Polyunsaturated fatty acid status in individuals with Poly Cystic Ovarian Syndrome

by
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Declaration

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M Bronkhorst

Date: 10 June 2018

Abstract

Introduction: Polycystic ovarian syndrome (PCOS) is the most common hormonal disorder among women of reproductive age and a leading cause of female infertility. PCOS patients are more susceptible to develop insulin resistance while hyperinsulinemia is known to aggravate reproductive dysfunction. Polyunsaturated fatty acids (PUFAs), have been shown to improve metabolic parameters. The purpose of the study was to determine whether there was a difference in PUFA status between women with PCOS who were struggling to conceive and a control group.

Methods: A quantitative, cross-sectional case control study including a total of 77 subjects was conducted. The study group (n = 39) was women with PCOS and infertility (> 6 months), and a control group (n = 38) was used. Independent t-tests, Levene's test and analysis of variance were used to analyse the data. Demographic information, anthropometric parameters, medical history, supplement history, fertility history, three-day food intake records and plasma phospholipids and polyunsaturated fatty acids (PUFA) red blood cell membranes were compared between the groups.

Results: Dietary intake of docosahexanoic acid (C22:6n3 DHA) ($p = 0.043$) and docosapentanoic acid (C22:5n3 DPA) ($p = 0.029$) were all significantly higher in the control group. Dietary eicosapentanoic acid (C20:5n3 EPA) did not differ significantly between the groups although a trend towards higher levels in the control group was observed ($p = 0.062$). Plasma phospholipid fatty acids with a significantly higher concentration in the study group were stearic acid (C18:0) ($p = 0.005$), elaidic acid (C18:1n9T) ($p = 0.042$), mead acid (C20:3n9) ($p = 0.039$) and C20:3n6 ($p = 0.013$). The plasma phospholipid omega-6:omega-3 ratio (n-6:n-3) was higher in women with PCOS (with a trend towards significance [$p = 0.071$]). The study group had significantly higher plasma phospholipid n-6:n-3 long-chain polyunsaturated fatty acids (LC-PUFAs) compared to the control group when adjusted for possible confounding of PUFA supplementation ($p = 0.039$) and PUFA supplementation with endometriosis ($p = 0.048$). Plasma phospholipid omega-3 fatty acids were higher in the control group compared to the study group for DHA ($p = 0.029$), total n-3 PUFAs ($p = 0.036$) and n3-LC-PUFAs ($p = 0.036$).

Conclusion: Significantly lower plasma phospholipid omega-3 PUFAs ($p = 0.036$), in particular DHA ($p = 0.029$), were observed in women with PCOS and infertility. Conversely, mead acid was significantly higher in this group. In addition, a higher plasma n6:n3 PUFA ratio was observed in women with PCOS (with a trend towards significance). The findings of this study demonstrate that plasma phospholipid fatty acid profiles differ between women with PCOS and infertility and controls and might provide a

complementary approach to treatment of PCOS. PUFA's could therefore potentially play a role in the management of PCOS and infertility.

Opsomming

Inleiding: Polisistiese ovariële sindroom (PSOS) is die algemeenste hormonale steuring onder vroue van voortplantingsouderdom en ’n hoofoorsaak van vroulike onvrugbaarheid. PSOS-pasiënte is vatbaarder daarvoor om insulienweerstand (IR) te ontwikkel, terwyl hiperinsulinemie daarvoor bekend is dat dit reproduktiewe disfunksie vererger. Daar is al aangetoon dat poli-onversadigde vetsure (POVS’e) metaboliese parameters verbeter. Die doel van die studie was om vas te stel of die POVS-status van vroue met PSOS wat sukkel om swanger te raak en ’n kontrolegroep verskil.

Metodes: ’n Kwantitatiewe, dwarsdeursnit geval-kontrolestudie is uitgevoer met ’n totaal van 77 proefpersone. Die studiegroep (n = 39) was vroue met PSOS en vrugbaarheidsprobleme (> 6 maande) en ’n kontrolegroep (n = 38). Onafhanklike t-toetse, Levene se toets en variansie-analise (ANOVA) is gebruik om die data te analiseer. Demografiese inligting, antropometriese parameters, mediese geskiedenis, voedingaanvullingsgeskiedenis, vrugbaarheidsgeskiedenis, drie-dae-voedselrekords sowel as die essensiële vetsuur fosfolipiede(EVS’e) in plasma en rooibloedsel membrane is tussen die groepe vergelyk.

Resultate: Dieetinname van dokosaheksanoësuur (C22:6n3 DHS) (p = 0.043) en dokosapentanoësuur (C22: 5n3 DPS) (p=0.029) was almal aansienlik hoër in die kontrolegroep. Dieet-eikosapentanoësuur (C20:5n3 EPA) het nie beduidend tussen die groepe verskil nie, hoewel ’n geneigdheid tot hoër vlakke in die kontrolegroep waargeneem is (p = 0.062). Die plasma fosfolipied vetsure met ’n beduidende hoër konsentrasie in die studiegroep was stearіensuur (C18:0) (p = 0.005), elaïdiensuur (C18:1n9T) (p = 0.042), eikosatriënoësuur (C20:3n9) en C20:3n6 (p = 0.013). Die omega-6:omega-3-verhouding in plasma fosfolipiede (n-6:n-3) was hoër by vroue met PSOS (met ’n beduidendheidstendens [p = 0.071]). Die studiegroep het aansienlik hoër plasma-n-6:n-3-langketting-poli-onversadigdevetsure (LKPOVS), vergeleke met die kontrolegroep, gehad nadat dit aangepas is vir die moontlike strengeling van EVS-aanvulling (p = 0.039) en EVS-aanvulling met endometriose (p = 0.048). Plasma fosfolipied -omega-3-vetsure was hoër in die kontrolegroep in vergelyking met die studiegroep vir DHS (p = 0.029), totale n-3-POVS (p = 0.036) en n3-LKPOVS (p = 0.036).

Samevatting: Beduidend laer plasma fosfolipied -omega-3-POVS (p = 0.036), veral DHA (p = 0.029), is by vroue met PSOS en vrugbaarheidsprobleme waargeneem. Daarteenoor was eikosatriënoësuur aansienlik hoër in hierdie groep. Daarbenewens is ’n hoër plasma-n6:n3-POVS-verhouding (met ’n beduidendheidstendens) by vroue met PSOS waargeneem. Die bevindings van hierdie studie toon dat daar ’n verskil in plasma-fosfolipiedvetsuurprofiele is tussen vroue met PSOS en vrugbaarheidsprobleme, en kontroles, en kan moontlik ’n komplementêre benadering tot die behandeling van PSOS bied. EVS’e kan dus potensieel ’n rol speel in die behandeling van PSOS en onvrugbaarheid.

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Contributions

The principal researcher (Maryke Bronkhorst) developed the idea and the protocol. The principal researcher planned the study, undertook data collection (without a research assistant), captured the data for analysis, analysed the data with the assistance of a statistician (Prof DG Nel), interpreted the data and drafted the thesis. Ms J Visser, Prof M Smuts and Dr J van Rensburg (supervisors) provided input at all stages and revised the protocol and thesis.

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List of text boxes

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List of abbreviations

Abbreviation	Description
Δ^5	delta 5
Δ^6	delta 6
AA	arachidonic acid
ACE	angiotensin-converting enzyme
ALA	α-linolenic acid
ANACOVA	analysis of covariance
ANOVA	analysis of variance
BMI	body mass index
CI	confidence interval
DGLA	dihomo-GLA
DHA	docosahexaenoic acid
DHEA	dehydroepiandrosterone
DMII	Type 2 diabetes mellitus
DPA	docosapentanoic acid
EFA	essential fatty acid
EPA	eicosapentaenoic acid
ESHRE/ASRM	European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine
FAMEs	fatty acid methyl esters
FFQ	food frequency questionnaire
GLA	γ-linolenic acid
HDL	high-density lipoprotein
HOMA	homeostasis model assessment
HOMA-IR	homeostasis model assessment – insulin resistance
IBS	Irritable bowel syndrome
IGT	impaired glucose tolerance
IR	Insulin resistance
IVF	in vitro fertilisation
LA	cis-linoleic acid
LC-MUFAs	long-chain monounsaturated fatty acids
LC-PUFAs	long-chain polyunsaturated fatty acids
LDL	low-density lipoprotein
LTs	leukotrienes
MD	mean absolute difference

Abbreviation	Description
MUFAs	monounsaturated fatty acids
MYO	myo-inositol
PCOS	polycystic ovarian syndrome
PGs	prostaglandins
PUFAs	polyunsaturated fatty acids
QUICKI	quantitative insulin sensitivity check index
RBCs	red blood cells
RDA	recommended daily allowance
RMANOVA	repeated measures analysis of variance
SD	standard deviation
SFAs	saturated fatty acids

List of key definitions

Word	Definition
Bioelectrical impedance analysis	The effective resistance of an electric circuit or component to alternating current, arising from the combined effects of ohmic resistance and reactance.(1)
Body mass index	An approximate measure of whether someone is over- or underweight, calculated by dividing the person's weight in kilograms by the square of her/his height in metres.(1)
Endometriosis	A condition resulting from the appearance of endometrial tissue outside the uterus and causing pelvic pain, especially associated with menstruation. (1)
Essential fatty acid	The 18-carbon fatty acids that are nutritionally required by humans and must be consumed though dietary sources. The EFA's are linoleic acid (C18:2n6) and linolenic acid (C18:2n3).(2)
Fertility	The capacity to conceive and bear offspring.(2)
Food frequency questionnaire	A set of printed or written questions with a choice of answers, devised for the purposes of a survey or statistical study. (1)
Infertility	Diminished or absent ability to produce offspring.(2)
Insulin	A hormone produced in the pancreas by the islets of Langerhans that regulates the amount of glucose in the blood. The lack of insulin causes a form of diabetes. (1)
Insulin resistance	An impaired response of the body to insulin, resulting in elevated levels of glucose in the blood. (1)
In vitro fertilisation	A medical procedure whereby an egg is fertilised by sperm in a test tube or elsewhere outside the body. (1)
Fatty acid	Any acid derived from fats by hydrolysis (e.g. oleic, palmitic or stearic acids).(2)

Word	Definition
Phenotypes	The set of observable characteristics of an individual resulting from the interaction of its genotype with the environment. [1]
Polycystic ovarian syndrome	A syndrome characterised by the presence of enlarged, polycystic ovaries and abnormal levels of gonadotropins, androgens and insulin that may cause such symptoms as menstrual disturbances, infertility, hirsutism and obesity: abbreviated as PCOS. [1]
Recommended daily allowance	The quantity of a particular nutrient that should be consumed daily in order to maintain good health. [1]
Reproduction	The production of offspring by a sexual or an asexual process. [1]
Saturated fatty acids	A fatty acid, the carbon chain of which contains no ethylenic or other unsaturated linkages between carbon atoms (e.g. stearic acid). Called saturated because it is incapable of absorbing any more hydrogen.[2]
Supplement	A substance taken to remedy the deficiencies in a person's diet. [1]
Type 2 diabetes mellitus	A disease in which the body's ability to produce or respond to the hormone insulin is impaired, resulting in abnormal metabolism of carbohydrates and elevated levels of glucose in the blood. There are two main types of diabetes. In Type 1 diabetes, the body lacks the cells that produce insulin in the pancreas. In Type 2 diabetes (which is more common and often develops later in life), the cells of the body fail to respond to insulin normally and the pancreas does not produce enough insulin. [1]
Unsaturated fatty acids	A fatty acid, the carbon chain of which possesses one or more double or triple bonds (e.g. oleic acid, with one double bond in the molecule, and linoleic acid, with two). Called unsaturated because it is capable of absorbing additional hydrogen.[2]
Pregnancy	The condition or period of being pregnant. [1]

CHAPTER 1

1 Introduction

Infertility is a global medical problem of the reproductive system that may affect the quality of a woman's life. Global fertility rates have fallen from 3.4 to 1.9 children per women in the period 1970–2010.[3] This can be due to many reasons, but infertility is definitely a major cause for concern. In 2015 infertility was estimated to affect as many as 186 million people globally.[4] Polycystic ovarian syndrome (PCOS) can affect up to 23% of women.[5] Assisted reproductive techniques are invasive, expensive and not easily accessible for all. Cheaper and easier accessible treatment options would therefore lessen this global burden.

PCOS is the most common hormonal disorder among women of reproductive age and is a leading cause in female infertility.[5-9] PCOS patients are more vulnerable to developing insulin resistance (IR) independent of obesity, at least 50% have metabolic syndrome, one-third have glucose intolerance and one out of five will develop Type 2 diabetes (DMII) before the age of 40 years.[5, 10] Hyperinsulinemia aggravates reproductive dysfunction by stimulating ovarian androgen production, causing a reduction of sex hormone-binding globulin (SHBG), which results in increased bioavailable testosterone. IR is a major factor in PCOS and can be indirectly related to poor fertility outcomes.[11]

Polyunsaturated fatty acids (PUFAs), in particular omega-6 and omega-3, have been shown to play an important role in reducing IR due to the impact that they have on cell membrane structure and their anti-inflammatory properties.[12-18] Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) omega-3 fatty acids can also improve insulin sensitivity by producing and secreting anti-inflammatory adipokins such as adiponectin and reducing pro-inflammatory cytokines.[19, 20] Dietary PUFAs are powerful modulators of lipid and glucose metabolism, which influences insulin secretion and resistance directly.[11] Many studies have shown that omega-3 fatty acids have positive effects on IR.[12-18] The role of omega-3 fatty acids in the reduction of IR in patients with PCOS have been identified and described.[21-23] PUFAs have been linked to significantly improved metabolic and endocrine effects in women with PCOS [19, 22, 24-26] and to a reduction in ovulatory infertility.[27, 28]

Against this background, the purpose of the study was to determine whether there was a difference in baseline PUFA status between women with PCOS who were struggling to conceive and a control group. Plasma phospholipids and red blood cell membranes (RBC) PUFAs, and dietary and supplemented PUFA intake were compared.

CHAPTER 2

2 LITERATURE REVIEW

2.1 Introduction

Infertility is defined as "diminished or absent ability to produce offspring"[5] or "the inability to conceive after 6-12 months of unprotected intercourse".[27] It is a common problem affecting 10–15% of couples.[29] To overcome infertility, assisted reproduction technologies have been developed, but to tackle infertility at a population level, these are not always a feasible option. It is therefore important to identify modifiable risk factors to prevent infertility.[9] Some evidence suggests that dietary factors affecting insulin sensitivity may have a role in the aetiology of some forms of infertility.[7, 29-31]

2.2 Polycystic ovarian syndrome

PCOS, or otherwise clinically known as Stein-Leventhal syndrome, is an endocrine disorder that affects many women.[7, 8, 32-34] It is the most common hormonal disorder among women of reproductive age, it is a leading cause in female infertility and it occurs amongst all nationalities and races.[6, 7] The syndrome can affect up to 23% of women, but the incidence in adolescence is still fairly unknown.[5, 34] It has a huge financial burden, and in the United States of America alone, it has been estimated at over 5 billion dollars a year.[5]

2.2.1 Pathophysiology

The disorder was previously considered an adult disorder only, but evidence suggests that it is in fact a lifelong disorder/syndrome and can manifest prenatally.[5] Prenatal risk factors include low birth weight infants who quickly catch up on their growth and high birth weight infants who continue to constantly increase their weight postnatally.[35, 36] Genetic susceptibility components along with these risk factors can lead to premature pubarche and adrenarche (increased laboratory dehydroepiandrosterone [DHEA]) and metabolic syndrome (that includes IR and visceral adiposity).[36] Signs and symptoms of hyperandrogenism and menstrual irregularities will develop in adolescence and will evolve into any of the various PCOS phenotypes in adulthood.

Possible pathogenesis of PCOS are described in Text box 2.1.

Text box 2.1: Possible pathogenesis of Poly cystic ovarian syndrome (PCOS)*

1) Hypothalamic-pituitary axis abnormalities cause abnormal secretion of gonadotropin-releasing hormone and luteinising hormone, resulting in increased ovarian androgen production.
2) An enzymatic defect of ovarian (adrenal) steroidogenesis favours excess androgen production.
3) IR drives the metabolic and reproductive abnormalities in PCOS.

*Data from PCOS Writing Committee.[9]

2.2.2 Diagnosis

Three different schools of thought for the diagnostic criteria for PCOS are commonly used (Figure 2.1):

1) In 1990 a consensus workshop sponsored by the National Institute of Health suggested that a patient had PCOS if:[34]
   a. She displayed signs of androgen excess (clinical or biochemical)
   b. She had oligo-ovulation
   c. Other entities that would cause polycystic ovaries were excluded
2) The European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM) sponsored a consensus workshop in 2003 in Rotterdam and concluded that for PCOS to be present, two out of three of the following criteria should be met, with the exclusion of other causes of PCOS:[8, 34, 37]
   a. Oligo-ovulation and/or anovulation
   b. Excess androgen action
   c. Polycystic ovaries (observed by gynaecologic ultrasound
3) In 2009 the Androgen Excess PCOS Society looked at all the peer-reviewed data to develop the following criteria:[38, 39]
   a. Hyperandrogenism (clinical and/or biochemical)
   b. Ovarian dysfunction (oligo-anovulation and/or polycystic ovaries)
   c. Exclusion of related disorders

All of the above are used in clinical settings. This study used the Rotterdam criteria for diagnosis.

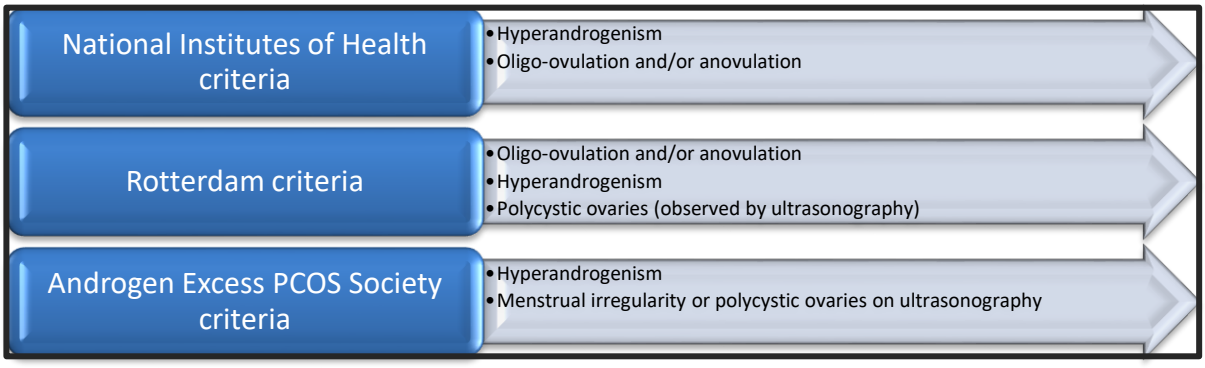


Figure 2.1: Guidelines for the diagnosis of Poly cystic ovarian syndrome (PCOS) [5, 8, 34]

2.2.3 Phenotypes

According to the Rotterdam criteria, PCOS is divided into four phenotypes because PCOS have a tendency to present as a spectrum of diseases:[37]

- Frank or classic PCOS – this is characterized by chronic anovulation, hyperandrogenism and polycystic ovaries.
- Classic non-polycystic ovary PCOS – this is classified by chronic anovulation, hyperandrogenism and normal ovaries.
- Non-classic ovulatory PCOS – this is classified by regular menstrual cycles, hyperandrogenism and polycystic ovaries.
- Non-classic mild or normoandrogenic PCOS – this is classified by chronic anovulation, normal androgens and polycystic ovaries.

Metabolic and cardiovascular risk factors are worse for women with the frank or classic phenotypes. In contrast, women with the normoandrogenic phenotype are less insulin resistant and tend to lack the metabolic features of PCOS when compared to women with the classic frank phenotype. These phenotypes were reviewed by the Amsterdam ESHRE/ASRM-sponsored Third PCOS Consensus Workshop Group.[40]

2.2.4 Signs and symptoms

The signs and symptoms of PCOS are summarised in Figure 2.2. The most common symptom definitions are provided in Text box 2.2.

Text box 2.2: Common definitions of Polycystic ovarian syndrome (PCOS) symptoms[5, 41, 42]

- Oligomenorrhea or amenorrhea – irregular, few or absent menstrual periods
- Infertility – generally resulting from chronic anovulation
- Hirsutism – unwanted body hair, typically in a male pattern, affecting face, chest and legs
- Dyspareunia – pain during sexual intercourse
- Androgenic alopecia – male-pattern baldness
- Acne, oily skin and seborrhoea
- Acanthosis nigrans – dark patches of skin, tan to dark brown or black, a sign of insulin resistance, which is associated with PCOS
- Acrochordons (skin tags) – tiny flaps of skin
- Prolonged periods of premenstrual stress-like symptoms (bloating, mood swings, pelvic pain and backaches)

2.2.4.1 Hyperandrogenism

Hyperandrogenism or androgen excess is a condition characterised by excessive levels of androgens (e.g. testosterone). Hyperandrogenism symptoms are a combination of acne, seborrhoea (inflamed skin), increased body and/or facial hair (hirsutism), hair loss on the scalp and an elevated libido.[5]

2.2.4.2 Menstrual irregularities

Irregular cycles or irregular periods are an abnormal variation in the length of a women's menstrual cycle. A menstruation cycle or period may be defined as irregular if it is shorter than 21 days or longer than 36 days. These are classified as polymenorrhea or oligomenorrhea respectively. Amenorrhea is the absence of menstrual period in women of reproductive age.[5]

2.2.4.3 Polycystic ovaries observed via ultrasonography

Ultrasonography or medical ultrasound is a diagnostic imaging technique based on the application of ultrasound.[7] Polycystic ovaries are diagnosed if there are 10 or more cysts or follicles of a diameter between 2 mm and 9 mm on one or both ovaries and the ovarian volume in at least one ovary exceeds 10 ml.[5]

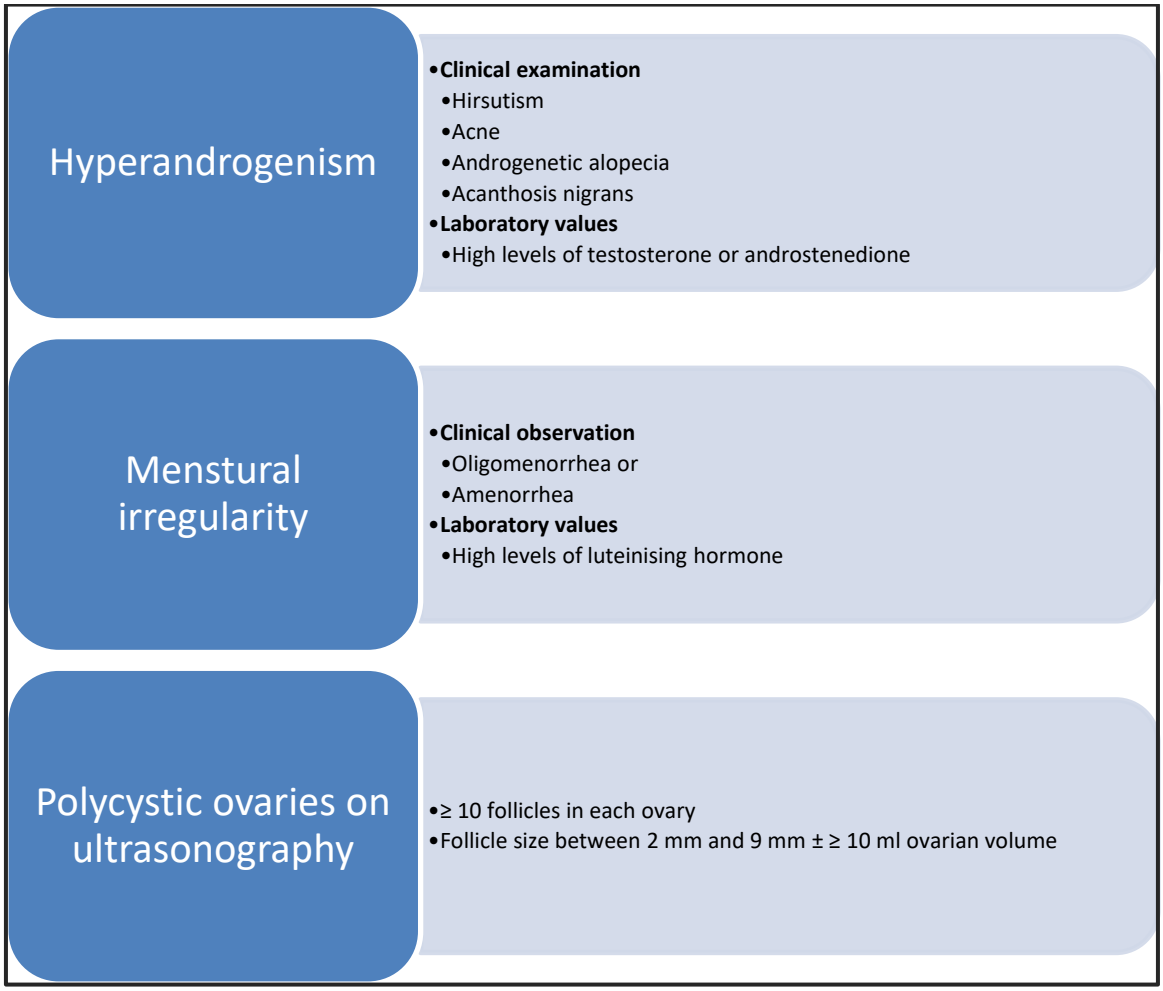


Figure 2.2: Signs and symptoms of patients with Poly cystic ovarian syndrome (PCOS) [5, 9, 42]

PCOS has multiple components, including reproductive, metabolic, cardiovascular and psychological.[6, 33]

2.2.5 Components of polycystic ovarian syndrome

2.2.5.1 Reproductive

Infertility is 10 times more common in women with PCOS compared to healthy controls.[43] Reduced fertility is a result of the oligo- or anovulation associated with PCOS. Extended absence of ovulation can also result in incessant endometrial stimulation by oestrogen, unhampered by progesterone. Women, therefore, have an increased risk of endometrial hyperplasia and possibly endometrial cancer. PCOS is also characterised by hyperandrogenism, which can cause, among others, acne, hirsutism or male-pattern hair loss.[33] Fertility may also be affected due to impaired implantation and higher rates of natural abortions.[7] High-risk pregnancies are also associated with PCOS due to a higher incidence of pre-eclampsia and gestational diabetes.[5]

Fulghesu et al[44]evaluated the influence of insulin level on ovarian response to ovulation induction by follicle-stimulating hormone (FSH) in 34 women with PCOS. They concluded that hyperinsulinemic patients may be at a greater risk for ovarian hyperstimulation.

2.2.5.2 Metabolic

IR is considered the main pathogenic factor of increased metabolic disturbances in women with PCOS.[45] The syndrome is marked as a prediabetic state due to the accompanying IR and hyperinsulinemia, and has a high incidence of impaired glucose tolerance (IGT), gestational diabetes and overt diabetes.[7] Both lean and obese women with PCOS can have increased IR and impaired beta-cell function. By their fourth decade, 31% have IGT and 7.5% have DMII. Almost 50% of them meet the criteria of the metabolic syndrome as defined by the National Cholesterol Education Program Adult Treatment Panel III.[32, 46] Studies with the euglycemic clamp technique indicate that woman with hyperinsulinemia and excess androgen activity have peripheral IR and a reduced insulin clearance rate due to decreased hepatic insulin extraction.[33]

PCOS is associated with numerous other metabolic complications including hypertension, central obesity, non-alcoholic fatty liver disease, dyslipidaemia and obstructive sleep apnoea.[6, 8, 33]

IR can be measured via several tests including the gold standard for direct measurement, the hyperinsulinemic euglycemic glucose clamp.[10] This is a very reliable test. The homeostasis model assessment-insulin resistance (HOMA-IR) (a fasting index used in the determination of basal IR, using only the fasting insulin and glucose values) and the quantitative insulin sensitivity check index (QUICKI) (another fasting index for IR, inverse logarithm of the HOMA-IR, also utilising fasting insulin and glucose measurements) are the most used methods because they are less invasive and simpler, though not necessarily as precise than other methods.[6]

Many new proteins are surfacing as potential markers of IR in PCOS. According to the literature, there is a strong connection between adipocytokines (in particular adiponectin, visfatin, vaspin and apelin), irisin, copeptin, plasminogen activator inhibitor-1 and zonulin, and IR and PCOS. The role of resistin, leptin, kisspetin and ghrelin is still controversial.[10, 47]

2.2.5.3 Cardiovascular

Cardiovascular disease risk is increased due to metabolic and biochemical changes such as dyslipidaemia and hypertension.[7, 33] In addition, hyperinsulinemia, either directly or more likely as a surrogate for IR, may independently contribute to the development of cardiovascular disease.[48]

All of the abovementioned effects may also be related to hyperinsulinemia.[5, 7]

2.2.5.4 Cancer

Women with PCOS may have many risk factors associated with the development of endometrial cancer, such as IR, DMII, anovulation and obesity.[49] Endometrial hyperplasia is triggered by unopposed uterine oestrogen exposure, which can be triggered by anovulation. This can ultimately trigger endometrial cancer.[43] Women with PCOS have a threefold increased risk of developing endometrial cancer.[40] There is, however very little data to support any association between PCOS and breast and ovarian cancer. [40]

2.2.5.5 Psychological issues

Exploration of psychological issues is very limited, but a few studies have found that women with PCOS have high incidence of depression, reduced health-related quality of life and deprived sexual satisfaction. In addition, eating disorders may be more prevalent.[9, 33]

2.2.6 Treatment options for polycystic ovarian syndrome

The optimal dietary treatment for PCOS is not known, despite the fact that the effect of weight loss is well known.[8, 32, 50], High-protein diets are being encouraged because of their beneficial effects on satiety, lean body mass, weight maintenance and lipid markers.[8] High-fat diets are suggested to reduce insulin response.[32] Treatment of this disorder should focus on reduction of androgen-associated symptoms, protection of the endometrium and reduction of the long-term risks of diabetes and cardiovascular complications.[8]

2.2.6.1 Lifestyle interventions

Lifestyle interventions such as diet and exercise are first-line treatments for women with PCOS, particularly if they are overweight. Several nonrandomised trials have shown that a reduction in body weight through diet and exercise improves insulin sensitivity and ovulation rate.[5, 51-54] Gambineri et al showed that weight loss of 5–7% body weight decreased the conversion from IGT to DMII by 58% over a three-year period.[51]

Intermittent fasting has recently been investigated as an alternative dietary treatment, but more research is needed in this area.[55]

2.2.6.2 Supplements

2.2.6.2.1 Chromium picolinate

Chromium picolinate has been shown to improve glucose tolerance compared with a placebo, but it did not improve ovulatory frequency or hormonal parameters according to Fogle in 2007.[50] In a more recent randomized control study (2016), chromium picolinate significantly increased the chances of ovulation and regular menstruation by almost twofold after five months of treatment. [56]

2.2.6.2.2 Inositol

Growing evidence suggests that inositol, an insulin-sensitising molecule, can be used to improve IR in women with PCOS.[57-62] Two stereoisomers of inositol, myo-inositol (MYO) and D-chiroinositol, are being investigated. IR might be the result of an alteration of metabolism of inositol phosphoglycans second messengers and mediators. It can also be due to a defect in their tissue availability. Many trials showed that MYO improved IR in women with PCOS.[57-60] More recent studies looked at the effect of MYO with a combination of other drugs or supplements. When combined with monacolin K and lipoic acid, inositol had a dose-dependent improvement in hyperandrogenism-associated symptoms and dyslipidaemia.[58] When inositol was combined with folic acid, hyperstimulation syndrome was decreased to a higher extent than with MYO alone.

2.2.6.3 Hormone therapy

If pregnancy is not desired, hormonal contraceptive agents can be used to provide endometrial protection and treat the symptoms of hyperandrogenism.[33]

Spironolactone, a competitive antagonist of aldosterone, effectively treats hirsutism. Spironolactone is often used in combination with oral contraceptives because of the additive effects of androgen suppression (oral contraceptives) and androgen blockade (spironolactone). Spironolactone is contraindicated during pregnancy because of potential teratogenicity.[33]

2.2.6.4 Other cosmetic treatments

In addition, permanent hair reduction can be achieved with laser or electrolysis therapy.[33]

2.2.6.5 Metformin

Metformin, a hypoglycaemic agent that potentiates the action of insulin, has become a popular treatment because it improves insulin sensitivity, hyperandrogenaemia and possibly ovulation, especially in overweight women with PCOS.[29, 33, 51, 59, 63-67] Metformin is commonly used to treat infertility, either alone or in

combination with clomiphene-citrate.[9, 51] Because it increases ovulation in some women, it can also increase the frequency of endometrial shedding and may help with cycle control. It is not known whether using metformin to treat IR in women with normal glucose levels improves long-term outcomes. In other populations, metformin decreases conversion from IGT to DMII. Thus, metformin may be useful in women with PCOS and hyperglycaemia. The decision to prescribe this drug should be made on an individual basis.[33]

2.2.6.6 Thiazolodinediones

Troglitazone, an oral hypoglycaemic agent, improved glycaemic measures, ovulation, hirsutism and free testosterone levels in women with PCOS in a large randomised controlled trial.[65] In 2002 troglitazone was withdrawn from the market secondary to hepatic toxicity seen in other trials.[9] More recently, smaller trials of newer glitazones, namely rosiglitazone and pioglitazone, have had promising results. However, these benefits need to be confirmed in larger trials. Because thiazolodinediones are Category C drugs, patients should be counselled to use contraception while taking the drugs.[33]

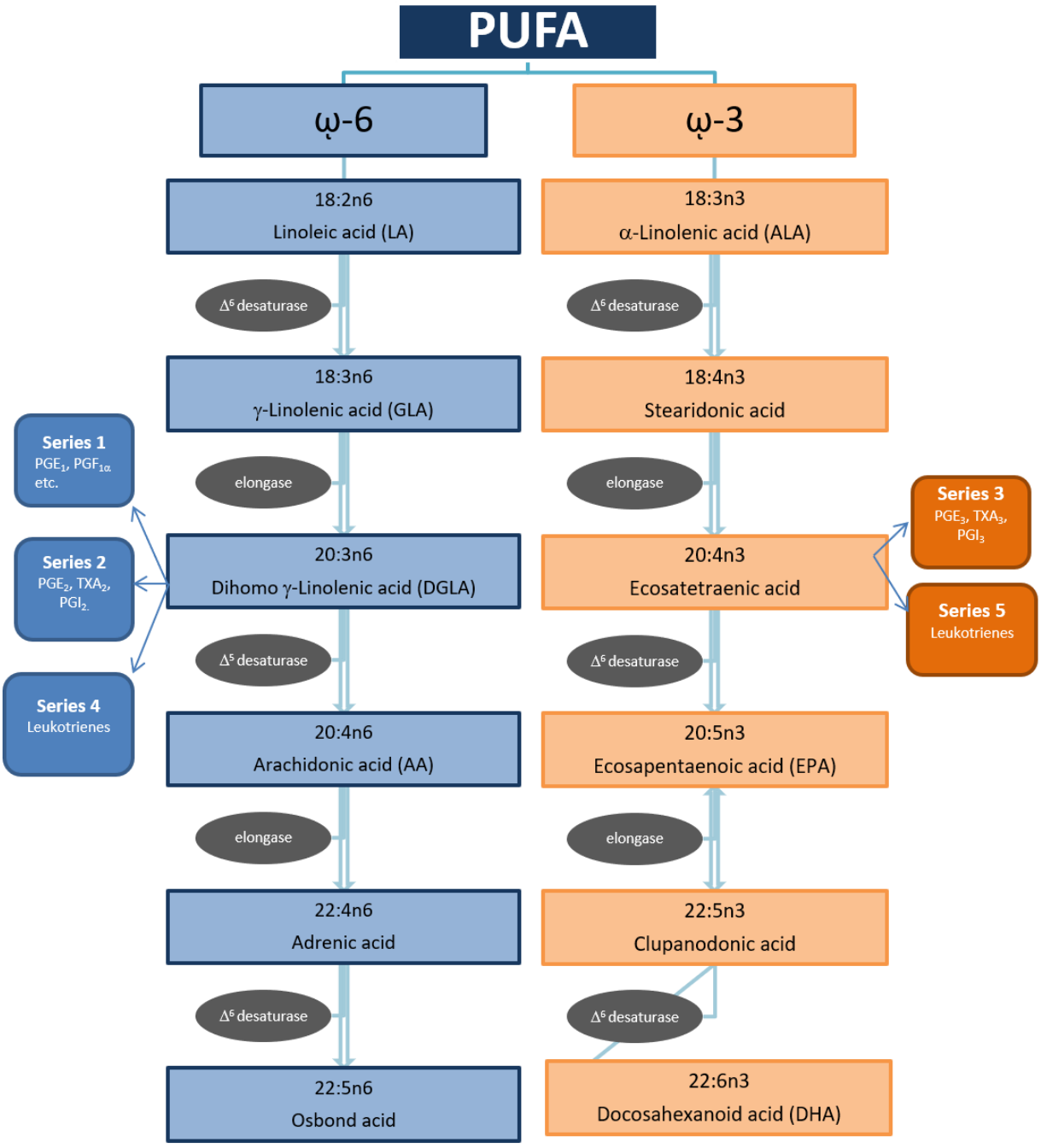
2.3 Essential fatty acids

2.3.1 Classification

EFAs are PUFAs since they contain two or more double bonds.[18, 68, 69] EFAs are essential for survival of humans and, as they are not synthesised in the body, they have to be consumed through the diet. EFAs are absolutely necessary for numerous processes including growth, reproduction, vision and brain development.[69] There are two EFAs in the body, in the omega-6 series cis-linoleic acid (LA, 18:2) and in the omega-3 series α-linolenic acid (ALA, 18:3). There is another sequence of fatty acids derived from oleic acid (18:1n-9), but oleic acid is not an EFA. The omega-9, omega-6 and omega-3 series of fatty acids are metabolised by the same set of enzymes to their respective long-chain metabolites.[18, 68, 69]

LA is converted to γ-linolenic acid (GLA, 18:3n-6) by the enzyme delta-6 (Δ^6) desaturase, and GLA is elongated to form dihomo-GLA (DGLA, 20:3n-6), the precursor of the 1 series of prostaglandins (PGs). DGLA can also be converted to arachidonic acid (AA, 20:4n-6) by the enzyme delta 5 (Δ^5) desaturase. AA forms the precursor of the 2 series of PGs, thromboxanes and the 4 series of leukotrienes (LTs). ALA is converted to eicosapentanoic acid (EPA, 20:5n-3) by Δ^6 desaturase and Δ^5 desaturase. EPA forms the precursor of the 3 series of PGs, TXs and the 5 series of LTs. LA, GLA, DGLA, AA, ALA, EPA and docosahexanoic acid (DHA, 22:6, n-3) are all PUFAs, but only LA and ALA are EFAs.[17, 18, 69, 70]

In the present discussion, the term 'PUFAs' is used to refer to all unsaturated fatty acids (UFAs): LA, GLA, DGLA, AA, ALA, EPA and DHA, and the term 'EFAs' refers to LA and ALA. The metabolism of PUFAs is illustrated in Figure 2.3.



PGE= prostaglandins, TXA = thromboxane's

Figure 2.3: Scheme to illustrate the biosynthesis of long chain polyunsaturated fatty acid pathway (LCPUFAs) [69, 71]

The common names, systematic names, structural formulas and lipid numbers are listed in tables 2.1 and 2.2 for saturated and unsaturated fatty acids respectively.

Table 2.1: List of saturated fatty acids[72]

Common name	Systematic name	Structural formula	Lipid numbers
Propionic acid	Propanoic acid	CH_3CH_2COOH	C3:0
Butyric acid	Butanoic acid	$CH_3(CH_2)_2COOH$	C4:0
Valeric acid	Pentanoic acid	$CH_3(CH_2)_3COOH$	C5:0
Caproic acid	Hexanoic acid	$CH_3(CH_2)_4COOH$	C6:0
Enanthic acid	Heptanoic acid	$CH_3(CH_2)_5COOH$	C7:0
Caprylic acid	Octanoic acid	$CH_3(CH_2)_6COOH$	C8:0
Pelargonic acid	Nonanoic acid	$CH_3(CH_2)_7COOH$	C9:0
Capric acid	Decanoic acid	$CH_3(CH_2)_8COOH$	C10:0
Undecylic acid	Undecanoic acid	$CH_3(CH_2)_9COOH$	C11:0
Lauric acid	Dodecanoic acid	$CH_3(CH_2)_{10}COOH$	C12:0
Tridecylic acid	Tridecanoic acid	$CH_3(CH_2)_{11}COOH$	C13:0
Myristic acid	Tetradecanoic acid	$CH_3(CH_2)_{12}COOH$	C14:0
Pentadecylic acid	Pentadecanoic acid	$CH_3(CH_2)_{13}COOH$	C15:0
Palmitic acid	Hexadecanoic acid	$CH_3(CH_2)_{14}COOH$	C16:0
Margaric acid	Heptadecanoic acid	$CH_3(CH_2)_{15}COOH$	C17:0
Stearic acid	Octadecanoic acid	$CH_3(CH_2)_{16}COOH$	C18:0
Nonadecylic acid	Nonadecanoic acid	$CH_3(CH_2)_{17}COOH$	C19:0
Arachidonic acid	Eicosanoic acid	$CH_3(CH_2)_{18}COOH$	C20:0
Heneicosylic acid	Heneicosanoic acid	$CH_3(CH_2)_{19}COOH$	C21:0
Behenic acid	Docosanoic acid	$CH_3(CH_2)_{20}COOH$	C22:0
Tricosylic acid	Tricosanoic acid	$CH_3(CH_2)_{21}COOH$	C23:0
Lignoceric acid	Tetracosanoic acid	$CH_3(CH_2)_{22}COOH$	C24:0
Pentacosylic acid	Pentacosanoic acid	$CH_3(CH_2)_{23}COOH$	C25:0
Cerotic acid	Hexacosanoic acid	$CH_3(CH_2)_{24}COOH$	C26:0
Heptacosylic acid	Heptacosanoic acid	$CH_3(CH_2)_{25}COOH$	C27:0
Montanic acid	Octacosanoic acid	$CH_3(CH_2)_{26}COOH$	C28:0
Nonacosylic acid	Nonacosanoic acid	$CH_3(CH_2)_{27}COOH$	C29:0
Melissic acid	Triacontanoic acid	$CH_3(CH_2)_{28}COOH$	C30:0
Henatriacontylic acid	Henatriacontanoic acid	$CH_3(CH_2)_{29}COOH$	C31:0
Lacceroic acid	Dotriacontanoic acid	$CH_3(CH_2)_{30}COOH$	C32:0
Psyllic acid	Tritriacontanoic acid	$CH_3(CH_2)_{31}COOH$	C33:0
Geddic acid	Tetratriacontanoic acid	$CH_3(CH_2)_{32}COOH$	C34:0
Ceroplastic acid	Pentatriacontanoic acid	$CH_3(CH_2)_{33}COOH$	C35:0
Hexatriacontylic acid	Hexatriacontanoic acid	$CH_3(CH_2)_{34}COOH$	C36:0
Heptatriacontanoic acid	Heptatriacontanoic acid	$CH_3(CH_2)_{35}COOH$	C37:0

Common name	Systematic name	Structural formula	Lipid numbers
Octatriacontanoic acid	Octatriacontanoic acid	$CH_3(CH_2)_{36}COOH$	C38:0

Table 2.2: List of unsaturated fatty acids[73]

Common name	ω–n	Lipid numbers	Trans or Cis	Food sources
α-Linolenic acid (ALA)	ω−3	C18:3	Cis	Flaxseeds, chia seeds, walnuts, hempseed oil, canola oil, hazelnuts, eggs, meat and dark green leafy vegetables
Stearidonic acid	ω−3	C18:4	Cis	Seed oils of hemp, blackcurrant and corn
Eicosatetraenoic acid	ω−3	C20:4	Cis	
Eicosapentaenoic acid (EPA)	ω−3	C20:5	Cis	Cod liver oil and fatty fish such as herring, mackerel, salmon, tuna and sardine, pilchards
Docosatrienoic acid	ω−3	C22:3	Cis	
Docosapentanoid acid (DPA)	ω−3	C22:5	Cis	
Docosahexaenoic acid (DHA)	ω−3	C22:6	Cis	Maternal milk and fish oil
cis-Linoleic acid (LA)	ω−6	C18:2	Cis	Peanut oil, chicken fat, olive oil and nuts, sunflower oil
γ-Linolenic acid (GLA)	ω−6	C18:3	Cis	Borage oil, blackcurrant oil, evening primrose oil and safflower oil
Eicosadienoic acid	ω−6	C20:2	Cis	
Dihomo-γ-linolenic acid (DGLA)	ω−6	C20:3	Cis	Only in trace amounts in animal products
Arachidonic acid (AA)	ω−6	C20:4	Cis	
Adrenic acid	ω−6	C22:4	Cis	
Osbond acid	ω−6	C22:5	Cis	
Palmitoleic acid	ω−7	C16:1	Cis	Macadamia nuts
Vaccenic acid	ω−7	C18:1	Cis	
Vaccenic acid	ω−7	C18:1	Trans	Dairy products such as milk, butter and yogurt.
Paullinic acid	ω−7	C20:1	Cis	Guarana
Oleic acid	ω−9	C18:1	Cis	Olive oil, pecan oil[14] and canola oil
Elaidic acid	ω−9	C18:1	Trans	Partially hydrogenated vegetable oil
Gondoic acid/eicosenoic acid	ω−9	C20:1	Cis	Jojoba oil (edible but noncaloric and nondigestible)
Mead acid	ω−9	C20:3	Cis	
Erucic acid	ω−9	C22:1	Cis	Wallflower seeds and mustard oil
Nervonic acid	ω−9	C24:1	Cis	King salmon, flaxseeds, sockeye salmon, sesame seeds and macadamia nuts

2.3.2 Dietary fatty acids

In westernised societies, the average consumption of omega-6 PUFAs far exceeds nutritional requirements (see dietary sources of PUFAs in Table 2.3). The ratio of omega-6 to omega-3 PUFAs now ranges from 10:1 to 25:1 in westernised human populations whereas in a primitive human diet, it was closer to 1:1.[12, 68, 69]

Table 2.3: Dietary sources of Polyunsaturated fatty acids (PUFAs)[69, 71]

Main dietary sources of EFAs and other PUFAs	
cis-Linoleic acid **(LA)**	Cereals, wholegrain breads, poultry, most vegetable oils, eggs, baked goods, margarine, sunflower oil, safflower oil and corn oil.
α-Linolenic acid **(ALA)**	Canola oil, flaxseed oil, linseed and grapeseed oil, chia seeds, hempseed oil, hazelnuts, walnuts, leafy green vegetables, human milk, eggs and meat.
γ-Linolenic acid **(GLA)**	Human milk, evening primrose oil, blackcurrant oil, borage oil, hempseed oil and some fungal sources.
Dihomo-γ-linolenic acid **(DGLA)**	Moderate amounts are found in human milk, liver, testes, adrenals and kidneys.
Arachidonic acid **(AA)**	Human milk contains modest amounts and cow's milk small amounts. Meat, egg yolks, some seaweeds and some shrimps contain substantial amounts.
Eicosapentaenoic acid **(EPA)** and docosahexaenoic acid **(DHA)**	The major source is marine fish.

2.3.3 Essential fatty acids and insulin resistance

IR is a generalised metabolic disorder and is defined as the decreased ability of insulin to stimulate glucose disposal into target tissues or a reduced glucose response to a specified amount of insulin.[10, 59] Chronic hyperinsulinemia is a compensatory response to this target tissue resistance. Several mechanisms have been suggested to explain IR, including decreased hepatic clearance, peripheral target tissue resistance and increased pancreatic sensitivity.[70, 74] It is associated with increased plasma triacylglycerol, elevated postprandial lipidaemia, low high-density lipoprotein (HDL) concentrations and a predominance of small low-density lipoprotein (LDL).[74]

There is a large body of evidence supporting the fact that a high intake of fat is associated with impaired insulin sensitivity.[13, 14, 70, 74] The quality of dietary fat also affects insulin sensitivity, independently of its effects on body weight.[75] Intervention studies and epidemiological evidence clearly show that in humans, MUFAs and PUFAs improve IR while saturated fat significantly worsens it.[12-18] Interestingly, insulin action is more improved with a higher content of PUFAs of a chain length of 20–22 carbons (long-chain PUFAs [LC-PUFAs]), more especially those belonging to the omega-3 fatty acid family.[14, 70, 74] In vitro studies, however, have not always corroborated the clinical evidence,[70] and one study actually found that moderate omega-3

fatty acid supplementation did not affect insulin sensitivity (it must be noted that this study was done in healthy individuals).[76]

It has been demonstrated that fish oil altered insulin secretion in women with PCOS.[11] Cross-sectional data showed that PUFAs modulated lipid and hormonal profiles and that supplementation with LC-PUFAs improved androgenic profiles in PCOS.[11]

2.3.4 Actions of polyunsaturated fatty acids and their metabolites

PUFAs play a significant role in collagen vascular diseases, hypertension, diabetes mellitus, IR, metabolic syndrome X, psoriasis, eczema, atopic dermatitis, coronary heart disease, atherosclerosis and cancer. This is in addition to the role of PGs and LTs in these conditions.[12]

Insulin sensitivity is affected by the dietary fatty acid profile. Multiple mechanisms may be involved in these effects:

2.3.4.1 Cell membrane fluidity

Lipid composition in a cell membrane determines the fluidity: increasing its content of saturated fatty acids (SFAs) and cholesterol yields a more rigid membrane whereas increasing UFAs makes it more fluid. The number of receptors and their affinity to their respective hormones/growth factors/proteins depend on the fluidity of the cell membrane. One example is that a rigid cell membrane has reduced the number of insulin receptors and their affinity to insulin, which causes IR. In contrast, increase in cell membrane fluidity, with an increased intake of UFAs, will increase the number of insulin receptors on the membrane and their affinity to insulin, which results in decreased IR.[13-15, 69, 77, 78]

2.3.4.2 Second messenger action

PUFAs inhibit leukocyte angiotensin-converting enzyme (ACE) activity, suggesting that they could function as endogenous regulators of ACE activity and thus regulate the formation of angiotensin-II. PUFAs enhance nitric oxide generation. Hence, when tissue/cell concentrations of PUFAs are low, the formation of angiotensin-II is high whereas that of endothelial nitric oxide is low. It has been noticed that plasma concentrations of PUFAs and endothelial nitric oxide are low in IR, diabetes mellitus and obesity, to name a few.[29]Low levels of endothelial nitric oxide (eNO), brain derived neurotrophic factor (BDNF) and inflammation can alter hypothalamic neurotransmitter activity, gut hormones, more free radicals as well as an imbalance of pro- and anti-inflammatory metabolites from bioactive lipids. DMII is caused by increased peripheral insulin resistance secondary to increased production of IL-6 and TNF-α. DMII is also associated with alteration in the production and action of hypothalamic neurotransmitters, eNO, BDNF, free radicals and gut hormones. Das et al suggest that bioactive lipids, such as AA, EPA, and DHA and their anti-inflammatory metabolites: lipoxin A4, resolvins, protectins, and maresins, may have antidiabetic actions.

These bioactive lipids have anti-inflammatory actions, enhance eNO, BDNF production, restore hypothalamic dysfunction, enhance vagal tone, modulate production and action of ghrelin, leptin and adiponectin, and influence gut microbiota that may explain their antidiabetic action.[79, 80]

2.3.4.3 Metabolic syndrome

Metabolic syndrome is characterised by IR, abdominal obesity, hyperinsulinemia, DMII, essential hypertension, hyperlipidaemia, atherosclerosis and coronary heart disease. Plasma levels of C-reactive protein (CRP), interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α), markers of inflammation, are increased in subjects with IR, obesity, DMII, essential hypertension and coronary heart disease, suggesting that metabolic syndrome might be a low-grade systemic inflammatory condition.[9, 12, 29, 81, 82] It is well known that PUFAs, especially those from the omega-3 series, can reduce inflammation.

An exciting new possibility is the polymorphisms noted in the peroxisome proliferator-activated receptor (PPARg-2) gene, which can possibly explain genetic predisposition to metabolic syndrome and also the potential linking of the immune system to fat-induced IR via the IkB kinase/nuclear factor-kappa B pathway.[70]

2.3.4.4 Triglycerides

In skeletal muscle tissue, IR can be due to triglyceride accumulation while in pancreatic β-cells, it causes cellular apoptosis with a consequent reduction of insulin secretion and ultimately frank diabetes mellitus.[15, 74] It is also well established that omega-3 PUFAs lower serum triglyceride levels.[15, 75]

2.4 Reproduction

There is evidence that both omega-6 and omega-3 PUFAs can influence reproductive processes through a variety of mechanisms, but the total effect is still unclear.[11, 69, 77] PUFAs provide the precursors for PG synthesis and can modulate the expression patterns of many key enzymes involved in both PG and steroid metabolism. Hardly any studies related to the reproductive system have actually measured 1- or 3-series PG production, in large part due to the limitations of appropriate methodology. Early enthusiasm for the possibility of using fish oil to improve fertility in cattle has not always been replicated in later studies. Some investigations have been underpowered. Others have lacked appropriate controls.[69] During fertilisation, the PUFA composition of the cell membranes of the sperm and oocyte is very important. Spermatozoa require a high PUFA content to provide the plasma membrane with the fluidity essential at fertilisation. However, this makes spermatozoa particularly vulnerable to be attacked by reactive oxygen species. Lifestyle factors promoting oxidative stress have clear associations with reduced fertility. Little is known about the overall effects of PUFAs on fertility, although theoretically, both positive and negative actions are possible.[13]

Factors known to increase IR, for instance decreased physical activity and increased body weight, have been associated with an elevated risk of infertility due to ovulatory dysfunction.[29]

2.5 Conclusion

From the literature, it is evident that IR is a common risk factor of PCOS. PCOS is the leading cause of female infertility due to an-ovulation. Reproduction and ovulation difficulties can be the result of IR. It has also been shown that EFAs can improve IR and androgenic profiles in PCOS. To the researcher's knowledge, no research has been done on the relationship between EFAs and pregnancy rates in individuals with PCOS. The motivation for this study was therefore to investigate the baseline PUFA status in women with PCOS and to compare that to women without PCOS.

CHAPTER 3

3 METHODS

3.1 Research question

What is the Polyunsaturated fatty acid (PUFA) status of women with Polycystic Ovarian Syndrome (PCOS) and infertility, compared to women without PCOS and infertility?

3.2 Aim and objectives

3.2.1 Research aim

To investigate the baseline PUFA status of women of child-bearing age (18–45 years) with PCOS who had been unable to conceive for at least six months and compare it to a control group without PCOS and infertility.

3.2.2 Specific objectives

To compare:

- Plasma phospholipids and RBC membranes PUFA status in women with PCOS and women without PCOS
- Dietary EFA and PUFA intake of women with PCOS and women without PCOS
- Supplemented PUFA intake of women with PCOS and women without PCOS
- Other dietary/nutritional supplement intake of women with PCOS and women without PCOS
- Basic anthropometry of women with PCOS and women without PCOS

To describe the fertility history of women with PCOS.

3.2.3 Hypothesis

There is no significant difference between women with PCOS and women without PCOS in terms of plasma phospholipids and RBC membranes PUFA status, dietary EFA and PUFA intake, supplemented PUFA intake and anthropometric parameters.

3.3 Definitions

3.3.1 Polycystic ovarian syndrome

According to the Rotterdam criteria of 2003, PCOS is present if two out of three of the following criteria are met, with the exclusion of other causes of PCOS:

- Oligo-ovulation and/or anovulation
- Excess androgen activity

- Polycystic ovaries[8]

These criteria were used because they were the criteria used by the doctors at Medfem Fertility Clinic.

3.3.2 Endometriosis

Endometriosis is an ectopic occurrence of endometrial tissue, frequently forming cysts containing blood.[2, 83] Laparoscopy is the gold standard diagnostic test.[83, 84] Currently, no noninvasive tests that accurately diagnose this condition are available in clinical practice.[84-88]

3.3.3 Nutritional supplement

A nutritional supplement, also known as a food supplement or dietary supplement, is a preparation intended to supplement the diet and provide nutrients, such as vitamins, minerals, PUFAs, fibre and amino acids.[2]

3.3.4 Dietary polyunsaturated fatty acids

These are PUFAs that are consumed through the diet. For the purpose of the study, the researcher used a three-day food intake record to measure PUFAs consumption.

3.3.5 Three-day food intake record

Food records ask participants to record all foods and beverages consumed over a specific period of time, usually 3 to 7 days or during multiple periods within a year.[89]

3.3.6 Serum phospholipid and RBC membrane polyunsaturated fatty acids

The following fatty acids were analysed: C14:0 Myristic, C16:0 Palmitic, C16:1n7 Palmitoleate, C18:0 Stearic, C18:1n9T Elaidic, C18:1n7T Vaccenic, C18:1n9 Oleic, C18:1n7 Vaccenic, C18:2n6 Linoleic, C20:0 Eicosenoic, C18:3n6 GLA, C18:3n3 ALA, C20:1n9 Eicosenoic, C18:4n3 Stearidonic, C20:2n6 Eicosadienoic, C20:3n9 Mead, C22:0 Behenic, C20:3n6, C20:3n3, C20:4n6 Arachidonic, C22:1n9 Erucic, C20:5n3 EPA, C24:0 Lignoceric, C22:3n3 Docosatranoic, C24:1n9 Nervonic, C22:4n6 Adrenic, C22:3n6 Osbond, C22:5n3 DPA, C22:6n3 DHA, SFAs, MUFAs, PUFAs, Trans fatty acids, n6 PUFAs, n3 PUFAs, n6 LC-PUFAs, n3 LC-PUFAs, n6:n3 PUFA ratio, n6:n3 LC-PUFA ratio.

3.3.7 Polyunsaturated fatty acid supplement

A PUFA supplement is a supplement that includes LA, ALA, GLA, EPA or DHA or a combination thereof.

3.3.8 Body mass index

Body mass index (BMI) is a definition of the degree of adiposity. The formula used is BMI $= \frac{weight\ (kg)}{height\ (cm)^2}$.

Cut-off values according to the World Health Organization are listed in Table 3.1.

Table 3.1: The international classification of adult underweight, overweight and obesity according to Body mass index (BMI) [90]

Classification	BMI (kg/m²)	
	Principal cut-off points	Additional cut-off points
Underweight	< 18.50	< 18.50
Severe thinness	< 16.00	< 16.00
Moderate thinness	16.00–16.99	16.00–16.99
Mild thinness	17.00–18.49	17.00–18.49
Normal range	18.50–24.99	18.50–22.99
		23.00–24.99
Overweight	≥ 25.00	≥ 25.00
Pre-obese	25.00–29.99	25.00–27.49
		27.50–29.99
Obese	≥ 30.00	≥ 30.00
Obese class I	30.00–34.99	30.00–32.49
		32.50–34.99
Obese class II	35.00–39.99	35.00–37.49
		37.50–39.99
Obese class III	≥ 40.00	≥ 40.00

3.3.9 Waist circumference

Waist circumference is the distance around the smallest firth below the rib cage and above the umbilicus. The circumference provides a risk prediction of obesity-related disease and can be used in patients with a BMI up to 35. Waist circumference measurement assesses abdominal fat content. A measurement of greater than 88 cm in women is an independent risk factor for disease. A circumference less than 80 cm is classified as normal.[91]

3.3.10 Medfem Fertility Clinic

A fertility clinic in Bryanston South Africa.

3.4 Conceptualisation

3.4.1 Conceptual framework

The conceptual framework for the study is shown in Figure 3.1.

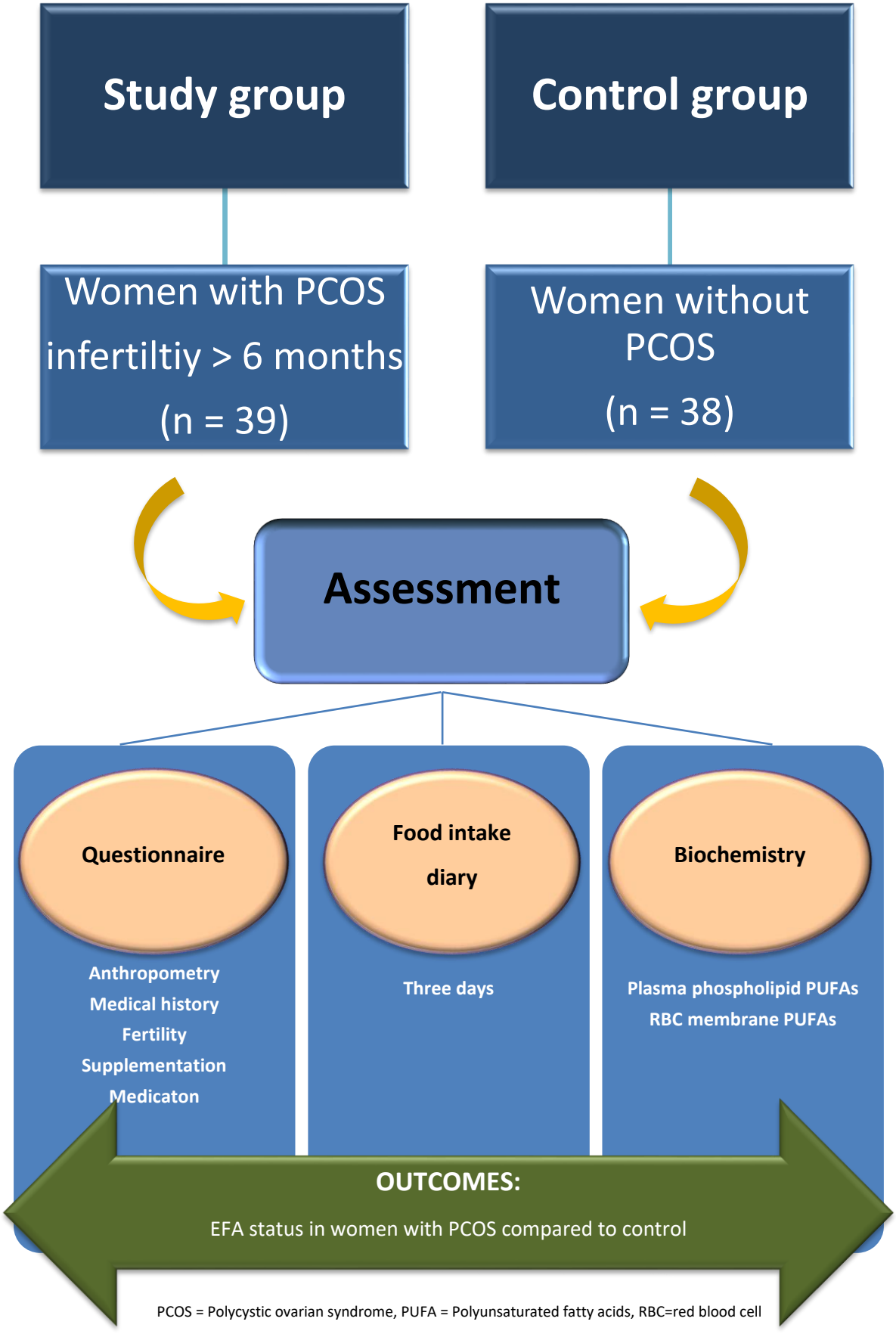


Figure 3.1: Conceptual framework for study

3.5 Operationalisation

3.5.1 Polycystic ovarian syndrome

PCOS was diagnosed by the gynaecologist according to the Rotterdam criteria:[92]

3.5.2 Polyunsaturated fatty acid supplement

Patients were asked to report which brand of PUFA they took and what the dosage was. To improve reliability, patients were asked to bring all supplements to the consultation.

3.5.3 Nutritional supplement

Patients were asked to report which nutritional supplements they took, which brands and what dosage. To improve reliability, patients were asked to bring all supplements to the consultation.

3.5.4 Three-day food intake record

Patients were asked to record an estimated food intake record (Addendum 1). Patients were asked to record two weekdays and one weekend day.

To determine PUFA intake, the researcher used an estimated food intake record (Addendum 1) as it was a valid dietary assessment tool, the subjects did not need to depend on their memory and they could provide detailed intake.[93] Multiple days are more representative of usual intake, and the researcher therefore asked two weekdays and one weekend day to be recorded. Food records are valid up to five days. The researcher was aware of the disadvantages, namely that a high degree of cooperation was required, that it was more time consuming and that the act of recording might alter the diet. Subjects also had to be literate, which the study population fortunately was. The three-day food intake record was analysed with FoodFinder3 software.[94]

The researcher acknowledges that a food frequency questionnaire (FFQ) may be an ideal tool to estimate PUFA intake because foods containing PUFAs are not always eaten on a daily or even weekly basis. As dietary PUFA intake was not the main focus of the study but was a mere addition to control for overall PUFA status and as no standardised and validated FFQ that specifically measured PUFA intake for this population was available, the researcher used a three-day food intake record to estimate PUFA intake.

3.5.5 Plasma polyunsaturated fatty acid blood sampling

At least 5 ml EDTA blood was drawn by qualified nurses. The plasma was separated from the RBCs by centrifugation. The RBCs were washed twice with 0.15 mol.L^{-1}NaCl and centrifuged at 1 800-x g for 10 minutes to remove the buffy coat. The plasma and RBCs were stored at -80 °C until analysis.

3.6 Study plan

3.6.1 Study design overview

3.6.1.1 Study domain

Quantitative domain.

3.6.1.2 Study design

Quantitative, analytical, case control.

3.6.1.3 Study techniques

General questionnaire, three-day food intake record and anthropometric measurements.

3.6.2 Study population

The study population was women (age 18-45 years) who had PCOS and fertility problems, seeking help at Medfem Fertility Clinic in Bryanston, South Africa. The control group was women without PCOS or fertility problems.

3.6.2.1 Sample selections

All newly diagnosed PCOS patients at Medfem Fertility Clinic who had been trying to conceive naturally for at least six months and complied with the inclusion criteria were included in the study.

- The control group was selected according to age (Women of child-bearing age (18–45 years). No previous medical history of PCOS or other infertility issues must've been present. They were recruited from the researcher's dietetic practice and gynaecologists in the area.
- Possible candidates were recruited as follows:
    - Doctors at Medfem clinic referred suitable candidates according to the referral sheet.
    - The nurses at Medfem clinic referred suitable candidates.
    - The researcher visited the Medfem clinic office on a regular basis to recruit patients. They were approached in the waiting room and asked if they would be willing to participate in the study. If they agreed the relevant process was followed.
    - The researcher asked patients who met the inclusion criteria at her Dietetic practice if they were willing to participate in the study. If they agreed, the relevant process was followed.

3.6.2.2 Sample size

Analysis of variance (ANOVA) with two groups, a study group and a control group, was done to detect the sample sizes necessary. A sample size of 40 for each group would yield 90% power to detect an effect size of $\delta = 0.52$ with an ANOVA test with significance level $\alpha = 0.05$.

3.6.2.3 Inclusion criteria

- Women diagnosed with PCOS who had been trying to conceive naturally without assistance for at least six months, including or excluding endometriosis (study group).
- Women not previously diagnosed with PCOS and who did not have any known fertility problems (control group).
- Women of child-bearing age (18–45).
- Women who might be going for artificial insemination, but not necessarily (study group).
- Women who might be going for in vitro fertilisation (IVF), but not necessarily (study group).
- Women who were literate.
- Women who could speak/understand English. If they could not speak/understand English, an interpreter was arranged. These patients generally attended the appointments with interpreters anyway.
- Women who were willing to comply with the study techniques.

3.6.2.4 Exclusion criteria

- Women who lived outside South Africa.
- Women who did not consent to participate in the study.
- Women who had fertility problems other than PCOS and endometriosis.

3.7 Methods of data collection

3.7.1 Logistical considerations

Various approaches were employed to recruit patients for the study. Newly diagnosed PCOS patients' file numbers were written onto a recording sheet (Addendum 2) by the three gynaecologists at Medfem Fertility Clinic – Dr Johan van Rensburg, Dr Antonio Rodrigues and Dr Johan van Schouwenburg. Each doctor then gave these patients a form (Addendum 3(a)) to fill in and leave at Reception. This form explained to the patients what the study entailed and clearly stated that participation was entirely voluntary and they could decline to fill in this form. Patients were then asked to leave the form at reception. The researcher collected the forms and recording sheets on a regular basis. All subjects eligible for the study were contacted telephonically to schedule an appointment either at Medfem or at the researcher's office (Suite 11 Sandton Medpark) (preferred times were 07:15–09:00 – Medfem Fertility Clinic opens at 07:00). The clinic works on a first-come-first-served basis, so there was ample time to see patients during this time period, especially while they were waiting for the gynaecologists). During the telephone call, subjects were asked to bring all supplements that they were currently taking to the meeting.

To help with the recruitment process, fertility nurses were also asked to help identify patients.

In addition, the researcher increased the recruitment of patients via the following methods:

- Printed and laminated reminders were placed inside the nurses' consulting room.
- Printed information leaflets were placed in the general waiting room, the day clinic waiting room and the fertility laboratory waiting room.
- Information leaflets were left in the general waiting room, the day clinic waiting room and the fertility laboratory waiting room.
- The researcher also visited the fertility centre on a regular basis and recruited patients.
- Additional adverts were placed on local fertility websites and newsletters to recruit more candidates.
- More doctors were approached and asked to refer suitable candidates.

Both venues had private rooms for the one-on-one meetings required between researcher and participant. At the meeting, the study was discussed *in detail* and at that point, the patient again had the chance to agree/decline to take part. The patient was asked to sign the informed consent form after she fully understood the study and what it entailed (Addendum 4(a)).

The control group was recruited from the researcher's practice, which is in the building next to Medfem. The control group generally consisted of healthy patients visiting the researcher's dietetic practice for nutritional advice. Eligible subjects were recruited during dietetic consultations. They were selected according to age and gender. Addendum 3(b) was then given to eligible subjects. Data was collected as follows from 2013 to 2017:

One-on one meeting (approximately 10 minutes):

This was conducted by the.

- The study was explained, and the consent form (Addendum 4(a) (study group) and (b) (control group)) was signed.
- Basic information was obtained and recorded on the basic questionnaire (Addendum 5(a) and Addendum 5(b) section 1) designed by the researcher, which included
  - demographic information;
  - anthropometric data (weight, height and waist circumference);
  - PUFA supplement usage; and
  - supplement usage.
- The food intake record was explained, and the forms were given to be filled in and returned to the researcher either via email or hard copy (Addendum 1). Patients were instructed to go to the Medfem clinic to have their blood drawn (Addendum 6).

3.7.2 Obtaining sociodemographic information

The following sociodemographic information was obtained by the researcher and documented (Addendum 5(a) and 5(b)):

- Age
- Race
- Nationality

3.7.3 Obtaining anthropometric data

The following anthropometric data was obtained by the researcher during the one-on-one meetings using standard equipment and techniques (Addendum 5(a) and (b)). To ensure privacy, all measurements were conducted in a private room:

3.7.3.1 Weight

Weight was measured using a standardised electronic scale to the nearest 0.2 kg. Validity could potentially have been influenced by the time of day that the measurement was taken and the clothing worn by the subject. Variation in clothing was controlled by asking the participant to remove all excess clothing. Measurements were done in the morning as far as possible. To improve reliability, weight was measured twice and an average was calculated. [16]

3.7.3.2 Height

Height was measured with a Stadiometer to the nearest 0.1 cm with the head in the Frankfort horizontal plane. Subjects were measured barefoot, with minimal clothing, heels together, arms to the side, legs straight, shoulders relaxed and heels, buttocks, scapulae and back of the head against a vertical surface. The measurement was taken after the subject had inhaled deeply and then held her breath. The measurement was repeated to improve accuracy.[16]

3.7.3.3 Waist circumference

Waist circumference was measured by placing the measuring tape in a horizontal plain around the abdomen at the level of the iliac crest. The measurement was taken at the end of normal expiration. It was repeated to ensure accuracy.[16]

3.7.4 Obtaining qualitative data

All qualitative data was based on self-reported data. Information was collected for the following:

3.7.4.1 Infertility time period

Subjects were asked how long they had been trying to conceive (in months), and it was recorded on the basic questionnaire by the researcher (Addendum 5(a))

3.7.4.2 Polyunsaturated fatty acid supplement usage

Subjects were asked whether they used PUFA supplements. If they did, type, brand name and dosage were recorded on the basic questionnaire by the researcher (Addendum 5(a) and 5(b)).

3.7.4.3 Other nutritional supplement usage

Subjects were asked whether they used any other nutritional supplements. If they did, type, brand name and dosage were recorded on the basic questionnaire by the researcher (Addendum 5(a) and 5(b)).

3.7.5 Obtaining data for food intake record

The researcher asked all patients to record an estimated three-day food intake record (Addendum 1 available as hard copy or electronically). The instructions were discussed and were as follows:

- Begin the food journal with documenting the 'Time' that you ate.
- In the column labelled 'Type of food', record what you ate. This includes any snack and/or meal items, even a piece of candy/sweets. Please be specific in describing the food. For example, instead of writing milk, please indicate the type: full cream, 2%, 1%, skim or fat free. If you consumed bread, was it whole wheat, white, rye, pumpernickel, and so on? If necessary, break down food items into different components. For instance, if you ate a chicken sandwich, write down rye bread, deli chicken, cheddar cheese, mayonnaise, lettuce and tomato. Feel free to send along a recipe or food label to improve the accuracy of your analysis. Also, please record brand names when possible.
- In the column labelled 'Method of preparation', document how the food was prepared (e.g. frying, grilling, baking, microwaving, steaming, etc.). Also, please indicate the name of the restaurant or eating place that you ate at in this column.
- In the column labelled 'Amount/Quantity', record the quantity of each food consumed, for example one teaspoon, one slice or one tablespoon. If you do not have access to measuring utensils, use the standard serving sizes or the portion control guide at the bottom of each food intake record page to describe amounts. Avoid subjective terms such as 'bowl', 'serving', 'plateful' or 'helping'.
- Do the same with the columns for 'Condiments' and 'Beverages'. Do not forget to write down beverages such as water, coffee, soda, tea, juices and alcohol.

After completion of the food intake record, patients had to send it back to the researcher either by fax, hard copy delivery or email. The food intake record was analysed with the FoodFinder 3 software program by the researcher.[94]

3.7.6 Obtaining percentage PUFA composition

Blood samples for the study group and control were taken by qualified nursing staff at Medfem Fertility Clinic. A stasis-free blood sample (± 5 ml) was collected by a nursing sister into evacuated glass tubes from the ante-

cubital vein of subjects. Blood samples were then taken to the Medfem laboratory where they were washed and stored appropriately by qualified biologists. The blood samples were then transported in appropriate containers with dry ice to the Centre of Excellence for Nutrition, North-West University, Potchefstroom. At the North-West University laboratory, the samples were stored at -80 °C. The blood samples where then analysed and quantified.

As documented by Smuts et al, fatty acid analyses in plasma and RBCs were performed within six months after collection. Phospholipids were extracted from plasma and RBCs membranes with chloroform:methanol (2:1 vol:vol; containing 0.01% butylated hydroxytoluene) by using a modification of the method of Folch et al.[95] Lipid extracts were concentrated, and neutral lipids were separated from phospholipids by using thin-layer chromatography (silica gel 60 plates, 10 x 20 cm; Merck) and eluted with diethyl ether:petroleum ether:acetic acid (30:90:1 vol:vol:vol). The lipid band that contained phospholipids was removed from the thin-layer chromatography plate and transmethylated with methanol:sulphuric acid (95:5 vol:vol) at 70 °C for two hours to yield fatty acid methyl esters (FAMEs). The resulting FAMEs were extracted with water and hexane. The organic layer was evaporated, redissolved in hexane and analysed by using gas chromatography electron impact mass spectrometry (GCMS) on an Agilent Technologies 7890A gas chromatograph system equipped with an Agilent Technologies 5975C VL mass selective detector (Agilent Technologies). The gas chromatography separation of FAMEs was carried out on a BPX 70 capillary column (60 m x 0.25 mm x 0.25 mm; SGE Analytic Sciences) by using helium as the carrier gas at a flow rate of 1.3 ml/min. The gas chromatography injector was held at a temperature of 280 °C, and the mass spectrometer source was maintained at a temperature of 230 °C. The injection volume of the sample solution was 1 µL with a split ratio of 1:1. The oven temperature was programmed to rise from 130 °C to 200 °C at 2 °C/min, held at 200 °C for four minutes, then raised at 5 °C/min to 220 °C. After the temperature had been held isothermal at 220 °C for five minutes, it was increased by 10 °C/min to 240 °C, where it was retained for five minutes. The total analysis time was 53 minutes. Mass spectrometry with 70 eV electron ionisation was carried out in full-scan acquisition mode, and all mass spectra were acquired over the m/z range of 50–550. Quantification of FAMEs was performed by using the selected-ion extraction method on the basis of the response of two diagnostic ions. Quantification of FAMEs was performed with Masshunter (B.06.00). FAME peaks were identified and calibrated against a standard reference mixture of 33 FAMEs (Nu-Check-Prep) and two single FAME standards (Larodan Fine Chemicals AB). Relative percentages of fatty acids were calculated by taking the concentration of a given fatty acid derivative as a percentage of the total concentration of all fatty acids identified in the sample.[96]

3.7.7 Research instruments

- Recording sheet for nurses (Addendum 2)
- Basic information leaflet (Addendum 3(a) and (b))

- Basic questionnaire (Addendum 5(a) and (b))
- Consent form (Addendum 4(a) and (b))
- Food intake record (Addendum 1)
- Patient information form for blood test (Addendum 6)
- Stadiometer
- Measuring tape
- Electronic scale
- MRC Foodfinder3[94]

3.8 Pilot study

A pilot study was conducted to test all questionnaires as research instruments. The pilot study included five subjects, conveniently selected by the researcher. The subjects were similar to the study subjects in terms of diagnosis and sociodemographic details. They had to comply with all inclusion and exclusion criteria. The subjects needed to fill in a basic information form (Addendum 3(a)). When all inclusion and exclusion criteria had been fulfilled, the researcher did the following:

- The study was explained in detail, and the consent form (Addendum 4(a)) was signed.
- Basic information was obtained, which included
  - demographic information;
  - anthropometric data; and
  - blood samples.

The pilot subjects were not included in the study results.

3.9 Data analysis

3.9.1 Analysis of data

A statistician, Prof DG Nel, from the Faculty of Medicine and Health Sciences, Stellenbosch University, assisted with the data analysis.

3.9.2 Statistical methods

MS Excel was used to capture the data, and Statistica data analysis software (StatSoft Inc., 2017) was used to analyse the data. Summary statistics were used to describe the variables. Distributions of variables were presented with histograms and/or frequency tables. Medians or means were used as the measures of central location for ordinal and continuous responses and standard deviations and quartiles as indicators of spread.

The relationships between two continuous variables were analysed with regression analysis, and the strength of the relationship was measured with Pearson correlation or Spearman correlation if the continuous

variables were not normally distributed. If one continuous response variable was related to several other continuous input variables, multiple regression analysis was used and the strength of the relationship was measured with multiple correlation.

The relationships between continuous response variables and nominal input variables (such as different diets) were analysed using ANOVA. To account for possible confounding variables, these variables could be included as covariates in analysis of covariance (ANACOVA). When ordinal response variables were compared to a nominal input variable, nonparametric ANOVA was used.

A p-value of $p < 0.05$ represented statistical significance in hypothesis testing, and 95% confidence intervals were used to describe the estimation of unknown parameters.

Independent t-tests were used to compare the study group with the control group. Levene's test was used to test homogeneity.

Relative percentages of fatty acids were calculated by taking the concentration of a given fatty acid derivative as a percentage of the total concentration of all fatty acids identified in the sample

3.10 Budget

The budget for the study is summarised in Table 3.2. The blood samples were drawn, free of charge, by the nurses at the Medfem clinic. The blood analysis was performed, free of charge, by the embryologists in the Medfem clinic laboratory. The test tubes were sponsored by Ampath laboratories. The analysis of blood samples was performed by Prof. M Smuts and his team, free of charge, at the Centre of Excellence for Nutrition, North-West University, Potchefstroom. The researcher carried the rest of the costs of the research herself.

Table 3.2: Budget

Description of expenses	Approximate cost
Printing	R2 000
Stationery	R100
Statistician	R0
Blood analysis	Sponsored
Telephone	R1 500
Test tubes	Sponsored
Dry ice	R600
Courier service	R600
Total	**R5 600**

3.11 Ethics

3.11.1 Ethics approval

The study was submitted for ethics approval at the Health Research Ethics Committee, Faculty of Medicine and Health Sciences, Stellenbosch University (HREC ref no: S12/05/134). Medfem Fertility Clinic also gave consent for the recruitment of patients at the clinic.

3.11.2 Informed consent

Participation in the study was voluntary. The researcher provided each participant with an informed consent form. The standard informed consent form used by the Faculty of Medicine and Health Sciences of Stellenbosch University was used. The informed consent form was adapted for this specific research study (Addendum 4(a) and (b)). The consent form was available only in English – this is an English private practice, and the vast majority of the population understand and speak English very well. In addition, all the doctors at the clinic are English and consultations are conducted in English. No interpreter was needed for any of the subjects, although one was available.

3.11.3 Patient confidentiality

Patient confidentiality was ensured through the omittance of all patient identification information from the study-related material. Each participant received a subject identification number that was used on most study-related material and documentation. Blood samples were analysed based on surnames and initials (clinic practice and preference) but were changed back to study numbers before the data was entered for analysis. All information provided to the researcher was used for the specific study only and was not shared for any other purposes or any other studies.

CHAPTER 4

4 RESULTS

4.1 Study population and demographics

A total of 80 subjects were recruited from Medfem Fertility Clinic in Bryanston and the researcher's practice to take part in the study. Three subjects failed to provide enough information and had to be excluded from data analysis. A total of 77 subjects were thus included in the study. Thirty-nine were part of the study group and 38 of the control group. The mean age for the total study population was 32.99 years (SD ± 6.7) while it was 32.72 years (SD ± 5.12) and 33.26 years (SD ± 6.97) for the study and control groups respectively. There was no significant difference in age between the two groups (p = 0.7) (see Table 4.1 for subject demographic information).

With regard to racial distribution, the majority of the subjects were white (n = 55), 7 were Asian, 3 were mixed ancestry and 11 were black. Seventy-five subjects (97.4%) were South African citizens, 1 was Indian and 1 was of another African nationality (Botswana). One subject did not provide demographic information and could not be used.

Table 4.1: Subject demographic information

	Total (n 77)	Study group (n 39)	Control group (n 38)
Age (yrs) (SD)	32.99 (6.07)	32.72 (5.11)	33.26 (6.97)
Race – white	55	30	25
Race – Asian	7	5	2
Race – mixed ancestry	3	1	2
Race – black	11	2	9
Nationality – South African	75	37	38
Nationality – African - Botswana	1	1	0
Nationality – Indian	1	1	0

4.2 Anthropometric data

Anthropometric data are presented in Table 4.2. The mean weight for both groups was 75.67 kg (SD ± 19.83), the mean height was 1.66 m (SD ± 0.07) and the mean waist circumference was 82.11 cm (SD ± 15.31). There were no significant differences between the groups for these parameters (Table 4.2). Mean waist circumference for the study group (83 cm [SD ± 17.42]) and for the control group (81.21 cm [SD ± 12.97]) fell

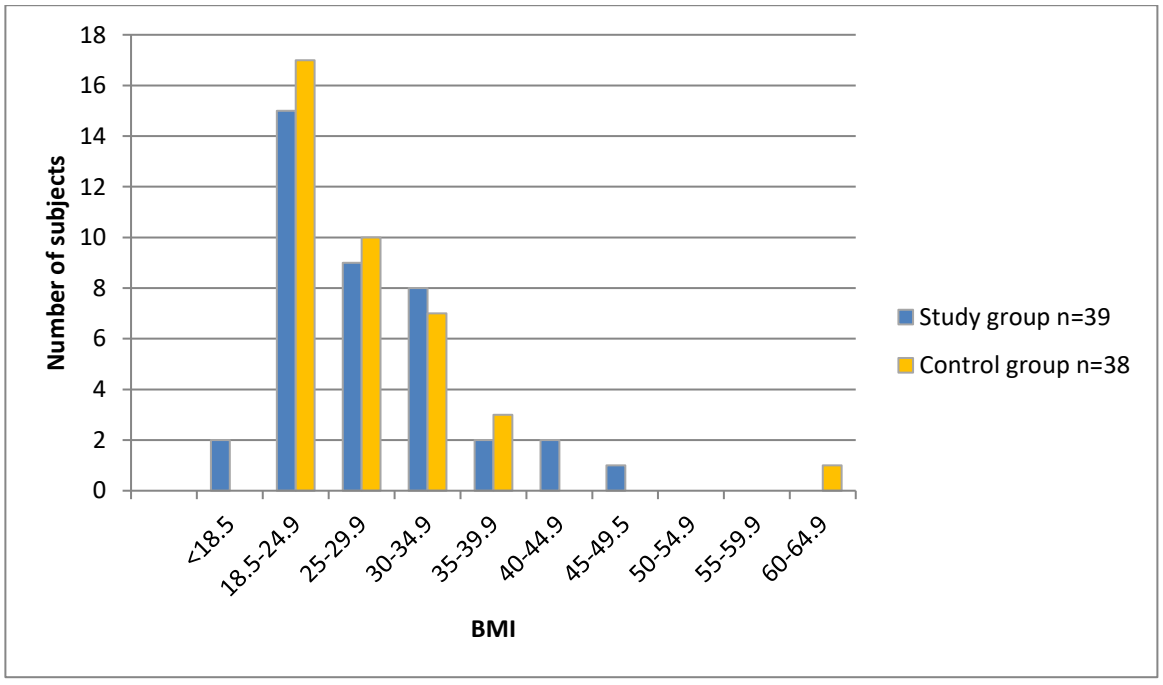
below the 88 cm cut-off that indicates a substantially increased risk for cardiovascular disease but was higher than the normal cut-off of 80 cm for women.

Table 4.2: Anthropometric data

	Total (n 77)	Study group (n 39)	Control group (n 38)	P-value
Mean weight (kg) (SD)	75.67 (19.83)	75.67 (19.29)	75.67 (20.63)	0.999
Mean height (m) (SD)	1.66 (0.07)	1.66 (0.07)	1.66 (0.08)	0.98
Mean BMI (kg/m²) (SD)	27.67 (7.41)	27.68 (7.42)	27.66 (7.51)	0.98
Mean waist circumference (cm)(SD)	82.12 (15.31)	83 (17.42)	81.97 (12.97)	0.61

SD = standard deviation

There was no significant difference between the two groups' mean BMI values (p = 0.98). The mean BMI for the study group was 27.68 kg/m^2 (SD ± 7.42) with a median of 27.38 kg/m^2. The mean BMI for the control group was 27.66 kg/m^2 (SD ± 7.51) with a median of 25.95 kg/m^2. It is worthy to note that there was a big outlier in the control group with a subject who had a BMI of 61.03 kg/m^2. If this outlier is excluded, the median for the control group is 26.76 kg/m^2, which does not significantly affect the result (p=0.089). Forty-three percent of the subjects in this study had a normal BMI. The BMI data is shown in Figure 4.1. Overall, there was no significant difference between the groups in terms of anthropometric data.



BMI = Body mass index (kg/m²)

Figure 4.1: Body mass index (BMI) comparison between the study group and control group, a categorised histogram

4.3 Pregnancy and fertility history

For the study group, only 6 subjects had a child or children. Thirty-three subjects had no children, 4 subjects had 1 child each and 2 subjects had 2 children each. For the control group, 27 subjects had no children, 5 subjects had 1 child each and 6 subjects had 2 children each. The 27 subjects in the control group who had no children had not started with family planning yet. The control group had a higher pregnancy rate.

The pregnancy history is presented in Table 4.3. As expected, there was a significant difference between the two groups with regard to the number of successful pregnancies (p = 0.002).

Table 4.3: Pregnancy history

	Total (n 77)	Study group (n 39)	Control group (n 38)	P-value
Mean Successful pregnancies total (SD)	0.32 (0.66)	0.21 (0.52)	0.45 (0.76)	0.002
0 children	60	33	27	
1 child	9	4	5	
2 children	8	2	6	

SD = standard deviation

For the study group, the infertility history is categorised in a histogram according to the number of months that a couple has been trying to fall pregnant (Figure 4.2). The largest group is 6–12 months (n = 20 cases). The reason for this might be that is normally when couples start to seek help when they are unable to fall pregnant. Eight couples had been trying to conceive for more than 24 months, with 3 couples trying for 6, 7 and 8 years respectively.

The mean infertility months were 22.59 (SD ± 25.01) and the median 12.

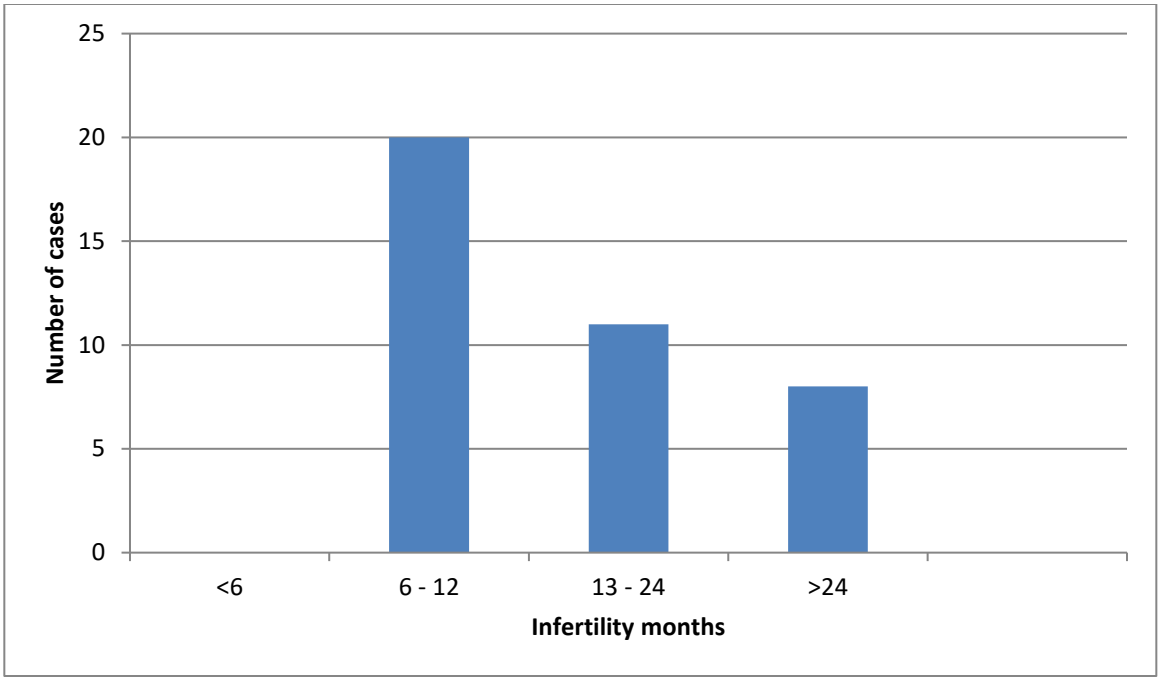


Figure 4.2: Fertility history: Categorised histogram for the study group (n = 39)

4.4 Medical history

The medical history of the study subjects is presented in Table 4.4. Overall, the most common diagnosis was IR (n = 12 study group, n = 0 control group) and endometriosis (n = 11 study group, n = 1 control group). Six subjects had hypothyroidism (n = 6 study group), and only 1 study group subject had a diagnosis of DMII. Other diagnoses reported were IBS (n = 2 study group), Hashimoto's (n = 1 study group), depression (n = 1 control group) and hyperthyroidism (n = 1 control group). Although not a medical diagnosis, 2 subjects reported that they were vegetarian.

It is interesting to observe that only 12 of the 39 PCOS subjects had been diagnosed with IR at the time of the study.

Table 4.4: Medical history of subjects

	Total (n 77)	Study group (n 39)	Control group (n 38)
PCOS	39	39	0
IR	12	12	0
Endometriosis	12	11	1
Hypothyroidism	6	6	0
DMII	1	1	0
Other – IBS	2	2	0
Other – hyperthyroidism	1	0	1
Other – depression	1	1	0
Other – Hashimoto's	1	0	1

PCOS = polycystic ovarian syndrome, IR = insulin resistance, DMII – type II diabetes mellitus, IBS – irritable bowel syndrome

4.5 Medication usage

The most common medication used by the study group was Glucophage (n = 21). The following fertility drugs were taken: Clomid, Postrinex and Provera. For hypothyroidism, 5 subjects in the study group took Eltroxin and 4 Euthyrox. A total number of 20 subjects from both groups used other medications. Medication usage is presented in Table 4.5.

Table 4.5: Medication use

	Total (n 77)	Study group (n 39)	Control group (n 38)
Glucophage	21	21	0
Eltroxin	5	5	0
Euthyrox	4	4	0
Melodene	3	1	2
Yaz	2	0	2
Yasmin	2	0	2
Clomid	1	1	0
Postrinex	1	1	0
Provera	1	1	0
Proginoval	1	1	0
Ecotrin	1	1	0
Desvelaxofine	2	1	1
Somnil	1	1	0
Venlor	1	1	0
Urbenol	1	1	0
Dorminot	1	1	0
Visanne	1	1	0
Femodene	1	0	1
Leximil	1	0	1
Cipralex	1	0	1
Venteeze	1	0	1
Tetralysil	1	0	1
Avandia	0	0	0
Parlodel	0	0	0

4.6 Supplement usage

The vitamin and mineral supplement usage of the study population is summarised in Table 4.6. The information was self-reported data. A total of 19 subjects used multivitamin supplements. The breakdown of the brands can also be viewed in Table 4.6. The highest consumption of supplements was documented for multivitamins (n = 19). StaminoGro was the most common multivitamin used, followed by calcium (n = 8). In the study group (n = 39), only 5 subjects used folic acid supplements. It should be noted that if StaminoGro is taken at the correct dosage, it contains sufficient folic acid for conception. Inofolic also contains folic acid and can be added to the equation. A total of 9 study group subjects took StaminoGro and 4 Inofolic. This means that only 18 study group subjects (46.15%) were taking any form of folic acid. This is very low considering that folic acid is an essential nutrient within the first few weeks after conception.

It is important to note that subjects did not always remember the brand names of the supplements used; brand names mentioned are thus limited. When a subject did not remember the brand name, it was only calculated under the totals.

Table 4.6: Supplement usage

	Total (n = 77)	Study group (n = 39)	Control group (n = 38)
Multivitamin – total	19	14	5
Multivitamin – StaminoGro	11	9	2
Multivitamin – Vital	1	1	0
Multivitamin – Solgar	1	1	0
Multivitamin – Solal	2	2	0
Multivitamin – Metagenics	1	1	0
Multivitamin – Foodstate	1	0	1
Multivitamin – Dischem	1	0	1
Multivitamin – Slender Wonder	1	0	1
Calcium – total	8	4	4
Calcium – Caltrate plus	1	1	0
Calcium – B-Cal-D	2	1	1
Calcium – Solal	1	1	0
Calcium – Cal-C-Vita	2	1	1
Calcium – Dischem	1	0	1
Calcium – Caltrate	1	0	1
Folic acid – total	6	5	1
Folic acid – Folic acid forte	1	1	0
Vit B – total	4	3	1
Vit B – Neurobion	1	1	0
Vit C – total	3	2	1
Vit C – Vita-thion	1	1	0
Vit C – Cal C vita	1	0	1
Immunity boosters – total	1	1	0
Immunity boosters – Viralgaurd	1	1	0
Probiotics – total	2	1	1
Probiotics – Dischem	1	1	0
Probiotics – Amipro	1	0	1
Inofolic	4	4	0
Vit D – total	3	0	3
Vit D – Foodstate	1	0	1
Vit D – Solal	1	0	1
Vit D – B-Cal-D	2	1	1
Fe – total	4	0	4
Fe – Floradix	1	0	1

	Total (n = 77)	Study group (n = 39)	Control group (n = 38)
Fe – Chelafer	1	0	1
Fe – Dischem	1	0	1
Other – total	5	0	5
Other – Magnesium	2	2	0
Other – Zinc	2	1	1
Other – Chromium	1	1	0
Other – DHEA	1	1	0
Other – Joint formula/collagen	1	0	1
Other – L-Lycine/L-Glutamine	1	0	1
Other – Muringa	1	0	1
Other – Athrochoice	1	0	1

4.7 Polyunsaturated fatty acid supplement usage

The PUFA usage of the study subjects is summarised in Table 4.7. A total of 16 (20.8%) (n = 10 study group, n = 6 control) subjects reported that they took PUFA supplements (p = 0.34). Only 13 of these subjects were able to report the type of supplements that they used (detail as reported in Table 4.7). A total of 5 subjects used a fish oil supplement, which was the most common. Flaxseed oil, omega-6 and a combination of omega-3, 6 and 9 were the least commonly reported PUFAs used.

Table 4.7: Polyunsaturated fatty acid (PUFA) supplement usage

	Total (n = 77)	Study group (n = 39)	Control group (n = 38)
PUFA use	16	10	6
ω-3 (fish oil)	5	3	2
ω-3 (krill oil)	2	1	1
ω-3 (flaxseed oil)	1	0	1
ω-6	1	1	0
ω-3 + ω-6	3	3	0
ω-3 + ω-6 + ω- 9	1	0	1

* Thirteen study subjects were able to report the type of supplements that they used.

The study objective to compare supplemented PUFA intake of women with PCOS and women without PCOS has thus been achieved. There was more supplement usage in the study group, but compared to the control group, it was not significant (p = 0.34).

4.8 Dietary intake

Of the 77 subjects, 50 (62.5%) (n = 27 study group, n = 23 control group) returned their three-day food intake records. The three-day food intake record analysis is summarised in Table 4.8. All datasets are reported, but the focus was on PUFAs. There was no significant difference in energy, fat and carbohydrate intake between the study and control groups. Total protein intake was significantly higher in the control group (p = 0.006) with the mean for the study group 74.55 g (SD ± 18.30) and for the control group 92.53 g (SD ± 26.12). There was no significant difference in plant protein intake, but animal protein intake was significantly higher in the control group (p = 0.008). Although not significant (p = 0.076), starch intake was higher in the study group.

Table 4.8: Average of three-day food intake record analysis (n = 50): Macronutrients

	Study (n = 27)		Control (n = 23)		F	P*
	Mean	SD	Mean	SD		
Energy (kJ)	6 229	4 098	5 872	1 527.003	0.156	0.695
Total protein (g)	74.565	18.296	92.526	26.115	**8.112**	**0.006**
Plant protein (g)	12.736	10.128	13.067	6.964	0.017	0.895
Animal protein (g)	58.886	20.165	77.012	26.113	**7.658**	**0.008**
Total fat (g)	71.398	67.742	59.271	18.296	0.692	0.410
Saturated fat (g)	25.555	31.262	17.396	6.727	1.503	0.226
MUFAs (g)	23.287	16.263	22.980	8.086	0.007	0.935
PUFAs (g)	14.872	22.002	11.811	5.205	0.424	0.518
Total trans fatty acids (g)	1.604	3.549	0.593	0.601	1.818	0.184
Cholesterol (mg)	313.407	169.267	390.754	141.996	3.001	0.090
Alcohol (g)	1.672	3.175	2.326	5.735	0.259	0.613
Total carbohydrate (g)	120.811	93.388	103.936	43.455	0.633	0.430

* ANOVA test

SD = standard deviation, KJ = kilojoules, g=grams, mg=milligrams, MUFA=monounsaturated fatty acid, PUFA=polyunsaturated fatty acid

Bold and shaded variables indicate statistical significance

The macronutrient mean intake and energy distribution is shown in Table 4.9. When compared to the prudent guidelines of 15% total energy protein, 55% total energy carbohydrate and 30% total energy fat, it is evident that fat and protein intake was higher and that mean carbohydrate intake was lower than the recommendation in the current study.

Table 4.9: Mean intake and energy distribution from macronutrients

	Study (n = 27)		Control (n = 23)	
	Mean	% of total energy	Mean	% of total energy
Energy (KJ)	6 229.07		5 872.14	
Protein (g)	74.56	20.1%	92.52	26.74%
Total fat (g)	71.398	43.33%	59.271	38.15%
Saturated fat (g)	25.56	15.15%	17.40	11.2%
MUFAs (g)	23.39	14.17%	22.98	14.79%
PUFAs (g)	14.87	9.02%	11.81	7.6%
Trans fatty acids (g)	1.604	1.7%	0.593	3.8%
Carbohydrate (g)	120.81	32.58%	103.94	29.74%

KJ = kilojoules, g=grams, mg=milligrams, MUFA=monounsaturated fatty acid, PUFA=polyunsaturated fatty acid

The following fatty acids were significantly higher in the control group: C15:0 ($p < 0.000$), C16:1 ($p = 0.009$), C20:2 ($p = 0.009$), C20:4 ($p = 0.031$), C22:5 ($p = 0.029$) and C22:6 ($p = 0.43$). Four of these fatty acids (C20:2, C20:4, C22:5 and C22:6) were UFAs that included DHA (C22:6). In addition, EPA (C20:5) was higher in the control group (trend towards significance [$p = 0.06$]). Only C17:1 was significantly higher ($p = 0.03$) in the study group. According to this study, the mean total dietary EPA and DHA intake was only 200 mg for the study group and 702 mg for the control group. Details are listed in Table 4.10.

Table 4.10: Average of three-day food intake record analysis (n = 50): Fatty acids

	Study (n = 27)		Control (n = 23)		F	P*
	Mean	SD	Mean	SD		
C4:0 (g)	0.246	0.186	0.281	0.279	0.283	0.597
C6:0 (g)	0.146	0.121	0.161	0.157	0.161	0.690
C8:0 (g)	0.159	0.208	0.137	0.112	0.214	0.646
C10:0 (g)	0.271	0.228	0.265	0.187	0.009	0.924
C12:0 (g)	1.088	2.051	0.487	0.428	1.897	0.175
C14:0 (g)	2.673	5.526	1.488	0.991	1.027	0.316
C15:0 (g)	0.019	0.017	0.045	0.031	**14.777**	**< 0.000**
C16:0 (g)	12.996	15.144	9.923	3.325	0.907	0.346
C17:0 (g)	0.009	0.007	0.012	0.008	1.141	0.291
C18:0 (g)	6.672	7.528	4.163	2.033	2.398	0.128
C20:0 (g)	0.080	0.127	0.055	0.041	0.816	0.371
C22:0 (g)	0.130	0.271	0.083	0.063	0.663	0.420
C24:0 (g)	0.048	0.083	0.041	0.026	0.144	0.706
C14:1 (g)	0.093	0.077	0.099	0.088	0.086	0.771

	Study (n = 27)		Control (n = 23)		F	P*
	Mean	SD	Mean	SD		
C16:1 (g)	1.074	0.651	1.542	0.544	**7.455**	**0.009**
C17:1 (g)	0.004	0.006	0.001	0.003	**4.789**	**0.034**
C18:1 (g)	21.422	16.192	20.657	7.433	0.044	0.836
C20:1 (g)	0.106	0.129	0.293	0.553	2.914	0.094
C20:3 (g)	0.010	0.018	0.031	0.060	2.892	0.096
C22:1 (g)	0.022	0.051	0.202	0.473	3.881	0.055
C18:2 (g)	13.863	21.954	10.176	4.852	0.621	0.435
C18:3 (g)	0.526	0.403	0.737	0.708	1.745	0.193
C18:4 (g)	0.005	0.015	0.048	0.133	2.762	0.103
C20:2 (g)	0.024	0.021	0.041	0.024	**7.306**	**0.009**
C20:4 (g)	0.099	0.059	0.134	0.054	**4.926**	**0.031**
C20:5 (g)	0.054	0.071	0.261	0.560	3.642	0.062
C22:2 (g)	0.009	0.018	0.005	0.008	1.327	0.255
C22:4 (g)	0.000	0.000	0.010	0.047	1.178	0.283
C22:5 (g)	0.018	0.014	0.076	0.133	**5.069**	**0.029**
C22:6 (g)	0.146	0.157	0.441	0.719	**4.326**	**0.043**
C24:6 (g)	0.000	0.000	0.000	0.000		

* ANOVA test
SD = standard deviation, g=grams
Bold and shaded variables indicate statistical significance

Thirteen amino acids were significantly higher in the control group, including the branched-chain amino acids isoleucine ($p = 0.004$), leucine ($p = 0.003$) and valine ($p = 0.002$) as well as lysine, methionine, threonine, phenylalanine, tryptophan, arginine, histidine, cystine, tyrosine and syrene (see Table 4.11 for details and relevant p-values).

Table 4.11: Average of three-day food intake record analysis (n = 50): Amino acids

	Study (n = 27)		Control (n = 23)		F	P*
	Mean	SD	Mean	SD		
Isoleucine (g)	2.993	0.807	3.802	1.098	**8.962**	**0.004**
Leucine (g)	5.556	1.469	7.129	2.080	**9.747**	**0.003**
Lysine (g)	5.320	1.566	6.780	2.072	**8.037**	**0.007**
Methionine (g)	1.682	0.523	2.196	0.669	**9.266**	**0.004**
Phenylalanine (g)	2.875	0.747	3.670	0.977	**10.603**	**0.002**
Threonine (g)	2.936	0.807	3.747	1.120	**8.819**	**0.005**
Tryptophan (g)	0.887	0.230	1.141	0.339	**9.871**	**0.003**
Valine (g)	3.312	0.882	4.288	1.198	**10.966**	**0.002**
Arginine (g)	4.107	1.252	5.109	1.422	**7.023**	**0.011**
Histidine (g)	2.130	0.609	2.872	0.954	**11.069**	**0.002**
Cystine (g)	0.692	0.304	0.897	0.406	**4.129**	**0.048**
Tyrosine (g)	1.796	0.659	2.338	1.080	**4.746**	**0.034**
Alanine (g)	2.833	1.171	3.425	1.781	1.981	0.166

* ANOVA test
SD = standard deviation, g=grams
Bold and shaded variables indicate statistical significance

The following nutrients were also significantly higher in the control group (Table 4.12): galactose (p = 0.006), lactose (p = 0.007), magnesium (p = 0.01), phosphorus (p = 0.002), potassium (p = 0.021), selenium (p = 0.014), iodine (p = 0.004), riboflavin (p = 0.005), vitamin B12 (p = 0.016), biotin (p = 0.041), fluorine (p = 0.001) and silicon (p = 0.022). Maltose was significantly higher in the study group (p = 0.038).

The total added sugar was significantly different (p = 0.042) between the two groups at 15.18 g (SD ± 21.29) for the study group and 5.52 g (SD ± 6.77) for the control group. The total sugar also had a trend towards significance (p = 0.064), being higher in the study group.

Calcium was also significantly higher in the control group (p = 0.008) with the mean calcium intake for the study group 489.80 mg (SD ± 555.45) and for the control group 696.64 mg (SD ± 848). Dietary vitamin D intake was also significantly lower in the study group (p = 0.01). Folate had a trend towards significance (p = 0.058) with a higher intake in the control group.

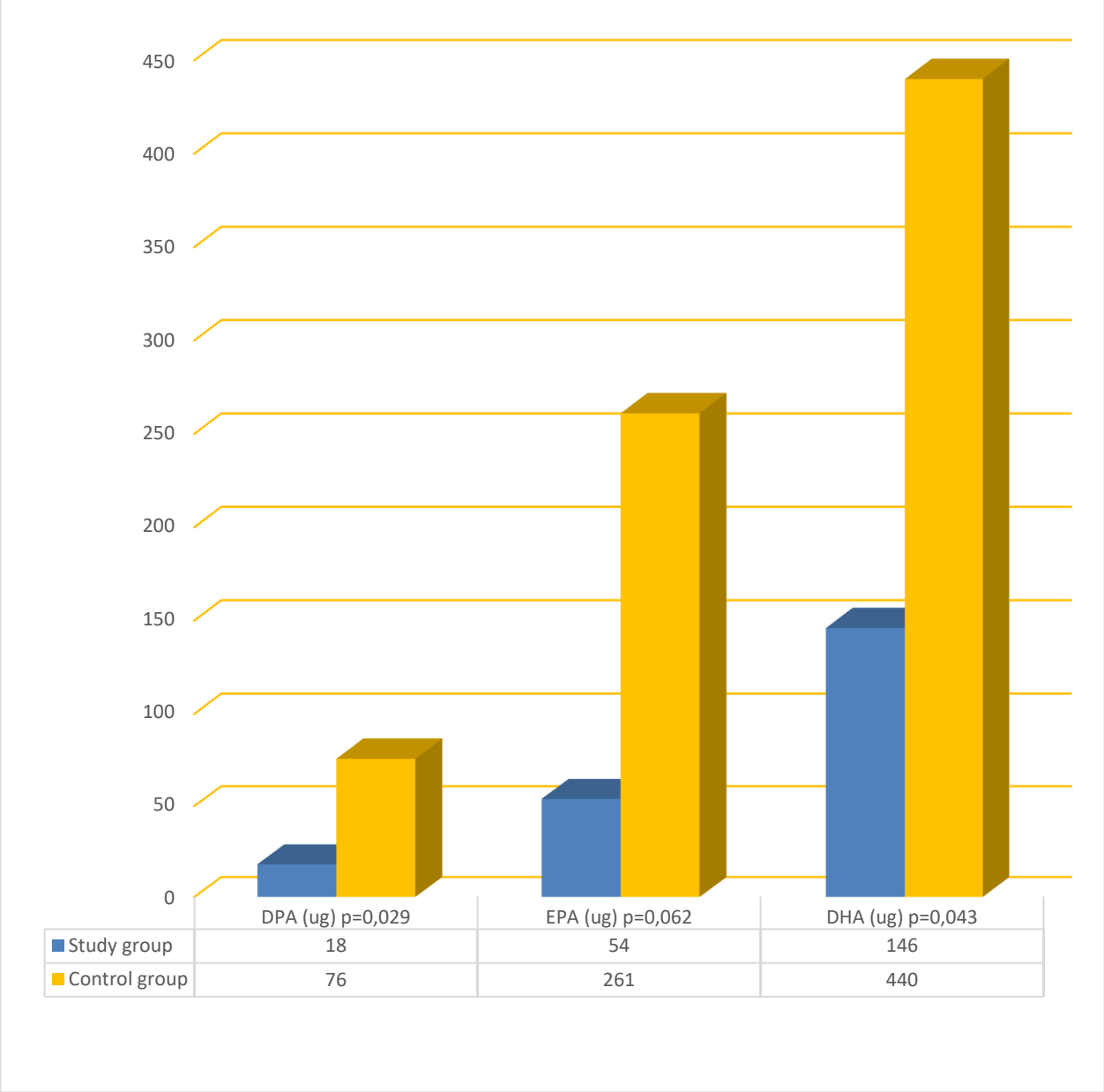
Table 4.12: Average of three-day food intake record analysis (n = 50): Micronutrients and other

	Study (n = 27)		Control (n = 23)		F	P*
	Mean	SD	Mean	SD		
Glucose (g)	5.812	4.456	7.510	3.693	2.105	0.153
Fructose (g)	7.594	6.867	10.987	7.658	2.728	0.105
Galactose (g)	0.932	1.278	2.071	1.535	**8.202**	**0.006**
Sucrose (g)	12.294	8.814	11.826	6.749	0.043	0.836
Maltose (g)	0.372	0.485	0.130	0.260	**4.570**	**0.038**
Lactose (g)	5.985	6.593	11.322	6.829	**7.874**	**0.007**
Total sugar (g)	33.359	20.417	44.513	21.058	3.601	0.064
Added sugar (g)	15.177	21.291	5.520	6.774	**4.344**	**0.042**
Total dietary fibre (g)	12.811	8.064	15.836	7.411	1.882	0.177
Insoluble dietary fibre (g)	4.053	2.684	4.372	1.754	0.238	0.628
Soluble dietary fibre (g)	3.131	2.136	3.681	1.460	1.090	0.302
Calcium (mg)	483.802	181.042	696.638	350.048	**7.612**	**0.008**
Iron (mg)	8.806	3.649	10.436	3.979	2.281	0.138
Haem iron (mg)	0.951	0.675	0.933	0.796	0.007	0.934
Non-haem iron (mg)	3.915	1.597	4.359	1.362	1.100	0.300
Magnesium (mg)	185.864	63.284	243.725	87.965	**7.275**	**0.010**
Phosphor (mg)	966.494	241.133	1 253.522	359.370	**11.283**	**0.002**
Potassium (mg)	1 836.605	622.532	2 223.623	509.139	**5.659**	**0.021**
Sodium (mg)	1 483.580	711.204	1 302.058	533.587	1.012	0.320
Chlorine (mg)	946.123	569.853	1 043.942	502.107	0.408	0.526
Zinc (mg)	9.022	3.176	9.640	3.125	0.477	0.493
Copper (mg)	1.007	0.424	1.070	0.424	0.270	0.605
Chromium (mcg)	42.114	16.785	37.597	16.582	0.909	0.345
Selenium (mcg)	42.435	18.193	57.730	24.313	**6.454**	**0.014**
Manganese (mcg)	1 512.309	983.757	1 732.246	793.529	0.739	0.394
Iodine (mcg)	38.704	18.673	56.522	23.255	**9.028**	**0.004**
Boron (mcg)	1 018.457	1 116.236	1 520.681	1 703.335	1.563	0.217
Vitamin A (RE) (mcg)	728.309	790.253	652.188	505.323	0.158	0.693
Retinol (mcg)	100.667	57.574	126.275	88.493	1.513	0.225
Total carotenoids (mcg)	2 671.383	2 493.633	2 301.957	2 104.951	0.314	0.578
B-carotene (mcg)	2 269.790	2 221.992	1 892.812	1 745.560	0.434	0.513
A-carotene (mcg)	561.654	629.395	637.551	790.605	0.143	0.707
Cryptoxanthin (mcg)	199.679	447.759	163.174	239.089	0.123	0.728
Thiamin (mg)	0.856	0.316	0.923	0.338	0.524	0.473
Riboflavin (mg)	1.044	0.340	1.467	0.642	**8.820**	**0.005**
Niacin (mg)	14.114	5.668	16.564	6.109	2.161	0.148
Vitamin B6 (mg)	1.290	0.582	1.428	0.532	0.751	0.390
Folate (mcg)	158.704	70.473	199.232	76.781	3.783	0.058

	Study (n = 27)		Control (n = 23)		F	P*
	Mean	SD	Mean	SD		
Vitamin B12 (mcg)	3.395	1.326	5.894	5.002	**6.245**	**0.016**
Pantothenate (mg)	4.020	2.095	4.947	1.876	2.671	0.109
Biotin (mcg)	30.343	15.266	38.154	10.063	**4.388**	**0.041**
Vitamin C (mg)	85.049	101.744	71.739	32.951	0.360	0.551
Vitamin D (mcg)	3.595	2.730	6.683	5.207	**7.197**	**0.010**
Vitamin E (mg)	11.006	19.714	10.069	4.296	0.050	0.824
Caffeine (mg)	0.000	0.000	0.174	0.834	1.178	0.283
Aspartic acid (g)	4.753	1.793	5.928	2.844	3.149	0.082
Glutamic acid (g)	8.418	2.919	10.378	4.773	3.167	0.081
Glycine (g)	2.413	1.079	2.632	1.393	0.391	0.535
Proline (g)	2.786	0.956	3.372	1.433	2.973	0.091
Serine (g)	2.385	0.878	3.061	1.323	**4.660**	**0.036**
Non-starch polysaccharides (g)	6.523	4.542	7.481	2.995	0.745	0.392
Lignin (g)	0.465	0.421	0.475	0.331	0.008	0.927
Fluorine (mcg)	111.728	51.913	175.522	58.852	**16.587**	**0.000**
Silicon (mcg)	3 193.580	3 139.723	5 567.652	3 970.009	**5.572**	**0.022**
Lycopene (mcg)	369.049	618.094	371.826	358.978	0.000	0.985
Lutein (mcg)	827.827	1 255.411	772.449	1 015.044	0.029	0.866
Vitamin K (mcg)	85.725	91.077	110.027	92.323	0.873	0.355
Hydroxyproline (g)	0.213	0.157	0.166	0.150	1.124	0.294

* ANOVA test
SD = standard deviation, g=grams, mg=milligrams, mcg=micrograms
Bold and shaded variables indicate statistical significance

Figure 4.3 compares statistically significant PUFAs between the study and the control group.



	DPA (ug) p=0,029	EPA (ug) p=0,062	DHA (ug) p=0,043
Study group	18	54	146
Control group	76	261	440

ug=micrograms, p=ANOVA test, DPA=docosapentanoic acid, EPA=eicosapentaenoic acid, DHA=docosahexaenoic acid, PUFA=polyunsaturated fatty acid

Figure 4.3: Summary of significant PUFAs from the three-day food intake records compared between the study group and the control group

4.9 Blood samples

Of the 77 subjects, 68 (85%) (n = 34 study group, n = 34 control group) blood samples were drawn and sent to the laboratory for analysis. If subjects were pressed for time and could not wait any longer, and nurses were unavailable to assist at a particular moment, subjects were requested to return to have their blood drawn. Nine subjects failed to return and provide the required blood samples during the study period, despite

efforts from the researcher. A total of 50 (n = 27 study group, n = 23 control group) RBC membranes samples and 54 (n = 30 study group, n = 24 control group) plasma phospholipid samples were analysed. Some blood samples were lost as one batch of samples defrosted during transportation from the laboratory at Medfem Fertility Clinic to the laboratory at North-West University. A reputable courier company was used to transport the frozen samples (as per standard practice), which were packed on dry ice to be kept frozen, but the courier company failed to deliver the samples the same day.

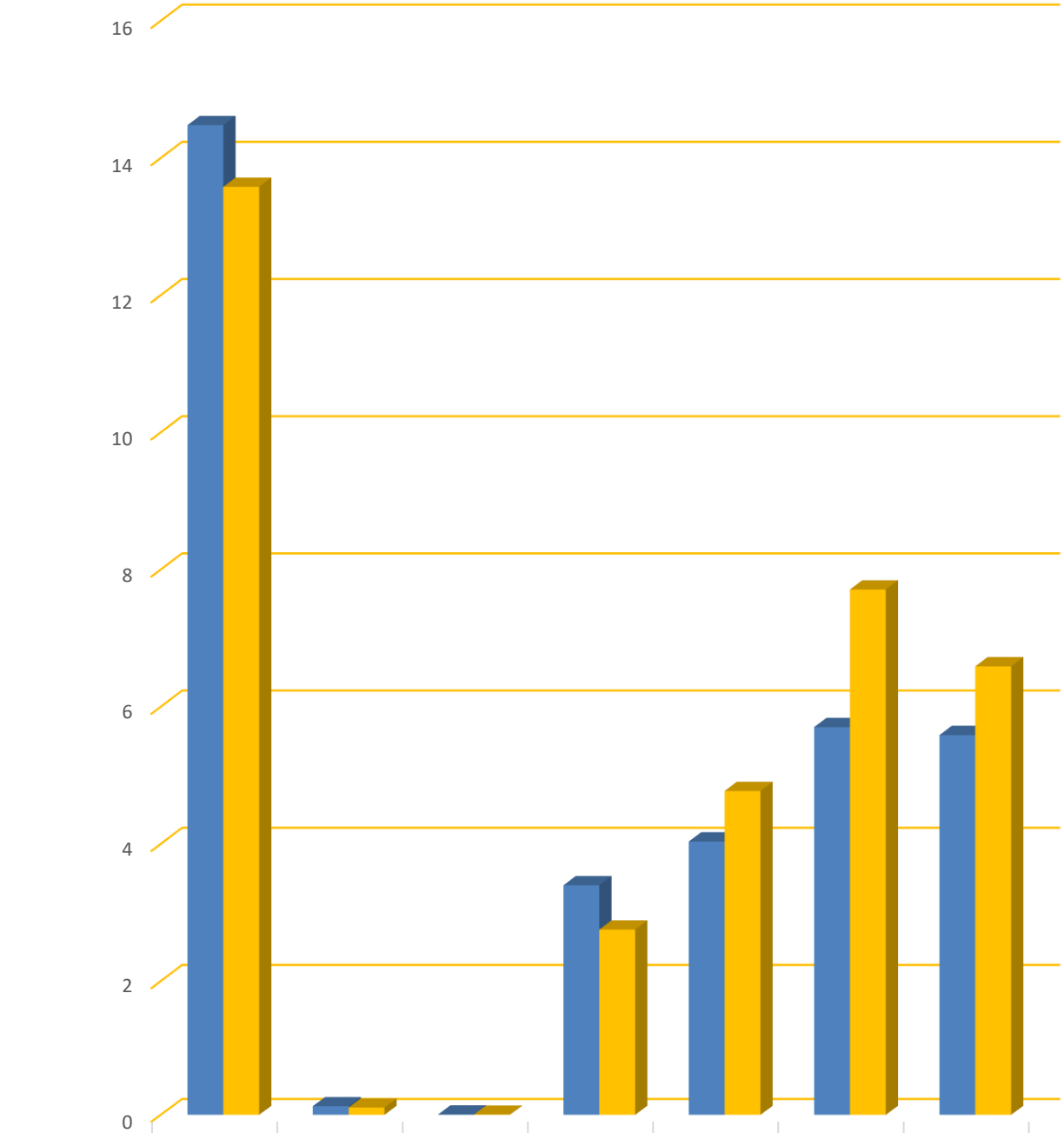
4.9.1 Plasma phospholipids

The plasma phospholipid blood results are summarised in Table 4.13. The following fatty acids had a significantly higher concentration in the study group (ANOVA) without adjusting for the possible confounders whose influence were investigated by treating them as covariates in the different ANACOVAS as reported in Table 4.13:

C18:0 stearic acid (p = 0.005), C18:1n9T elaidic acid (p = 0.042), C20:3n9 mead acid (p = 0.039) and C20:3n6 (p = 0.013).

From Table 4.13 we see that eicosadienoic acid (C20:2n6) was not significant when compared between the study and the control group in the ANOVA (p = 0.077) but became significant when adjusting for each of the possible confounding factors of PUFA supplementation (p = 0.005) and endometriosis (p = 0.049) and both (p = 0.004). Adrenic acid (C22:4n6) was higher in the study group and was not significant when compared between the study and the control group in the ANOVA (p = 0.121) but became significant when adjusting for possible confounding factors of PUFA supplementation and endometriosis (p = 0.044). SFAs were higher in the study group but were not significant when compared between the study and the control group in the ANOVA (p = 0.081) yet were statistically significant with possible confounding of endometriosis alone (p = 0.05). The n-6:n-3 PUFA ratio and the n-6:n-3 LC-PUFA ratio were not significant when compared between the study and the control group in the ANOVA (p = 0.071) and (p = 0.075) respectively. The n-6:n-3 LC-PUFA ratio was, however, significantly higher in the study group when adjusted for possible confounding of PUFA supplementation (p = 0.039) and PUFA supplementation with endometriosis (p = 0.048). In the study group, the main fatty acids that were higher were the omega-6 fatty acids. The following fatty acids were statistically lower in the study group: vaccenic acid (C18:1n7) (p = 0.036), DHA (C22:6n3) (p = 0.029), n-3 PUFAs (p = 0.036) and n3-LC-PUFAs (p = 0.036).

Figure 4.4 compares the statistically significant plasma fatty acids between the control group and the study group.



	Stearic acid p=0,005	Eladic acid p=0,042	Mead acid p=0,039	C20:5n6 p=0,013	DHA p=0,029	n3 PUFA p=0,036	n3 LC PUFA p=0,036
Study group	14,48	0,131	0,007	3,398	4,036	5,704	5,588
Control group	13,58	0,11	0,005	2,752	4,773	7,71	6,59

g=grams, mg=milligrams, ug=micrograms, p=ANOVA test, DHA=docosahexaenoic acid, PUFA=polyunsaturated fatty acid

Relative percentages of fatty acids were calculated by taking the concentration of a given fatty acid derivative as a percentage of the total concentration of all fatty acids identified in the sample

Figure 4.4: Summary of statistically significant fatty acids from the plasma phospholipid blood analysis compared between the study group and the control group

Table 4.13: Percentage polyunsaturated fatty acid (PUFA) plasma phospholipid composition statistical differences between study and control group

	All results					Confounding of PUFAs			Confounding of endometriosis				Confounding of PUFAs and endometriosis		
	Study (n = 30)		Control (n = 24)		P*	Study mean (n = 24)	Control mean (n = 21)	P**	Study mean (n = 21)	Control mean (n = 24)	P**		Study mean (n = 18)	Control mean (n = 21)	P**
	Mean	SD	Mean	SD											
C14:0 Myristic	0.340	0.110	0.305	0.125	0.281	0.346	0.300	0.212	0.343	0.305	0.293	0.346	0.300	0.256	
C16:0 Palmitic	27.849	1.725	27.831	1.311	0.966	27.778	27.800	0.962	28.190	27.831	0.448	28.038	27.800	0.656	
C16:1n7 Palmitoleate	0.406	0.139	0. 424	0.155	0.645	0.415	0.447	0.486	0.438	0.424	0.763	0.441	0.447	0.908	
C18:0 Stearic	14.486	1.135	13.585	1.134	**0.005**	14.596	13.571	**0.004**	14.325	13.585	**0.033**	14.460	13.571	**0.017**	
C18:1n9T Elaidic	0.131	0.043	0.110	0.028	**0.042**	0.131	0.111	**0.038**	0.124	0.110	0.138	0.130	0.111	0.068	
C18:1n7T Vaccenic	0.492	0.419	0.383	0.296	0.286	0.471	0.353	0.182	0.434	0.383	0.583	0.475	0.353	0.197	
C18:1n9 Oleic	7.466	1.217	7.263	1.026	0.518	7.418	7.301	0.735	7.345	7.263	0.798	7.364	7.301	0.860	
C18:1n7 Vaccenic	1.246	0.219	1.373	0.212	**0.036**	1.222	1.388	**0.018**	1.265	1.373	0.122	1.242	1.388	0.062	
C18:2n6 Linoleic	18.784	3.104	18.629	3.033	0.854	18.785	18.501	0.763	18.588	18.629	0.966	18.634	18.501	0.894	
C20:0 Eicosenoic	0.487	0.081	0.488	0.116	0.951	0.488	0.496	0.793	0.489	0.488	0.974	0.492	0.496	0.904	
C18:3n6 GLA	0.071	0.054	0.055	0.040	0.222	0.077	0.059	0.228	0.078	0.055	0.144	0.083	0.059	0.167	
C18:3n3 ALA	0.116	0.057	0.120	0.042	0.818	0.124	0.115	0.588	0.122	0.120	0.895	0.128	0.115	0.491	
C20:1n9 Eicosenoic	0.177	0.036	0.178	0.035	0.871	0.176	0.174	0.812	0.175	0.178	0.758	0.175	0.174	0.941	
C18:4n3 Stearidonic	0.000	0.000	0.000	0.000	0.573	0.000	0.000	0.347	0.000	0.000	0.474	0.000	0.000	0.317	
C20:2n6 Eicosadienoic	0.423	0.093	0.380	0.078	0.077	0.439	0.370	**0.005**	0.433	0.380	**0.049**	0.446	0.370	**0.004**	
C20:3n9 Mead	0.007	0.005	0.005	0.001	**0.039**	0.008	0.005	**0.049**	0.007	0.005	**0.042**	0.008	0.005	**0.044**	
C22:0 Behenic	1.484	0.245	1.505	0.270	0.766	1.473	1.541	0.391	1.479	1.505	0.727	1.469	1.541	0.375	
C20:3n6	3.398	0.913	2.752	0.918	**0.013**	3.531	2.855	**0.020**	3.525	2.752	**0.010**	3.665	2.855	**0.013**	
C20:3n3	0.016	0.005	0.019	0.015	0.447	0.017	0.016	0.671	0.016	0.019	0.477	0.017	0.016	0.756	
C20:4n6 Arachidonic	11.859	1.901	12.600	2.217	0.192	11.915	12.886	0.117	11.959	12.600	0.318	11.927	12.886	0.155	
C22:1n9 Erucic	0.032	0.049	0.028	0.052	0.735	0.036	0.027	0.547	0.034	0.028	0.668	0.039	0.027	0.449	
C20:5n3 EPA	0.630	0.434	0.900	1.110	0.226	0.598	0.563	0.748	0.552	0.900	0.171	0.507	0.563	0.471	
C24:0 Lignoceric	1.405	0.249	1.440	0.250	0.612	1.380	1.459	0.303	1.411	1.440	0.683	1.374	1.459	0.251	
C22:3n3 Docosatranoic	0.007	0.005	0.009	0.004	0.380	0.008	0.009	0.457	0.007	0.009	0.316	0.007	0.009	0.319	
C24:1n9 Nervonic	2.581	0.584	2.905	0.807	0.093	2.581	2.979	0.073	2.559	2.905	0.128	2.526	2.979	0.074	
C22:4n6 Adrenic	0.582	0.177	0.506	0.176	0.121	0.626	0.541	0.085	0.606	0.506	0.065	0.647	0.541	**0.044**	
C22:3n6 Osbond	0.567	0.224	0.528	0.218	0.522	0.606	0.560	0.484	0.611	0.528	0.225	0.654	0.560	0.189	
C22:5n3 DPA	0.899	0.200	0.890	0.230	0.877	0.895	0.856	0.523	0.878	0.890	0.862	0.892	0.856	0.579	
C22:6n3 DHA	4.036	1.226	4.773	1.164	**0.029**	3.833	4.701	**0.021**	3.979	4.773	**0.033**	3.784	4.701	**0.023**	
SFAs	46.051	2.088	45.154	1.472	0.081	46.060	45.167	0.121	46.238	45.154	**0.050**	46.180	45.167	0.110	
MUFAs	11.907	1.279	12.171	1.155	0.436	11.849	12.315	0.213	11.816	12.171	0.320	11.787	12.315	0.178	

PUFAs	41.396	1.939	42.164	1.626	0.127	41.462	42.037	0.329	41.362	42.164	0.133	41.399	42.037	0.290
Trans fatty acids	0.623	0.456	0.493	0.310	0.237	0.603	0.464	0.146	0.558	0.493	0.512	0.605	0.464	0.167
n6 PUFAs	35.684	2.436	35.449	2.415	0.725	35.980	35.771	0.774	35.800	35.449	0.631	36.056	35.771	0.707
n3 PUFAs	5.704	1.545	6.710	1.882	**0.036**	5.475	6.260	0.080	5.555	6.710	**0.027**	5.335	6.260	**0.041**
n6 LC-PUFAs	16.828	2.049	16.765	2.721	0.923	17.118	17.211	0.892	17.134	16.765	0.624	17.339	17.211	0.869
n3 LC-PUFAs	5.588	1.554	6.590	1.876	**0.036**	5.350	6.144	0.077	5.433	6.590	**0.027**	5.207	6.144	**0.039**
n6:n3 PUFA ratio	6.750	2.011	5.753	1.920	0.071	7.085	6.072	0.088	6.914	5.753	0.053	7.198	6.072	0.070
n6:n3 LC-PUFA ratio	3.227	0.917	2.768	0.934	0.075	3.419	2.957	0.083	3.371	2.768	**0.039**	3.534	2.957	**0.048**

* ANOVA test

** ANACOVA test

SD = standard deviation, PUFA=polyunsaturated fatty acid, MUFA=monounsaturated fatty acid, DHA=docosahexaenoic acid, DPA=docosapentanoic acid, EPA, Eicosapentaenoic acid

Relative percentages of fatty acids were calculated by taking the concentration of a given fatty acid derivative as a percentage of the total concentration of all fatty acids identified in the sample

Bold and shaded variables indicate statistical significance

4.9.2 Red blood cell membranes

The RBC membranes data is summarised in Table 4.14. Three fatty acids, namely eicosenoic (C20:1n9) ($p = 0.032$), C20:2n6 ($p = 0.039$) and adrenic acid (C22:4n6) ($p = 0.024$) were all significantly higher in the study group.

When adjusting for possible confounding factors of PUFA supplementation and endometriosis, stearic acid (C18:0) ($p = 0.017$), eicosadeinoic acid (C20:2n6) ($p = 0.004$) and mead acid (C20:3n9) ($p = 0.044$) were all significantly higher in the study group. The n-6:n-3 LC-PUFA ratio was also significantly higher in the study group ($p = 0.048$), and there was a trend towards significance for the n-6:n-3 PUFA ratio ($p = 0.07$).

Again, when adjusting for possible confounding factors of PUFA supplementation and endometriosis, the n-3 PUFAs ($p = 0.041$) and the n-3 LC-PUFAs ($p = 0.039$) were significantly lower in the study group.

Table 4.14: Percentage polyunsaturated fatty acid (PUFA) red blood cell membranes (RBC) composition statistical comparison between study and control group

	All results					Confounding of PUFAs			Confounding of endometriosis			Confounding of PUFAs and endometriosis		
	Study (n = 30)		Control (n = 24)		P*	Study mean (n = 24)	Control mean (n = 21)	P**	Study mean (n = 21)	Control mean (n = 24)	P**	Study mean (n = 18)	Control mean (n = 21)	P**
	Mean	SD	Mean	SD										
C14:0 Myristic	0.271	0.122	0.249	0.193	0.616	0.258	0.236	0.676	0.265	0.249	0.756	0.253	0.236	0.256
C16:0 Palmitic	22.888	2.354	24.188	3.209	0.106	22.705	24.110	0.115	22.864	24.188	0.154	22.454	24.110	0.656
C16:1n7 Palmitoleate	0.331	0.128	0.329	0.167	0.972	0.319	0.332	0.784	0.337	0.329	0.881	0.319	0.332	0.908
C18:0 Stearic	16.263	1.842	15.397	2.360	0.152	16.044	15.277	0.283	16.100	15.397	0.314	15.884	15.277	**0.017**
C18:1n9T Elaidic	0.270	0.258	0.318	0.251	0.504	0.273	0.306	0.675	0.244	0.318	0.326	0.232	0.306	0.068
C18:1n7T Vaccenic	0.445	0.411	0.347	0.319	0.357	0.467	0.326	0.178	0.431	0.347	0.432	0.487	0.326	0.197
C18:1n9 Oleic	10.935	1.204	10.652	1.174	0.407	10.989	10.660	0.410	11.144	10.652	0.190	11.174	10.660	0.860
C18:1n7 Vaccenic	0.929	0.141	0.938	0.148	0.842	0.926	0.930	0.920	0.945	0.938	0.867	0.937	0.930	0.062
C18:2n6 Linoleic	10.503	1.662	11.120	2.217	0.268	10.783	11.264	0.447	10.620	11.120	0.417	10.828	11.264	0.894
C20:0 Eicosenoic	0.377	0.106	0.350	0.115	0.393	0.373	0.341	0.394	0.375	0.350	0.492	0.376	0.341	0.904
C18:3n6 GLA	0.054	0.030	0.041	0.014	0.061	0.057	0.043	0.076	0.057	0.041	**0.035**	0.060	0.043	0.167
C18:3n3 ALA	0.083	0.035	0.097	0.048	0.238	0.086	0.099	0.333	0.083	0.097	0.321	0.088	0.099	0.491
C20:1n9 Eicosenoic	0.258	0.036	0.235	0.039	**0.032**	0.252	0.229	**0.027**	0.259	0.235	0.055	0.250	0.229	0.941
C18:4n3 Stearidonic	0.000	0.000	0.000	0.000	0.176	0.000	0.000	0.134	0.000	0.000	0.109	0.000	0.000	0.317
C20:2n6 Eicosadienoic	0.329	0.108	0.324	0.075	0.850	0.342	0.325	0.598	0.335	0.324	0.720	0.343	0.325	**0.004**
C20:3n9 Mead	0.002	0.002	0.001	0.001	0.289	0.002	0.001	0.266	0.002	0.001	0.139	0.002	0.001	**0.044**
C22:0 Behenic	1.449	0.334	1.328	0.355	0.223	1.424	1.309	0.328	1.453	1.328	0.280	1.445	1.309	0.375
C20:3n6	1.683	0.513	1.385	0.468	**0.039**	1.786	1.435	**0.027**	1.771	1.385	**0.021**	1.883	1.435	**0.013**
C20:3n3	0.009	0.008	0.013	0.013	0.190	0.011	0.013	0.508	0.010	0.013	0.304	0.012	0.013	0.756
C20:4n6 Arachidonic	14.525	2.899	14.473	3.918	0.957	14.916	15.043	0.905	14.600	14.473	0.912	15.092	15.043	0.155
C22:1n9 Erucic	0.060	0.061	0.054	0.053	0.739	0.070	0.056	0.457	0.066	0.054	0.495	0.078	0.056	0.449
C20:5n3 EPA	0.424	0.223	0.620	0.626	0.134	0.391	0.432	0.436	0.373	0.620	0.106	0.367	0.432	0.471
C24:0 Lignoceric	3.929	1.080	3.828	1.304	0.767	3.722	3.723	0.996	3.903	3.828	0.848	3.698	3.723	0.251
C22:3n3 Docosatreinoic	0.004	0.004	0.005	0.003	0.230	0.004	0.005	0.175	0.004	0.005	0.372	0.004	0.005	0.319
C24:1n9 Nervonic	4.040	0.844	3.964	0.934	0.766	3.877	3.878	0.996	4.006	3.964	0.882	3.821	3.878	0.074
C22:4n6 Adrenic	3.099	0.803	2.600	0.692	**0.024**	3.233	2.694	**0.030**	3.059	2.600	0.075	3.186	2.694	**0.044**
C22:3n6 Osbond	0.829	0.265	0.733	0.239	0.188	0.881	0.778	0.180	0.839	0.733	0.209	0.896	0.778	0.189
C22:5n3 DPA	1.707	0.443	1.693	0.655	0.927	1.669	1.532	0.316	1.633	1.693	0.736	1.656	1.532	0.579
C22:6n3 DHA	4.242	1.398	4.657	1.224	0.274	4.070	4.559	0.270	4.153	4.657	0.241	4.095	4.559	**0.023**
SFAs	45.177	5.118	45.341	6.521	0.921	44.525	44.997	0.803	44.960	45.341	0.844	44.109	44.997	0.110

MUFAs	16.552	1.303	16.172	1.299	0.308	16.431	16.085	0.415	16.758	16.172	0.173	16.578	16.085	0.178
PUFAs	37.493	5.889	37.762	7.107	0.884	38.230	38.223	0.997	37.539	37.762	0.918	38.512	38.223	0.290
Trans fatty acids	0.715	0.423	0.665	0.336	0.654	0.739	0.632	0.331	0.675	0.665	0.930	0.719	0.632	0.167
n6 PUFAs	31.022	4.955	30.675	6.230	0.827	31.997	31.582	0.810	31.280	30.675	0.746	32.288	31.582	0.707
n3 PUFAs	6.470	1.866	7.086	2.127	0.280	6.231	6.639	0.480	6.257	7.086	0.200	6.222	6.639	**0.041**
n6 LC-PUFAs	20.465	3.912	19.515	4.817	0.445	21.157	20.276	0.512	20.603	19.515	0.461	21.399	20.276	0.869
n3 LC-PUFAs	6.387	1.851	6.989	2.107	0.287	6.145	6.541	0.488	6.174	6.989	0.203	6.134	6.541	**0.039**
n6/n3 PUFA ratio	5.124	1.467	4.651	1.382	0.249	5.479	4.980	0.232	5.334	4.651	0.134	5.559	4.980	0.070
n6/n3 LC-PUFA ratio	3.415	1.006	2.977	0.883	0.111	3.662	3.208	0.098	3.551	2.977	0.063	3.724	3.208	**0.048**

*ANOVA test

** ANACOVA test

SD = standard deviation, PUFA=polyunsaturated fatty acid, MUFA=monounsaturated fatty acid, DHA=docosahexaenoic acid, DPA=docosapentanoic acid, EPA, Eicosapentaenoic acid

Relative percentages of fatty acids were calculated by taking the concentration of a given fatty acid derivative as a percentage of the total concentration of all fatty acids identified in the sample

Bold and shaded variables indicate statistical significance

4.10 Results summary

A total of 77 subjects were included in the study, 39 for the study group with 38 controls. Anthropometric data did not differ significantly. Only 16 subjects used PUFA supplements. These were fairly evenly spread between the study and control groups. The most common PUFA supplement was fish oil (n = 5), and the second was a combination supplement of omega-3 and omega-6 (n = 3).

For the food intake records, 50 were analysed and there was no significant difference in the total energy, fat and carbohydrate intake between the study and control groups. Four UFAs were significantly higher in the control group, including DHA and DPA. EPA had a trend towards significance.

Fifty-four plasma phospholipid blood samples were analysed. Stearic acid, mead acid and elaidic acid were significantly higher in the study group while C20:3n6, vaccenic acid, DHA, n3-PUFAs and n3-LC-PUFAs had significantly lower concentrations in the study group. When adjusting for possible confounding for endometriosis, SFAs were significantly higher in the study group. When adjusting for possible confounding of endometriosis and PUFA supplementation, eicosadienoic acid and the n6:n3 LC-PUFA ratio were higher in the study group and adrenic acid was lower in the study group.

Fifty RBC membrane samples were analysed. For the RBC membranes analysis, eicosenoic and adrenic acid and C20:3n6 were significantly higher in the study group. When adjusting for possible confounding factors of PUFA supplementation and endometriosis, stearic acid, mead acid and the n6:n3 LC-PUFA ratio were all significantly higher in the study group while DHA, n3 PUFAs and n3 LC-PUFAs were lower in the study group. When adjusting for possible confounding factors of endometriosis, GLA was significantly higher in the study group.

CHAPTER 5

5 DISCUSSION

Infertility is a worldwide problem that is placing a burden on the economy and an emotional burden on couples. PCOS is one of the most common infertility conditions with hormonal disruption, a very high rate of IR, independence from obesity, metabolic syndrome, glucose intolerance and DMII. Treating IR increases fertility rates. [2-5] PUFAs have been shown to reduce IR due to the action on phospholipids and the ability to reduce inflammation.[97] PUFAs can also reduce secretion of insulin and can therefore reduce IR.(98)

5.1 Study population and demographics

In this study, subjects were recruited from a private fertility clinic (study group) and a dietetic practice (control group). Whilst the required number of subjects were recruited per group, three subjects failed to provide sufficient information and were excluded from the study, leading to a 3.75% attrition rate. Groups had no significant difference in baseline demographics.

The average age of the subjects was 32.99 years (n = 77). This is in line with the literature that indicates that women who delay childbearing (willingly or not) until 30 or 40 years of age constitute the largest portion of the total infertility population.[99] There was no significant difference between the groups with relation to basic demographics.

5.2 Anthropometric data

The study and control groups were similar at baseline in terms of anthropometric data, with no significant differences between the groups. The mean BMI for both groups (27.68 kg/m^2 for the study group and 27.66 kg/m^2 for the control group) fell within the overweight category. According to the literature, PCOS and metabolic impairment seems to be independent of the total fat mass content of the body and is not necessarily related to obesity.[100]

Increased androgen production rates can also increase visceral and subcutaneous body fat distribution. The frank phenotype can explain the contradiction.[5, 81] In many other studies, subjects with PCOS have a higher BMI, but a high BMI or increased weight is not indicative of infertility. The anthropometric data had no statistical differences between the two groups, which is not uncommon.[101]

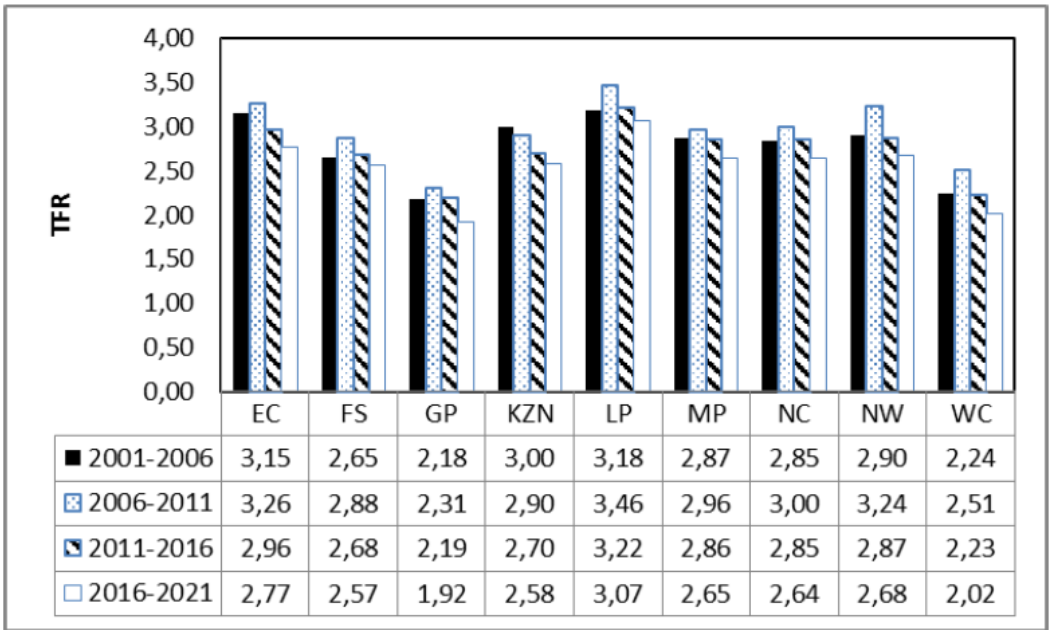
According to a study done by Langley et al with 300 fertility patients, 43% of the women had a BMI < 20 or ≥ 25 kg/m^2.[102] This was higher in the current study with 66% for the study group. The current study had a total of 22 subjects in the study group with a BMI above normal (≥ 25 kg/m^2) and 2 subjects with a BMI below the normal range (< 18.5 kg/m^2).

Sundaram et al studied the association between a couple's BMI and time to pregnancy.[103] When modelled individually, neither the male nor the female partner's BMI was associated with a longer time to pregnancy, but when looking at couples' BMI together, obese class II (BMI ≥ 35.0 kg/m^2) couples experienced a longer time to pregnancy compared to couples with a normal BMI.[90]

Groups were well matched for height (both at 1.66 m), and mean waist circumference was 82 cm (n = 77). The World Health Organization criteria indicate > 80 cm as an increased risk for metabolic complications and > 88 cm as a substantially increased risk.[63] This group therefor has some risk for metabolic complications, and can be seen in the rate of IR of the study group.

5.3 Pregnancy rates and infertility history

The average number of children born to a woman has been declining progressively during recent decades.[104] The mean pregnancy rate for the study group was only 0.21, but this could be expected because they were patients from a fertility clinic. It is possible to conceive a child naturally initially and then to struggle with conception afterwards. The fertility rate for South Africa is shown in Figure 5.1. The statistics for Gauteng Province (where the current study took place) show a total estimated fertility rate of 1.92, which is the lowest in the country.[105] In this study, the average time that subjects in the study group struggled to conceive was 22.59 months (SD ± 25.01) with a median of 12 months.



	EC	FS	GP	KZN	LP	MP	NC	NW	WC
2001-2006	3,15	2,65	2,18	3,00	3,18	2,87	2,85	2,90	2,24
2006-2011	3,26	2,88	2,31	2,90	3,46	2,96	3,00	3,24	2,51
2011-2016	2,96	2,68	2,19	2,70	3,22	2,86	2,85	2,87	2,23
2016-2021	2,77	2,57	1,92	2,58	3,07	2,65	2,64	2,68	2,02

TFR = total fertility rate

EC = Eastern Cape, FS = Free State, GP = Gauteng, KZN = Kwa-Zulu Natal, LP = Limpopo, MP = Mpumalanga, NC = Northern Cape, NW = North West, WC = Western Cape

Figure 5.1: South African provincial average fertility rate

5.4 Medical and medication usage history

For the study group, 31% of women presented with IR. IR is considered the main pathogenic factor for PCOS and causes increased metabolic disturbances.[106] This can explain hyperandrogenism, menstrual infrequency and other metabolic manifestations.[3] Recent research found that hyperinsulinemia is present in 85% of patients with PCOS.[107, 108] Thirty percent is thus a low prevalence for IR in PCOS, but as mentioned before, the reason might be underreporting, misdiagnosis or women being undiagnosed. It should be noted that 21 subjects in the study group were taking Glucophage. Metformin, marketed under the trade name Glucophage, is in the biguanide class of drugs. It works by decreasing glucose production by the liver and increasing insulin sensitivity of body tissues. Glucophage can also increase ovulation and the frequency of endometrial shedding in some women, thus helping with cycle control.[29, 33, 51, 59, 63-67] Only 12 study subjects reported a medical history of IR. This could be an indication of underreporting of IR in this group. It was also noted that 1 subject had DMII, which means that she previously had IR. It could thus be concluded that the prevalence of IR was probably higher than officially reported in the study group.

Conditions that are often related to infertility were noted in the study group; 30.8% presented with endometriosis and 15.4% with hypothyroidism. According to the literature, about 10% of women of reproductive age suffer from endometriosis.[86] It is a condition in which the endometrium or layer of tissue that covers the inside of the uterus grows outside it, most often on the ovaries, fallopian tubes, and tissue around the uterus and ovaries. In rare cases it may also grow on other parts of the body such as the bowel and kidneys.[77] It is a chronic disease that causes pelvic pain and subfertility. Laparoscopy is the gold standard diagnostic test. Currently, there are no noninvasive tests available to diagnose endometriosis.[86] In this fertility clinic, it is not standard practice to do laparoscopies for all patients, and it is therefore an assumption that this condition might also be underdiagnosed.

Thyroid hormones act on nearly every cell in the human body. Moreover, the thyroid gland interacts with the ovaries and thyroid hormones are involved in almost all phases of reproduction, including acting on the oocytes, sperm and embryo during fertilisation, implantation and placentation.[109] Thyroid dysfunction, specifically hypothyroidism, is relatively common among women of reproductive age. It can affect fertility in numerous ways, resulting in anovulatory cycles, sex hormone imbalances and high prolactin levels. Untreated and undiagnosed thyroid disease can be a cause of subfertility.[109, 110] The second most frequently used medication in the study was for hypothyroidism. This included Euthyrox (n = 4) and Eltroxin (n = 5). A total of six subjects reported hypothyroidism, but according to the medication usage, there might again have been underreporting because more subjects were on thyroid hormone medication than actually reported the condition.

The total number of subjects on specific fertility drugs was surprisingly low. Only four subjects were taking fertility drugs, including Clomid, Postrinex, Provera and Proginoval. Since many subjects were still in the initial stages of their fertility treatment and these drugs are not first-line treatment for infertility, it could be that these medications had not yet been initiated at the time of the study. During the recruitment process, the researcher noticed that many patients at the fertility clinic were reluctant to take part in the study, possibly because fertility treatment is a difficult, emotional and cumbersome process. Patients further down the fertility treatment process might have been in the low numbers, and that might explain the low usage of fertility drugs reported.

5.5 Supplement usage

Supplement usage is extremely common nowadays. In 2016, the supplement industry contributed $122 billion to the United States of America economy. The dietary supplementary market is projected to be worth $278 billion by the end of 2024.[111]

In this study, a total of 59 supplements were reported to be taken by the subjects. The most common was multivitamins (n = 19), with the most frequently used brand name StaminoGro (n = 11). StaminoGro is a 5-in-1 combination supplement, containing amino acids, antioxidants, B-complex vitamins, calcium and vitamin D as well as other essential vitamins and minerals (detailed nutritional information is provided in Table 5.1. These ingredients all act in synergy to promote natural growth hormone release and enhance mitochondrial function.[99] As the body grows older, it secretes less natural growth hormone. The amino acid L-arginine helps the body to secrete more of its own natural growth hormone, leading to antiaging effects on the body's cells.[99] StaminoGro may be of benefit to persons suffering from subfertility, immune problems, depression, insomnia and fatigue.[112]

Medfem Fertility Clinic recommends StaminoGro as a multivitamin to most of its patients, and this might have caused biased towards the specific brand.

Table 5.1: Nutritional information for StaminoGro

Each tablet contains	Per tablet	% RDA
Amino acids		
L-arginine	187.5 mg	*
L-glutamine	150 mg	*
Glycine	75 mg	*
L-lysine	50 mg	*
L-ornithine	45 mg	*
Antioxidants		*
Beta carotene (10%)	4.5 mg	7.5
Lipoic acid	5 mg	*
Vitamin C	75 mg	125
Vitamin E	5 iu	50
Selenium	37.5 ug	*
Zinc	5.62 mg	37.5
B-complex vitamins		
Folic acid	198 ug	99
Vitamin B1 – thiamine	0.75 mg	53.6
Vitamin B2 – riboflavine	1.25 mg	78.1
Vitamin B3 – nicotinamide	6 mg	3.33
Vitamin B5 – pantothenic acid	6 mg	100
Vitamin B6 – pyridoxine	6 mg	300
Vitamin B12 – methylcobalamin	6 mg	600
Calcium and vitamin D		
Elemental calcium	100 mg	12.5
Vitamin D	75 IU	37.5
Other vitamins and minerals		
Biotin	20 ug	20
Choline bitartrate	10 mg	*
Copper	500 ug	25
Magnesium	60 mg	20
Manganese	125 mg	*

RDA: recommended daily allowance, mg=milligrams, ug=micrograms, IU=international units

*RDA not available

Ten percent of the study group subjects took Inofolic. Inofolic is a supplement that contains 2 g MYO and 200 mcg folic acid. Inositol is an insulin-sensitising molecule that can be used to improve IR in women with PCOS. [57-62] Two stereoisomers of inositol, MYO and D-chiroinositol, are being investigated. IR might be the result of an alteration of metabolism of inositol phosphoglycans second messengers and mediators. It can also be due to a defect in their tissue availability. Many trials showed that MYO improved IR in women with

PCOS.[57-60] More recent studies looked at the effect of MYO in combination with other drugs or supplements. When combined with monacolin K and lipoic acid, inositol had a dose-dependent improvement in hyperandrogenism-associated symptoms and dyslipidaemia.[58] When inositol was combined with folic acid, hyperstimulation syndrome was decreased to a greater extent than with MYO alone.[44]

A concern is that very few subjects were taking folic acid supplements (five women in the study group). Even if taking into consideration the folic acid content in StaminoGro and Inofolic, it adds up to only 46% of study group participants. The other five multivitamins used by the study group had minimal folic acid content and were below the RDA for pregnancy. Folic acid is an essential micronutrient that prevents neural tube defects.[113-115] Intake of supplemental folic acid has also been consistently related to lower frequency of infertility, lower risk of pregnancy loss and greater success in infertility treatment.[113] As all the women in the study group were hoping to conceive, it would be expected that most if not all would be taking sufficient folic acid.

According to a recent review by Wilson et al, folic acid supplementation dosages are recommended as tabulated in Table 5.2.

Table 5.2: Recommended folic acid intake before, during and after pregnancy[114]

Risk classification	Amount	Time period
Women with *low risk* of a neural tube defect or other folic acid-sensitive congenital anomaly	Diet rich in folate-containing food Supplement containing 0.4 mg folic acid	Two to three months before conception Throughout pregnancy Four to six weeks postpartum or as long as breastfeeding continues
Women with *medium risk* of a neural tube defect or other folic acid-sensitive congenital anomaly	Diet rich in folate-containing food Supplement containing 1 mg folic acid	Three months before conception Throughout pregnancy Twelve weeks postpartum or as long as breastfeeding continues
Women with *high risk* of a neural tube defect or other folic acid-sensitive congenital anomaly	Diet rich in folate-containing food Supplement containing ≥ 1 mg folic acid	Three months before conception Throughout pregnancy Twelve weeks postpartum or as long as breastfeeding continues

The role of vitamin D in bone metabolism and calcium and phosphorus homeostasis is very well known. [116] Over the past few decades, the importance of vitamin D in non-skeletal actions has been studied. Vitamin D is an important secosteroid hormone in non-skeletal as well as skeletal systems and plays a key role in autoimmune diseases, metabolic syndromes, cardiovascular disease, cancer, pregnancy and all-cause mortality. Recent evidence has demonstrated an association between low vitamin D status and

autoimmune thyroid disease as well as the development of gestational diabetes mellitus and foetal growth.[117, 118] Vitamin D supplementation can also significantly reduce the risk of preeclampsia.[119] There is a dramatic two- to three-fold increase in active vitamin D (1.25(OH)2D) concentrations during the early weeks of gestation, which is sustained throughout pregnancy until the time of gestation in both the foetus and the mother. This suggests an immunomodulatory role in preventing foetal rejection by the mother.[120] Because a crucial issue is the initiation or timing of vitamin D supplementation and given the potential effects of vitamin D on placental gene expression and on inflammation within the placenta, it appears important to start vitamin D treatment before placentation as well as trophoblast invasion.[79][121, 122]

According to Lerchbaum et al, vitamin D supplementation can improve metabolic parameters in women with PCOS specifically and a high vitamin D intake might provide protection against endometriosis.[110] A sufficient level of ≥ 30 ng/ml should be maintained.[123] This can be achieved either through natural sun exposure or supplementation. Even though South Africa has lots of sunny weather, people wear sunblock that blocks 99% of the vitamin D and do not spend enough time in full sunlight.[120, 124] Although there is vitamin D in StaminoGro, the dosage is fairly small at only 75 IU per tablet. There were only three subjects who used vitamin D supplementation, and they were all part of the control group.

5.6 Polyunsaturated fatty acid usage

Only 25.6% of the study group took some form of omega-3 or omega-6 supplement at the time of the study. The lack of sufficient supplement usage as well as brand name and dosage data is acknowledged, and it could've affected the outcome of the results when comparing the serum phospholipids and RBC membranes. According to the researcher's knowledge, there is no data for PUFA supplement usage in women with PCOS or infertility. Data on the importance of PUFAs in general health has shown that PUFAs play a significant role in collagen vascular diseases, hypertension, DMII, IR, metabolic syndrome, psoriasis, eczema, atopic dermatitis, coronary heart disease, atherosclerosis and cancer. This is in addition to the role of PGs and LTs in these conditions.[12]

Research focusing on the role of PUFAs during pregnancy showed that women who had high DHA intakes either through their diet (four fish servings per week) or through supplementation with up to 1 100 mg DHA plus 800 mg EPA per day gave birth to infants with higher cognitive development scores and had young children with higher IQ scores and mental processing scores up to four years of age.[125-127] There is also published evidence that links higher maternal DHA intake with improved sleeping patterns in babies, enhanced infant immunity and possibly reduced risk of infant allergies.[128] DHA intake has also been linked with lower risk of postpartum depression, moderately prolonged gestation, reduction in risk of preterm delivery and increased birth weight.[129, 130]

PUFA supplementation during infertility or subfertility is not as well documented. A few studies done on animals (including rams, cows, pigs, mice rats, stallions and cockerels), mainly the male counterpart, have shown positive results in enhancing reproduction.[20, 131-138] There is also no evidence to confirm the recommended dosage for subfertility.

A recent meta-analysis of randomised control trials (n = 3) that looked at the effect of omega-3 fatty acid supplementation on IR in women with PCOS found that there was no significant effect of omega-3 fatty acid supplements compared to placebo on IR (MD: 6.18%; CI = -3.347, 15.382; p = 0.208) and HOMA-IR (MD: 0.276; 95% CI = -1.428, 1.981; p = 0.751) in women with PCOS.[98] The meta-analysis, or rather the trials included, had some limitations, and thus the findings should be interpreted with caution. The supplements used contained a combination of EPA andDHA, but the control groups received a combination of soy bean oil and olive oil. It should be noted that MUFAs can also have a positive effect on IR. As shown in recent research by Karakis et al, soy bean oil acts similarly to fish oil.[11] The question needs to be asked whether soy bean oil is a good option as a placebo. It is also worthy to note that in the end, only three randomised control trials were included in the meta-analysis, so the sample size was very small. Only one study showed negative results[26] while two studies had a positive outcome. Also, in two studies serum omega-3 levels were not tested. Only one study received a Jadad score of > 3, which indicated a high-quality study. One study only looked at non-alcoholic fatty liver disease in PCOS and not specifically at IR.[21] This meta-analysis is thus not very valuable due to poor comparisons and a very small sample size.

PUFA supplement recommendations are available in the literature and are listed in Table 5.3.

Table 5.3: Recommended dosage of Polyunsaturated fatty acids (PUFA) supplementation during life stages

Life stage or disease	Dosage	Source
Cardiovascular disease – general	1 000 mg/day (DHA/EPA) Prevention 250–500 mg (DHA/EPA)	(139)
Cardiovascular disease – to reduce triglycerides	2 000–4 000 mg/day (DHA/EPA)	(17, 23, 97)
Pregnancy	300–1 000 mg/day (DHA/EPA)	(71, 140)
Adult	250–500 mg/day (DHA/EPA)	(71, 141, 142)

DHA = docosahexanoic acid, EPA = ecosapentanoic acid

5.7 Dietary intake

The study and control group had a relatively low-calorie intake compared to the BMI. No activity data was collected, it would be difficult to draw a conclusion in this regard. The participants in the study group had a significantly lower protein intake, specifically animal protein, compared to the control group, even though

it was still above the prudent guidelines. According to Chavarro et al, women who adhere to a 'fertility diet' (higher intake of MUFAs compared to trans fatty acids, higher vegetable intake compared to animal protein sources, more low-glycaemic carbohydrates, higher intake of full-fat dairy products, and higher intake of multivitamins and iron from plants and supplements) have a lower risk of ovulatory disorder infertility.[27] Szczuko et al analysed the diets of 54 Polish women with PCOS and found that 36.7% had insufficient protein intake.[143] The current study also showed a lower protein intake for the study group and higher MUFA intake for the control group.

The study group had a higher fat intake, although not significantly, than the control group. This is also seen in other research studies investigating dietary intake in PCOS.[70, 101] DPA and DHA intake was significantly higher in the control group, and EPA intake was higher in the control group (although not significant at $p = 0.062$), findings that are supported by the literature.[24, 70, 113]

The South African food-based dietary guidelines recommend two to three fish servings per week, preferably oily fish, such as sardines, pilchards, tuna, anchovies and mackerel (including tinned versions).[144] This should provide ± 250 mg EPA and DHA. According to the American Heart Association, the general adult recommendation is actually ± 500 mg EPA andDHA, which is the equivalent to four to five servings of oily fish per week.[139, 141, 142] A healthy ratio of n-6:n-3 in the human diet is said to range from 1:1 to 4:1.[71] According to this study, the mean total dietary EPA andDHA intake was only 200 mg for the study group and 702 mg for the control group, excluding the PUFA supplements recorded. It should be noted that this was a very small study sample and only a three-day food intake record was used, which could have skewed the results. In comparison with a National Health and Nutrition Examination Survey study between 2003 and 2012 that looked at omega-3 fatty acid intake in women of childbearing age (n = 6 478), the mean EPA andDHA intake was only 89 mg.[145] Another large study by Richter et al showed that more than 90% of the study population (n = 24 621) consumed less than the recommended 500 mg EPA andDHA per day (median = 0.11 g/day; mean = 0.17 g/day).[142] According to the South African Food Consumption Survey (2000–2010), the average fish intake for adults is only 15 g per day.[144] This study therefore found much higher intakes compared to the average intake reported in other research papers.

Moran et al investigated preconception fatty acid intake during IVF and what effect it had on fertility rates (n = 46). Women who became pregnant had higher levels of PUFA intake ($p = 0.03$), specifically omega-6 PUFAs and linoleic acid ($p = 0.045$) with a trend towards an elevated intake of omega-3 PUFAs ($p = 0.06$). The study concluded that preconception PUFA intake, and specifically omega-3 and linoleic acid intake, was associated with improved pregnancy rates in overweight and obese women undergoing IVF, and this was confirmed by more studies.[25, 146] Following a 'Mediterranean' diet high in fish, vegetable oils, vegetables

and legumes and low in carbohydrate-rich snacks increased the probability of pregnancy during IVF treatment by 40%.[146-148]

Mumford et al investigated dietary fat intake through 2 cycles of 24-hour recalls (n = 259) and also concluded that PUFAs were associated with small increases in testosterone concentrations and that increased DPA (C22:5n3) was associated with a lower risk of anovulation.[28] In Mumford's study, the total PUFA intake did not differ significantly (p = 0.518), but the DPA (p = 0.029) and DHA (p = 0.043) were significantly lower in the study group and the EPA (p = 0.062) had a trend towards significance, which is in line with the results of this study.

Different types of PUFAs have different effects.[18, 71] Women with a frank phenotype have a worse profile of cardiovascular and metabolic risk factors (i.e. higher IR and poorer lipid panel) than those with the nonclassic phenotype, even when the control groups have a comparable BMI.[5] Some studies have shown a cardio-protective effect from omega-3 fatty acids, specifically a reduction in hypertriglyceridemia, in high doses (2–2.4 g per day) and increasing HDL cholesterol levels.[17, 19, 22, 97, 149, 150] Women with PCOS often have hypercholesterolaemia, and a high-omega 3 PUFA diet might help to reduce this complication.

Ebrahimi et al did a randomised, double-blind placebo control trial (n = 68) with flaxseed oil and vitamin E supplementation. After 12 weeks of intervention, omega-3 fatty acids (1 000 mg flaxseed oil providing 400 mg α-linolenic acid) and 400 IU vitamin E co-supplementation resulted in a significant reduction in insulin (-1.0 ± 3.5 vs. + 2.7 ± 6.6 µIU/mL, p = 0.004) and serum total testosterone (-0.5 ± 0.7 vs. -0.1 ± 0.5 ng/mL, p = 0.008) as well as free testosterone (-1.2 ± 2.1 vs. -0.2 ± 1.7, p = 0.04) compared to the placebo group.[22] Further tests were done on the same group of subjects, investigating the effects on gene expression of lipoprotein(a) and oxidised low-density lipoprotein, lipid profiles and biomarkers of oxidative stress. The co-supplementation downregulated expressed levels of lipoprotein(a) mRNA (p < 0.001) and oxidised low-density lipoprotein mRNA (p < 0.001) in peripheral blood mononuclear cells. In addition, a significant decrease in serum triglycerides (p < 0.001), very-low-density lipoprotein (p < 0.001), LDL (p < 0.001) and total HDL cholesterol (p < 0.001) was observed.[23]

Vargas et al demonstrated that fish oil altered insulin secretion and resistance in women with PCOS but flaxseed oil did not. The effects of fish oil were similar to those of omega-6 PUFA-rich soybean oil.[26]

In the current study, the dietary intake data showed that 13 amino acids were significantly higher in the control group, including the branched-chain amino acids isoleucine, leucine and valine as well as lysine, methionine, threonine, phenylalanine, tryptophan, arginine, histidine, cystine, tyrosine and syrene. Since increased branched-chain and aromatic amino acids also affect insulin secretion and resistance, Karakis et al investigated whether fish oil, flaxseed oil and/or soybean oil affected plasma metabolites of amino acids, especially aromatic amino acids. The results showed that the effects of fish oil and soybean oil on plasma

aromatic amino acids were similar but differed significantly from those of flaxseed oil.[11] A significant difference for branched-chain amino acids was observed in a few other studies.[11, 106, 151] A decrease in serum branched-chain amino acid and aromatic amino acid ratio was directly correlated with the development of PCOS.[11, 106, 151]

The study group had a significantly higher intake of total added sugar. This can contribute to hyperinsulinemia, IR, hyperglycaemia and DMII. Again, this is in line with the common dietary habits of women with PCOS.[101, 152]

The highest risk of deficiency in minerals in women with PCOS in a Polish study was related to calcium, potassium and magnesium. With reference to vitamin deficiency, as much as 70% of tested women were at risk of insufficient intake of folic acid, 36.7% for vitamin C and 26.7% for vitamin B12.[143] The current study also confirmed a significantly lower intake of magnesium (mean = 185.86 mg, RDA = 320 mg) and calcium for the study group (mean = 483.8 mg, RDA = 1 000 mg). Folate had a trend towards significance (p = 0.058) with a lower mean intake of 158.7 mcg (RDA = 400 mcg) for the study group.

Folic acid, as discussed under supplement usage, is of vital importance in the maternal preconception diet. The RDA for preconception is a diet rich in folate plus 1 mg supplemented per day.[114, 115] The mean folate intake for the study group was 158.7 mcg per day (SD ± 70.47). If it is taken into consideration that only 41.15% of the study group subjects were taking some sort of folic acid supplement, it means that more than half of the study group might go into their pregnancy, if and when they conceive, with low folic acid levels.

Calcium intake was also significantly lower in the study group. A recent study investigating dairy intake in women undergoing IVF treatment (n = 323) found higher chances of live birth with higher dairy intake.[153] High-fat dairy intake was also associated with higher fertility rates in a large (n = 17 544) Harvard study in 2007.[27] It is uncertain whether the calcium in dairy has an impact on increased fertility, but it is a possible association that can be studied in future..

Vitamin D deficiency was discussed previously. Dietary vitamin D intake was significantly lower in the study group. Vitamin D deficiency (25O HD < 20 ng/ml) is extremely common in women with PCOS and has been calculated to be prevalent in as much as 67–85% of women with PCOS.[154, 155] Low vitamin D levels could contribute to the development of obesity and IR.[156, 157] It has been reported that gene polymorphism might be partly linked with PCOS through the role of the gene on insulin blood levels and IR.[154] The mechanisms by which low vitamin D levels can cause IR are still unclear.

Mean total dietary fibre intake was 12.81 g (SD ± 8.06) for the study group and 15.84 g (SD ± 7.41) for the control group. This is considerably lower than the recommendation of > 25 g per day. This was also observed in other studies.[143]

The RDA for iron is 18 mg/day. In this study, the mean iron intake for the study group was 8.81 mg (SD ± 3.65) and for the control group 10.47 mg (SD ± 3.98). This constitutes an insufficient iron intake for both groups.

5.8 Blood samples for plasma phospholipids and red blood cell membranes

A total of 30 fatty acids were analysed and quantified in this study. Research on specific serum and plasma fatty acids and PCOS is scarce. Chen et al did a very interesting study in which they investigated plasma metabolomics by comparing plasma between IR-PCOS (n = 21) and non-IR-PCOS women (n = 19). They found that there was a significant increase in the levels of SFAs (palmitic acid and stearic acid) in a group of non-IR PCOS (n = 19) women compared to a control group.[158] This was confirmed in the present study with a significant difference for stearic acid (p = 0.005) between groups. Metabolomics network pathway analysis suggested a profound association of the abnormalities of fatty acids as well as glycerophospholipid and glycerolipid metabolisms in the pathogenesis of PCOS and IR complications.[106, 158]

Zhang et al used a gas chromatography mass spectrometry approach to characterise plasma phospholipid fatty acid profiles of women with PCOS with and without insulin.[159] Patients with IR-PCOS had notably decreased levels of DPA, DHA, total MUFAs and total omega-3 PUFAs, and dramatically elevated behenic acid, DGLA and n-6:n-3 PUFA ratio. Zhang et al also identified that nervonic acid and DGLA were potential fatty acid biomarkers of PCOS and its IR complication. Pearson correlation analysis indicated that nervonic acid and C20:3n6 correlated well with the clinical characteristics of PCOS and IR indicators, respectively. These findings demonstrated that plasma phospholipid fatty acid profiles might provide a complementary approach for clinical diagnosis of PCOS and its IR complication.[159] The current study also showed a significant difference in plasma C20:3n6, RBC C20:3n6, DHA (p = 0.029), omega-3 PUFAs and omega-3 LC-PUFAs. The n-6:n-3 PUFA ratio and the n-6:n-3 LC-PUFA ratio had a trend towards significance, but after adjusting for possible confounding factors of endometriosis and PUFA supplement usage, it was significant. Nervonic acid had a trend towards significance.

Zhao et al specifically looked at the metabolic profiles of the different PCOS phenotypes and found a significantly positive association of linoleic acid and stearic acid concentrations with the occurrence of PCOS. A decrease of branched-chain amino acid/aromatic amino acid ratio was directly correlated with the development of PCOS.[11, 106, 151] Plasma stearic acid was also significantly higher in the study group (p = 0.005), and RBC stearic acid was significantly higher after adjusting for possible confounding of endometriosis and PUFA supplementation.

Cross-sectional data showed that a higher plasma n-6 PUFA concentration and n-6:n-3 PUFA ratio were associated with higher circulating androgens in women with PCOS (n = 104).[60] Plasma LC omega-3 PUFA status was associated with a decreased atherogenic lipid profile. LC omega-3 PUFA supplementation reduced plasma bioavailable testosterone concentrations ($p < 0.05$), with the greatest reductions in subjects who exhibited greater reductions in plasma n-6:n-3 PUFA ratios.[77] The present study can support this evidence with significantly higher amounts in the control group for omega-3 PUFAs and omega-3 LC-PUFAs ($p = 0.036$) and a trend towards significance with n-6:n-3 PUFA and n-6:n-3 LC-PUFA ratios. The n-6:n-3 LC-PUFA ratio was significant after adjusting for possible confounders of PUFA supplementation and endometriosis.

In a PUFA-rich diet, plasma linoleic acid (C18:2n6) and plasma linolenic acid (C18:3n3) increased significantly.[24] No significant difference was seen between LA and ALA in this study, but the diets were not specifically PUFA rich.

PCOS is associated with inflammation, and it therefore valuable to note that the omega-3 PUFA levels were significantly higher in the control group and that the n-6:n-3 PUFA ratio was higher in the study group.[160] PUFAs are important precursors of eicosanoids, which function as signalling molecules. An excess of n-6 PUFAs and a high n-6:n-3 PUFA ratio will cause an overproduction of omega-6 eicosanoids from AA, leading to the production of more pro-inflammatory PGs and thromboxanes.[142]

Elevated serum mead acid (C20:3n9) is an indirect marker of an EFA deficiency.[161, 162] Mead acid is synthesised from oleic acid during a state of PUFA deficiency. Mead acid is thought to be produced by the same enzymes that synthesise AA and EPA.[153] The genes and pathways involved in the conversion of oleic acid to mead acid have not been fully elucidated.[163] The study group had a significantly higher mead acid level compared to the control group. This is an indication that the study group could have been PUFA deficient.

CHAPTER 6

6 CONCLUSIONS AND RECOMMENDATIONS

6.1 Hypotheses acceptance/rejection

Rejected:

There is no significant difference between women with PCOS and women without PCOS in terms of plasma phospholipid n-3 PUFA.

There is no significant difference between women with PCOS and women without PCOS in terms of plasma phospholipid and RBC membrane n-6/n-3 LC PUFA ratio (when adjusted for possible confounding of EFA supplementation and Endometriosis).

Accepted:

There is no significant difference between women with PCOS and women without PCOS in terms of anthropometric parameters and supplemented PUFA.

There is no significant difference between women with PCOS and women without PCOS in terms of plasma phospholipids and RBC membranes n-6.

Partially accepted/rejected:

There is no significant difference between women with PCOS and women without PCOS in terms of dietary PUFA's. Four dietary PUFA's were significantly different between groups, but no significant differences were found for the others

6.2 Conclusions

PUFAs, in particular omega-3, have been shown to play a significant role in reducing IR due to the impact that they have on cell membrane structure and their anti-inflammatory properties. Dietary PUFAs are powerful modulators of lipid and glucose metabolism, which influences insulin secretion and resistance directly and alters plasma amino acids indirectly. Omega-3 fatty acids can also improve insulin sensitivity by producing and secreting anti-inflammatory adipokins such as adiponectin and reducing pro-inflammatory cytokines. [18, 19] [18, 19] Many studies have shown that omega-3 fatty acids have positive effects on IR and that EPA supplementation has a meaningful effect with the reduction of serum insulin and HOMA-IR levels. The role of omega-3 fatty acids in the reduction of IR in patients with PCOS, with a definite trend towards omega-3 PUFAs, has been identified. More specifically, PUFAs have been linked to significantly improved metabolic and endocrine effects in women with PCOS and to a reduction in ovulatory infertility.

It seems that differences related to these results are due to supplementation dosage, trial duration, lack of control groups, obesity, severity of IR and the presence of other medical conditions that can affect IR.

Although several nutritional studies have demonstrated that omega-3 LC-PUFA supplementation exhibited beneficial effects on the endocrine and metabolic profiles in PCOS women, it is not clear whether the recruited PCOS subjects in these studies had IR complications. Therefore, the specific role of PUFAs in the pathogenesis of PCOS remains to be revealed.

In the present study, 54 plasma phospholipid blood samples were analysed. Stearic acid, mead acid and elaidic acid were significantly higher in the study group while C20:3n6, vaccenic acid, DHA, n3-PUFAs and n3-LC-PUFAs had significantly lower concentrations in the study group when compared to the control group. When adjusting for possible confounding for endometriosis, SFAs were significantly higher in the study group. When adjusting for possible confounding of endometriosis and PUFA supplementation, eicosadienoic acid and the n6:n3 LC-PUFA ratio were higher in the study group and adrenic acid was lower in the study group when compared to the control group.

Fifty RBC membrane samples were analysed. For the RBC membranes analysis, eicosenoic and adrenic acid and C20:3n6 were significantly higher in the study group. When adjusting for possible confounding factors of PUFA supplementation and endometriosis, stearic acid, mead acid and the n6:n3 LC-PUFA ratio were all significantly higher in the study group while DHA, n3 PUFAs and n3 LC-PUFAs were lower in the study group. When adjusting for possible confounding factors of endometriosis, GLA was significantly higher in the study group when compared to the control group.

In the present study, the researcher demonstrated that a plasma phospholipid fatty acid profile could discriminate between the controls and PCOS patients. There were significantly lower plasma omega-3 PUFAs, in particular DHA, in the study group. Women with PCOS had a higher plasma n6:n3 PUFA ratio, which had a trend towards significance. Mead acid was significantly higher in the study group, which indicated a PUFA deficiency in the PCOS group.

These findings demonstrate that plasma phospholipid fatty acid profiles may provide a complementary approach for clinical treatment and assessment of women with PCOS. PUFAs therefore play a role in the management of PCOS and infertility.

6.3 Recommendations

Based on the literature and findings of this study, the following recommendations are made for the management of individuals with PCOS:

- Different phenotypes for PCOS should be distinguished for all patients because this will influence the treatment approach.

- IR testing should be considered for all PCOS patients, regardless of weight or BMI. An insulin level below 10.4 mmol/L and a normal HOMA-IR should be aimed for.
- A healthy Mediterranean or 'fertility' diet regime should be recommended to all women with PCOS regardless of their plans to start with conception. This includes
  - higher intake of MUFAs rather than trans fatty acids;
  - vegetable protein rather than animal protein sources;
  - low-glycaemic carbohydrates; and
  - high-fat dairy products.
- It is important to determine micronutrient status and intake during preconception.
- A healthy diet should aim to provide all micronutrients needed for preconception. Supplementation should be recommended to all fertility patients who are micronutrient deficient to ensure adequate intake of micronutrients; specific emphasis should be put on folic acid, vitamin D and PUFAs.
- When recommending a PUFA supplement, it is important to look at the dosage and EPA:DHA content and to avoid additional n-6 PUFAs.

6.4 Future research

- Because not all patients with PCOS present with IR, it may prove prudent to distinguish between IR-PCOS and non-IR-PCOS when analysing data.
- It would be even more beneficial to distinguish between the different phenotypes when collecting and analysing data.
- Because the effect of PUFAs on amino acids, specifically branched-chain amino acids and aromatic amino acids, seems to be significantly different between women with PCOS and those without PCOS, this field of study might seem fruitful.
- Fatty acids can possibly be more intensely investigated as possible metabolomics for PCOS diagnosis.

6.5 Study limitations

The study design as well as the methodology did have limitations, which may have influenced the results obtained. The study sample was small, which limits the generalisation of the results to larger groups. The researcher acknowledges that a FFQ might be an ideal tool to estimate PUFA intake because foods containing PUFAs are not always eaten on a daily or even weekly basis. As dietary PUFA intake was not the main focus of the study but was a mere addition to control for overall PUFA status and as there is no available standardised and validated FFQ that specifically measures PUFA intake for this population, the researcher used a three-day food intake record to estimate PUFA intake.

An estimated food intake record is a valid dietary assessment tool; subjects did not need to depend on memory and could provide details regarding intake.[93] Multiple days are more representative of usual intake, and the researcher therefore asked for two weekdays and one weekend day to be recorded. Food intake records are valid up to five days.[70] The researcher is aware of the disadvantages, namely that a high degree of cooperation is required, that it is time-consuming and that the act of recording may alter the diet. Subjects must also be literate, which the study population fortunately was. The lack of cooperation did, unfortunately, influence the return of the food diaries. The collection of dietary intake data was not the main focus of this research. Despite this, dietary intake data was reported in detail, especially non-fatty acid related data. The possible relationship between nutrients, other than fatty acids, and POCS is however interesting and indicates how important it was to use the correct dietary intake methodology in this study, but it is still a shortcoming of this study.

All the information gathered also required cooperation and relying on the memory of the subjects. Because the subjects were only seen once most of the time (steps 1 and 2 were done simultaneously if the subjects had been recruited by the researcher), the opportunity to bring all supplements and medications to the follow-up meeting was unfortunately forgone. This resulted relying on the subjects' memory. There were times when the subjects could not remember supplements or brand names, which could have caused underreporting. Information regarding who prescribed supplements was not collected and might have given insight into supplement usage. It would've been useful to have done a telephonic follow up to collect information regarding supplement brands.

Diagnosis of medical conditions was done on a reporting basis only. This could have resulted in an underreporting scenario because the subjects had forgotten their diagnosis, did not understand their diagnosis or had not been properly diagnosed. This could clearly be seen in the number of subjects taking Glucophage versus the number of subjects who reported IR as a medical condition. The original idea was to collect data from patient files, but the doctors at the Medfem clinic felt that it would've been unethical.

The FoodFinder3 software program has limitations, and not all 'modern' foods are available as choices (for instance, chia seeds are a fairly new food source in South Africa but are not listed in the database). Dishes and meals are difficult to enter and analyse.

As the study was primarily planned and executed by the researcher, logistics only allowed one study site to be used for recruitment, which could have impacted on the generalisability of the results.

Although not a primary objective of the study as no stratification was done, caution needed to be exercised in the assessment of the demographic findings because this could limit the reliability of any associations drawn from a heterogeneous study population.

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ADDENDA

Addendum 1	**Food diary**
Addendum 2	**Record form**
Addendum 3(a)	**Basic information – study group**
Addendum 3(b)	**Basic information – control group**
Addendum 4 (a)	**Consent form – study group**
Addendum 4 (b)	**Consent form – control group**
Addendum 5 (a)	**Basic questionnaire – study group**
Addendum 5 (b)	**Basic questionnaire – control group**
Addendum 6	**Patient information form for blood test**

ADDENDUM 1

Food diary

Patient ID:

ADDENDUM 1

FOOD INTAKE RECORD

Name:
Tel work:
Cell:

Instructions:

- Begin the food journal with documenting the "Time" that you ate.
- In the column labeled "Type of food," record what you ate. This includes any snack and/or meal items, even a piece of candy/sweets. Please be specific in describing the food. For example, instead of writing milk, please indicate the type- full cream, 2%, 1%, skim, or fat free. If you consumed bread, was it whole wheat, white, rye, pumpernickel, etc. If necessary, break down food items into different components. For instance, if you ate a chicken sandwich, write down rye bread, deli chicken, cheddar cheese, mayonnaise, lettuce, and tomato. Feel free to send along a recipe or food label to improve the accuracy of your analysis. Also, please record brand names when possible.
- In the column labeled "Method of Preparation," document how the food was prepared (eg. frying, grilling, baking, microwaving, steamed, etc). Also, please indicate the name of the restaurant or dining hall that you ate at in this column.
- In the column labeled "Amount/Quantity," record the quantity of each food consumed, eg. 1 tsp, 1 slice, 1 tbsp. If you don't have access to measuring utensils, use the standard serving sizes or the portion control guide at the bottom of each food intake record page to describe amounts. Avoid subjective terms like "bowl", "serving", "plateful" or "helping.
- Do the same with the column for "Condiments" and "Beverage". Don't forget to write down beverages such as water, coffee, soda, tea, juices, and alcohol.
- When you are finished, please send it to maryke@dietitiansa.co.za, or fax to 011 7069749, or drop it off at my office – Suite 11 Sandton Medpark, Peter Place, Sandton.

The more details you can provide, the more accurate your analysis will be!

Call or email if you have any questions about documenting your food intake.

Maryke Bronkhorst

0834408143

maryke@dietitiansa.co.za

Sample day: Food intake record Date:_____

Name:_____

Use standard measures such as cups, grams, teaspoons and tablespoons for amount/quantity. Unsure? Use the portion control guide below to describe your serving sizes.

Time	Type of food	Method of preparation	Amount/ Quantity	Condiment	Amount/ Quantity	Beverage	Amount/ Quantity
07:30	Kelloggs All bran flakes		1 cup	Milk – low fat Sugar	½ cup ½ tsp	Rooibos tea (1 sugar, low fat milk)	1 cup
10:00	Apple		1				
11:30	Strawberry low fat Yoghurt – Danone		175ml			Water	500ml
13:00	Sandwhich – chicken mayonnaise Side salad – lettuce, cucumber, tomato	Toasted`	1 ½ cup	Salad dressing	1 tbsp	Cappucino – skinny	300ml
14:30	Nestle kit kat		30g			Coke	340ml
17:00	Cheddar cheese Provita crackers		Matchbox 3	Butter	1 tsp		
19:45	Chicken (no skin) Green beans with potato Rice Salad – tomato, cucumber, lettuce	Grilled Boiled Boiled	Skin and thigh ½ cup 1 cup ½ cup	Salad dressing	1 tbsp	Fruit juice – orange	200ml

Potion control guide	¼ cup = golf ball	1/2cup = tennis ball	1 cup = small fist	30g = matchbox
	1 teaspoon = tip of your thumb	3 teaspoons = 1 tablespoon	1 cup = 250ml	90g cooked meat = deck of playing cards

Day 1 Food intake record Date:_____

Name:_____

Use standard measures such as cups, grams, teaspoons and tablespoons for amount/quantity. Unsure? Use the portion control guide below to describe your serving sizes.

Time	Type of food	Method of preparation	Amount/ Quantity	Condiment	Amount/ Quantity	Beverage	Amount/ Quantity

Potion control guide			
¼ cup = golf ball	1/2cup = tennis ball	1 cup = small fist	30g = matchbox
1 teaspoon = tip of your thumb	3 teaspoons = 1 tablespoon	1 cup = 250ml	90g cooked meat = deck of playing cards

Day 2 Food intake record Date:_____

Name:_____

Use standard measures such as cups, grams, teaspoons and tablespoons for amount/quantity. Unsure? Use the portion control guide below to describe your serving sizes.

Time	Type of food	Method of preparation	Amount/ Quantity	Condiment	Amount/ Quantity	Beverage	Amount/ Quantity

Potion control guide	¼ cup = golf ball	1/2cup = tennis ball	1 cup = small fist	30g = matchbox
	1 teaspoon = tip of your thumb	3 teaspoons = 1 tablespoon	1 cup = 250ml	90g cooked meat = deck of playing cards

Day 3 Food intake record Date:_____

Name:_____

Use standard measures such as cups, grams, teaspoons and tablespoons for amount/quantity. Unsure? Use the portion control guide below to describe your serving sizes.

Time	Type of food	Method of preparation	Amount/ Quantity	Condiment	Amount/ Quantity	Beverage	Amount/ Quantity

Potion control guide	¼ cup = golf ball	1/2cup = tennis ball	1 cup = small fist	30g = matchbox
	1 teaspoon = tip of your thumb	3 teaspoons = 1 tablespoon	1 cup = 250ml	90g cooked meat = deck of playing cards

ADDENDUM 2

Record form

ADDENDUM 2:

Record sheet

Dear Hannelie

Please write down all possible PCOS patients which might be willing to participate in the study.

Date	Name of patient	File number	Basic information form given? (yes/no)	Basic information form collected? (yes/no)

Thank you very much.

Maryke Bronkhorst

ADDENDUM 2:

Record sheet

Dear Dr. Rodriques

Please write down all possible PCOS patients which might be willing to participate in the study.
Please hand in at reception every Friday.

				To be recorded by reception
Date	**Name of patient**	**File number**	**Basic information form given? (yes/no)**	**Basic information form collected? (yes/no)**

Thank you very much.

Maryke Bronkhorst

ADDENDUM 2

Record sheet

Dear Dr. Van Scouwenburg

Please write down all possible PCOS patients which might be willing to participate in the study.
Please hand in at reception every Friday.

				To be recorded by reception
Date	**Name of patient**	**File number**	**Basic information form given? (yes/no)**	**Basic information form collected? (yes/no)**

Thank you very much.

Maryke Bronkhorst

ADDENDUM 2

Record sheet

Dear Dr. Divanovic

Please write down all possible PCOS patients which might be willing to participate in the study.
Please hand in at reception every Friday.

				To be recorded by reception
Date	**Name of patient**	**File number**	**Basic information form given? (yes/no)**	**Basic information form collected? (yes/no)**

Thank you very much.

Maryke Bronkhorst

ADDENDUM 2

Record sheet

Dear Dr. R Janse van Rensburg

Please write down all possible PCOS patients which might be willing to participate in the study.
Please hand in at reception every Friday.

				To be recorded by reception
Date	**Name of patient**	**File number**	**Basic information form given? (yes/no)**	**Basic information form collected? (yes/no)**

Thank you very much.

Maryke Bronkhorst

ADDENDUM 2

Record sheet

Dear Dr. Clark

Please write down all possible PCOS patients which might be willing to participate in the study.
Please hand in at reception every Friday.

				To be recorded by reception
Date	**Name of patient**	**File number**	**Basic information form given? (yes/no)**	**Basic information form collected? (yes/no)**

Thank you very much.

Maryke Bronkhorst

ADDENDUM 2

Record sheet

Dear Dr. H Allan-Gauld

Please write down all possible PCOS patients which might be willing to participate in the study.
Please hand in at reception every Friday.

				To be recorded by reception
Date	**Name of patient**	**File number**	**Basic information form given? (yes/no)**	**Basic information form collected? (yes/no)**

Thank you very much.

Maryke Bronkhorst

ADDENDUM 2

Record sheet

Dear Dr. Barrow

Please write down all possible PCOS patients which might be willing to participate in the study.
Please hand in at reception every Friday.

				To be recorded by reception
Date	**Name of patient**	**File number**	**Basic information form given? (yes/no)**	**Basic information form collected? (yes/no)**

Thank you very much.

Maryke Bronkhorst

ADDENDUM 2

PCOS patient record sheet

Date	Name of patient	Cell number

ADDENDUM 3(a)

Basic information – study group

ADDENDUM 3 (a)

Basic information: Study group

Dear patient

You are being invited to take part in a research project which will evaluate the Polyunsaturated Fatty Acid status of women with Poly Cystic Ovarian Syndrome. Your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the **Health Research Ethics Committee at Stellenbosch University** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

I would like to ask you a few basic questions, after which I will contact you telephonically to arrange an appointment. At this meeting the study will be discussed **in detail** and at that point you will, again have the chance to agree/decline to take part.

Name:	
Telephone numbers:	(please provide all possible numbers and also please tick the box of the number you prefer me to use)
Home:	☐
Office:	☐
Cell:	☐
Diagnosis:	
Previous medical history:	

(6)If you do not wish to participate, please tick the box below:

☐ I do not wish to be contacted about this study

If you have any questions please do not hesitate to contact me on **0834408143**.

Thank you very much

Maryke Bronkhorst

Registered Dietitian

PLEASE LEAVE THIS FORM AT RECEPTION WHEN YOU ARE FINISHED.

ADDENDUM 3 (b)

Basic information – control group

ADDENDUM 3 (b)

Basic information: Control group

Dear patient

You are being invited to take part in a research project which will evaluate the Polyunsaturated Fatty Acid status of women with Poly Cystic Ovarian Syndrome. You have been selected as part of the **control group** of the study. Your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the **Health Research Ethics Committee at Stellenbosch University** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

I would like to ask you a few basic questions, after which I will contact you telephonically to arrange an appointment. At this meeting the study will be discussed **in detail** and at that point you will, again have the chance to agree/decline to take part.

Name:	
Telephone numbers:	(please provide all possible numbers and also please tick the box of the number you prefer me to use)
Home:	☐
Office:	☐
Cell:	☐
Medical history:	

(6)If you do not wish to participate, please tick the box below:

☐ I do not wish to be contacted about this study

If you have any questions please do not hesitate to contact me on **0834408143**.

Thank you very much

Maryke Bronkhorst

Registered Dietitian

PLEASE LEAVE THIS FORM AT RECEPTION WHEN YOU ARE FINISHED.

ADDENDUM 4 (a)

Consent form – study group

Addendum 4 (a)

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM: Study group

TITLE OF THE RESEARCH PROJECT:

Polyunsaturated Fatty Acid status in individuals with PCOS

REFERENCE NUMBER:

PRINCIPAL INVESTIGATOR: Maryke Bronkhorst

ADDRESS: Suite 11 Sandton Medpark, Peter Place,Sandton

CONTACT NUMBER: 0834408143

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the researcher or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the **Health Research Ethics Committee at Stellenbosch University** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

What is this research study all about?

- This study will be conducted at the Medfem clinic only. A total number of 40 cases and 40 controls will be recruited for the study.
- The aim of the study is to investigate the baseline PUFA status of women with PCOS.
- The procedures will be as follows:
  - ✓ Whilst seeing the gynaecologist you will be identified as a possible candidate for the study.
  - ✓ You will be given a basic form to ask for your details.
  - ✓ You will then be contacted by the researcher to set up an appointment.
  - ✓ **One-on-one meeting:** You will be contacted via telephone to meet either at Medfem or the researchers' office. The study will be explained. You will then be asked a few basic questions, you will be weighed and your height and waist circumference will be measured. You will also be asked to fill out a 3 day food intake

record and return it to the researchers office via fax, email, or post. A qualified nurse will take some blood to do a simple blood test. This will only take about 15 minutes. You are then free to go home.

Why have you been invited to participate?

- You have been invited because you are diagnosed with Polycystic ovarian syndrome and therefore fall within the group of patients that we would like to investigate.

Who will be included in the study?

- Women diagnosed with PCOS who have been trying to conceive naturally without assistance for at least 6 months (including or excluding endometriosis) – (study group)
- Women not previously diagnosed with PCOS and who does not have any known fertility problems – (control/reference group)
- Women who are willing to answer the initial questionnaire.
- Women who are willing to do a simple blood test.
- Women who are willing to fill out a 3 day food intake record.
- Women who are willing to undergo anthropometric measurements including weight, height and waist circumference.
- Women of child bearing age (20 – 45).
- Women who might be going for artificial insemination (but not necessarily) (study group).
- Women who might be going for in vitro fertilization (but not necessarily) (study group).
- Women who are literate.
- Women who can speak/understand English. If they cannot speak/understand English, an interpreter will be arranged

Who will be excluded from the study?

- Women who live outside South Africa.
- Women not providing consent to participate in the study.
- Women who have fertility problems other than PCOS and Endometriosis

What will your responsibilities be?

- You will be expected to meet the researcher for one meeting where you need to answer a few basic questions. You will be weighed and measured. This will be a time which is convenient to you, e.g. while you wait to see your doctor at Medfem in the morning.

- You will also be expected to give one blood sample.
- You will be expected to fill out a 3 day food intake record.

Will you benefit from taking part in this research?

- This study might benefit you and all PCOS patients in future, as the relationship between polyunsaturated fatty acids (PUFA), fertility and PCOS will be better understood. The results will guide the researchers in the treatment of this condition, but further research will be needed to confirm this study.

Are there in risks involved in your taking part in this research?

- The procedures followed in this study are generally safe and without side effects but you may experience some discomfort when blood is drawn (needle prick and bruising).

If you do not agree to take part, what alternatives do you have?

- If you do not agree to take part, or want to leave the study at any time during the meeting, you can leave the study immediately.

Who will have access to your medical records?

- All the information collected will be treated as confidential and protected. If it is used in a publication or thesis, the identity of all the subjects will remain anonymous. The researcher herself, as well as your gynecologist (either Dr Johan van Rensburg or **Dr Antonio Rodrigues** or **Dr Johan van Schouwenburg) are the only people who will have access to your medical records for the purpose of this study.**

Will you be paid to take part in this study and are there any costs involved?

- No you will not be paid to take part in the study.
- There will be no costs involved for you, if you do take part.

Is there anything else that you should know or do?

- You can contact Dr Johan van Rensburg at tel 011 463 2244 if you have any further queries or encounter any problems.
- You can contact the Committee for Human Research at 021-938 9207 if you have any concerns or complaints that have not been adequately addressed by your study doctor.
- You will receive a copy of this information and consent form for your own records.

Declaration by participant

By signing below, I …… agree to take part in a research study entitled

*(*Polyunsaturated Fatty Acid status in individuals with PCOS*)*.

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Signed at (*place*) ..... on (*date*) ..... 20__.

....

Signature of participant ***Signature of witness***

Declaration by investigator

I *Maryke Bronkhorst* declare that:

- I explained the information in this document to ……..
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use a Interpreter. (*If a Interpreter is used then the Interpreter must sign the declaration below.*

Signed at (*place*) ..... on (*date*) ..... 20__.

....

Signature of participant ***Signature of witness***

Declaration by Interpreter

- I *(name)* …… declare that:
- I assisted the investigator (*name*) ……. to explain the information in this document to (*name of participant*) …….. using the language medium of Afrikaans/Xhosa.
- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

Signed at (*place*) ..... on (*date*) ..... 20__.

....

Signature of participant ***Signature of witness***

ADDENDUM 4 (b)

Consent form – control group

Addendum 4 (b)

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM: Control group

TITLE OF THE RESEARCH PROJECT:

Polyunsaturated Fatty Acid status in individuals with PCOS

REFERENCE NUMBER:

PRINCIPAL INVESTIGATOR: Maryke Bronkhorst

ADDRESS: Suite 11 Sandton Medpark, Peter Place,Sandton

CONTACT NUMBER: 0834408143

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the researcher or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the **Health Research Ethics Committee at Stellenbosch University** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

What is this research study all about?

- This study will be conducted at the Medfem clinic, Sandton Mediclinic and Sandton Medpark only. A total number of 40 cases and 40 controls will be recruited for the study.
- The aim of the study is to investigate the baseline PUFA status of women with PCOS.
- The procedures will be as follows:
  - ✓ Whilst seeing the gynaecologist or dietitian you will be identified as a possible candidate for the study.
  - ✓ You will be given a basic form to ask for your details.
  - ✓ You will then be contacted by the researcher to set up an appointment.
  - ✓ **One-on-one meeting:** You will be contacted via telephone to meet either at Medfem or the researchers' office. The study will be explained. You will then be asked a few basic questions, you will be weighed and your height and waist circumference will be measured. You will also be asked to fill out a 3 day food intake

record and return it to the researchers' office via fax, email, or post. A qualified nurse will take some blood to do a simple blood test. This will only take about 15 minutes. You are then free to go home.

Why have you been invited to participate?

- You have been invited because you fall within the group of patients that we would like to investigate, according to your age and medical history of no fertility problems.

Who will be included in the study?

- Women diagnosed with PCOS who have been trying to conceive naturally without assistance for at least 6 months (including or excluding endometriosis) – (study group)
- Women not previously diagnosed with PCOS and who does not have any known fertility problems – (control/reference group)
- Women who are willing to answer the initial questionnaire.
- Women who are willing to do a simple blood test.
- Women who are willing to fill out a 3 day food intake record.
- Women who are willing to undergo anthropometric measurements including weight, height and waist circumference.
- Women of child bearing age (20 – 45).
- Women who might be going for artificial insemination (but not necessarily) (study group).
- Women who might be going for in vitro fertilization (but not necessarily) (study group).
- Women who are literate.
- Women who can speak/understand English. If they cannot speak/understand English, an interpreter will be arranged

Who will be excluded from the study?

- Women who live outside South Africa.
- Women not providing consent to participate in the study.
- Women who have fertility problems other than PCOS and Endometriosis

What will your responsibilities be?

- You will be expected to meet the researcher for one meeting where you need to answer a few basic questions. You will be weighed and measured. This will be a time which is convenient to you.

- You will also be expected to give one blood sample.
- You will be expected to fill out a 3 day food intake record.

Will you benefit from taking part in this research?

- This study might benefit you and all PCOS patients in future, as the relationship between polyunsaturated fatty acids (PUFA), fertility and PCOS will be better understood. The results will guide the researchers in the treatment of this condition, but further research will be needed to confirm this study.

Are there in risks involved in your taking part in this research?

- The procedures followed in this study are generally safe and without side effects but you may experience some discomfort when blood is drawn (needle prick and bruising).

If you do not agree to take part, what alternatives do you have?

- If you do not agree to take part, or want to leave the study at any time during the meeting, you can leave the study immediately.

Who will have access to your medical records?

- All the information collected will be treated as confidential and protected. If it is used in a publication or thesis, the identity of all the subjects will remain anonymous. The researcher herself, as well as your gynecologist (either Dr Barrow, Dr Janse van Rensburg, Dr Divanovic, Dr Russouw**) are the only people who will have access to your medical records for the purpose of this study.**

Will you be paid to take part in this study and are there any costs involved?

- No you will not be paid to take part in the study.
- There will be no costs involved for you, if you do take part.

Is there anything else that you should know or do?

- You can contact Dr Johan van Rensburg at tel 011 463 2244 if you have any further queries or encounter any problems.
- You can contact the Committee for Human Research at 021-938 9207 if you have any concerns or complaints that have not been adequately addressed by your study doctor.
- You will receive a copy of this information and consent form for your own records.

Declaration by participant

By signing below, I …… agree to take part in a research study entitled

*(*Polyunsaturated Fatty Acid status in individuals with PCOS*)*.

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Signed at (*place*) ..... on (*date*) ..... 20__.

....

Signature of participant ***Signature of witness***

Declaration by investigator

I *Maryke Bronkhorst* declare that:

- I explained the information in this document to ……..
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use a Interpreter. (*If a Interpreter is used then the Interpreter must sign the declaration below.*

Signed at (*place*) ..... on (*date*) ..... 20__.

....

Signature of participant ***Signature of witness***

Declaration by Interpreter

- I *(name)* …… declare that:
- I assisted the investigator (*name*) ……. to explain the information in this document to (*name of participant*) …….. using the language medium of Afrikaans/Xhosa.
- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

Signed at (*place*) ..... on (*date*) ..... 20__.

....

Signature of participant ***Signature of witness***

ADDENDUM 5 (a)

Basic questionnaire – study group

Patient ID:
Tel home:
Tel work:
Cell:

ADDENDUM 5

BASIC INFORMATION: Study group

Section 1:

Subject information	
File number	
Name	
Date of birth	
Diagnosis	
Fertility: How long (m)	
Preferred time to phone	☐ Day ☐ Evening
	☐ Anytime

Anthropometric data	
Weight (kg)	
Height (m)	
Waist (cm)	

PUFA use	
☐ (1)No	**If yes**
☐ (2)Yes	☐ (1)ω-3 (fish oil)
	☐ (2)ω-3 (krill oil)
	☐ (3)ω-3 (flax seed)
	☐ (4)ω-6
	☐ (5) ω-3 and ω-6
	☐ (6) ω-3, ω-6 and ω-9
	☐ (7) other
Brand name:	
Dosage:	

Demographic information	
Race	☐ (1)White ☐ (2)Black ☐ (3)Asian ☐ (4)Coloured ☐ (5)other
Nationality	☐ (1)South African ☐ (2)African other ☐ (3)European ☐ (4)American ☐ (5)other

Medication	
☐ (1) Glucophage	☐ (6)Provera
☐ (2) Avandia	☐ (7)Eltroxin
☐ (3) Clomid	☐ (8)Euthyrox
☐ (4) Postrinex	☐ (9)Fertimed
☐ (5) Parlodel	☐ (10) Other

Supplements	
	Brand name
☐ (1) Multivitamin	
☐ (2) Antioxidant	
☐ (3) Calcium	
☐ (4) Folic Acid	
☐ (5) B vitamins	
☐ (6) Vitamin C	
☐ (7) Immune boosters	
☐ (8) Probiotics	
☐ (9) Herbal suppl	
☐ (10) Inofolic	
☐ (11) Other	

Section 2

Serum PUFA	
DHA (mmol/l)	
EPA (mmol/l)	

ADDENDUM 5 (b)

Basic questionnaire – control group

Patient ID:
Tel home:
Tel work:
Cell:

ADDENDUM 5

BASIC INFORMATION: Control group

Section 1:

Subject information	
File number	
Name	
Date of birth	
Diagnosis	
Successful pregnancies	
Preferred time to phone	☐ Day ☐ Evening
	☐ Anytime
Anthropometric data	
Weight (kg)	
Height (m)	
Waist (cm)	
PUFA use	
☐ (1)No	**If yes**
☐ (2)Yes	☐ (1)ω-3 (fish oil)
	☐ (2)ω-3 (krill oil)
	☐ (3)ω-3 (flax seed)
	☐ (4)ω-6
	☐ (5) ω-3 and ω-6
	☐ (6) ω-3, ω-6 and ω-9
	☐ (7) other
Brand name:	
Dosage:	

Demographic information	
Race	☐ (1)White ☐ (2)Black ☐ (3)Asian ☐ (4)Coloured ☐ (5)other
Nationality	☐ (1)South African ☐ (2)African other ☐ (3)European ☐ (4)American ☐ (5)other

Medication	
☐ (1)	☐ (6)
☐ (2)	☐ (7)
☐ (3)	☐ (8)
☐ (4)	☐ (9)
☐ (5)	☐ (10)

Supplements	
	Brand name
☐ (1) Multivitamin	
☐ (2) Antioxidant	
☐ (3) Calcium	
☐ (4) Folic Acid	
☐ (5) B vitamins	
☐ (6) Vitamin C	
☐ (7) Immune boosters	
☐ (8) Probiotics	
☐ (9) Herbal suppl	
☐ (10) Inofolic	
☐ (11) Other	

Section 2

Serum PUFA	
DHA (mmol/l)	
EPA (mmol/l)	

ADDENDUM 6

Patient information form for blood test

Maryke BRONKHORST
live life the healthy weigh

Dear Patient

You have been selected to participate in my research study POLYUNSATURATED FATTY ACID STATUS IN INDIVIDUALS WITH POLY CYSTIC OVARIAN SYNDROME.

You will need to please do the following:

1. **Complete a 3 day food intake record**
   When completed, please email to maryke@dietitiansa.co.za, fax to 011 706 9749, or drop off at my office, 11 Sandton Medpark, Peter Place.

2. **Do a blood test**
   For your blood test, please go to the **Medfem Clinic**, corner Peter Place and Nursery lane. On the first floor, at Dr van Rensurg, Rodrigues, Clark and van Scouwenburg's rooms. Ask one of the nurses (Cornie) to assist and draw a small blood sample.

If you have any queries, please do not hesitate to contact me.

Thank you so much!

Maryke Bronkhorst

0834408143