid retention or appetite stimulation (Rosenberg & Waugh, 1998; Rickenlund *et al.,* 2004; Myllyaho, 2016).

The hormonal effect of oral contraceptive treatment was investigated in female athletes, and the results for the athlete groups and the control groups were quite similar (Rickenlund *et al.,* 2004; Myllyaho, 2016). Marked changes in body composition were recorded only among the oligo-/amenorrheic athletes. The increase in body weight was mainly caused by an increase in body fat, and there was no change in lean body mass (Rickenlund *et al.,* 2004; Myllyaho, 2016). Hormonal contraceptive treatment significantly increased bone mineral density (BMD) in those with low BMD at baseline. It was concluded that hormonal contraceptive treatment in female athletes has primarily beneficial effects on body composition without adverse effects on physical performance (Rickenlund *et al.,* 2004; Myllyaho, 2016).

5.3.6 Skin fold

The subdermal implant contraceptive group displayed the highest average skin fold measurement, though there was no significant difference between any of the groups in the study.

Among the main methods used to assess body composition, the skinfold thickness method stands out, as it is easy to apply, is of low operational cost, and provides valid and reliable results. The skin thickness method is considered to be doubly indirect, as it is structured on the assumptions of hydrostatic weighing, which, in spite of being an indirect method, has long been considered a gold standard for the study of body composition in humans. Similar to hydrostatic weighing, the skin thickness method enables the assessment of both fat and lean body mass, as body composition assessment is performed from body density estimates generated from specific or general regression equations. It is believed that in healthy adults, one third of total fat is found in the subcutaneous area. Furthermore, there seems to be a good relationship between fat found in subcutaneous deposits and body density. As the sites where there is subcutaneous fat are not uniform, one must measure skinfold

thickness at different anatomic sites from different body segments (upper limbs, lower limbs, trunk), in order to obtain a clear overall and regional perception of fat distribution (Cyrino *et al*., 2003). A number of formulae have been proposed whereby skinfold measurement can be used to predict total body fat (Feldman *et al.,* 1969; Dauncey *et al*., 1977).

5.3.7 Waist to hip ratio

The implant contraceptive group displayed the highest average waist to hip ratio while the non-contraceptive group demonstrated the lowest average waist to hip ratio. This amounted to a significant difference between these groups, with no significant differences among any of the other groups.

According to the World Health Organisation's (2011) cut-off points for waist-hip ratio, a measurement of 0.85 and higher substantially increases the risk of metabolic complications. The measurements in this research indicated that all the groups were under the cut-off points.

Waist-hip ratio (i.e. the waist circumference divided by the hip circumference) was suggested as an additional measure of body fat distribution. In populations with a predisposition to central (i.e. abdominal or visceral) obesity and the related increased risk of developing a metabolic syndrome, it was recommended that, where possible, waist circumference should be used to refine action levels based on body mass index (Wei *et al.,* 1997; De Koning *et al*., 2007; World Health Organisation, 2011; Kharbanda *et al.,* 2014).

Sex steroids have been shown to be associated with metabolic function and mechanisms of regulation (Rickenlund *et al.,* 2004; Myllyaho, 2016). Because of the regional distribution of receptors for sex steroid hormones, there is a gender difference in fat accumulation. In premenopausal women, for example, oestrogens increase the amount of fat accumulation in the subcutaneous tissues (Borer, 2003; Myllyaho, 2016). In the current study there was a relationship between higher waist-to-hip ratio and hormonal contraceptive usage.

5.3.8 Water content

In this study, the monophasic contraceptive group showed the highest percentage of water content and implant contraceptive group the lowest percentage of water content. No significant differences were found among any of the groups.

Water is the largest component in the body, making up about 62% of the body weight of an adult. Water is one of the chief regulators of homeostasis, and the body cannot function without it (Van Wynsburghe *et al.*, 1995). It is crucial in maintaining optimal physical and mental functioning (Shanholtzer & Patterson, 2002). Previous studies have indicated that progesterone may have effects on body weight because of water regulation and fluid retention via aldosterone (Burrows & Peters, 2007; Myllyaho, 2016).

There are potential changes in the distribution of body fluids throughout the menstrual cycle since many women report changes in body weight and a bloated feeling (De Jonge, 2003; Myllyaho, 2016). These changes are usually observed alongside high doses of glucocorticoid-like activity, leading to salt and water retention (Sitruk-Ware, 2006). In addition, androgenic progestins stimulate insulin secretion, which may be responsible for true weight gain (Sitruk-Ware, 2006; Batista *et al*., 2017). However, most studies with hormone specifications have not found significant changes in body weight over the normal menstrual cycle (Lebrun *et al.,* 1995; Casazza *et al.*, 2002; Myllyaho, 2016). It is therefore suggested that oestrogen and progesterone changes during the menstrual cycle do not significantly affect fluid regulation and body weight (De Jonge, 2003).

CHAPTER SIX

CONCLUSION

This study was based on a cross-sectional research sampling and investigated the effects of different contraceptive methods on oxidative stress status, skin parameters and anthropometric parameters.

With regard to oxidative stress status, the results showed that superoxide dismutase (SOD) activities in the triphasic contraceptive group were significantly higher than those in monophasic contraceptive group. There was also an increase in lipid peroxidation (TBARS) in the triphasic contraceptive group in comparison to the control group, the monophasic contraceptive group and the injectable contraceptive group, indicative of increased oxidative stress levels in the triphasic contraceptive group.

Skin parameters in this study indicated that there was an increase in the incidence of erythema in the monophasic contraceptive group as compared to the control group, the injectable contraceptive group, the implant contraceptive group and the triphasic contraceptive group, symptomatic of higher vascular activity in the monophasic group. Melanocyte activity measured in the forehead, cheek and chin areas was also significantly higher in the monophasic contraceptive group than in the control group, the injectable contraceptive group, the implant contraceptive group and the triphasic contraceptive group. This represented the pigmentation pattern of chloasma/melasma known to be caused by hormones. The hydration measurements were significantly higher in the implant contraceptive group than in the control and monophasic contraceptive groups. Furthermore, a significant increase in hydration was evident in the injectable contraceptive group in comparison to the control and monophasic contraceptive groups. Injectable contraceptives and implant contraceptives mainly contain progesterone which has been proven to combat signs of aging and increase collagen and elastin in the skin.

Results obtained from anthropometric measurements showed a significantly higher waist-to-hip ratio in the implant contraceptive group compared to the control group (non-contraceptive). Progesterone's influence on adipose tissue distribution points to an increase of adipose tissue in the abdominal region.

In future studies, the effect of the different hormonal contraceptives should be compared to the baseline values gathered before the participants commence the use of the contraceptives. This will provide a more accurate representation of the changes with regard to oxidative status, skin and anthropometric parameters that may occur during the use of different contraceptives. This study included participants from many different racial groups, which might also have affected the outcome of certain parameters, for example the pigmentation patterns. It was not clear whether the individuals experienced any significant changes caused specifically by the contraceptives. As this study was a pilot study, recommendation fo further studies is to consider the use of medication (e.g. antibiotics), diet, exercise, skin care regime and lifestyle factors.

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APPENDICES

APPENDIX A: QUESTIONNAIRE

INTRODUCTION TO QUESTIONNAIRE

This survey is part of a research study titled " **The effects of contraceptives on the anti-oxidant status, skin parameters and anthropometric indicators in female students: a pilot study**" that will be conducted at the Department of Wellness Sciences, Cape Peninsula University of Technology (CPUT). The study will be conducted in fulfillment of a dissertation for the Master of Technology: Biomedical Technology.

The body cells use oxygen to produce energy. In the process, oxygen sometimes reacts with body compounds to produce highly unstable molecules known as free radicals. In addition to normal body processes, environmental factors such as radiation, pollution and tobacco smoke can act as oxidants and cause free radical formation. The trouble begins when free radicals in the body exceed its defences against free radicals and a condition known as oxidative stress sets in. Antioxidants significantly decrease the adverse effects of oxidants on human physical and biological functions.

The purpose of this study is to determine:

- The differences in skin health (level of inflammation, pigmentation, hydration, sebaceous activity and transepidermal water loss) between females using hormonal contraceptives and non-contraceptive users.
- The differences in body weight status (body mass index and hip-to-waist ratio) between females using hormonal contraceptives and non-contraceptives users.
- The differences in body composition (moisture content, lean body mass and adipose tissue) between females using hormonal contraceptives and non-contraceptives users.
- Selected oxidative stress markers (lipid peroxidation, oxygen radical absorbance capacity and GSH: GSSG ratios, enzyme measurements); vitamin A and E as well as zinc and selenium in blood samples from females using hormonal contraceptives versus non contraceptive users.

The sample group will be determined by the outcome of the survey according to age, gender and lifestyle.

Therefore, participants will have to enclose contact details in order to schedule an appointment for a once off gathering of research information (blood collection, skin analysis and body analysis).

The participants will receive the results of the research information collected.

The identity of participants will at all times be protected and codes will be used to analyse data. No individually-identifiable information about any participant will be shared with others without written permission, except if it is necessary to protect the welfare of the participant (for example, if the participant were injured and in need physician care) or if required by law.

Name:						**Today's date:**			
Email Address:									
Telephone	**Home:**				**Date of birth:**				
	Work:				**Cell phone:**				
							RESEARCH CODE:		
Background									
1. Ethnic origin: (tick only one)									
☐	Asian / Indian		☐	White					
☐	Black		☐	Other:_____					
☐	Coloured								
2. Are you currently (tick one only):									
☐	Married		☐	Separated			☐	Widowed	
☐	Single		☐	Divorced					
3. Please indicate below if you have any of the following chronic conditions(									
☐	Diabetes				☐	Asthma			
☐	Low blood pressure				☐	High blood pressure			
☐	Heart disease: Type of heart disease: _____								
☐	Cancer: Type of cancer: _____								
☐	Other chronic condition: Specify:_____								

Physical Activities

During the past week, even if it was not a typical week for you, how much total time (for the entire week) did you spend on each of the following? (*Please circle one number for each question*.)

	None	Less than 30 min/wk	30-60 min/wk	1-3hrs per week	More than 3 hrs/wk
1. Stretching or strengthening exercises (range of motion, using weights etc.)	0	1	2	3	4
2. Walk for exercise (including treadmill)	0	1	2	3	4
3. Swimming or aquatic exercise	0	1	2	3	4
4. Cycling (including stationary exercise bikes)	0	1	2	3	4
5. Other aerobic exercise equipment (Stairmaster, rowing, etc.)	0	1	2	3	4
6. Other aerobic exercise Specify:	0	1	2	3	4

Diet

During the past week, even if it was not a typical week for you, please indicate how much of the following you consume. (*Please circle one number for each question*.)

Beverages

1. Caffeinated beverages: Coffee or tea (one cup = 250 ml)	None	1-2 cup /day	3-4 cups/day	5-6 cups/day	More than 7cups a day	
2. Water	Less than 500 ml	500ml /day	1 litre /day	2 litres /day	More than 2 litres/ day	
3. Alcohol Beverages (one glass = 120 ml)	None	1-2 glasses/ w eek	3-4 glasses/ w eek	5-6 glasses/ w eek	More than 7 glasses a w eek	

Food sources

dark red vegetables, orange vegetables, yellow vegetables). One portion = 100g	None	1- 2 portions / week	3-4 portions / week	5-6 portions / day	More than 7 portions /week
2. Berries (blue berries, strawberries, grapes, cranberries etc.) One portion = 100g	None	1- 2 portions / week	3-4 portions / week	5-6 portions / day	More than 7 portions /week
3. Fruits (oranges, apples, kiwi fruit, grape fruit, grape, plum, guava). One portion = 100 - 150 g	None	1- 2 portions / week	3-4 portions / week	5-6 portions / day	More than 7 portions /week
4. Legumes (Broad beans, pinto beans, soybeans). One portion = 100 g	None	1- 2 portions / week	3-4 portions / week	5-6 portions / day	More than 7 portions /week
5. Red meat (Lamb, beef, lamb, venison etc). One portion = 60 - 90 g	None	1- 2 portions / week	3-4 portions / week	5-6 portions / day	More than 7 portions /week
6. White meat (Fish, poultry, pork). One portion = 60 - 90 g	None	1- 2 portions / week	3-4 portions / week	5-6 portions / day	More than 7 portions /week
7. Nuts and seed (Pecans, walnuts, hazelnuts, ground nuts or peanuts, sunflower seeds, pumpkin seed, sesame seed, linseed ect.). One portion = 30 g	None	1- 2 portions / week	3-4 portions / week	5-6 portions / day	More than 7 portions /week

Supplements

1. Vitamin A	None	Unsure	350 µg/d	700 µg/d	1400 µg/d	2100 µg/d
2. Vitamin C	None	Unsure	35 mg/d	75 mg/d	150 mg/d	225 mg/d
3. Vitamin E	None	Unsure	7mg/d	15 mg/d	30 mg/d	45 mg/d
4. Selenium	None	Unsure	25 µg/d	55 µg/d	110 µg/d	165 µg/d
5. Zinc	None	Unsure	4 mg/d	8 mg/d	16 mg/d	24 mg/d

name)_____

Life style

During the past week, even if it was not a typical week for you, please indicate which of the following is applicable to your life style.(*Please circle one number for each question*.)

1. In general, would you say your health is:	*Circle only one:*	
	Excellent	1
	Very good	2
	Good	3
	Fair	4
	Poor	5

2. How many hours do you sleep per day?		Less than 4 hours	5-6 hours	7-8 hours	More than 8 hours
3. How much sun exposure do you get per day?	None	10 min	20 min	30 min	More than 30 min
4. Do you smoke?				Yes	No

If your answer is no, please continue with question 6.

5. How many cigarettes do you smoke on average per day?	N/A	Less than 5	10	20	More than 20
6. How would you rate your stress levels?	Highly stressful	Stressful	Manageabl	No stress/ Balanced	
7. How would you describe your energy levels?			Good	Adequate	Low

Contraceptive use

Please select the option most applicable to you (please tick):

1. Do you use a contraceptive?			Yes	No

If your answer is no, please continue with the skin care section.

2. What is the main reason for the use of contraceptive?	Family planning	Hormonal therapy	PMS relief	Acne treatment
	Other:_____			

3. What type of contraceptive do you use?	Hormonal	IUD (Intrauterine device)	Barrier methods (male condom, female condom, spermicide etc.)	Periodic abstinence	Sterilisation (Tubal occlusion, hysteroscopic sterilisation)

If you selected hormonal, please answer the following questions. If you selected another option please continue to question 8.

4. If you are using a hormonal contraceptive, what type of method do you use?	Oral Combination hormonal method (contains oestrogen and progesterone)
	Name of contraceptive:
	Oral Progestin only hormonal method
	Name of contraceptive:
	Injection:
	Name of contraceptive:
	Other (please specify):

5. How long have you been using the hormonal contraceptive?	Less than 6 months	6-12 months	1-2 years	3-4 years	More than 4 years

6. Have you experienced any complications while using the contraceptive pill?	Yes	No

7. If yes to question 6, please specify:_____

8. Where do you usually get your contraceptive from?	Clinic	Doctor	Pharmacy
	Other:		

Skin care

1. Do you use skin care products on a regular basis (day cream, night cream etc)?	Yes	No

2. Please select (tick) from the list the product/s you use on a weekly basis:

Day cream	Daily	Weekly	Monthly
Toner	Daily	Weekly	Monthly
Exfoliator / scrub	Daily	Weekly	Monthly
Mask	Daily	Weekly	Monthly
Night cream	Daily	Weekly	Monthly
Other (please specify): _____	Daily	Weekly	Monthly

3. How long have you been using these products?

Day cream	Unsure	Less than 1 month	1-2 months	2-3 months	4-5 months	5-6 months	More than 6 months
Toner	Unsure	Less than 1 month	1-2 months	2-3 months	4-5 months	5-6 months	More than 6 months
Exfoliator / scrub	Unsure	Less than 1 month	1-2 months	2-3 months	4-5 months	5-6 months	More than 6 months
Mask	Unsure	Less than 1 month	1-2 months	2-3 months	4-5 months	5-6 months	More than 6 months
Night cream	Unsure	Less than 1 month	1-2 months	2-3 months	4-5 months	5-6 months	More than 6 months
Other (please specify): _____	Unsure	Less than 1 month	1-2 months	2-3 months	4-5 months	5-6 months	More than 6 months

4. Do you use a Sun Protector Factor (SPF) every day?	Yes	No

5. How long have you been using the SPF?

Unsure	2 months	3-6 months	6-9 months	10-12 months	More than 12 months

6. Please indicate the SPF level you use.	None	SPF less than 10	SPF 10-20	SPF 20-30	SPF higher than 30

Thank you for taking time out of your day to complete this questionnaire.

APPENDIX B: ETHICAL APPROVAL

Cape Peninsula University of Technology

HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE (HW-REC)
Registration Number NHREC: REC- 230408-014

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Symphony Road Bellville 7535
•Tel: +27 21 959 6917 • Fax +27 21 953 8490
Email: lebenyat@cput.ac.za

08 October 2014
CPUT/HW-REC 2014/H13

Faculty of Health and Wellness Sciences – Biomedical Sciences Department

Dear Mrs. Engelbrecht (Germishuys)

YOUR APPLICATION TO THE HW-REC FOR EXTENSION

Approval was granted by the Health and Wellness Sciences-REC on 02 October 2014 to Mrs. Martha P. Engelbrecht for ethical clearance. This approval is for research activities related to your MTech Biomedical Technology at CPUT.

TITLE: The effects of contraceptives on the anti-oxidant status, skin parameters and anthropometric indicators in female students: a pilot study

SUPERVISOR: Dr. N Brooks

Comment:
Approval will not extend beyond 09 October 2015. An extension should be applied for 6 weeks before this expiry date should data collection and use/analysis of data, information and/or samples for this study continue beyond this date.

The investigator(s) should understand the conditions under which they are authorized to carry out this study and they should be compliant to these conditions. **It is required that the investigator(s) complete an annual progress report that should be submitted to the HW-REC in December of that particular year, for the HW-REC to be kept informed of the progress and of any problems you may encounter.**

Kind Regards

APPENDIX C: INFORMED CONSENT FORM

INFORMED CONSENT FORM

I, _____, agree to participate in a research study titled " **The effects of contraceptives on the anti-oxidant status, skin parameters and anthropometric indicators in female students: a pilot study"** conducted by the Martha Petronella Engelbrecht in the Department of Wellness Sciences, Cape Peninsula University of Technology (CPUT). The study will be conducted in fulfillment of a dissertation for the Master of Technology: Biomedical Technology. The contact details of the researcher: Room 4.10; Tel: (021)460-8317 and e-mail: engelbrechtm@cput.ac.za.

The body's cells use oxygen to produce energy. In the process, oxygen sometimes reacts with body compounds to produce highly unstable molecules known as free radicals. In addition to normal body processes, environmental factors such as radiation, pollution and tobacco smoke can act as oxidants and cause free radical formation. The trouble begins when free radicals in the body exceed its defences against them, a condition known as oxidative stress. Antioxidants significantly decrease the adverse effects of oxidants on human physical functions.

The purpose of this study is to determine:

- The differences in skin health (level of inflammation, pigmentation, hydration, sebaceous activity and transepidermal water loss) between females using hormonal contraceptives and non-contraceptive users.
- The differences in body weight status (body mass index and hip-to-waist ratio) between females using hormonal contraceptives and non-contraceptives users.
- The differences in body composition (moisture content, lean body mass and adipose tissue) between females using hormonal contraceptives and non-contraceptives users.
- Selected oxidative stress markers (lipid peroxidation, oxygen radical absorbance capacity and GSH: GSSG ratios, enzyme measurements); vitamin A and E as well as zinc and selenium in blood samples from females using hormonal contraceptives versus non contraceptive users.

I understand that my participation is voluntary. I can refuse to participate or stop taking part at any point without giving any reason, and without any penalty. I can ask to have all information obtained about me in the study to be returned to me, removed from the research records, or destroyed.

If I volunteer to take part in this study, I will be asked to do the following:

1. I will have a blood sample taken by a qualified nurse in the morning before I have eaten.
2. I will have a non-invasive skin analysis performed by a qualified somatologist to establish skin parameters that will form part of the investigation.
3. I will have photographs taken with the Visioscope® Imager Dual and digital camera to record the appearance of my skin and any signs of pigmentation and premature aging.
4. I will have a non-invasive manual skin analysis by a qualified somatologist to rate the age of my skin by using the Rao-Goldman 5-point Facial Wrinkle Scale.
5. If my entire face will be photographed, I will be allowed to choose whether or not I want my eyes blocked out on the photographs to protect my identity, which I will indicate on this form after viewing the photographs.
6. I will have a non-invasive body composition analysis by a qualified somatologist to establish my anthropometric indicators relative to this study.
7. I will have my weight and height measurements taken by a qualified somatologist to determine my body mass index (BMI).
8. I will have hip and waist circumference measurements taken by a qualified somatologist to determine my hip-to-waist ratio.
9. I will have my blood pressure taken by a registered qualified nurse to determine if my blood pressure is normal, high or low.
10. I will have a skin fold test done by a qualified somatologist to determine the subcutaneous adipose tissue percentage.

No individually-identifiable information about me, or provided by me during the study, will be shared with others without my written permission, except if it is necessary to protect my welfare (for example, if I were injured and need physician care) or if required by law. All the blood samples will be disposed of after oxidative stress status analysis is completed.

I understand that there will be no costs to me for participation in this study and that I will not be compensated to participate in the study.

I declare that I take part in this study at my own risk and that CPUT, any of its workers or students are not responsible if anything should happen to me during the course of the study. All risks associated with blood sample collection have been explained to me.

I declare that I will not to sue the CPUT for any personal losses or damage that might occur during the project due to negligence from the CPUT or persons that take part in this study.

I declare that I have read the details of the project or have listened to the oral explanation thereof, and declare that I understand these. I have had the opportunity to discuss relevant aspects with the researcher and declare that I voluntarily give consent to participate in this study.

_____	_____	_____
Name of Participant	**Signature**	**Date**

Telephone: _____

Email: _____

_____	_____	_____
Name of Researcher	**Signature**	**Date**

CONSENT FOR RELEASE OF PHOTOGRAPHS:

(To be completed before commencement of the study)

I hereby state that:

I will have the opportunity to review the final photographs selected by the researcher. ☐

I consent for these photographs to be published by the researcher. ☐

I choose to have my eyes blocked out on the photographs *(if applicable)* YES ☐

NO ☐

N/A ☐

_____	_____	_____
Name of Participant	**Signature**	**Date**

_____	_____	_____
Name of Researcher	**Signature**	**Date**

Please sign both copies, keep one and return one to the researcher.

APPENDIX D: STATISTICAL DATA COLLECTED: ANOVA

One way ANOVA

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Catalase	Monophasic oral contraceptive	20	8.23496	1.928192	.431157	7.33254	9.13738	5.219	12.176
	Non contraceptive	20	8.17054	1.922780	.429947	7.27065	9.07043	4.262	12.465
	Injectable contraceptive	20	6.99316	2.459388	.549936	5.84213	8.14419	3.446	13.156
	Implantable contraceptive	21	7.53359	2.027526	.442442	6.61067	8.45651	2.729	10.835
	Triphasic oral contraceptive	20	6.95753	2.252913	.503767	5.90314	8.01193	1.409	12.177
	Total	101	7.57752	2.155686	.214499	7.15196	8.00307	1.409	13.156
SOD	Monophasic oral contraceptive	20	36.55246	17.098582	3.823359	28.55007	44.55484	23.998	80.853
	Non contraceptive	20	46.78548	19.122428	4.275905	37.83591	55.73506	23.956	93.397
	Injectable contraceptive	20	50.22963	22.931806	5.127708	39.49722	60.96205	23.162	93.602
	Implantable contraceptive	21	52.27384	25.663983	5.600340	40.59174	63.95595	22.747	109.685
	Triphasic oral contraceptive	20	61.55728	16.443022	3.676772	53.86171	69.25285	32.271	96.503
	Total	101	49.50740	21.767832	2.165980	45.21016	53.80465	22.747	109.685
ORAC	Monophasic oral contraceptive	20	2718.10746	855.979682	191.402876	2317.49663	3118.71828	1226.657	4411.900
	Non contraceptive	20	2686.42543	1052.292185	235.299686	2193.93753	3178.91334	1311.432	4472.480
	Injectable contraceptive	20	2256.55502	863.701536	193.129535	1852.33026	2660.77978	1226.657	3576.281
	Implantable contraceptive	21	2996.96095	1141.723078	249.144401	2477.25483	3516.66706	1459.089	4853.203
	Triphasic oral contraceptive	20	3411.09473	1420.912885	317.725780	2746.08702	4076.10243	1543.253	5602.237
	Total	101	2815.64191	1131.914150	112.629668	2592.18785	3039.09596	1226.657	5602.237
GSH	Monophasic oral contraceptive	20	1086.85521	281.747808	63.000725	954.99318	1218.71724	571.424	1728.836
	Non contraceptive	20	988.52812	162.250749	36.280371	912.59244	1064.46381	706.442	1362.551
	Injectable contraceptive	20	1007.13787	169.984369	38.009661	927.58274	1086.69301	663.852	1302.663
	Implantable contraceptive	21	1088.72005	177.828572	38.805376	1007.77346	1169.66665	849.723	1602.086
	Triphasic oral contraceptive	20	1058.68870	207.422650	46.381115	961.61191	1155.76549	708.701	1688.912
	Total	101	1046.40910	204.414538	20.340007	1006.05511	1086.76310	571.424	1728.836
GSSG	Monophasic oral contraceptive	20	17.13893	9.374210	2.096137	12.75166	21.52619	4.922	39.215
	Non contraceptive	20	17.88256	11.241908	2.513767	12.62119	23.14394	3.652	48.789
	Injectable contraceptive	20	12.58173	9.199774	2.057132	8.27611	16.88736	3.554	43.904
	Implantable contraceptive	21	13.14728	5.514745	1.203416	10.63700	15.65756	4.238	24.853

Descriptives									
		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
	Triphasic oral contraceptive	20	12.87556	4.642901	1.038184	10.70262	15.04850	.387	22.820
	Total	101	14.70959	8.500146	.845796	13.03155	16.38763	.387	48.789
GSH/GSSG	Monophasic oral contraceptive	20	88.13687	58.529834	13.087669	60.74407	115.52968	17.647	225.761
	Non contraceptive	20	82.56020	61.923203	13.846449	53.57925	111.54115	24.627	285.264
	Injectable contraceptive	20	110.67270	60.225087	13.466739	82.48649	138.85890	21.488	219.718
	Implantable contraceptive	21	101.15616	59.216811	12.922168	74.20099	128.11133	34.929	296.095
	Triphasic oral contraceptive	20	212.55266	585.630387	130.950935	-61.53079	486.63612	54.113	2699.369
	Total	101	118.83889	264.990710	26.367561	66.52640	171.15138	17.647	2699.369
TBARS	Monophasic oral contraceptive	20	.29565	.014402	.003220	.28891	.30239	.261	.325
	Non contraceptive	20	.29177	.019694	.004404	.28255	.30099	.258	.346
	Injectable contraceptive	20	.29460	.013217	.002956	.28842	.30079	.267	.318
	Implantable contraceptive	21	.31971	.066324	.014473	.28952	.34990	.270	.538
	Triphasic oral contraceptive	20	19.00049	83.463339	18.662970	-20.06155	58.06254	.277	373.597
	Total	101	4.00361	37.143686	3.695935	-3.32902	11.33623	.258	373.597
FRAP	Monophasic oral contraceptive	20	412.48939	57.773120	12.918462	385.45074	439.52804	289.413	544.389
	Non contraceptive	20	389.67244	61.434419	13.737154	360.92025	418.42464	297.654	553.150
	Injectable contraceptive	20	424.50136	96.380515	21.551338	379.39389	469.60883	288.235	626.875
	Implantable contraceptive	21	431.84473	83.191869	18.153954	393.97625	469.71321	277.422	619.219
	Triphasic oral contraceptive	20	368.37351	64.950542	14.523383	337.97572	398.77130	263.592	494.869
	Total	101	405.63835	76.520640	7.614088	390.53222	420.74448	263.592	626.875
Average: Erythema	Monophasic oral contraceptive	20	37.31667	6.045128	1.351732	34.48746	40.14587	26.000	45.000
	Non contraceptive	20	43.43333	4.622725	1.033673	41.26983	45.59684	31.000	50.667
	Injectable contraceptive	20	47.86667	4.725692	1.056697	45.65497	50.07836	38.333	53.333
	Implantable contraceptive	21	46.19048	6.167632	1.345888	43.38300	48.99795	30.333	54.667
	Triphasic oral contraceptive	20	44.81667	4.476678	1.001016	42.72152	46.91182	36.333	51.667
	Total	101	43.94719	6.307796	.627649	42.70196	45.19243	26.000	54.667
Forehead: Melanin percentage	Monophasic oral contraceptive	20	27.80	22.287	4.983	17.37	38.23	8	96
	Non contraceptive	20	54.10	23.595	5.276	43.06	65.14	17	94
	Injectable contraceptive	20	64.70	23.618	5.281	53.65	75.75	23	99
	Implantable contraceptive	21	60.14	25.833	5.637	48.38	71.90	12	94
	Triphasic oral contraceptive	20	60.15	27.865	6.231	47.11	73.19	15	99
	Total	101	53.45	27.613	2.748	47.99	58.90	8	99
Cheek: Melanin	Monophasic oral contraceptive	20	23.45	15.619	3.492	16.14	30.76	7	64

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
percentage	Non contraceptive	20	46.35	21.700	4.852	36.19	56.51	18	95
	Injectable contraceptive	20	57.90	21.465	4.800	47.85	67.95	19	99
	Implantable contraceptive	21	54.43	25.748	5.619	42.71	66.15	7	87
	Triphasic oral contraceptive	20	57.40	25.968	5.807	45.25	69.55	11	99
	Total	101	47.97	25.517	2.539	42.93	53.01	7	99
Chin: Melanin percentage	Monophasic oral contraceptive	20	33.10	20.906	4.675	23.32	42.88	13	86
	Non contraceptive	20	64.30	25.477	5.697	52.38	76.22	17	99
	Injectable contraceptive	20	75.35	21.256	4.753	65.40	85.30	21	99
	Implantable contraceptive	21	72.33	27.726	6.050	59.71	84.95	15	99
	Triphasic oral contraceptive	20	68.35	24.743	5.533	56.77	79.93	21	99
	Total	101	62.78	28.212	2.807	57.21	68.35	13	99
Average: Hydration levels	Monophasic oral contraceptive	20	33.11500	3.259704	.728892	31.58941	34.64059	27.833	42.667
	Non contraceptive	20	33.60667	3.778496	.844897	31.83828	35.37506	25.600	39.700
	Injectable contraceptive	20	37.82667	5.062725	1.132060	35.45724	40.19609	31.167	47.767
	Implantable contraceptive	21	39.16825	5.022787	1.096062	36.88191	41.45460	31.900	52.333
	Triphasic oral contraceptive	20	35.73833	4.292592	.959853	33.72934	37.74733	27.833	46.933
	Total	101	35.92343	4.872518	.484834	34.96154	36.88533	25.600	52.333
Average: Sebum	Monophasic oral contraceptive	20	24.96667	10.820113	2.419451	19.90270	30.03064	9.333	50.667
	Non contraceptive	20	32.11667	16.571403	3.705478	24.36101	39.87232	8.333	60.000
	Injectable contraceptive	20	33.70167	14.526078	3.248130	26.90325	40.50008	4.333	62.000
	Implantable contraceptive	21	28.36508	12.180542	2.658012	22.82056	33.90960	9.000	66.000
	Triphasic oral contraceptive	20	27.48333	13.090095	2.927034	21.35698	33.60969	9.667	51.667
	Total	101	29.31716	13.667552	1.359972	26.61902	32.01531	4.333	66.000
Average TEWL	Monophasic oral contraceptive	20	6.73333	1.945455	.435017	5.82283	7.64383	3.333	11.333
	Non contraceptive	20	6.73333	1.576880	.352601	5.99533	7.47134	3.333	10.333
	Injectable contraceptive	20	6.55000	1.864645	.416947	5.67732	7.42268	3.667	10.000
	Implantable contraceptive	21	6.66667	2.945807	.642828	5.32575	8.00758	3.000	14.000
	Triphasic oral contraceptive	20	7.06667	2.180724	.487625	6.04606	8.08728	3.333	12.333
	Total	101	6.74917	2.126241	.211569	6.32943	7.16892	3.000	14.000
Average Elasticity	Monophasic oral contraceptive	20	77.86667	7.563687	1.691292	74.32675	81.40658	64.667	90.333
	Non contraceptive	20	81.31667	5.910121	1.321543	78.55064	84.08269	71.333	91.667
	Injectable contraceptive	20	80.06667	5.986925	1.338717	77.26470	82.86863	66.333	89.333
	Implantable contraceptive	21	81.49206	9.067414	1.978672	77.36463	85.61950	64.333	95.667
	Triphasic oral contraceptive	20	80.40000	6.796628	1.519772	77.21908	83.58092	70.667	94.667

Descriptives									
		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
	Total	101	80.24092	7.157920	.712240	78.82786	81.65399	64.333	95.667
Roa-Goldman scale eye area	Monophasic oral contraceptive	20	1.55	.605	.135	1.27	1.83	1	3
	Non contraceptive	20	1.50	.513	.115	1.26	1.74	1	2
	Injectable contraceptive	20	1.50	.513	.115	1.26	1.74	1	2
	Implantable contraceptive	21	1.57	.598	.130	1.30	1.84	1	3
	Triphasic oral contraceptive	20	1.50	.688	.154	1.18	1.82	1	3
	Total	101	1.52	.576	.057	1.41	1.64	1	3
Roa-Goldman scale mouth area	Monophasic oral contraceptive	20	1.75	.444	.099	1.54	1.96	1	2
	Non contraceptive	20	1.50	.513	.115	1.26	1.74	1	2
	Injectable contraceptive	20	1.65	.489	.109	1.42	1.88	1	2
	Implantable contraceptive	21	1.52	.602	.131	1.25	1.80	1	3
	Triphasic oral contraceptive	20	1.40	.598	.134	1.12	1.68	1	3
	Total	101	1.56	.537	.053	1.46	1.67	1	3
Systolic Blood pressure	Monophasic oral contraceptive	20	128.20	17.386	3.888	120.06	136.34	106	180
	Non contraceptive	20	125.05	13.632	3.048	118.67	131.43	106	159
	Injectable contraceptive	20	132.75	15.525	3.472	125.48	140.02	112	174
	Implantable contraceptive	21	131.05	14.333	3.128	124.52	137.57	110	173
	Triphasic oral contraceptive	20	126.00	9.889	2.211	121.37	130.63	109	147
	Total	101	128.63	14.381	1.431	125.79	131.47	106	180
Diastolic Blood pressure	Monophasic oral contraceptive	20	89.50	16.631	3.719	81.72	97.28	70	129
	Non contraceptive	20	80.65	7.748	1.732	77.02	84.28	67	98
	Injectable contraceptive	20	84.45	10.231	2.288	79.66	89.24	66	109
	Implantable contraceptive	21	85.43	9.610	2.097	81.05	89.80	62	102
	Triphasic oral contraceptive	20	84.50	9.434	2.110	80.08	88.92	60	99
	Total	101	84.91	11.278	1.122	82.68	87.14	60	129
Heart rate	Monophasic oral contraceptive	20	80.00	9.131	2.042	75.73	84.27	61	98
	Non contraceptive	20	81.10	13.696	3.062	74.69	87.51	66	110
	Injectable contraceptive	20	86.65	14.929	3.338	79.66	93.64	62	117
	Implantable contraceptive	21	83.76	15.703	3.427	76.61	90.91	52	108
	Triphasic oral contraceptive	20	85.75	14.661	3.278	78.89	92.61	65	115
	Total	101	83.46	13.805	1.374	80.73	86.18	52	117
Weight	Monophasic oral contraceptive	20	60.51000	13.421267	3.001087	54.22865	66.79135	44.500	106.500
	Non contraceptive	20	60.52500	13.653258	3.052961	54.13508	66.91492	40.400	94.000
	Injectable contraceptive	20	69.29500	17.076468	3.818414	61.30297	77.28703	45.000	115.700

Descriptives									
		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
	Implantable contraceptive	21	73.60476	21.762203	4.748902	63.69873	83.51080	45.600	147.000
	Triphasic oral contraceptive	20	69.80000	21.342693	4.772371	59.81131	79.78869	45.500	116.500
	Total	101	66.81485	18.299467	1.820865	63.20231	70.42740	40.400	147.000
Min recommended weight	Monophasic oral contraceptive	20	53.60	8.165	1.826	49.78	57.42	44	74
	Non contraceptive	20	51.95	5.356	1.198	49.44	54.46	44	65
	Injectable contraceptive	20	54.50	6.809	1.523	51.31	57.69	45	72
	Implantable contraceptive	21	58.24	11.661	2.545	52.93	63.55	44	91
	Triphasic oral contraceptive	20	54.10	6.078	1.359	51.26	56.94	44	68
	Total	101	54.51	8.098	.806	52.92	56.11	44	91
Max recommended weight	Monophasic oral contraceptive	20	58.60	8.506	1.902	54.62	62.58	49	80
	Non contraceptive	20	57.00	5.410	1.210	54.47	59.53	49	70
	Injectable contraceptive	20	59.35	7.118	1.592	56.02	62.68	50	78
	Implantable contraceptive	21	62.86	12.511	2.730	57.16	68.55	48	98
	Triphasic oral contraceptive	20	58.95	6.245	1.396	56.03	61.87	48	74
	Total	101	59.39	8.455	.841	57.72	61.06	48	98
Waist to hip ratio	Monophasic oral contraceptive	20	.71900	.050877	.011376	.69519	.74281	.630	.810
	Non contraceptive	20	.70650	.052342	.011704	.68200	.73100	.630	.850
	Injectable contraceptive	20	.74750	.071147	.015909	.71420	.78080	.630	.890
	Implantable contraceptive	21	.77429	.079597	.017370	.73805	.81052	.630	.930
	Triphasic oral contraceptive	20	.73650	.068462	.015309	.70446	.76854	.600	.870
	Total	101	.73713	.068532	.006819	.72360	.75066	.600	.930
Skin fold measurement	Monophasic oral contraceptive	20	22.14500	10.948659	2.448195	17.02087	27.26913	12.000	50.000
	Non contraceptive	20	20.35000	8.499381	1.900519	16.37217	24.32783	10.000	35.000
	Injectable contraceptive	20	26.45000	9.795138	2.190260	21.86573	31.03427	10.000	47.000
	Implantable contraceptive	21	27.47619	11.944116	2.606420	22.03929	32.91309	12.000	50.000
	Triphasic oral contraceptive	20	25.65000	11.202796	2.505021	20.40693	30.89307	12.000	48.000
	Total	101	24.44455	10.702546	1.064943	22.33174	26.55737	10.000	50.000
Adipose tissue	Monophasic oral contraceptive	20	32.33500	6.542837	1.463023	29.27286	35.39714	17.700	44.400
	Non contraceptive	20	33.20500	6.337730	1.417159	30.23885	36.17115	23.400	49.700
	Injectable contraceptive	20	36.85500	9.396274	2.101071	32.45741	41.25259	21.700	57.100
	Implantable contraceptive	21	35.29048	11.595814	2.530414	30.01212	40.56883	13.700	53.500
	Triphasic oral contraceptive	20	35.41500	8.796367	1.966928	31.29817	39.53183	22.700	53.300
	Total	101	34.62673	8.763503	.872001	32.89671	36.35676	13.700	57.100
Lean tissue	Monophasic oral contraceptive	20	67.63224	6.547203	1.463999	64.56806	70.69643	55.587	82.332

Descriptives									
		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
	Non contraceptive	20	65.13294	9.721072	2.173698	60.58334	69.68254	39.681	76.600
	Injectable contraceptive	20	63.10627	9.396610	2.101146	58.70852	67.50402	42.947	78.349
	Implantable contraceptive	21	64.71204	11.610307	2.533577	59.42709	69.99699	46.514	86.275
	Triphasic oral contraceptive	20	64.56688	8.794752	1.966566	60.45081	68.68295	46.695	77.336
	Total	101	65.02693	9.310869	.926466	63.18884	66.86501	39.681	86.275
Water content	Monophasic oral contraceptive	20	47.63000	5.251175	1.174198	45.17237	50.08763	38.400	58.500
	Non contraceptive	20	46.20000	7.096997	1.586937	42.87850	49.52150	28.000	55.700
	Injectable contraceptive	20	44.27000	6.967300	1.557936	41.00920	47.53080	30.900	56.700
	Implantable contraceptive	21	45.61905	9.210028	2.009793	41.42669	49.81140	30.400	64.900
	Triphasic oral contraceptive	20	45.56500	7.020442	1.569819	42.27933	48.85067	33.000	56.700
	Total	101	45.85446	7.179589	.714396	44.43711	47.27180	28.000	64.900
Estimated resting metabolic rate	Monophasic oral contraceptive	20	1405.45	152.783	34.163	1333.95	1476.95	1165	1857
	Non contraceptive	20	1390.70	147.982	33.090	1321.44	1459.96	1105	1675
	Injectable contraceptive	20	1455.90	147.281	32.933	1386.97	1524.83	1225	1819
	Implantable contraceptive	21	1535.81	185.697	40.522	1451.28	1620.34	1223	2182
	Triphasic oral contraceptive	20	1489.75	189.703	42.419	1400.97	1578.53	1204	1855
	Total	101	1456.32	171.408	17.056	1422.48	1490.15	1105	2182
Estimated average energy required	Monophasic oral contraceptive	20	2266.15	244.178	54.600	2151.87	2380.43	1981	2786
	Non contraceptive	20	2193.40	279.606	62.522	2062.54	2324.26	1547	2680
	Injectable contraceptive	20	2291.15	319.437	71.428	2141.65	2440.65	1838	2966
	Implantable contraceptive	21	2452.38	342.639	74.770	2296.41	2608.35	1957	3491
	Triphasic oral contraceptive	20	2270.35	253.422	56.667	2151.74	2388.96	1805	2787
	Total	101	2296.25	298.007	29.653	2237.42	2355.08	1547	3491
BMI	Monophasic oral contraceptive	20	24.11000	5.214040	1.165895	21.66975	26.55025	19.700	41.600
	Non contraceptive	20	24.15000	5.590264	1.250021	21.53368	26.76632	17.500	41.800
	Injectable contraceptive	20	27.72500	7.028654	1.571655	24.43549	31.01451	20.000	42.000
	Implantable contraceptive	21	28.71429	6.984074	1.524050	25.53517	31.89340	20.800	48.000
	Triphasic oral contraceptive	20	27.57500	7.861825	1.757957	23.89555	31.25445	18.700	43.900
	Total	101	26.47723	6.767258	.673367	25.14129	27.81317	17.500	48.000
Pigmentation Forehead to Cheek	Monophasic oral contraceptive	20	1.27930	.184559	.041269	1.19293	1.36568	1.036	1.714
	Non contraceptive	20	1.23812	.279604	.062521	1.10726	1.36898	1.033	2.278
	Injectable contraceptive	20	1.17984	.166100	.037141	1.10210	1.25758	1.018	1.619
	Implantable contraceptive	21	1.19385	.253435	.055304	1.07849	1.30922	1.000	2.042
	Triphasic oral contraceptive	20	1.10654	.110861	.024789	1.05465	1.15842	1.000	1.364

Descriptives									
		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
	Total	101	1.19947	.212445	.021139	1.15753	1.24141	1.000	2.278
Pigmentation Forehead to Chin	Monophasic oral contraceptive	20	1.48178	.321198	.071822	1.33145	1.63210	1.061	2.143
	Non contraceptive	20	1.46348	.322553	.072125	1.31252	1.61444	1.042	2.556
	Injectable contraceptive	20	1.36396	.289389	.064709	1.22852	1.49940	1.000	2.095
	Implantable contraceptive	21	1.44988	.331856	.072417	1.29882	1.60094	1.050	2.333
	Triphasic oral contraceptive	20	1.28596	.252409	.056440	1.16783	1.40409	1.000	1.909
	Total	101	1.40942	.307976	.030645	1.34862	1.47021	1.000	2.556
Pigmentation Chin to Cheek	Monophasic oral contraceptive	20	1.34085	.301640	.067449	1.19968	1.48202	1.038	2.250
	Non contraceptive	20	1.22860	.159346	.035631	1.15403	1.30318	1.000	1.577
	Injectable contraceptive	20	1.21174	.194173	.043418	1.12087	1.30262	1.000	1.714
	Implantable contraceptive	21	1.26409	.172295	.037598	1.18566	1.34252	1.049	1.643
	Triphasic oral contraceptive	20	1.21273	.206971	.046280	1.11587	1.30960	1.000	1.818
	Total	101	1.25173	.213783	.021272	1.20952	1.29393	1.000	2.250
Max ratio of Pigmentation	Monophasic oral contraceptive	20	1.57931	.303454	.067854	1.43729	1.72133	1.280	2.250
	Non contraceptive	20	1.48593	.316653	.070806	1.33773	1.63413	1.076	2.556
	Injectable contraceptive	20	1.39265	.274484	.061376	1.26418	1.52111	1.031	2.095
	Implantable contraceptive	21	1.47410	.322600	.070397	1.32726	1.62095	1.113	2.333
	Triphasic oral contraceptive	20	1.31291	.252046	.056359	1.19495	1.43087	1.000	1.909
	Total	101	1.44923	.303128	.030162	1.38939	1.50907	1.000	2.556

Post Hoc Tests

Multiple Comparisons							
Bonferroni							
Dependent Variable	(I) GroupCode	(J) GroupCode	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Catalase	Monophasic oral contraceptive	Non contraceptive	.064423	.672729	1.000	-1.86857	1.99742
		Injectable contraceptive	1.241802	.672729	.680	-.69119	3.17480
		Implantable contraceptive	.701372	.664673	1.000	-1.20847	2.61122
		Triphasic oral contraceptive	1.277429	.672729	.606	-.65556	3.21042
	Non contraceptive	Monophasic oral contraceptive	-.064423	.672729	1.000	-1.99742	1.86857
		Injectable contraceptive	1.177379	.672729	.833	-.75562	3.11037
		Implantable contraceptive	.636949	.664673	1.000	-1.27289	2.54679
		Triphasic oral contraceptive	1.213006	.672729	.745	-.71999	3.14600
	Injectable contraceptive	Monophasic oral contraceptive	-1.241802	.672729	.680	-3.17480	.69119
		Non contraceptive	-1.177379	.672729	.833	-3.11037	.75562
		Implantable contraceptive	-.540430	.664673	1.000	-2.45027	1.36941
		Triphasic oral contraceptive	.035627	.672729	1.000	-1.89737	1.96862

Multiple Comparisons

Bonferroni

Dependent	(I) GroupCode	(J) GroupCode	Mean	Std. Error	Sig.	95% Confidence Interval	
	Implantable contraceptive	Monophasic oral contraceptive	-.701372	.664673	1.000	-2.61122	1.20847
		Non contraceptive	-.636949	.664673	1.000	-2.54679	1.27289
		Injectable contraceptive	.540430	.664673	1.000	-1.36941	2.45027
		Triphasic oral contraceptive	.576057	.664673	1.000	-1.33379	2.48590
	Triphasic oral contraceptive	Monophasic oral contraceptive	-1.277429	.672729	.606	-3.21042	.65556
		Non contraceptive	-1.213006	.672729	.745	-3.14600	.71999
		Injectable contraceptive	-.035627	.672729	1.000	-1.96862	1.89737
		Implantable contraceptive	-.576057	.664673	1.000	-2.48590	1.33379
SOD	Monophasic oral contraceptive	Non contraceptive	-10.233027	6.519516	1.000	-28.96594	8.49989
		Injectable contraceptive	-13.677176	6.519516	.385	-32.41009	5.05574
		Implantable contraceptive	-15.721385	6.441435	.165	-34.22995	2.78718
		Triphasic oral contraceptive	-25.004825*	6.519516	.002	-43.73774	-6.27191
	Non contraceptive	Monophasic oral contraceptive	10.233027	6.519516	1.000	-8.49989	28.96594
		Injectable contraceptive	-3.444149	6.519516	1.000	-22.17706	15.28877
		Implantable contraceptive	-5.488358	6.441435	1.000	-23.99692	13.02020
		Triphasic oral contraceptive	-14.771797	6.519516	.257	-33.50471	3.96112
	Injectable contraceptive	Monophasic oral contraceptive	13.677176	6.519516	.385	-5.05574	32.41009
		Non contraceptive	3.444149	6.519516	1.000	-15.28877	22.17706
		Implantable contraceptive	-2.044209	6.441435	1.000	-20.55277	16.46435
		Triphasic oral contraceptive	-11.327648	6.519516	.855	-30.06056	7.40527
	Implantable contraceptive	Monophasic oral contraceptive	15.721385	6.441435	.165	-2.78718	34.22995
		Non contraceptive	5.488358	6.441435	1.000	-13.02020	23.99692
		Injectable contraceptive	2.044209	6.441435	1.000	-16.46435	20.55277
		Triphasic oral contraceptive	-9.283440	6.441435	1.000	-27.79200	9.22512
	Triphasic oral contraceptive	Monophasic oral contraceptive	25.004825*	6.519516	.002	6.27191	43.73774
		Non contraceptive	14.771797	6.519516	.257	-3.96112	33.50471
		Injectable contraceptive	11.327648	6.519516	.855	-7.40527	30.06056
		Implantable contraceptive	9.283440	6.441435	1.000	-9.22512	27.79200
ORAC	Monophasic oral contraceptive	Non contraceptive	31.682022	343.943907	1.000	-956.59260	1019.95664
		Injectable contraceptive	461.552433	343.943907	1.000	-526.72218	1449.82705
		Implantable contraceptive	-278.853490	339.824669	1.000	-1255.29206	697.58508
		Triphasic oral contraceptive	-692.987268	343.943907	.467	-1681.26189	295.28735
	Non contraceptive	Monophasic oral contraceptive	-31.682022	343.943907	1.000	-1019.95664	956.59260
		Injectable contraceptive	429.870412	343.943907	1.000	-558.40421	1418.14503

Multiple Comparisons

Bonferroni

Dependent	(I) GroupCode	(J) GroupCode	Mean	Std. Error	Sig.	95% Confidence Interval	
		Implantable contraceptive	-310.535512	339.824669	1.000	-1286.97408	665.90305
		Triphasic oral contraceptive	-724.669289	343.943907	.377	-1712.94391	263.60533
	Injectable contraceptive	Monophasic oral contraceptive	-461.552433	343.943907	1.000	-1449.82705	526.72218
		Non contraceptive	-429.870412	343.943907	1.000	-1418.14503	558.40421
		Implantable contraceptive	-740.405923	339.824669	.318	-1716.84449	236.03264
		Triphasic oral contraceptive	-1154.539701*	343.943907	.011	-2142.81432	-166.26508
	Implantable contraceptive	Monophasic oral contraceptive	278.853490	339.824669	1.000	-697.58508	1255.29206
		Non contraceptive	310.535512	339.824669	1.000	-665.90305	1286.97408
		Injectable contraceptive	740.405923	339.824669	.318	-236.03264	1716.84449
		Triphasic oral contraceptive	-414.133778	339.824669	1.000	-1390.57234	562.30479
	Triphasic oral contraceptive	Monophasic oral contraceptive	692.987268	343.943907	.467	-295.28735	1681.26189
		Non contraceptive	724.669289	343.943907	.377	-263.60533	1712.94391
		Injectable contraceptive	1154.539701*	343.943907	.011	166.26508	2142.81432
		Implantable contraceptive	414.133778	339.824669	1.000	-562.30479	1390.57234
GSH	Monophasic oral contraceptive	Non contraceptive	98.327086	64.609029	1.000	-87.31790	283.97208
		Injectable contraceptive	79.717337	64.609029	1.000	-105.92765	265.36233
		Implantable contraceptive	-1.864842	63.835240	1.000	-185.28646	181.55678
		Triphasic oral contraceptive	28.166514	64.609029	1.000	-157.47848	213.81150
	Non contraceptive	Monophasic oral contraceptive	-98.327086	64.609029	1.000	-283.97208	87.31790
		Injectable contraceptive	-18.609750	64.609029	1.000	-204.25474	167.03524
		Implantable contraceptive	-100.191928	63.835240	1.000	-283.61354	83.22969
		Triphasic oral contraceptive	-70.160572	64.609029	1.000	-255.80556	115.48442
	Injectable contraceptive	Monophasic oral contraceptive	-79.717337	64.609029	1.000	-265.36233	105.92765
		Non contraceptive	18.609750	64.609029	1.000	-167.03524	204.25474
		Implantable contraceptive	-81.582179	63.835240	1.000	-265.00380	101.83944
		Triphasic oral contraceptive	-51.550823	64.609029	1.000	-237.19581	134.09417
	Implantable contraceptive	Monophasic oral contraceptive	1.864842	63.835240	1.000	-181.55678	185.28646
		Non contraceptive	100.191928	63.835240	1.000	-83.22969	283.61354
		Injectable contraceptive	81.582179	63.835240	1.000	-101.83944	265.00380
		Triphasic oral contraceptive	30.031356	63.835240	1.000	-153.39026	213.45297
	Triphasic oral contraceptive	Monophasic oral contraceptive	-28.166514	64.609029	1.000	-213.81150	157.47848

Multiple Comparisons

Bonferroni

Dependent	(I) GroupCode	(J) GroupCode	Mean	Std. Error	Sig.	95% Confidence Interval	
		Non contraceptive	70.160572	64.609029	1.000	-115.48442	255.80556
		Injectable contraceptive	51.550823	64.609029	1.000	-134.09417	237.19581
		Implantable contraceptive	-30.031356	63.835240	1.000	-213.45297	153.39026
GSSG	Monophasic oral contraceptive	Non contraceptive	-.743636	2.641175	1.000	-8.33268	6.84541
		Injectable contraceptive	4.557195	2.641175	.877	-3.03185	12.14624
		Implantable contraceptive	3.991651	2.609543	1.000	-3.50651	11.48981
		Triphasic oral contraceptive	4.263369	2.641175	1.000	-3.32568	11.85242
	Non contraceptive	Monophasic oral contraceptive	.743636	2.641175	1.000	-6.84541	8.33268
		Injectable contraceptive	5.300830	2.641175	.476	-2.28822	12.88988
		Implantable contraceptive	4.735287	2.609543	.727	-2.76287	12.23344
		Triphasic oral contraceptive	5.007004	2.641175	.610	-2.58204	12.59605
	Injectable contraceptive	Monophasic oral contraceptive	-4.557195	2.641175	.877	-12.14624	3.03185
		Non contraceptive	-5.300830	2.641175	.476	-12.88988	2.28822
		Implantable contraceptive	-.565544	2.609543	1.000	-8.06370	6.93261
		Triphasic oral contraceptive	-.293826	2.641175	1.000	-7.88287	7.29522
	Implantable contraceptive	Monophasic oral contraceptive	-3.991651	2.609543	1.000	-11.48981	3.50651
		Non contraceptive	-4.735287	2.609543	.727	-12.23344	2.76287
		Injectable contraceptive	.565544	2.609543	1.000	-6.93261	8.06370
		Triphasic oral contraceptive	.271718	2.609543	1.000	-7.22644	7.76987
	Triphasic oral contraceptive	Monophasic oral contraceptive	-4.263369	2.641175	1.000	-11.85242	3.32568
		Non contraceptive	-5.007004	2.641175	.610	-12.59605	2.58204
		Injectable contraceptive	.293826	2.641175	1.000	-7.29522	7.88287
		Implantable contraceptive	-.271718	2.609543	1.000	-7.76987	7.22644
GSH/GSSG	Monophasic oral contraceptive	Non contraceptive	5.576671	84.120989	1.000	-236.13320	247.28654
		Injectable contraceptive	-22.535823	84.120989	1.000	-264.24570	219.17405
		Implantable contraceptive	-13.019284	83.113515	1.000	-251.83432	225.79575
		Triphasic oral contraceptive	-124.415793	84.120989	1.000	-366.12566	117.29408
	Non contraceptive	Monophasic oral contraceptive	-5.576671	84.120989	1.000	-247.28654	236.13320
		Injectable contraceptive	-28.112494	84.120989	1.000	-269.82237	213.59738
		Implantable contraceptive	-18.595955	83.113515	1.000	-257.41099	220.21908
		Triphasic oral contraceptive	-129.992464	84.120989	1.000	-371.70234	111.71741
	Injectable contraceptive	Monophasic oral contraceptive	22.535823	84.120989	1.000	-219.17405	264.24570
		Non contraceptive	28.112494	84.120989	1.000	-213.59738	269.82237

Multiple Comparisons							
Bonferroni							
Dependent	(I) GroupCode	(J) GroupCode	Mean	Std. Error	Sig.	95% Confidence Interval	
		Implantable contraceptive	9.516539	83.113515	1.000	-229.29850	248.33158
		Triphasic oral contraceptive	-101.879970	84.120989	1.000	-343.58984	139.82990
	Implantable contraceptive	Monophasic oral contraceptive	13.019284	83.113515	1.000	-225.79575	251.83432
		Non contraceptive	18.595955	83.113515	1.000	-220.21908	257.41099
		Injectable contraceptive	-9.516539	83.113515	1.000	-248.33158	229.29850
		Triphasic oral contraceptive	-111.396509	83.113515	1.000	-350.21155	127.41853
	Triphasic oral contraceptive	Monophasic oral contraceptive	124.415793	84.120989	1.000	-117.29408	366.12566
		Non contraceptive	129.992464	84.120989	1.000	-111.71741	371.70234
		Injectable contraceptive	101.879970	84.120989	1.000	-139.82990	343.58984
		Implantable contraceptive	111.396509	83.113515	1.000	-127.41853	350.21155
TBARS	Monophasic oral contraceptive	Non contraceptive	.003878	11.741866	1.000	-33.73473	33.74248
		Injectable contraceptive	.001048	11.741866	1.000	-33.73756	33.73965
		Implantable contraceptive	-.024057	11.601239	1.000	-33.35859	33.31048
		Triphasic oral contraceptive	-18.704841	11.741866	1.000	-52.44345	15.03376
	Non contraceptive	Monophasic oral contraceptive	-.003878	11.741866	1.000	-33.74248	33.73473
		Injectable contraceptive	-.002830	11.741866	1.000	-33.74144	33.73578
		Implantable contraceptive	-.027935	11.601239	1.000	-33.36247	33.30660
		Triphasic oral contraceptive	-18.708720	11.741866	1.000	-52.44732	15.02989
	Injectable contraceptive	Monophasic oral contraceptive	-.001048	11.741866	1.000	-33.73965	33.73756
		Non contraceptive	.002830	11.741866	1.000	-33.73578	33.74144
		Implantable contraceptive	-.025105	11.601239	1.000	-33.35964	33.30943
		Triphasic oral contraceptive	-18.705889	11.741866	1.000	-52.44449	15.03272
	Implantable contraceptive	Monophasic oral contraceptive	.024057	11.601239	1.000	-33.31048	33.35859
		Non contraceptive	.027935	11.601239	1.000	-33.30660	33.36247
		Injectable contraceptive	.025105	11.601239	1.000	-33.30943	33.35964
		Triphasic oral contraceptive	-18.680785	11.601239	1.000	-52.01532	14.65375
	Triphasic oral contraceptive	Monophasic oral contraceptive	18.704841	11.741866	1.000	-15.03376	52.44345
		Non contraceptive	18.708720	11.741866	1.000	-15.02989	52.44732
		Injectable contraceptive	18.705889	11.741866	1.000	-15.03272	52.44449
		Implantable contraceptive	18.680785	11.601239	1.000	-14.65375	52.01532
FRAP	Monophasic oral	Non contraceptive	22.816946	23.500709	1.000	-44.70905	90.34294

Multiple Comparisons

Bonferroni

Dependent	(I) GroupCode	(J) GroupCode	Mean	Std. Error	Sig.	95% Confidence Interval	
	contraceptive	Injectable contraceptive	-12.011972	23.500709	1.000	-79.53797	55.51402
		Implantable contraceptive	-19.355340	23.219253	1.000	-86.07261	47.36193
		Triphasic oral contraceptive	44.115882	23.500709	.635	-23.41011	111.64188
	Non contraceptive	Monophasic oral contraceptive	-22.816946	23.500709	1.000	-90.34294	44.70905
		Injectable contraceptive	-34.828918	23.500709	1.000	-102.35491	32.69708
		Implantable contraceptive	-42.172286	23.219253	.725	-108.88956	24.54499
		Triphasic oral contraceptive	21.298936	23.500709	1.000	-46.22706	88.82493
	Injectable contraceptive	Monophasic oral contraceptive	12.011972	23.500709	1.000	-55.51402	79.53797
		Non contraceptive	34.828918	23.500709	1.000	-32.69708	102.35491
		Implantable contraceptive	-7.343368	23.219253	1.000	-74.06064	59.37390
		Triphasic oral contraceptive	56.127854	23.500709	.189	-11.39814	123.65385
	Implantable contraceptive	Monophasic oral contraceptive	19.355340	23.219253	1.000	-47.36193	86.07261
		Non contraceptive	42.172286	23.219253	.725	-24.54499	108.88956
		Injectable contraceptive	7.343368	23.219253	1.000	-59.37390	74.06064
		Triphasic oral contraceptive	63.471222	23.219253	.075	-3.24605	130.18849
	Triphasic oral contraceptive	Monophasic oral contraceptive	-44.115882	23.500709	.635	-111.64188	23.41011
		Non contraceptive	-21.298936	23.500709	1.000	-88.82493	46.22706
		Injectable contraceptive	-56.127854	23.500709	.189	-123.65385	11.39814
		Implantable contraceptive	-63.471222	23.219253	.075	-130.18849	3.24605
Average: Erythema	Monophasic oral contraceptive	Non contraceptive	-6.116667*	1.666529	.004	-10.90520	-1.32813
		Injectable contraceptive	-10.550000*	1.666529	.000	-15.33854	-5.76146
		Implantable contraceptive	-8.873810*	1.646570	.000	-13.60500	-4.14262
		Triphasic oral contraceptive	-7.500000*	1.666529	.000	-12.28854	-2.71146
	Non contraceptive	Monophasic oral contraceptive	6.116667*	1.666529	.004	1.32813	10.90520
		Injectable contraceptive	-4.433333	1.666529	.092	-9.22187	.35520
		Implantable contraceptive	-2.757143	1.646570	.973	-7.48833	1.97405
		Triphasic oral contraceptive	-1.383333	1.666529	1.000	-6.17187	3.40520
	Injectable contraceptive	Monophasic oral contraceptive	10.550000*	1.666529	.000	5.76146	15.33854
		Non contraceptive	4.433333	1.666529	.092	-.35520	9.22187
		Implantable contraceptive	1.676190	1.646570	1.000	-3.05500	6.40738
		Triphasic oral contraceptive	3.050000	1.666529	.703	-1.73854	7.83854
	Implantable contraceptive	Monophasic oral contraceptive	8.873810*	1.646570	.000	4.14262	13.60500
		Non contraceptive	2.757143	1.646570	.973	-1.97405	7.48833

Multiple Comparisons							
Bonferroni							
Dependent	(I) GroupCode	(J) GroupCode	Mean	Std. Error	Sig.	95% Confidence Interval	
		Injectable contraceptive	-1.676190	1.646570	1.000	-6.40738	3.05500
		Triphasic oral contraceptive	1.373810	1.646570	1.000	-3.35738	6.10500
	Triphasic oral contraceptive	Monophasic oral contraceptive	7.500000*	1.666529	.000	2.71146	12.28854
		Non contraceptive	1.383333	1.666529	1.000	-3.40520	6.17187
		Injectable contraceptive	-3.050000	1.666529	.703	-7.83854	1.73854
		Implantable contraceptive	-1.373810	1.646570	1.000	-6.10500	3.35738
Forehead: Melanin percentage	Monophasic oral contraceptive	Non contraceptive	-26.300*	7.820	.011	-48.77	-3.83
		Injectable contraceptive	-36.900*	7.820	.000	-59.37	-14.43
		Implantable contraceptive	-32.343*	7.727	.001	-54.54	-10.14
		Triphasic oral contraceptive	-32.350*	7.820	.001	-54.82	-9.88
	Non contraceptive	Monophasic oral contraceptive	26.300*	7.820	.011	3.83	48.77
		Injectable contraceptive	-10.600	7.820	1.000	-33.07	11.87
		Implantable contraceptive	-6.043	7.727	1.000	-28.24	16.16
		Triphasic oral contraceptive	-6.050	7.820	1.000	-28.52	16.42
	Injectable contraceptive	Monophasic oral contraceptive	36.900*	7.820	.000	14.43	59.37
		Non contraceptive	10.600	7.820	1.000	-11.87	33.07
		Implantable contraceptive	4.557	7.727	1.000	-17.64	26.76
		Triphasic oral contraceptive	4.550	7.820	1.000	-17.92	27.02
	Implantable contraceptive	Monophasic oral contraceptive	32.343*	7.727	.001	10.14	54.54
		Non contraceptive	6.043	7.727	1.000	-16.16	28.24
		Injectable contraceptive	-4.557	7.727	1.000	-26.76	17.64
		Triphasic oral contraceptive	-.007	7.727	1.000	-22.21	22.19
	Triphasic oral contraceptive	Monophasic oral contraceptive	32.350*	7.820	.001	9.88	54.82
		Non contraceptive	6.050	7.820	1.000	-16.42	28.52
		Injectable contraceptive	-4.550	7.820	1.000	-27.02	17.92
		Implantable contraceptive	.007	7.727	1.000	-22.19	22.21
Cheek: Melanin percentage	Monophasic oral contraceptive	Non contraceptive	-22.900*	7.101	.017	-43.30	-2.50
		Injectable contraceptive	-34.450*	7.101	.000	-54.85	-14.05
		Implantable contraceptive	-30.979*	7.016	.000	-51.14	-10.82
		Triphasic oral contraceptive	-33.950*	7.101	.000	-54.35	-13.55
	Non contraceptive	Monophasic oral contraceptive	22.900*	7.101	.017	2.50	43.30
		Injectable contraceptive	-11.550	7.101	1.000	-31.95	8.85
		Implantable contraceptive	-8.079	7.016	1.000	-28.24	12.08
		Triphasic oral contraceptive	-11.050	7.101	1.000	-31.45	9.35

Multiple Comparisons							
Bonferroni							
Dependent	(I) GroupCode	(J) GroupCode	Mean	Std. Error	Sig.	95% Confidence Interval	
	Injectable contraceptive	Monophasic oral contraceptive	34.450*	7.101	.000	14.05	54.85
		Non contraceptive	11.550	7.101	1.000	-8.85	31.95
		Implantable contraceptive	3.471	7.016	1.000	-16.69	23.63
		Triphasic oral contraceptive	.500	7.101	1.000	-19.90	20.90
	Implantable contraceptive	Monophasic oral contraceptive	30.979*	7.016	.000	10.82	51.14
		Non contraceptive	8.079	7.016	1.000	-12.08	28.24
		Injectable contraceptive	-3.471	7.016	1.000	-23.63	16.69
		Triphasic oral contraceptive	-2.971	7.016	1.000	-23.13	17.19
	Triphasic oral contraceptive	Monophasic oral contraceptive	33.950*	7.101	.000	13.55	54.35
		Non contraceptive	11.050	7.101	1.000	-9.35	31.45
		Injectable contraceptive	-.500	7.101	1.000	-20.90	19.90
		Implantable contraceptive	2.971	7.016	1.000	-17.19	23.13
Chin: Melanin percentage	Monophasic oral contraceptive	Non contraceptive	-31.200*	7.653	.001	-53.19	-9.21
		Injectable contraceptive	-42.250*	7.653	.000	-64.24	-20.26
		Implantable contraceptive	-39.233*	7.561	.000	-60.96	-17.51
		Triphasic oral contraceptive	-35.250*	7.653	.000	-57.24	-13.26
	Non contraceptive	Monophasic oral contraceptive	31.200*	7.653	.001	9.21	53.19
		Injectable contraceptive	-11.050	7.653	1.000	-33.04	10.94
		Implantable contraceptive	-8.033	7.561	1.000	-29.76	13.69
		Triphasic oral contraceptive	-4.050	7.653	1.000	-26.04	17.94
	Injectable contraceptive	Monophasic oral contraceptive	42.250*	7.653	.000	20.26	64.24
		Non contraceptive	11.050	7.653	1.000	-10.94	33.04
		Implantable contraceptive	3.017	7.561	1.000	-18.71	24.74
		Triphasic oral contraceptive	7.000	7.653	1.000	-14.99	28.99
	Implantable contraceptive	Monophasic oral contraceptive	39.233*	7.561	.000	17.51	60.96
		Non contraceptive	8.033	7.561	1.000	-13.69	29.76
		Injectable contraceptive	-3.017	7.561	1.000	-24.74	18.71
		Triphasic oral contraceptive	3.983	7.561	1.000	-17.74	25.71
	Triphasic oral contraceptive	Monophasic oral contraceptive	35.250*	7.653	.000	13.26	57.24
		Non contraceptive	4.050	7.653	1.000	-17.94	26.04
		Injectable contraceptive	-7.000	7.653	1.000	-28.99	14.99
		Implantable contraceptive	-3.983	7.561	1.000	-25.71	17.74
Average: Hydration levels	Monophasic oral contraceptive	Non contraceptive	-.491667	1.374929	1.000	-4.44233	3.45900
		Injectable contraceptive	-4.711667*	1.374929	.009	-8.66233	-.76100
		Implantable contraceptive	-6.053254*	1.358462	.000	-9.95660	-2.14990

Multiple Comparisons

Bonferroni

Dependent	(I) GroupCode	(J) GroupCode	Mean	Std. Error	Sig.	95% Confidence Interval	
		Triphasic oral contraceptive	-2.623333	1.374929	.594	-6.57400	1.32733
	Non contraceptive	Monophasic oral contraceptive	.491667	1.374929	1.000	-3.45900	4.44233
		Injectable contraceptive	-4.220000*	1.374929	.028	-8.17067	-.26933
		Implantable contraceptive	-5.561587*	1.358462	.001	-9.46494	-1.65824
		Triphasic oral contraceptive	-2.131667	1.374929	1.000	-6.08233	1.81900
	Injectable contraceptive	Monophasic oral contraceptive	4.711667*	1.374929	.009	.76100	8.66233
		Non contraceptive	4.220000*	1.374929	.028	.26933	8.17067
		Implantable contraceptive	-1.341587	1.358462	1.000	-5.24494	2.56176
		Triphasic oral contraceptive	2.088333	1.374929	1.000	-1.86233	6.03900
	Implantable contraceptive	Monophasic oral contraceptive	6.053254*	1.358462	.000	2.14990	9.95660
		Non contraceptive	5.561587*	1.358462	.001	1.65824	9.46494
		Injectable contraceptive	1.341587	1.358462	1.000	-2.56176	5.24494
		Triphasic oral contraceptive	3.429921	1.358462	.132	-.47343	7.33327
	Triphasic oral contraceptive	Monophasic oral contraceptive	2.623333	1.374929	.594	-1.32733	6.57400
		Non contraceptive	2.131667	1.374929	1.000	-1.81900	6.08233
		Injectable contraceptive	-2.088333	1.374929	1.000	-6.03900	1.86233
		Implantable contraceptive	-3.429921	1.358462	.132	-7.33327	.47343
Average: Sebum	Monophasic oral contraceptive	Non contraceptive	-7.150000	4.290754	.989	-19.47888	5.17888
		Injectable contraceptive	-8.735000	4.290754	.445	-21.06388	3.59388
		Implantable contraceptive	-3.398413	4.239366	1.000	-15.57964	8.78281
		Triphasic oral contraceptive	-2.516667	4.290754	1.000	-14.84555	9.81221
	Non contraceptive	Monophasic oral contraceptive	7.150000	4.290754	.989	-5.17888	19.47888
		Injectable contraceptive	-1.585000	4.290754	1.000	-13.91388	10.74388
		Implantable contraceptive	3.751587	4.239366	1.000	-8.42964	15.93281
		Triphasic oral contraceptive	4.633333	4.290754	1.000	-7.69555	16.96221
	Injectable contraceptive	Monophasic oral contraceptive	8.735000	4.290754	.445	-3.59388	21.06388
		Non contraceptive	1.585000	4.290754	1.000	-10.74388	13.91388
		Implantable contraceptive	5.336587	4.239366	1.000	-6.84464	17.51781
		Triphasic oral contraceptive	6.218333	4.290754	1.000	-6.11055	18.54721
	Implantable contraceptive	Monophasic oral contraceptive	3.398413	4.239366	1.000	-8.78281	15.57964
		Non contraceptive	-3.751587	4.239366	1.000	-15.93281	8.42964
		Injectable contraceptive	-5.336587	4.239366	1.000	-17.51781	6.84464
		Triphasic oral contraceptive	.881746	4.239366	1.000	-11.29948	13.06297
	Triphasic oral contraceptive	Monophasic oral contraceptive	2.516667	4.290754	1.000	-9.81221	14.84555
		Non contraceptive	-4.633333	4.290754	1.000	-16.96221	7.69555

Multiple Comparisons							
Bonferroni							
Dependent	(I) GroupCode	(J) GroupCode	Mean	Std. Error	Sig.	95% Confidence Interval	
		Injectable contraceptive	-6.218333	4.290754	1.000	-18.54721	6.11055
		Implantable contraceptive	-.881746	4.239366	1.000	-13.06297	11.29948
Average TEWL	Monophasic oral contraceptive	Non contraceptive	.000000	.683989	1.000	-1.96535	1.96535
		Injectable contraceptive	.183333	.683989	1.000	-1.78201	2.14868
		Implantable contraceptive	.066667	.675798	1.000	-1.87514	2.00848
		Triphasic oral contraceptive	-.333333	.683989	1.000	-2.29868	1.63201
	Non contraceptive	Monophasic oral contraceptive	.000000	.683989	1.000	-1.96535	1.96535
		Injectable contraceptive	.183333	.683989	1.000	-1.78201	2.14868
		Implantable contraceptive	.066667	.675798	1.000	-1.87514	2.00848
		Triphasic oral contraceptive	-.333333	.683989	1.000	-2.29868	1.63201
	Injectable contraceptive	Monophasic oral contraceptive	-.183333	.683989	1.000	-2.14868	1.78201
		Non contraceptive	-.183333	.683989	1.000	-2.14868	1.78201
		Implantable contraceptive	-.116667	.675798	1.000	-2.05848	1.82514
		Triphasic oral contraceptive	-.516667	.683989	1.000	-2.48201	1.44868
	Implantable contraceptive	Monophasic oral contraceptive	-.066667	.675798	1.000	-2.00848	1.87514
		Non contraceptive	-.066667	.675798	1.000	-2.00848	1.87514
		Injectable contraceptive	.116667	.675798	1.000	-1.82514	2.05848
		Triphasic oral contraceptive	-.400000	.675798	1.000	-2.34181	1.54181
	Triphasic oral contraceptive	Monophasic oral contraceptive	.333333	.683989	1.000	-1.63201	2.29868
		Non contraceptive	.333333	.683989	1.000	-1.63201	2.29868
		Injectable contraceptive	.516667	.683989	1.000	-1.44868	2.48201
		Implantable contraceptive	.400000	.675798	1.000	-1.54181	2.34181
Average Elasticity	Monophasic oral contraceptive	Non contraceptive	-3.450000	2.271589	1.000	-9.97709	3.07709
		Injectable contraceptive	-2.200000	2.271589	1.000	-8.72709	4.32709
		Implantable contraceptive	-3.625397	2.244383	1.000	-10.07432	2.82352
		Triphasic oral contraceptive	-2.533333	2.271589	1.000	-9.06042	3.99376
	Non contraceptive	Monophasic oral contraceptive	3.450000	2.271589	1.000	-3.07709	9.97709
		Injectable contraceptive	1.250000	2.271589	1.000	-5.27709	7.77709
		Implantable contraceptive	-.175397	2.244383	1.000	-6.62432	6.27352
		Triphasic oral contraceptive	.916667	2.271589	1.000	-5.61042	7.44376
	Injectable contraceptive	Monophasic oral contraceptive	2.200000	2.271589	1.000	-4.32709	8.72709
		Non contraceptive	-1.250000	2.271589	1.000	-7.77709	5.27709
		Implantable contraceptive	-1.425397	2.244383	1.000	-7.87432	5.02352
		Triphasic oral contraceptive	-.333333	2.271589	1.000	-6.86042	6.19376

Multiple Comparisons							
Bonferroni							
Dependent	(I) GroupCode	(J) GroupCode	Mean	Std. Error	Sig.	95% Confidence Interval	
	Implantable contraceptive	Monophasic oral contraceptive	3.625397	2.244383	1.000	-2.82352	10.07432
		Non contraceptive	.175397	2.244383	1.000	-6.27352	6.62432
		Injectable contraceptive	1.425397	2.244383	1.000	-5.02352	7.87432
		Triphasic oral contraceptive	1.092063	2.244383	1.000	-5.35686	7.54098
	Triphasic oral contraceptive	Monophasic oral contraceptive	2.533333	2.271589	1.000	-3.99376	9.06042
		Non contraceptive	-.916667	2.271589	1.000	-7.44376	5.61042
		Injectable contraceptive	.333333	2.271589	1.000	-6.19376	6.86042
		Implantable contraceptive	-1.092063	2.244383	1.000	-7.54098	5.35686
Roa-Goldman scale eye area	Monophasic oral contraceptive	Non contraceptive	.050	.186	1.000	-.48	.58
		Injectable contraceptive	.050	.186	1.000	-.48	.58
		Implantable contraceptive	-.021	.183	1.000	-.55	.51
		Triphasic oral contraceptive	.050	.186	1.000	-.48	.58
	Non contraceptive	Monophasic oral contraceptive	-.050	.186	1.000	-.58	.48
		Injectable contraceptive	.000	.186	1.000	-.53	.53
		Implantable contraceptive	-.071	.183	1.000	-.60	.46
		Triphasic oral contraceptive	.000	.186	1.000	-.53	.53
	Injectable contraceptive	Monophasic oral contraceptive	-.050	.186	1.000	-.58	.48
		Non contraceptive	.000	.186	1.000	-.53	.53
		Implantable contraceptive	-.071	.183	1.000	-.60	.46
		Triphasic oral contraceptive	.000	.186	1.000	-.53	.53
	Implantable contraceptive	Monophasic oral contraceptive	.021	.183	1.000	-.51	.55
		Non contraceptive	.071	.183	1.000	-.46	.60
		Injectable contraceptive	.071	.183	1.000	-.46	.60
		Triphasic oral contraceptive	.071	.183	1.000	-.46	.60
	Triphasic oral contraceptive	Monophasic oral contraceptive	-.050	.186	1.000	-.58	.48
		Non contraceptive	.000	.186	1.000	-.53	.53
		Injectable contraceptive	.000	.186	1.000	-.53	.53
		Implantable contraceptive	-.071	.183	1.000	-.60	.46
Roa-Goldman scale mouth area	Monophasic oral contraceptive	Non contraceptive	.250	.169	1.000	-.23	.73
		Injectable contraceptive	.100	.169	1.000	-.38	.58
		Implantable contraceptive	.226	.167	1.000	-.25	.71
		Triphasic oral contraceptive	.350	.169	.408	-.13	.83
	Non contraceptive	Monophasic oral contraceptive	-.250	.169	1.000	-.73	.23
		Injectable contraceptive	-.150	.169	1.000	-.63	.33
		Implantable contraceptive	-.024	.167	1.000	-.50	.46

Multiple Comparisons							
Bonferroni							
Dependent	(I) GroupCode	(J) GroupCode	Mean	Std. Error	Sig.	95% Confidence Interval	
		Triphasic oral contraceptive	.100	.169	1.000	-.38	.58
	Injectable contraceptive	Monophasic oral contraceptive	-.100	.169	1.000	-.58	.38
		Non contraceptive	.150	.169	1.000	-.33	.63
		Implantable contraceptive	.126	.167	1.000	-.35	.61
		Triphasic oral contraceptive	.250	.169	1.000	-.23	.73
	Implantable contraceptive	Monophasic oral contraceptive	-.226	.167	1.000	-.71	.25
		Non contraceptive	.024	.167	1.000	-.46	.50
		Injectable contraceptive	-.126	.167	1.000	-.61	.35
		Triphasic oral contraceptive	.124	.167	1.000	-.36	.60
	Triphasic oral contraceptive	Monophasic oral contraceptive	-.350	.169	.408	-.83	.13
		Non contraceptive	-.100	.169	1.000	-.58	.38
		Injectable contraceptive	-.250	.169	1.000	-.73	.23
		Implantable contraceptive	-.124	.167	1.000	-.60	.36
Systolic Blood pressure	Monophasic oral contraceptive	Non contraceptive	3.150	4.544	1.000	-9.91	16.21
		Injectable contraceptive	-4.550	4.544	1.000	-17.61	8.51
		Implantable contraceptive	-2.848	4.489	1.000	-15.75	10.05
		Triphasic oral contraceptive	2.200	4.544	1.000	-10.86	15.26
	Non contraceptive	Monophasic oral contraceptive	-3.150	4.544	1.000	-16.21	9.91
		Injectable contraceptive	-7.700	4.544	.934	-20.76	5.36
		Implantable contraceptive	-5.998	4.489	1.000	-18.90	6.90
		Triphasic oral contraceptive	-.950	4.544	1.000	-14.01	12.11
	Injectable contraceptive	Monophasic oral contraceptive	4.550	4.544	1.000	-8.51	17.61
		Non contraceptive	7.700	4.544	.934	-5.36	20.76
		Implantable contraceptive	1.702	4.489	1.000	-11.20	14.60
		Triphasic oral contraceptive	6.750	4.544	1.000	-6.31	19.81
	Implantable contraceptive	Monophasic oral contraceptive	2.848	4.489	1.000	-10.05	15.75
		Non contraceptive	5.998	4.489	1.000	-6.90	18.90
		Injectable contraceptive	-1.702	4.489	1.000	-14.60	11.20
		Triphasic oral contraceptive	5.048	4.489	1.000	-7.85	17.95
	Triphasic oral contraceptive	Monophasic oral contraceptive	-2.200	4.544	1.000	-15.26	10.86
		Non contraceptive	.950	4.544	1.000	-12.11	14.01
		Injectable contraceptive	-6.750	4.544	1.000	-19.81	6.31
		Implantable contraceptive	-5.048	4.489	1.000	-17.95	7.85
Diastolic Blood pressure	Monophasic oral contraceptive	Non contraceptive	8.850	3.524	.137	-1.28	18.98
		Injectable contraceptive	5.050	3.524	1.000	-5.08	15.18

Multiple Comparisons							
Bonferroni							
Dependent	(I) GroupCode	(J) GroupCode	Mean	Std. Error	Sig.	95% Confidence Interval	
		Implantable contraceptive	4.071	3.482	1.000	-5.93	14.08
		Triphasic oral contraceptive	5.000	3.524	1.000	-5.13	15.13
	Non contraceptive	Monophasic oral contraceptive	-8.850	3.524	.137	-18.98	1.28
		Injectable contraceptive	-3.800	3.524	1.000	-13.93	6.33
		Implantable contraceptive	-4.779	3.482	1.000	-14.78	5.23
		Triphasic oral contraceptive	-3.850	3.524	1.000	-13.98	6.28
	Injectable contraceptive	Monophasic oral contraceptive	-5.050	3.524	1.000	-15.18	5.08
		Non contraceptive	3.800	3.524	1.000	-6.33	13.93
		Implantable contraceptive	-.979	3.482	1.000	-10.98	9.03
		Triphasic oral contraceptive	-.050	3.524	1.000	-10.18	10.08
	Implantable contraceptive	Monophasic oral contraceptive	-4.071	3.482	1.000	-14.08	5.93
		Non contraceptive	4.779	3.482	1.000	-5.23	14.78
		Injectable contraceptive	.979	3.482	1.000	-9.03	10.98
		Triphasic oral contraceptive	.929	3.482	1.000	-9.08	10.93
	Triphasic oral contraceptive	Monophasic oral contraceptive	-5.000	3.524	1.000	-15.13	5.13
		Non contraceptive	3.850	3.524	1.000	-6.28	13.98
		Injectable contraceptive	.050	3.524	1.000	-10.08	10.18
		Implantable contraceptive	-.929	3.482	1.000	-10.93	9.08
Heart rate	Monophasic oral contraceptive	Non contraceptive	-1.100	4.378	1.000	-13.68	11.48
		Injectable contraceptive	-6.650	4.378	1.000	-19.23	5.93
		Implantable contraceptive	-3.762	4.325	1.000	-16.19	8.67
		Triphasic oral contraceptive	-5.750	4.378	1.000	-18.33	6.83
	Non contraceptive	Monophasic oral contraceptive	1.100	4.378	1.000	-11.48	13.68
		Injectable contraceptive	-5.550	4.378	1.000	-18.13	7.03
		Implantable contraceptive	-2.662	4.325	1.000	-15.09	9.77
		Triphasic oral contraceptive	-4.650	4.378	1.000	-17.23	7.93
	Injectable contraceptive	Monophasic oral contraceptive	6.650	4.378	1.000	-5.93	19.23
		Non contraceptive	5.550	4.378	1.000	-7.03	18.13
		Implantable contraceptive	2.888	4.325	1.000	-9.54	15.32
		Triphasic oral contraceptive	.900	4.378	1.000	-11.68	13.48
	Implantable contraceptive	Monophasic oral contraceptive	3.762	4.325	1.000	-8.67	16.19
		Non contraceptive	2.662	4.325	1.000	-9.77	15.09
		Injectable contraceptive	-2.888	4.325	1.000	-15.32	9.54
		Triphasic oral contraceptive	-1.988	4.325	1.000	-14.42	10.44

Multiple Comparisons

Bonferroni

Dependent	(I) GroupCode	(J) GroupCode	Mean	Std. Error	Sig.	95% Confidence Interval	
	Triphasic oral contraceptive	Monophasic oral contraceptive	5.750	4.378	1.000	-6.83	18.33
		Non contraceptive	4.650	4.378	1.000	-7.93	17.23
		Injectable contraceptive	-.900	4.378	1.000	-13.48	11.68
		Implantable contraceptive	1.988	4.325	1.000	-10.44	14.42
Weight	Monophasic oral contraceptive	Non contraceptive	-.015000	5.648689	1.000	-16.24571	16.21571
		Injectable contraceptive	-8.785000	5.648689	1.000	-25.01571	7.44571
		Implantable contraceptive	-13.094762	5.581037	.210	-29.13109	2.94157
		Triphasic oral contraceptive	-9.290000	5.648689	1.000	-25.52071	6.94071
	Non contraceptive	Monophasic oral contraceptive	.015000	5.648689	1.000	-16.21571	16.24571
		Injectable contraceptive	-8.770000	5.648689	1.000	-25.00071	7.46071
		Implantable contraceptive	-13.079762	5.581037	.212	-29.11609	2.95657
		Triphasic oral contraceptive	-9.275000	5.648689	1.000	-25.50571	6.95571
	Injectable contraceptive	Monophasic oral contraceptive	8.785000	5.648689	1.000	-7.44571	25.01571
		Non contraceptive	8.770000	5.648689	1.000	-7.46071	25.00071
		Implantable contraceptive	-4.309762	5.581037	1.000	-20.34609	11.72657
		Triphasic oral contraceptive	-.505000	5.648689	1.000	-16.73571	15.72571
	Implantable contraceptive	Monophasic oral contraceptive	13.094762	5.581037	.210	-2.94157	29.13109
		Non contraceptive	13.079762	5.581037	.212	-2.95657	29.11609
		Injectable contraceptive	4.309762	5.581037	1.000	-11.72657	20.34609
		Triphasic oral contraceptive	3.804762	5.581037	1.000	-12.23157	19.84109
	Triphasic oral contraceptive	Monophasic oral contraceptive	9.290000	5.648689	1.000	-6.94071	25.52071
		Non contraceptive	9.275000	5.648689	1.000	-6.95571	25.50571
		Injectable contraceptive	.505000	5.648689	1.000	-15.72571	16.73571
		Implantable contraceptive	-3.804762	5.581037	1.000	-19.84109	12.23157
Min recommended weight	Monophasic oral contraceptive	Non contraceptive	1.650	2.524	1.000	-5.60	8.90
		Injectable contraceptive	-.900	2.524	1.000	-8.15	6.35
		Implantable contraceptive	-4.638	2.493	.659	-11.80	2.53
		Triphasic oral contraceptive	-.500	2.524	1.000	-7.75	6.75
	Non contraceptive	Monophasic oral contraceptive	-1.650	2.524	1.000	-8.90	5.60
		Injectable contraceptive	-2.550	2.524	1.000	-9.80	4.70
		Implantable contraceptive	-6.288	2.493	.133	-13.45	.88
		Triphasic oral contraceptive	-2.150	2.524	1.000	-9.40	5.10
	Injectable contraceptive	Monophasic oral contraceptive	.900	2.524	1.000	-6.35	8.15
		Non contraceptive	2.550	2.524	1.000	-4.70	9.80
		Implantable contraceptive	-3.738	2.493	1.000	-10.90	3.43

Multiple Comparisons							
Bonferroni							
Dependent	(I) GroupCode	(J) GroupCode	Mean	Std. Error	Sig.	95% Confidence Interval	
		Triphasic oral contraceptive	.400	2.524	1.000	-6.85	7.65
	Implantable contraceptive	Monophasic oral contraceptive	4.638	2.493	.659	-2.53	11.80
		Non contraceptive	6.288	2.493	.133	-.88	13.45
		Injectable contraceptive	3.738	2.493	1.000	-3.43	10.90
		Triphasic oral contraceptive	4.138	2.493	1.000	-3.03	11.30
	Triphasic oral contraceptive	Monophasic oral contraceptive	.500	2.524	1.000	-6.75	7.75
		Non contraceptive	2.150	2.524	1.000	-5.10	9.40
		Injectable contraceptive	-.400	2.524	1.000	-7.65	6.85
		Implantable contraceptive	-4.138	2.493	1.000	-11.30	3.03
Max recommended weight	Monophasic oral contraceptive	Non contraceptive	1.600	2.655	1.000	-6.03	9.23
		Injectable contraceptive	-.750	2.655	1.000	-8.38	6.88
		Implantable contraceptive	-4.257	2.623	1.000	-11.79	3.28
		Triphasic oral contraceptive	-.350	2.655	1.000	-7.98	7.28
	Non contraceptive	Monophasic oral contraceptive	-1.600	2.655	1.000	-9.23	6.03
		Injectable contraceptive	-2.350	2.655	1.000	-9.98	5.28
		Implantable contraceptive	-5.857	2.623	.279	-13.39	1.68
		Triphasic oral contraceptive	-1.950	2.655	1.000	-9.58	5.68
	Injectable contraceptive	Monophasic oral contraceptive	.750	2.655	1.000	-6.88	8.38
		Non contraceptive	2.350	2.655	1.000	-5.28	9.98
		Implantable contraceptive	-3.507	2.623	1.000	-11.04	4.03
		Triphasic oral contraceptive	.400	2.655	1.000	-7.23	8.03
	Implantable contraceptive	Monophasic oral contraceptive	4.257	2.623	1.000	-3.28	11.79
		Non contraceptive	5.857	2.623	.279	-1.68	13.39
		Injectable contraceptive	3.507	2.623	1.000	-4.03	11.04
		Triphasic oral contraceptive	3.907	2.623	1.000	-3.63	11.44
	Triphasic oral contraceptive	Monophasic oral contraceptive	.350	2.655	1.000	-7.28	7.98
		Non contraceptive	1.950	2.655	1.000	-5.68	9.58
		Injectable contraceptive	-.400	2.655	1.000	-8.03	7.23
		Implantable contraceptive	-3.907	2.623	1.000	-11.44	3.63
Waist to hip ratio	Monophasic oral contraceptive	Non contraceptive	.012500	.020746	1.000	-.04711	.07211
		Injectable contraceptive	-.028500	.020746	1.000	-.08811	.03111
		Implantable contraceptive	-.055286	.020498	.083	-.11418	.00361
		Triphasic oral contraceptive	-.017500	.020746	1.000	-.07711	.04211
	Non contraceptive	Monophasic oral contraceptive	-.012500	.020746	1.000	-.07211	.04711
		Injectable contraceptive	-.041000	.020746	.510	-.10061	.01861

Multiple Comparisons							
Bonferroni							
Dependent	(I) GroupCode	(J) GroupCode	Mean	Std. Error	Sig.	95% Confidence Interval	
		Implantable contraceptive	-.067786*	.020498	.013	-.12668	-.00889
		Triphasic oral contraceptive	-.030000	.020746	1.000	-.08961	.02961
	Injectable contraceptive	Monophasic oral contraceptive	.028500	.020746	1.000	-.03111	.08811
		Non contraceptive	.041000	.020746	.510	-.01861	.10061
		Implantable contraceptive	-.026786	.020498	1.000	-.08568	.03211
		Triphasic oral contraceptive	.011000	.020746	1.000	-.04861	.07061
	Implantable contraceptive	Monophasic oral contraceptive	.055286	.020498	.083	-.00361	.11418
		Non contraceptive	.067786*	.020498	.013	.00889	.12668
		Injectable contraceptive	.026786	.020498	1.000	-.03211	.08568
		Triphasic oral contraceptive	.037786	.020498	.684	-.02111	.09668
	Triphasic oral contraceptive	Monophasic oral contraceptive	.017500	.020746	1.000	-.04211	.07711
		Non contraceptive	.030000	.020746	1.000	-.02961	.08961
		Injectable contraceptive	-.011000	.020746	1.000	-.07061	.04861
		Implantable contraceptive	-.037786	.020498	.684	-.09668	.02111
Skin fold measurement	Monophasic oral contraceptive	Non contraceptive	1.795000	3.340234	1.000	-7.80269	11.39269
		Injectable contraceptive	-4.305000	3.340234	1.000	-13.90269	5.29269
		Implantable contraceptive	-5.331190	3.300230	1.000	-14.81394	4.15156
		Triphasic oral contraceptive	-3.505000	3.340234	1.000	-13.10269	6.09269
	Non contraceptive	Monophasic oral contraceptive	-1.795000	3.340234	1.000	-11.39269	7.80269
		Injectable contraceptive	-6.100000	3.340234	.709	-15.69769	3.49769
		Implantable contraceptive	-7.126190	3.300230	.333	-16.60894	2.35656
		Triphasic oral contraceptive	-5.300000	3.340234	1.000	-14.89769	4.29769
	Injectable contraceptive	Monophasic oral contraceptive	4.305000	3.340234	1.000	-5.29269	13.90269
		Non contraceptive	6.100000	3.340234	.709	-3.49769	15.69769
		Implantable contraceptive	-1.026190	3.300230	1.000	-10.50894	8.45656
		Triphasic oral contraceptive	.800000	3.340234	1.000	-8.79769	10.39769
	Implantable contraceptive	Monophasic oral contraceptive	5.331190	3.300230	1.000	-4.15156	14.81394
		Non contraceptive	7.126190	3.300230	.333	-2.35656	16.60894
		Injectable contraceptive	1.026190	3.300230	1.000	-8.45656	10.50894
		Triphasic oral contraceptive	1.826190	3.300230	1.000	-7.65656	11.30894
	Triphasic oral contraceptive	Monophasic oral contraceptive	3.505000	3.340234	1.000	-6.09269	13.10269
		Non contraceptive	5.300000	3.340234	1.000	-4.29769	14.89769
		Injectable contraceptive	-.800000	3.340234	1.000	-10.39769	8.79769
		Implantable contraceptive	-1.826190	3.300230	1.000	-11.30894	7.65656
Adipose	Monophasic	Non contraceptive	-.870000	2.778910	1.000	-8.85481	7.11481

Multiple Comparisons							
Bonferroni							
Dependent	(I) GroupCode	(J) GroupCode	Mean	Std. Error	Sig.	95% Confidence Interval	
tissue	oral contraceptive	Injectable contraceptive	-4.520000	2.778910	1.000	-12.50481	3.46481
		Implantable contraceptive	-2.955476	2.745629	1.000	-10.84466	4.93370
		Triphasic oral contraceptive	-3.080000	2.778910	1.000	-11.06481	4.90481
	Non contraceptive	Monophasic oral contraceptive	.870000	2.778910	1.000	-7.11481	8.85481
		Injectable contraceptive	-3.650000	2.778910	1.000	-11.63481	4.33481
		Implantable contraceptive	-2.085476	2.745629	1.000	-9.97466	5.80370
		Triphasic oral contraceptive	-2.210000	2.778910	1.000	-10.19481	5.77481
	Injectable contraceptive	Monophasic oral contraceptive	4.520000	2.778910	1.000	-3.46481	12.50481
		Non contraceptive	3.650000	2.778910	1.000	-4.33481	11.63481
		Implantable contraceptive	1.564524	2.745629	1.000	-6.32466	9.45370
		Triphasic oral contraceptive	1.440000	2.778910	1.000	-6.54481	9.42481
	Implantable contraceptive	Monophasic oral contraceptive	2.955476	2.745629	1.000	-4.93370	10.84466
		Non contraceptive	2.085476	2.745629	1.000	-5.80370	9.97466
		Injectable contraceptive	-1.564524	2.745629	1.000	-9.45370	6.32466
		Triphasic oral contraceptive	-.124524	2.745629	1.000	-8.01370	7.76466
	Triphasic oral contraceptive	Monophasic oral contraceptive	3.080000	2.778910	1.000	-4.90481	11.06481
		Non contraceptive	2.210000	2.778910	1.000	-5.77481	10.19481
		Injectable contraceptive	-1.440000	2.778910	1.000	-9.42481	6.54481
		Implantable contraceptive	.124524	2.745629	1.000	-7.76466	8.01370
Lean tissue	Monophasic oral contraceptive	Non contraceptive	2.499304	2.967385	1.000	-6.02706	11.02567
		Injectable contraceptive	4.525972	2.967385	1.000	-4.00039	13.05234
		Implantable contraceptive	2.920201	2.931846	1.000	-5.50405	11.34445
		Triphasic oral contraceptive	3.065359	2.967385	1.000	-5.46101	11.59172
	Non contraceptive	Monophasic oral contraceptive	-2.499304	2.967385	1.000	-11.02567	6.02706
		Injectable contraceptive	2.026668	2.967385	1.000	-6.49970	10.55303
		Implantable contraceptive	.420897	2.931846	1.000	-8.00335	8.84515
		Triphasic oral contraceptive	.566055	2.967385	1.000	-7.96031	9.09242
	Injectable contraceptive	Monophasic oral contraceptive	-4.525972	2.967385	1.000	-13.05234	4.00039
		Non contraceptive	-2.026668	2.967385	1.000	-10.55303	6.49970
		Implantable contraceptive	-1.605771	2.931846	1.000	-10.03002	6.81848
		Triphasic oral contraceptive	-1.460613	2.967385	1.000	-9.98698	7.06575
	Implantable contraceptive	Monophasic oral contraceptive	-2.920201	2.931846	1.000	-11.34445	5.50405
		Non contraceptive	-.420897	2.931846	1.000	-8.84515	8.00335
		Injectable contraceptive	1.605771	2.931846	1.000	-6.81848	10.03002

Multiple Comparisons							
Bonferroni							
Dependent	(I) GroupCode	(J) GroupCode	Mean	Std. Error	Sig.	95% Confidence Interval	
		Triphasic oral contraceptive	.145158	2.931846	1.000	-8.27909	8.56941
	Triphasic oral contraceptive	Monophasic oral contraceptive	-3.065359	2.967385	1.000	-11.59172	5.46101
		Non contraceptive	-.566055	2.967385	1.000	-9.09242	7.96031
		Injectable contraceptive	1.460613	2.967385	1.000	-7.06575	9.98698
		Implantable contraceptive	-.145158	2.931846	1.000	-8.56941	8.27909
Water content	Monophasic oral contraceptive	Non contraceptive	1.430000	2.290415	1.000	-5.15119	8.01119
		Injectable contraceptive	3.360000	2.290415	1.000	-3.22119	9.94119
		Implantable contraceptive	2.010952	2.262984	1.000	-4.49142	8.51332
		Triphasic oral contraceptive	2.065000	2.290415	1.000	-4.51619	8.64619
	Non contraceptive	Monophasic oral contraceptive	-1.430000	2.290415	1.000	-8.01119	5.15119
		Injectable contraceptive	1.930000	2.290415	1.000	-4.65119	8.51119
		Implantable contraceptive	.580952	2.262984	1.000	-5.92142	7.08332
		Triphasic oral contraceptive	.635000	2.290415	1.000	-5.94619	7.21619
	Injectable contraceptive	Monophasic oral contraceptive	-3.360000	2.290415	1.000	-9.94119	3.22119
		Non contraceptive	-1.930000	2.290415	1.000	-8.51119	4.65119
		Implantable contraceptive	-1.349048	2.262984	1.000	-7.85142	5.15332
		Triphasic oral contraceptive	-1.295000	2.290415	1.000	-7.87619	5.28619
	Implantable contraceptive	Monophasic oral contraceptive	-2.010952	2.262984	1.000	-8.51332	4.49142
		Non contraceptive	-.580952	2.262984	1.000	-7.08332	5.92142
		Injectable contraceptive	1.349048	2.262984	1.000	-5.15332	7.85142
		Triphasic oral contraceptive	.054048	2.262984	1.000	-6.44832	6.55642
	Triphasic oral contraceptive	Monophasic oral contraceptive	-2.065000	2.290415	1.000	-8.64619	4.51619
		Non contraceptive	-.635000	2.290415	1.000	-7.21619	5.94619
		Injectable contraceptive	1.295000	2.290415	1.000	-5.28619	7.87619
		Implantable contraceptive	-.054048	2.262984	1.000	-6.55642	6.44832
Estimated resting metabolic rate	Monophasic oral contraceptive	Non contraceptive	14.750	52.492	1.000	-136.08	165.58
		Injectable contraceptive	-50.450	52.492	1.000	-201.28	100.38
		Implantable contraceptive	-130.360	51.863	.136	-279.38	18.66
		Triphasic oral contraceptive	-84.300	52.492	1.000	-235.13	66.53
	Non contraceptive	Monophasic oral contraceptive	-14.750	52.492	1.000	-165.58	136.08
		Injectable contraceptive	-65.200	52.492	1.000	-216.03	85.63
		Implantable contraceptive	-145.110	51.863	.062	-294.13	3.91
		Triphasic oral contraceptive	-99.050	52.492	.622	-249.88	51.78
	Injectable contraceptive	Monophasic oral contraceptive	50.450	52.492	1.000	-100.38	201.28
		Non contraceptive	65.200	52.492	1.000	-85.63	216.03

Multiple Comparisons							
Bonferroni							
Dependent	(I) GroupCode	(J) GroupCode	Mean	Std. Error	Sig.	95% Confidence Interval	
		Implantable contraceptive	-79.910	51.863	1.000	-228.93	69.11
		Triphasic oral contraceptive	-33.850	52.492	1.000	-184.68	116.98
	Implantable contraceptive	Monophasic oral contraceptive	130.360	51.863	.136	-18.66	279.38
		Non contraceptive	145.110	51.863	.062	-3.91	294.13
		Injectable contraceptive	79.910	51.863	1.000	-69.11	228.93
		Triphasic oral contraceptive	46.060	51.863	1.000	-102.96	195.08
	Triphasic oral contraceptive	Monophasic oral contraceptive	84.300	52.492	1.000	-66.53	235.13
		Non contraceptive	99.050	52.492	.622	-51.78	249.88
		Injectable contraceptive	33.850	52.492	1.000	-116.98	184.68
		Implantable contraceptive	-46.060	51.863	1.000	-195.08	102.96
Estimated average energy required	Monophasic oral contraceptive	Non contraceptive	72.750	91.999	1.000	-191.60	337.10
		Injectable contraceptive	-25.000	91.999	1.000	-289.35	239.35
		Implantable contraceptive	-186.231	90.897	.432	-447.41	74.95
		Triphasic oral contraceptive	-4.200	91.999	1.000	-268.55	260.15
	Non contraceptive	Monophasic oral contraceptive	-72.750	91.999	1.000	-337.10	191.60
		Injectable contraceptive	-97.750	91.999	1.000	-362.10	166.60
		Implantable contraceptive	-258.981	90.897	.054	-520.16	2.20
		Triphasic oral contraceptive	-76.950	91.999	1.000	-341.30	187.40
	Injectable contraceptive	Monophasic oral contraceptive	25.000	91.999	1.000	-239.35	289.35
		Non contraceptive	97.750	91.999	1.000	-166.60	362.10
		Implantable contraceptive	-161.231	90.897	.793	-422.41	99.95
		Triphasic oral contraceptive	20.800	91.999	1.000	-243.55	285.15
	Implantable contraceptive	Monophasic oral contraceptive	186.231	90.897	.432	-74.95	447.41
		Non contraceptive	258.981	90.897	.054	-2.20	520.16
		Injectable contraceptive	161.231	90.897	.793	-99.95	422.41
		Triphasic oral contraceptive	182.031	90.897	.480	-79.15	443.21
	Triphasic oral contraceptive	Monophasic oral contraceptive	4.200	91.999	1.000	-260.15	268.55
		Non contraceptive	76.950	91.999	1.000	-187.40	341.30
		Injectable contraceptive	-20.800	91.999	1.000	-285.15	243.55
		Implantable contraceptive	-182.031	90.897	.480	-443.21	79.15
BMI	Monophasic oral contraceptive	Non contraceptive	-.040000	2.091363	1.000	-6.04924	5.96924
		Injectable contraceptive	-3.615000	2.091363	.871	-9.62424	2.39424
		Implantable contraceptive	-4.604286	2.066316	.282	-10.54156	1.33298
		Triphasic oral contraceptive	-3.465000	2.091363	1.000	-9.47424	2.54424
	Non contraceptive	Monophasic oral contraceptive	.040000	2.091363	1.000	-5.96924	6.04924

Multiple Comparisons							
Bonferroni							
Dependent	(I) GroupCode	(J) GroupCode	Mean	Std. Error	Sig.	95% Confidence Interval	
		Injectable contraceptive	-3.575000	2.091363	.906	-9.58424	2.43424
		Implantable contraceptive	-4.564286	2.066316	.296	-10.50156	1.37298
		Triphasic oral contraceptive	-3.425000	2.091363	1.000	-9.43424	2.58424
	Injectable contraceptive	Monophasic oral contraceptive	3.615000	2.091363	.871	-2.39424	9.62424
		Non contraceptive	3.575000	2.091363	.906	-2.43424	9.58424
		Implantable contraceptive	-.989286	2.066316	1.000	-6.92656	4.94798
		Triphasic oral contraceptive	.150000	2.091363	1.000	-5.85924	6.15924
	Implantable contraceptive	Monophasic oral contraceptive	4.604286	2.066316	.282	-1.33298	10.54156
		Non contraceptive	4.564286	2.066316	.296	-1.37298	10.50156
		Injectable contraceptive	.989286	2.066316	1.000	-4.94798	6.92656
		Triphasic oral contraceptive	1.139286	2.066316	1.000	-4.79798	7.07656
	Triphasic oral contraceptive	Monophasic oral contraceptive	3.465000	2.091363	1.000	-2.54424	9.47424
		Non contraceptive	3.425000	2.091363	1.000	-2.58424	9.43424
		Injectable contraceptive	-.150000	2.091363	1.000	-6.15924	5.85924
		Implantable contraceptive	-1.139286	2.066316	1.000	-7.07656	4.79798
Pigmentation Forehead to Cheek	Monophasic oral contraceptive	Non contraceptive	.041185	.065946	1.000	-.14830	.23067
		Injectable contraceptive	.099464	.065946	1.000	-.09002	.28895
		Implantable contraceptive	.085449	.065156	1.000	-.10177	.27267
		Triphasic oral contraceptive	.172767	.065946	.102	-.01672	.36225
	Non contraceptive	Monophasic oral contraceptive	-.041185	.065946	1.000	-.23067	.14830
		Injectable contraceptive	.058279	.065946	1.000	-.13121	.24776
		Implantable contraceptive	.044264	.065156	1.000	-.14295	.23148
		Triphasic oral contraceptive	.131582	.065946	.488	-.05790	.32107
	Injectable contraceptive	Monophasic oral contraceptive	-.099464	.065946	1.000	-.28895	.09002
		Non contraceptive	-.058279	.065946	1.000	-.24776	.13121
		Implantable contraceptive	-.014015	.065156	1.000	-.20123	.17320
		Triphasic oral contraceptive	.073303	.065946	1.000	-.11618	.26279
	Implantable contraceptive	Monophasic oral contraceptive	-.085449	.065156	1.000	-.27267	.10177
		Non contraceptive	-.044264	.065156	1.000	-.23148	.14295
		Injectable contraceptive	.014015	.065156	1.000	-.17320	.20123
		Triphasic oral contraceptive	.087318	.065156	1.000	-.09990	.27453
	Triphasic oral contraceptive	Monophasic oral contraceptive	-.172767	.065946	.102	-.36225	.01672
		Non contraceptive	-.131582	.065946	.488	-.32107	.05790
		Injectable contraceptive	-.073303	.065946	1.000	-.26279	.11618
		Implantable contraceptive	-.087318	.065156	1.000	-.27453	.09990

Multiple Comparisons

Bonferroni

Dependent	(I) GroupCode	(J) GroupCode	Mean	Std. Error	Sig.	95% Confidence Interval	
Pigmentation Forehead to Chin	Monophasic oral contraceptive	Non contraceptive	.018299	.096508	1.000	-.25900	.29560
		Injectable contraceptive	.117821	.096508	1.000	-.15948	.39512
		Implantable contraceptive	.031898	.095352	1.000	-.24208	.30588
		Triphasic oral contraceptive	.195824	.096508	.452	-.08148	.47313
	Non contraceptive	Monophasic oral contraceptive	-.018299	.096508	1.000	-.29560	.25900
		Injectable contraceptive	.099522	.096508	1.000	-.17778	.37682
		Implantable contraceptive	.013599	.095352	1.000	-.26038	.28758
		Triphasic oral contraceptive	.177525	.096508	.689	-.09978	.45483
	Injectable contraceptive	Monophasic oral contraceptive	-.117821	.096508	1.000	-.39512	.15948
		Non contraceptive	-.099522	.096508	1.000	-.37682	.17778
		Implantable contraceptive	-.085923	.095352	1.000	-.35990	.18806
		Triphasic oral contraceptive	.078003	.096508	1.000	-.19930	.35530
	Implantable contraceptive	Monophasic oral contraceptive	-.031898	.095352	1.000	-.30588	.24208
		Non contraceptive	-.013599	.095352	1.000	-.28758	.26038
		Injectable contraceptive	.085923	.095352	1.000	-.18806	.35990
		Triphasic oral contraceptive	.163926	.095352	.888	-.11005	.43791
	Triphasic oral contraceptive	Monophasic oral contraceptive	-.195824	.096508	.452	-.47313	.08148
		Non contraceptive	-.177525	.096508	.689	-.45483	.09978
		Injectable contraceptive	-.078003	.096508	1.000	-.35530	.19930
		Implantable contraceptive	-.163926	.095352	.888	-.43791	.11005
Pigmentation Chin to Cheek	Monophasic oral contraceptive	Non contraceptive	.112250	.067200	.981	-.08084	.30534
		Injectable contraceptive	.129110	.067200	.577	-.06398	.32220
		Implantable contraceptive	.076760	.066395	1.000	-.11402	.26754
		Triphasic oral contraceptive	.128121	.067200	.596	-.06497	.32121
	Non contraceptive	Monophasic oral contraceptive	-.112250	.067200	.981	-.30534	.08084
		Injectable contraceptive	.016860	.067200	1.000	-.17623	.20995
		Implantable contraceptive	-.035490	.066395	1.000	-.22627	.15529
		Triphasic oral contraceptive	.015871	.067200	1.000	-.17722	.20896
	Injectable contraceptive	Monophasic oral contraceptive	-.129110	.067200	.577	-.32220	.06398
		Non contraceptive	-.016860	.067200	1.000	-.20995	.17623
		Implantable contraceptive	-.052350	.066395	1.000	-.24313	.13843
		Triphasic oral contraceptive	-.000989	.067200	1.000	-.19408	.19210
	Implantable contraceptive	Monophasic oral contraceptive	-.076760	.066395	1.000	-.26754	.11402
		Non contraceptive	.035490	.066395	1.000	-.15529	.22627
		Injectable contraceptive	.052350	.066395	1.000	-.13843	.24313

Multiple Comparisons							
Bonferroni							
Dependent	(I) GroupCode	(J) GroupCode	Mean	Std. Error	Sig.	95% Confidence Interval	
		Triphasic oral contraceptive	.051361	.066395	1.000	-.13941	.24214
	Triphasic oral contraceptive	Monophasic oral contraceptive	-.128121	.067200	.596	-.32121	.06497
		Non contraceptive	-.015871	.067200	1.000	-.20896	.17722
		Injectable contraceptive	.000989	.067200	1.000	-.19210	.19408
		Implantable contraceptive	-.051361	.066395	1.000	-.24214	.13941
Max ratio of Pigmentation	Monophasic oral contraceptive	Non contraceptive	.093383	.093400	1.000	-.17499	.36176
		Injectable contraceptive	.186665	.093400	.485	-.08171	.45504
		Implantable contraceptive	.105209	.092282	1.000	-.15995	.37037
		Triphasic oral contraceptive	.266399	.093400	.053	-.00197	.53477
	Non contraceptive	Monophasic oral contraceptive	-.093383	.093400	1.000	-.36176	.17499
		Injectable contraceptive	.093281	.093400	1.000	-.17509	.36165
		Implantable contraceptive	.011826	.092282	1.000	-.25333	.27698
		Triphasic oral contraceptive	.173016	.093400	.670	-.09536	.44139
	Injectable contraceptive	Monophasic oral contraceptive	-.186665	.093400	.485	-.45504	.08171
		Non contraceptive	-.093281	.093400	1.000	-.36165	.17509
		Implantable contraceptive	-.081455	.092282	1.000	-.34661	.18370
		Triphasic oral contraceptive	.079735	.093400	1.000	-.18864	.34811
	Implantable contraceptive	Monophasic oral contraceptive	-.105209	.092282	1.000	-.37037	.15995
		Non contraceptive	-.011826	.092282	1.000	-.27698	.25333
		Injectable contraceptive	.081455	.092282	1.000	-.18370	.34661
		Triphasic oral contraceptive	.161190	.092282	.839	-.10397	.42635
	Triphasic oral contraceptive	Monophasic oral contraceptive	-.266399	.093400	.053	-.53477	.00197
		Non contraceptive	-.173016	.093400	.670	-.44139	.09536
		Injectable contraceptive	-.079735	.093400	1.000	-.34811	.18864
		Implantable contraceptive	-.161190	.092282	.839	-.42635	.10397

*. The mean difference is significant at the 0.05 level.