

Polycystic Ovary Syndrome and Associated Metabolic Features in Indigenous Women in the Northern Territory

By

Jacqueline Boyle
MBBS, FRANZCOG, MPH&TM

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Menzies School of Health Research
Darwin, Australia

and

Faculty of Medicine
University of Adelaide
South Australia, Australia

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Originality Statement

I hereby declare that this submission is my own work and to the best of my knowledge it contains no materials previously published or written by another person, or substantial proportions of material which have been accepted for the award of any other degree or diploma at the University of Adelaide or any other educational institution, except where due acknowledgement is made in the thesis. Any contribution made to the research by others, with whom I have worked at the University of Adelaide or elsewhere, is explicitly acknowledged in the thesis. I also declare that the intellectual content of this thesis is the product of my own work, except to the extent that assistance from others in the project's design and conception or in style, presentation and linguistic expression is acknowledged.

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April 2010

Abstract

Polycystic Ovary Syndrome (PCOS), the most common endocrinological problem in reproductive aged women, has been found in population based studies to be present in 4–8% of women from Caucasian, African American and Sri Lankan backgrounds (Asunción et al. 2000, Diamanti-Kandarakis et al. 1999, Knochenhauer et al. 1998, Kumarapeli et al. 2008). Australian Indigenous women would be anticipated to be more at risk of PCOS due to rising obesity, diabetes and associated components of metabolic syndrome. A small study of Australian Indigenous women in Victoria and Western Australia appears to support this hypothesis reporting a prevalence of PCOS of 18% (Davis et al. 2002).

This study aimed, therefore, to assess the reproductive characteristics, the prevalence of PCOS and the associated burden of diabetes, obesity, dyslipidaemia and hypertension in a group of urban Indigenous women living in Darwin, Northern Territory (NT).

A number of issues in this study warrant further attention: a high proportion of early teenage pregnancy, significant infertility, high testosterone measures and a high proportion with PCOS. Of the 424 women screened, 248 met the study inclusion criteria and of these, 38 (15.3%) had PCOS. The frequency of PCOS increased in those women who were overweight or obese by BMI; in women with a BMI $\geq 30\text{kg/m}^2$ the prevalence was 29.9%. The frequency of PCOS did not change with central obesity probably because it was the typical pattern of fat distribution in this group.

This research highlights the importance of awareness of PCOS in Indigenous women among health providers and policy makers. Whilst the majority of women with metabolic or glucose abnormalities were overweight or obese and ≥ 35 years, a significant minority were younger with normal BMI. Screening therefore should be considered for all women with PCOS for dyslipidaemia and IGT/diabetes.

Potential future research includes exploration of knowledge and attitudes to family planning and reproductive health; optimum ways to provide education and health services to Indigenous women; the identification of young women at risk of future metabolic complications and their prevention, and a comparison of androgens in Indigenous and non-Indigenous women.

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- Fertility Society, Hobart 2007: “Fertility in Indigenous Women”
- RANZCOG ASM, Gold Coast 2007: The Dame Ella Macknight Lecture.

“Polycystic ovary syndrome and associated metabolic complications in urban Indigenous women in Darwin.”

List of Abbreviations

Abbreviation	Definition
AES	Androgen excess society
ACTH	adrenocorticotrophic hormone
ACR	albumin creatinine ratio
AIHW	Australian Institute of Health and Welfare
AMH	anti-mullerian hormone
ART	Assisted reproductive technology
ASRM	American Society of Reproductive Medicine
ATSI	Aboriginal and Torres Strait Islander
BMI	body mass index
BP	blood pressure
CAD	coronary artery disease
CHD	coronary heart disease
CI	confidence interval
CIMT	carotid intima-media thickness
COPD	chronic obstructive pulmonary disease
CRP	C-reactive protein
CVD	cardiovascular disease
DALY	disability adjusted life years
DBP	diastolic blood pressure
DEXA	dual energy x-ray absorptiometry
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulphate
DHT	dihydrotestosterone
DM	diabetes mellitus
DRUID	Diabetes and related disorders in urban Indigenous people in Darwin
ESHRE	European Society of Human Reproduction and Embryology
FT	free testosterone
FAI	free androgen index
FFA	free fatty acid
FSH	follicle stimulating hormone

GDM	gestational diabetes mellitus
GnRH	gonadotrophin releasing hormone
HDL-C	high density lipoprotein cholesterol
HOMA-IR	homeostasis model assessment of insulin resistance
HRT	hormone replacement therapy
hsCRP	highly sensitive C-reactive protein
IFG	impaired fasting glucose
IGF	insulin like growth factor
IGM	impaired glucose metabolism
IGT	impaired glucose tolerance
IHD	ischaemic heart disease
IL-6	interleukin 6
IMT	intima media thickness
IR	insulin resistance
IUGR	intra-uterine growth restriction
LBW	low birth weight
LDL-C	low density lipoprotein cholesterol
LH	luteinising hormone
LVH	left ventricular hypertrophy
MSHR	Menzies School of Health Research
NATSIHS	National Aboriginal and Torres Strait Islander Health Survey
NCAH	non-classic adrenal hyperplasia
NCEP	National Cholesterol Education Panel
NHF	National Heart Foundation
NIH	National Institute of Health
NICHD	National Institutes of Child Health and Development
NHMRC	National Health and Medical Research Council
NT	Northern Territory
OCP	oral contraceptive pill
OGTT	oral glucose tolerance test
PAI-1	plasminogen activator inhibitor-1
PCOS	Polycystic Ovary Syndrome
PCOS-NIH	Polycystic ovary syndrome as defined by the NIH criteria

PCOS-R	Polycystic ovary syndrome as defined by the ESHRE/ASRM Rotterdam consensus
PCO	polycystic ovaries
PID	pelvic inflammatory disease
SES	socioeconomic status
SBP	systolic blood pressure
SHBG	sex hormone binding globulin
STI	sexually transmitted infection
TFR	total fertility rate
TG	triglycerides
TNF α	tumour necrosis factor α
TSH	thyroid stimulating hormone
TVS	transvaginal scan
UKPDS	United Kingdom Prospective Diabetes Study
VAT	visceral adipose tissue
VEGF	vascular endothelial growth factor
WC	waist circumference
WHO	World Health Organisation
WHR	waist–hip ratio
WHtR	waist–height ratio
YLD	years lived with disability
YLL	years of life lost

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Chapter 1 Introduction

1.1 Demography of Aboriginal and Torres Strait Islander people in the NT

The study was conducted with Indigenous women situated in the city of Darwin in the Top End of the Northern Territory (NT) of Australia. The Northern Territory has a population of 198,351 and is the most sparsely populated jurisdiction in Australia at 0.2 persons per square kilometre, compared to the national average of 2.7 people per square kilometre (Australian Bureau of Statistics 2008a). Darwin also has the lowest capital city population density at 926/km² in contrast to areas of Sydney which peak at 8,100 people per square kilometre (Australian Bureau of Statistics 2008a, Australian Bureau of Statistics 2008b, Australian Bureau of Statistics 2008c) The NT has the highest proportion of Indigenous residents to total population of any other state or Territory; just over 30% in 2006 compares to a national proportion of Indigenous residents of 2.5% (Australian Bureau of Statistics & Australian Institute of Health and Welfare 2008) and Darwin has the highest proportion of any city in Australia at 11.1% (Australian Bureau of Statistics 2008b, Australian Bureau of Statistics 2008c). The mean age of non-Indigenous people in the NT at 32.4 years is younger than the national population and that of the Indigenous population is 21.8 years, similar to other figures for Indigenous people in Australia (Australian Bureau of Statistics 2004, Australian Bureau of Statistics & Australian Institute of Health and Welfare 2008). This is due to higher fertility and mortality rates in the Indigenous people and a large transient non-Indigenous population in defence and mining. The majority, 79.3%, of Indigenous people in the NT live in remote or very remote areas (Australian Bureau of Statistics 2008b).

For Indigenous people in Darwin there is high unemployment, poor educational attainments, overcrowding of housing and many other social difficulties that impact on both acute and chronic health.

1.2 Aboriginal and Torres Strait Islander Women's Health

1.2.1 Major causes of morbidity and mortality

Indigenous Australians continue to have higher death rates and lower life expectancies compared to non-Indigenous Australians due to chronic and infectious diseases as well as

injury. Life expectancy for Indigenous women in 1996–2001 was 65.0 years, 17 years less than the national rate (Australian Bureau of Statistics & Australian Institute of Health and Welfare 2008). The five major causes of mortality in Indigenous women in 2003 were ischaemic heart disease (IHD), Type 2 diabetes, stroke, lung cancer and chronic obstructive pulmonary disease (COPD) (Appendix 1). In addition to higher mortality, there is also an increase in years lived with disability (YLD) due to disease or injury. In order to assess the total burden of disease and injury experienced a measure called the DALY¹ is employed (disability-adjusted life years or years of life lost through premature death or disability). Overall, for Indigenous women, many of the top 20 causes of mortality and DALY are the same but there are some differences, such as the prominence of mental health disorders in the DALY. The top five leading causes of DALY in 2003 were anxiety and depression; Type 2 diabetes, IHD, Asthma and COPD (Appendix 1) (Vos T et al. 2007).

The DALY rate ratio² for ischaemic heart disease in Indigenous women compared to all Australian women is 6.6 and for Type 2 diabetes 6.3 whilst the mortality rate ratio is 5.0 for IHD and 18.9 for Type 2 diabetes.

1.2.2 Metabolic syndrome

The metabolic syndrome is a constellation of health risk factors including central obesity (elevated waist circumference (WC) or waist: hip ratio (WHR)), dyslipidaemia (elevated triglycerides and/or low HDL – cholesterol), hypertension and insulin resistance. The primary purpose of diagnosing metabolic syndrome is to identify those individuals at increased risk of cardiovascular disease (CVD) and diabetes which are significant health issues for Indigenous women. Identification of those individuals can potentially lead to modification of risk with lifestyle change and medication. In those with CVD or diabetes already, the number of components of metabolic syndrome present contributes to risk of disease progression (Alberti et al 2009).

There are a number of definitions of metabolic syndrome that are used clinically and in research. It has proven difficult to define in a manner that is acceptable and applicable internationally. The three definitions most commonly referred to are: the National

¹ DALY= YLL(years of life lost) + YLD (years lived with disability)

² Age standardized to the total Indigenous population

Cholesterol Education Program Adult Treatment Panel III (NCEP) (Grundy et al. 2005, Third Report National Cholesterol Education Program (NCEP) Expert Panel on Detection 2002), the World Health Organisation (WHO) (Alberti & Zimmet 1998, WHO consultation 1999) and the International Diabetes Federation (Alberti et al. 2006) (Table 1.1).

The prevalence of metabolic syndrome has been reported from values as low as 8% in adult rural Chinese populations to one quarter of Iranians and one third of adults from diverse backgrounds including India, the USA and the Seychelles (Ford 2005, Kelliny et al 2008, Misra & Khurana 2009, Yan et al. 2005). Prevalence varies depending on the definition of metabolic syndrome used, the population studied and the age surveyed. Metabolic syndrome increases with age and obesity and physical inactivity; in the USA the prevalence was 5%, 22% and 60% in normal, overweight and obese adults respectively and in Iran the prevalence increased from 7.5% in those under 30 years to 45.6% in those greater than 50 years (Ford 2005, Ford & Li 2006, Sharifi et al. 2009).

Higher socioeconomic status is associated with lower risk in developed nations, but increases the risk in developing countries. Higher education appears to be protective in women (Buckland et al. 2008, Chichlowska et al. 2009, Ntandou et al. 2009, Rosero-Bixby & Dow 2009, Scuteri et al. 2008).

Table 1.1 Definitions of the Metabolic Syndrome

	NCEP ATP III 2005	WHO 1998	WHO modified 1999 EGIR†	IDF 2005
	≥ 3 of the following	IFG/IGT/diabetes +/insulin resistance [#] + 2 of the following	Insulin resistance [‡] + 2 of the following (dyslipidaemia can only be one)	Central obesity essential WC≥80 cm ^{**} for Europid women [§] + 2 of the following
Central obesity	WC >88 cm in women	WHR >0.85 in women	WC ≥ 80 cm in women	
Triglycerides mmol/L	≥1.7	≥1.7	>2.0 or treated for dyslipidaemia	≥1.7 or specific treatment for this lipid abnormality
HDL-C mmol/L	≤1.29 in women	<1.0 in women	<1.0 or treated for dyslipidaemia	<1.29 in women or specific treatment for this lipid abnormality
Glucose mmol/L	Fasting glucose ≥ 5.6*		fasting glucose ≥6.1 but non-diabetic	Fasting glucose ≥5.6 or previously diagnosed type 2 diabetes
Blood Pressure mmHg	≥130/≥85	≥160/≥90	≥140/≥90 or treated for hypertension	SBP≥130mmHg or DBP≥85mmHg or treatment of previously diagnosed hypertension
Microalbuminuria		Urine Albumin creatinine ratio (ACR) ≥20mg/g		

glucose uptake in the lowest quartile of the background population under hyperinsulinaemic euglycaemic clamp conditions

‡ hyperinsulinaemia defined as the 25% of the population with the highest fasting insulins

†European Group for the Study of Insulin Resistance (EGIR)

§other WC values ethnic specific

*earlier definition used fasting blood glucose of 6.1 mmol/L

** if BMI>30.0kg/m² can assume WC>80cm

Correlations between definitions vary in different populations screened. Some have shown little difference, for example in Arab Americans metabolic syndrome was found in 23% using NCEP and 28% using WHO (Jaber et al. 2004). Others however have shown marked differences such as in Mexican adults where 14% had metabolic syndrome according to WHO and 27% by NCEP (Aguilar-Salinas et al. 2003).

Within Australia there have been several studies of metabolic syndrome with both Indigenous and non-Indigenous people. Studies not specific to Indigenous people include Ausdiab, a national study that was however predominantly urban, which found a prevalence of metabolic syndrome of 22.1% by NCEP, 21.7% by WHO and 30.7% by the IDF definition and figures were slightly lower for women at 19.9%, 18.2% and 27.2% respectively (Cameron et al 2007). Similar prevalence between definitions was reported in a community survey of non-Indigenous women in South Australia; 14.4% by NCEP and 19.4% by IDF (Adams et al. 2005). Metabolic syndrome was present in nearly one third of rural residents assessed from two shires in South Australia and Victoria and there was consistency between the IDF (30.0%) and NCEP (28.3%) figures (Janus et al. 2007) (Table 1.2).

Metabolic syndrome has been found to be more frequent in Indigenous Australians. A review of data from adult health checks on Central Australian Aboriginals and North Queensland Torres Strait Islanders (Schutte et al. 2005) found metabolic syndrome was present in 28–44 %. In the NT, Maple-Brown assessed metabolic syndrome by NCEP and a modified WHO definition in urban and remote Aboriginal and Torres Strait Islanders and compared them with a group of non-Indigenous urban adults. There was only a small difference of prevalence between the definitions and metabolic syndrome, by either definition, was found to be highest in the remote Indigenous adults (50%), followed by the urban Indigenous adults (30–38%) and lastly the urban non-Indigenous adults (20–22%) (Maple-Brown 2005).

Table 1.2 Prevalence of metabolic syndrome (MS) by NCEP, WHO and IDF definitions in Australians

Author	Population	N	Age (years)	Prevalence by NCEP III	MS ATP	Prevalence MS by WHO	Prevalence by IDF ^{###}
Maple-Brown (2005)	Urban Aboriginal	144	15+	30%		38%*	-
	Remote Aboriginal	119	15+	46%		50%*	-
	Urban non-Indigenous	122	15 +	20%		22%*	-
Schutte et al. (2005)	Central Australian Aboriginal	675	18+	44%		28%	-
	North Queensland – Torres Strait Islanders	369	18+	43.7%		34%	-
Adams et al. (2005) #	South Australia non-Indigenous	2072 (49% of eligible sample [‡])	18+	14.4%		-	19.4%
Cameron et al (2007)	National Australian Survey (Ausdiab)	11,247	25+	19.3%		18.2%	27.2%
Janus et al (2007) #	Rural people from electoral role in two shires from South Australia and Victoria	423 women (42–51% of those eligible)	25–74	28.3% [†]		-	30.1%

*modified WHO criteria , did not use an essential component of insulin resistance

results presented are for women only

† fasting glucose of 5.6 mmol/l compared to the other studies which used the definition of 6.1 mmol/l

of studies that assessed by IDF definition

‡ 49% is for proportion of all participants, male and female for all 8213 eligible adults, no separate figures for proportion of male and female participants

1.2.3 Cardiovascular and metabolic health risk factors

There are many potential reasons for the discrepancy in cardiovascular disease (CVD) and diabetes risk in the Indigenous population. Vos et al. 2007 identified eleven selected risk factors under the broad categories of lifestyle behaviours, physiological states and social and environmental factors that together were responsible for half the discrepancy of disease burden between Indigenous and non-Indigenous people³ (Vos et al. 2007). Tobacco was the leading cause of Indigenous burden of disease in 2003 at 12.1%, closely followed by high body mass at 11.4%. The ten risk factors associated with cardiovascular disease together explained 68.9% of this burden in Indigenous Australians. Tobacco contributed most to this cause, followed closely by high body mass, high blood cholesterol, physical inactivity and high blood pressure and low fruit and vegetable intake. However, this may vary according to location as the Framingham coronary heart disease absolute risk function, incorporating these traditional risk factors of high cholesterol and hypertension, has been shown to underestimate coronary heart disease in Aboriginal people in Western Australia and the Northern Territory (Knuiman & Vu 1997, Wang & Hoy 2005). Other lipid abnormalities are important with a high proportion of Indigenous Australians having low HDL-C and high triglycerides (O'Neal et al. 2008).

Other more novel factors that are associated with and may contribute to excess cardiac disease are also emerging and include the long term effects of low birth weight, high C-reactive protein (CRP) and albuminuria (Leonard et al. 2002, McDonald et al. 1999, Rowley et al. 2003).

For diabetes, strikingly just two factors from the eleven proposed by Vos et al, high body mass and physical inactivity, contributed to 68.8% of Type 2 diabetes in Indigenous Australians.

³ Lifestyle behaviours: tobacco, alcohol, physical inactivity, illicit drugs, low fruit and vegetable consumption and unsafe sex. Physiological states: high BMI, high blood pressure and high cholesterol. Social and Environmental factors: Intimate partner violence and child sexual abuse. Risk factors chosen on basis of evidence of causal link to disease, reliable estimates of exposure and importance to policy making.

1.2.3.1 Diet

Within the risk factors described by Vos et al. above, the largest differences in the contribution of risk factor for disease burden between Indigenous and non-Indigenous Australians were low fruit and vegetable intake, high body mass and high tobacco intake (Vos et al. 2007). The disease most impacted by low fruit and vegetable intake is ischaemic heart disease. This is partly due to the low intake of these substances but also the fact that the replacement foods are often high in saturated fat and refined carbohydrates, which also have a negative effect on risk factors for cardiovascular disease. There are many reasons why diet quality is poor for many Indigenous people, certainly the high cost of fresh fruit and vegetables compared to processed foods rich in saturated fat, sugars and salt plays a significant role in food choices in remote communities (Brimblecombe & O'Dea 2009).

Diet is an important factor influencing all lipids. A diet high in fruit and vegetables decreases low density lipoprotein cholesterol (LDL-C) and total cholesterol in some studies (Mirmiran et al. 2009, Suido et al. 2002). High carbohydrate diets have been shown to decrease HDL-C and increase triglycerides possibly by inducing fatty acid production in the liver and inhibiting the action of lipoprotein lipase (LPL), particularly in the presence of insulin resistance. Therefore lean people without insulin resistance may not respond in the same way as overweight/obese people on the same diet. The type of carbohydrate is probably important; simple sugars (and particularly those containing fructose) have more effect than starch and dietary fibre has a triglyceride lowering effect (Albrink & Ullrich 1986). Hence, sugar-containing soft drinks, juices and snacks are among the most harmful (Merchant et al. 2007, Yang et al. 2003). One particular sugar, fructose, a component of most food and beverage sweeteners, has been shown in high doses to increase hepatic de-novo lipogenesis, increase fasting and postprandial triglycerides, decrease insulin sensitivity and increase visceral adiposity (Stanhope et al. 2009).

High saturated fat diets can decrease insulin sensitivity and increase the risk of diabetes as well as adversely affect lipid profiles, increasing total and LDL - cholesterol (Babio et al. 2009).

1.2.3.2 Physical activity

A lack of physical activity is an independent risk factor for both heart disease and diabetes. Physical activity alone, decreases diastolic blood pressure, triglycerides and fasting blood glucose and benefits can be seen even without an accompanying loss of weight (Shaw et al. 2006). There is little information about the patterns of physical activity in Indigenous Australians. However, the National Aboriginal and Torres Strait Islander Health Survey (NATSIHS) 2004–2005 reported that fewer than half of those surveyed aged ≥ 15 years had played sport or participated in any physical activity in the last 12 months and that the amount of activity decreased with age and was less in women than men (Australian Bureau of Statistics 2005).

Knowledge of the health benefits of physical activity among urban Indigenous adults appears to be well understood and most people would like to be more active than they are. However, a number of barriers to physical activity have been identified. For Indigenous women, as for those from other backgrounds and cultures, domestic work takes up much of their time and having children negatively affects the physical activity of mothers. Cost and safety concerns have also been identified as barriers to activity (Hunt et al. 2008).

In attempting to address the lack of activity, consideration should be given to findings from studies with urban Aboriginal adults which report that family/community activity is more important than individual physical activity and that there is a lack of opportunity to participate in ongoing team activities (Hunt et al. 2008, Thompson et al. 2000).

1.2.3.3 Obesity

For females aged 35–54 years, high body mass was responsible for the largest amount of burden among the 11 risks examined by Vos et al., and Type 2 diabetes and ischaemic heart disease accounted for 89% of the total burden due to high body mass in Indigenous Australians, over half of this burden experienced by females.

Change in lifestyle since colonisation with poor quality diet and decreased physical exercise has contributed to obesity, an established risk in the high rates of premature

diabetes and related complications. Prior to colonisation, the hunter gatherer lifestyle of Indigenous Australians comprised a high quality diet derived from non domesticated animals and uncultivated plant foods. It was nutrient rich and low in fat (particularly saturated fat) and refined carbohydrate. Regular physical activity was built into daily routines (O'Dea 1991). Changes in lifestyle have subsequently occurred with factors such as a move to urban or settled environments, poor socioeconomic circumstances, a lack of recreational opportunities and consumption of affordable store foods rather than bush food. Most store food of high nutrient value such as fresh fruit and vegetables is expensive and food such as flour, sugar and cheap cuts of fatty meat have therefore become dietary staples (Brimblecombe & O'Dea 2009).

The prevalence of obesity ranges from zero in communities with a traditionally orientated lifestyle to well over 50% in the worst affected communities (Rowley et al. 1997). In general, the prevalence of obesity is related to the duration and intensity of exposure to European influences and tends to be more pronounced in women than men.

Aboriginal Australians preferentially deposit fat abdominally when they gain weight. With their traditionally linear body build for any given body mass index (BMI) they have a greater central distribution of body fat than Australians of European background (Piers et al. 2003, Rowley et al. 1997). Thus, even in the “healthy” BMI range for Europeans ($20\text{--}25\text{kg/m}^2$) they can have excess central fat. Central fat conveys a higher health risk with greater insulin resistance and cardiovascular risk than peripheral fat distribution (Després 2006). Remaining lean ($\text{BMI} < 20\text{kg/m}^2$) has been shown, to a large extent, to protect even older Aboriginal people from dyslipidaemia, insulin resistance and diabetes (Rowley et al. 1997). Therefore it may be that due to differences in body build the “healthy” BMI may be lower in Aboriginal people (Daniel et al. 1999, Kondalsamy-Chennakesavan et al. 2008). This is similar to findings in other populations such as South Indians and Asians (Deurenberg et al. 2002, Deurenberg-Yap et al. 2002, Ko et al. 2001).

1.2.3.4 Smoking

Nationally, half of Indigenous adults, males and females, are regular smokers and after adjusting for age differences in the populations this is more than twice that of non-Indigenous Australians in every age group. One in ten of Indigenous adults who are daily smokers began smoking regularly before the age of 13 and two thirds before 18 years. Whilst smoking rates have decreased in the non-Indigenous population they have not changed significantly in Indigenous adults (Australian Bureau of Statistics & Australian Institute of Health and Welfare 2008). There is evidence smoking rates may be even higher, up to 70%, in some remote Northern Territory communities (Australian Bureau of Statistics & Australian Institute of Health and Welfare 2008, Burgess P 2007). Many Indigenous women also continue to smoke in pregnancy (Leeds KL et al. 2007).

Some factors that sustain high smoking rates may be similar to those from other disadvantaged backgrounds; these include such issues as stress, boredom and quitting being a low health priority (Australian Bureau of Statistics & Australian Institute of Health and Welfare 2008, Johnston & Thomas 2008). Other factors identified in two remote Aboriginal communities suggest that the unique social and cultural context of normalisation of smoking and the importance of reciprocity and sharing within Aboriginal families may play a part in such high rates of smoking (Johnston & Thomas 2008). In this context, smoking is important in socialising, aids social cohesion through sharing and can lead to non-smokers feeling excluded, which increases the difficulties of quitting (Brady 1993, Briggs et al. 2003, Johnston & Thomas 2008).

1.2.3.5 Dyslipidaemia

Lipid abnormalities are important risk factors for coronary artery disease and stroke. Increased total cholesterol, decreased HDL-C, elevated triglycerides (TG) and raised small LDL-C particles are all well established as risk factors for atherosclerotic vascular disease (Austin et al. 1988, Berneis & Krauss 2002, Gardner et al. 1996). Aboriginal people have been shown to have a characteristic profile encompassing components of this risk profile (Leonard et al. 2002, McDermott et al. 2000, O'Neal D.N. et al. 2008).

Many epidemiological studies have demonstrated the link between low levels of HDL-C and increased risk of cardiovascular disease. HDL-C is thought to be protective against cardiovascular disease through its antioxidant, anti-inflammatory and anti-thrombotic actions. However, HDL-C particles are heterogeneous in size due to variations in the composition of particle components and it is possible the smaller, denser HDL-C particles may not have the same protective effect and may in fact contribute to the risk of cardiovascular disease (Dodani 2008).

HDL-C has been noted to be low in a number of studies in Aboriginal and Torres Strait Islanders and to be inversely related to triglyceride level, LDL particle size and WHR, as has been reported in other populations (Leonard et al. 2002, McDermott et al. 2000, O'Neal et al. 2008). Further, O'Neal et al. also found for any given level of these factors, the corresponding HDL-C was disproportionately reduced in Indigenous participants compared to non-Indigenous (O'Neal et al. 2008). This did not appear to be related to heavy ethanol consumption and, whilst not measured in this study, may possibly have been related to chronic inflammation as has been demonstrated in other studies (Shemesh et al. 2007). In populations of European origin, women have higher HDL-C than men but Indigenous men and women have similarly low levels of HDL-C (O'Neal et al. 2008). This may be related to the higher insulin levels and insulin resistance in the Indigenous women, secondary to high levels of abdominal obesity.

LDL particles are heterogeneous and whilst total levels of LDL-C may be normal the proportion of smaller, atherogenic particles can be increased. Triglyceride levels are usually inversely related to HDL-C and positively correlated with the number of small LDL particles. LDL particles are comprised predominantly of cholesterol esters packaged with apoprotein B100 and a small component of triglycerides. In the presence of high plasma triglycerides LDL particles can become depleted of cholesterol esters and enriched with triglyceride; this creates a smaller, denser LDL particle (Chan et al. 2004, Genest J et al. 2005, Packard 2003). Excess adiposity and insulin resistance contribute to this lipid profile. They increase the delivery of non-esterified fatty acids to the liver and this combined with other mechanisms lead to a preferential production of small, dense LDL and a decrease in HDL size (Chan & Watts 2004).

Whilst elevated triglycerides are a powerful determinant of a high prevalence of small, dense LDL particles, this may occur at different thresholds for different ethnic groups. In non-diabetic Aboriginal and Torres Strait Islander women from Central Australia and Cape York, compared to non-Indigenous women in Melbourne, there was no difference in triglycerides but the Aboriginal and Torres Strait Islander women had smaller LDL size (O'Neal et al. 2008). Racial differences have also been found with Japanese and Koreans having a significantly higher prevalence of small LDL particles compared to Mongolians, at a triglyceride level less than 1.5 mmol/l (Anuurad et al. 2004). The authors of the study speculated that dietary differences may play a role in LDL-C.

1.2.3.6 C-Reactive Protein (CRP)

Inflammation is recognised as a central feature of atherogenesis and CRP as a marker of inflammation may identify people at risk of cardiovascular disease. CRP is positively associated with age, BMI, smoking, Type 2 diabetes and central obesity. In Aboriginal people CRP is high, indeed the mean CRP in an Indigenous West Australian community was within the range associated with the highest risk of ischaemic heart disease mortality, and has been shown to be related to BMI in remote Aboriginal women (McDonald et al. 2004, Shemesh et al. 2007, Shemesh et al. 2008 Rowley et al. 2003).

Infectious disease increases CRP production and a high prevalence of infectious disease has been reported in Indigenous communities often due to overcrowding and generally poor living conditions (Gillen et al. 2002, Krause et al. 2000, Maguire et al. 1996, McDonald et al. 2004).

Chronic life stress, anxiety and depression, significant problems in Aboriginal and Torres Strait Islander women, may be associated with cardiovascular disease and their effect may be mediated in part through their influence on inflammatory responses (Black 2003). Low socioeconomic status has also been found to be inversely associated with CRP and fibrinogen after adjustment for smoking, waist:hip ratio and concurrent long standing disease (Jousilahti et al. 2003).

Poor quality diet with low fruit and vegetables intake, common in many Aboriginal and Torres Strait Islander communities, may also amplify a pro-inflammatory state (Brimblecombe & O'Dea 2009, Lee et al. 1994). This has been demonstrated with an inverse relationship between CRP and plasma diet-derived antioxidants in a West Australian Aboriginal population (Cohen et al. 2002, Rowley et al. 2003, Rowley et al. 2001).

1.2.3.7 Fetal origins of disease

Adverse environmental factors at critical times of intrauterine development and in early life are associated with programming of the child for increased risks of obesity and other cardiovascular risk factors in adulthood. This may occur through change in structure or function of organs and/or systems (Barker et al. 1993, Barker 1995, Cheung et al. 2000, Ravelli et al. 1999, Roseboom et al. 2000, Taylor & Poston 2007, Yajnik 2000, Yajnik et al. 1995). Initial work in this area proposed that fetal undernutrition was the key factor, but paradoxically overnutrition in utero (secondary to diabetes or impaired glucose tolerance in the mothers) may also contribute to excess risk of adult disease; evidence supports a U shaped curve relationship between birth weight and adult obesity and diabetes (Beall et al. 2004, Curhan et al. 1996, Pettitt & Jovanovic 2001).

Animal models suggest that through a variety of mechanisms both over and under nutrition in embryogenesis through to early childhood may alter significant processes including adipocyte development, nephron number, glucocorticoid signaling, and altered hypothalamic activity which may be important programmers of appetite and adult body weight (Taylor & Poston 2007)

Indian researchers have also noted that Indian babies born with a low birth weight have similar proportions of subcutaneous fat as their higher birth weight counterparts. This pattern has also been observed in Indian adults, who have more body fat at a given BMI relative to Caucasians and African Americans (Yajnik 2004). This has been described as the “thin-fat” body composition and is not dissimilar to the build of Aboriginal Australians as described earlier. They have proposed that these babies are at risk of developing obesity and insulin resistance later in life and those most at risk

are the ones who have a positive energy balance later in life as obesity, particularly central obesity, becomes exaggerated.

1.2.3.8 Social factors

Ischaemic heart disease, metabolic syndrome, obesity and Type 2 diabetes mellitus are all increased in people of low socioeconomic status in developed countries (Kunst & Mackenbach 1994). Part of the association is explained by higher prevalence of smoking, high blood pressure, abnormal serum lipid concentrations and lack of exercise (McFadden et al. 2008, Pekkanen et al. 1995). Risk behaviours such as poor diet, lack of exercise and substance abuse are associated with poverty and may be underpinned by hopelessness, lack of empowerment and internalised racism (Borrell et al. 2007, Choi et al. 2006, Landrine & Klonoff 2000). Internalised racism and associated stress with disruption of the hypothalamic-pituitary-adrenal axis and abnormal cortisol secretion has been proposed to lead to an increased waist circumference, insulin resistance and diabetes (Butler et al. 2002, Tull et al. 1999). Elevations in catecholamines in response to repeated acute and chronic stress, due to any cause, may induce an acute phase response mediating inflammatory and metabolic pathways and contributing to metabolic syndrome, diabetes and atherosclerosis (Black 2003).

There is evidence of intergenerational effects, with women from low socioeconomic backgrounds more likely to have babies with low birth weight who are at higher risk of chronic cardiovascular and other disease as adults (Barker et al. 1993, Kelly et al. 2009).

1.2.4 Reproductive health in Indigenous women

Nationally, there is some data on fertility rates, sexually transmitted infections (STIs) and pregnancy outcomes, but there is a lack of information on infertility, contraceptive practices, or Indigenous women's beliefs about these health issues.

1.2.4.1 Fertility

Indigenous women tend to have more babies and to have them at a younger age. Indigenous women in the Northern Territory have the highest total fertility rate (TFR)

of 2.6 babies per woman. Nationally, more than one fifth of Indigenous births were to women aged less than 20 years compared to 4% of non-Indigenous women (Leeds et al. 2007). The highest fertility rates in Indigenous women are for the 20–24 age group compared with 30–34 years for non-Indigenous women (Australian Bureau of Statistics & Australian Institute of Health and Welfare 2008). Among all women, Indigenous and non-Indigenous, fertility is higher in those who live in rural areas and those who leave school early. Fertility rates in Indigenous women have fallen since the 1960's, with the main difference being a decline in fertility in the late 30's (Kinfu & Taylor 2002). There are a number of possibilities for this: less desire to have children after 30 years when many women become grandmothers, tubal ligation and long acting contraceptive availability, increased exposure to STIs with increasing age, increasing prevalence of obesity with increasing age leading to increased infertility and the effect of polycystic ovary syndrome (PCOS). Further, in 2000 the single year estimate of total fertility rates in Indigenous women was 2.1, a figure very close to the replacement fertility level with the greatest decline in teenage pregnancies. TFR among Indigenous women is lowest in the south and east and metropolitan areas of the country. It is possible that this decline is related to increased economic participation in society as has happened in Australian women generally, but this has yet to be explored (Kinfu & Taylor 2002).

There is little information on infertility in Indigenous women. A study in a remote NT community by Kildea based on a retrospective review of 342 women's health records found that total infertility was 26.3% (Kildea & Bowden 2000). Of this, 8.2% was classified as primary and 18.1% secondary. Infertility was classified as three years of infertility, which is longer than most assessments. An additional 3.3% had resolved (spontaneously or medically). Only 35% of these women had a causal diagnosis; infectious tubal damage in 20% and 15% hormone irregularities or PCOS. However, there were no specified criteria for diagnosing PCOS. After one episode of PID the odds ratio for infertility was 8.5 and the risk of infertility increased with the number of episodes of STI or pelvic inflammatory disease (PID). STIs were possibly underestimated due to inefficient tests at the time and there were no specific protocols for bacterial vaginosis diagnosis.

The Tri-State project, in the late 1990's, examined notes from four health clinics in communities across South Australia, Northern Territory and Western Australia to describe infertility in the region. They found 28% of women had received fertility investigations, 54% for primary infertility and 46% for secondary infertility. Only one male partner was found to have had any investigations. The main causes of infertility noted were PID, PCOS and hypothyroidism. Interestingly they found that the overall diagnosis of PID was uncommon despite increasing rates of gonorrhoea and chlamydia. Some women were noted to have been treated for urinary tract infection and not investigated appropriately for STI (Menon & Coppola 2009).

From the early to mid 1990's there was also an increased number of women in the reproductive age group in the NT but a corresponding decrease in births, generally and in the communities surveyed, reflecting lower total fertility rates. Concurrent contraceptive data was not available and it is possible higher contraception use may have contributed to decreased fertility. However another implication is that infertility may have increased (Menon & Coppola 2009).

Information on STI is limited nationally but there is reliable data from the NT, Western Australia and South Australia. These show the incidence of gonorrhoea, chlamydia and syphilis among Indigenous people to be up to 100 times higher than the general population (Skov & Murray 2003). In the Top End of the NT, where 10–25% of women were screened in remote communities: 11% had Chlamydia, 25% had trichomonas and 17% had gonorrhoea (Bowden et al. 1999). Not surprisingly pelvic inflammatory disease was also common: 14% of all admission of Indigenous women to Royal Darwin Hospital in the NT were for PID compared with 2% of non-Indigenous women (Mein & Bowden 1997). Studies of populations with similar rates of STIs have attributed as much as 60% of infertility to STIs (Cates et al. 1985).

Many health providers working in Indigenous women's health suggest anecdotally that PCOS seems to be a significant problem in this group and a pilot study from Davis et al. who screened 38 Indigenous women in the Kimberley and Victoria would seem to support this (Davis et al. 2002). The study only assessed a small number of women and there was probable selection bias, but they found an 18% prevalence of PCOS and 39% prevalence of hirsutism in their study participants. Certainly as insulin

resistance and obesity continue to increase in Indigenous women it is anticipated that chronic disease including PCOS will increase and the associated metabolic complications will be exacerbated as it has in other populations (Pasquali et al. 2007a).

Obesity, even without PCOS, has also been shown to increase cycle irregularity and amenorrhoea and to decrease fecundity even in regularly cycling women (Gesink Law et al. 2007, Hartz et al. 1979, Ramlau-Hansen et al. 2007). It decreases ovarian response to clomiphene citrate and gonadotrophins in assisted reproductive technology (ART) and is associated with a decreased live birth rate in both spontaneous and assisted conceptions (Balen et al. 2006, Fedorcsák et al. 2004, Wang et al. 2002).

Smoking has been associated with variation in menstrual cycle patterns, with heavy smokers having shorter and more variable menstrual cycles with the main effect being a shorter follicular phase (Liu et al. 2004, Windham et al. 1999, Windham et al. 2005). Menopause has also been reported to occur earlier in smokers and is an important factor in complications of pregnancy such as antepartum haemorrhage and low birthweight. Smoking, as already discussed, is about 50% higher in Indigenous people and many Indigenous women smoke in pregnancy (Leeds KL et al. 2007). It is therefore an extremely important health behaviour that needs to be addressed in order to improve reproductive, maternal, child and general adult health.

1.2.4.2 Pregnancy

Pregnancy is another time of life where health discrepancies are seen between Indigenous and non-Indigenous women. Maternal mortality is three times that of non-Indigenous women, and neonatal mortality is also higher, as are premature delivery, low birth weight, anaemia, urinary tract infections and STI's in pregnancy (de Costa & Child 1996, Hunt 2004, Laws & Hilder 2009, Leeds et al. 2007, Panaretto et al. 2002, Panaretto et al. 2006).

In the NT in 2004, 60% of first time Indigenous mothers were under 20 years compared with 11% of non-Indigenous. The perinatal mortality (27.8/1000) and low birth weight (10%) were twice as high for Indigenous babies (Zhang & Johnstone

2009). There are a number of reasons for this: increased smoking in pregnancy, prematurity, diabetes, late presentation to antenatal care and few visits, often due to a lack of appropriate culturally secure care models and suitable care providers (Hancock 2006, Hunt 2004, Zhang & Johnstone 2009).

Diabetes in pregnancy is likely to be higher in Indigenous women, particularly in rural and remote areas, due to high rates of obesity, increasingly high rates of early onset Type 2 diabetes during the child bearing years, and presence of PCOS (Davis et al. 2009, Graham et al. 2007). Diabetes in pregnancy can cause problems both antenatally and at delivery for mother and baby. Complications may include: macrosomia or conversely intra-uterine growth retardation (IUGR), increased rate of fetal anomalies, increased risk of induction of labour and complicated delivery. There is also evidence that diabetes in pregnancy can increase the risk of early onset obesity and diabetes in the adult offspring (Dabelea et al. 2000, Fetita et al. 2006).

Adequate maternal care and understanding of pregnancy in Indigenous women is therefore very important as a child born prematurely and/or with low birth weight to a mother with suboptimal health has a disadvantaged start to life and the effects may persist well into adulthood when considering the risk of chronic disease.

1.2.4.3 Contraception

Little is known about contraceptive use in Indigenous women as most data does not separate Indigenous and non-Indigenous status. It is known that remote women tend to have more tubal ligations and use less condoms (Richters et al. 2003). Anecdotally, tubal ligations would seem to be more common in remote Indigenous women in the NT. Remote Indigenous women report higher use of long acting injectable progestagens (DepoProvera) or implantable progestagens (Implanon) compared to urban women (Australian Bureau of Statistics 2006). This may be due to factors such as lack of easy access to a doctor or pharmacist for scripts for the oral contraceptive pill, difficulties with storage of pills and difficulty remembering pills as many women tend to move location both within and between communities. Factors affecting contraceptive use are not understood.

1.3 Polycystic ovary syndrome (PCOS)

PCOS was first popularised by Stein and Leventhal in 1935. They described a syndrome of abnormal, enlarged and sclerocystic ovaries; menstrual irregularity; sterility; masculine type hirsutism and, less consistently, retarded breast development and obesity.

Since then interest in this syndrome has grown. There has been debate about the diagnosis and subsequent prevalence and associated features. It is agreed however that PCOS is the most common endocrine problem in women of reproductive years affecting between 4–8% of reproductive age women in western societies (Asunción et al. 2000, Diamanti-Kandarakis et al. 1999, Knochenhauer et al. 1998).

PCOS is generally accepted to have three key components including: oligomenorrhoea, polycystic ovaries (PCO) on ultrasound and evidence of hyperandrogenism (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004, The Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group 2004). There is probably a spectrum of disease seen in women with a propensity to PCOS. This ranges from the classic triad described of PCO, hyperandrogenism and oligomenorrhoea, with infertility at one extreme through to regularly cycling women with PCO only.

The aetiology of PCOS is complex with a heritable component, probably due to a number of genes, interacting with multiple environmental factors. There is evidence that PCOS runs in families, suggesting genetic susceptibility (Kahsar-Miller et al. 2001, Legro et al. 1998). Despite extensive research in this area no predominant genes have been identified as yet as key candidates.

Other factors predisposing to increased risk of PCOS may be low birth weight and perhaps antenatal androgen exposure (Abbott et al. 2002, Abbott et al 2005). For asymptomatic women, with a predisposition to PCOS, environmental factors such as excessive energy intake and low physical activity leading to obesity trigger the development of the set of symptoms associated with typical PCOS (Laitinen et al. 2003, Legro et al. 2002).

In many young women, PCOS becomes evident shortly after menarche. It has been suggested that premature adrenarche⁴ is the earliest recognizable PCOS phenotype. Affected girls display hyperinsulinaemia and elevated dehydroepiandrosterone sulphate (DHEAS) levels, many have an elevated BMI and after menarche they become oligomenorrhoeic (Ibanez et al. 1993, Ibanez et al. 1999a, Oppenheimer et al. 1995).

Apart from the immediate problems of irregular menses, acne, alopecia, hirsutism and infertility there are significant longer term health issues associated with PCOS including: hyperinsulinaemia and insulin resistance, impaired glucose tolerance (IGT), diabetes, dyslipidaemia (↓ HDL, ↑ small, dense LDL, ↓triglycerides), sleep apnoea and possibly endometrial cancer and cardiovascular disease (Azziz et al. 2008, Salley et al. 2007).

There is a tendency to obesity in PCOS with up to 50% of women being obese (Azziz et al. 2008). Irrespective of obesity, women with PCOS tend to have central fat deposition (Dunaif et al. 1987, Kirchengast & Huber 2001). Obesity may be involved through its association with hyperinsulinaemia and its attendant consequences and increased peripheral oestrogen production. Given the rising prevalence of obesity in many parts of the world there is concern for women's reproductive health in the future. In particular, the epidemic of metabolic syndrome and diabetes that is occurring particularly in populations making a rapid change from traditional to more Westernised lifestyles and associated with rapidly changing body shapes leads to particular concerns for these women (Zimmet et al. 2001). Such groups include women from the Indian subcontinent, Pacific islands, and Mauritius and Indigenous populations in Australia, New Zealand, Canada and the USA.

1.4 PCOS definition

Whilst there is general agreement about the components of PCOS, there are diagnostic differences between studies, which make direct comparisons between studies of prevalence and associated features of PCOS difficult. It is therefore important to discuss this issue in some detail. Following is a description of the individual

⁴ The development of pubic hair less than 8 years of age

components of the diagnostic criteria for PCOS, and the combinations of these used in various studies to diagnose PCOS.

1.4.1 Diagnostic criteria for PCOS

The diagnosis has changed over time. In its early days following its original description by Stein and Leventhal, diagnosis was largely clinical, with oligomenorrhoea and excessive body hair. Whilst a clinical assessment of hirsutism and a history of oligomenorrhoea remain important aspects in the diagnosis of PCOS, over time urinary and more recently, serum measurements of various hormones and ultrasound assessment of ovarian follicles have come to be vital in establishing a diagnosis.

1.4.1.1 Gonadotropins

Women with PCOS tend to have higher mean luteinizing hormone (LH) concentrations but normal or low levels of follicle stimulating hormone (FSH), with a corresponding increase in the LH:FSH ratio on serum testing in 50–70% of women (Conway et al. 1989, Rebar et al. 1976, Taylor et al. 1997). The increased production of LH is due to both increased pulse frequency and amplitude (Rebar et al. 1976, Venturoli et al. 1988, Waldstreicher et al. 1988).

However, a lack of sensitivity and specificity precludes the use of LH as a diagnostic tool (Polson et al. 1988). LH can be raised in normal women and it can also be normal in obese women with PCOS. Obesity, as already noted, is present in up to 50% of women with PCOS in developed countries such as the USA and this is likely to increase with increasing global obesity (Gambineri A et al. 2002). Pulse frequency of LH is increased in obesity but pulse amplitude is inversely proportional to body mass index and therefore is lower (Arroyo et al. 1997, Taylor et al. 1997).

1.4.1.2 Ultrasound

Historically, polycystic ovaries (PCO) were recognised at surgery and histologically but with the ascendancy of ultrasound, ovarian morphology has become a clinically useful diagnostic measure.

Several features on ultrasound have been used to define polycystic ovaries. These include multiple small cysts/follicles, increased ovarian stroma or increased ovarian volume.

“Cysts”/Follicles⁵

Whilst there is general consensus that ovarian cysts seen for a diagnosis of polycystic ovaries should be between 2–9 mm in size, there has been debate about the number of these cysts that are required for a diagnosis of PCO as well as their placement. Some use peripheral placement of cysts giving the classical “pearl necklace” appearance while others accept cysts scattered throughout the ovary. The most referenced study, by Adams et al. in 1985, defined ≥ 10 cysts 2–8mm in diameter arranged either peripherally around a dense core of stroma or scattered throughout an increased amount of stroma or both (Adams et al. 1985). Many subsequent studies used a modified Adams criteria for the definition of polycystic ovaries seen on ultrasound (Balen et al. 1995, Clayton et al. 1992, Farquhar et al. 1994) Recommendations have subsequently varied but the European Society of human reproduction and embryology/American Society of reproductive medicine (ESHRE/ASRM) consensus statement 2004 proposed that ≥ 12 follicles of 2–9mm in any part of the ovary and/or increased ovarian volume would constitute a diagnosis of PCO (The Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group 2004).

It is contentious as to whether one or both ovaries are required to have a polycystic appearance or whether one is sufficient. Bilaterality has traditionally been considered essential for diagnosis yet left and right ovaries have been shown to have different patterns in the same women (Atiomo et al. 2000, Battaglia et al. 1999).

Increased Ovarian Stroma

The finding of increased stroma has largely been based on increased stromal brightness. This is quite subjective relying on a comparison with uterine brightness and is further affected by such factors as operator interpretation, machine settings and structures such as bowel nearby. Computer assisted technology and pixilation have

⁵ Whilst the term “cysts” is commonly used, and will be used in this thesis, to describe the characteristic appearance of the polycystic ovary, they are more accurately follicles.

been employed to try and improve the reproducibility of these findings but still need to be validated (Khalid 2004).

The ESHRE/ASRM guidelines do not include ovarian stroma or echogenicity as it is not specific to polycystic ovaries. Increased ovarian volume relates to increased stroma.

Additionally, in obese women, an accurate assessment requires a vaginal probe to be used rather than an abdominal one. There have not been any defined criteria for transvaginal scans (TVS).

Further complicating the use of ultrasound in diagnosis of PCOS is that the finding of polycystic ovaries on ultrasound is not specific to PCOS. Up to 30% of normal cycling women with normal androgens have polycystic ovaries on ultrasound and women who are on the oral contraceptive pill or have other conditions such as hypothalamic amenorrhoea and hyperprolactinaemia can have PCO on ultrasound (Ardaens et al. 1991, Futterweit et al. 1988, Polson et al. 1988).

In summary, ultrasound findings consistent with PCO have been found in many normal women. There are difficulties distinguishing between multifollicular ovaries, PCO and other anovulatory states. There are also technical issues including reproducibility, interobserver variability and lack of validation of transvaginal scanning which is the most accurate means of assessment in obese women but is considered by some women to be quite invasive (Amer et al. 2002, Lujan et al. 2009). It is important to be aware that PCO alone is not consistent with the endocrine syndrome of PCOS.

1.4.1.3 Hyperandrogenism

In women, androgens play a role in both normal and abnormal physiology. Androgens are essential in the normal menstrual cycle and ovulation and play a role in bone metabolism and possibly cognition and libido. Low androgens can be related to lower bone density and possibly low libido (Davison & Davis 2003). An increase in androgens and associated decrease in sex hormone binding globulin (SHBG) are linked to anovulation and PCOS in pre-menopausal women and an increase in

diabetes and cardiovascular risk factors in both pre and post-menopausal women (Ding et al. 2006, Sutton-Tyrrell et al. 2005). These risk factors include central obesity, increased triglycerides and decreased HDL-C levels. These effects have been reported in women from diverse ethnic groups (Sutton-Tyrrell et al. 2005).

Hyperandrogenism is used as a diagnostic criterion in PCOS. Some definitions use clinical assessments of hyperandrogenism, others use biochemical assessment, and some both.

1.4.1.3.1 Clinical criteria

The hyperandrogenism seen in PCOS may manifest clinically as hirsutism, male pattern balding or acne. Hirsutism is defined as the presence of terminal hairs (coarse, pigmented hair >1cm length) in a male like pattern. When assessing hirsutism most clinicians would use one of several modifications of the Ferriman Galwey (FG) assessment of hirsutism (Ferriman & Gallwey 1961). This measure initially included eleven body areas but modifications subsequently excluded the lower leg and forearm as these did not show significant correlation with hair in the other nine areas assessed (Hatch et al. 1981). Differences arise between what is deemed the cut off for clinical hirsutism. Typically, this is a score of ≥ 6 or ≥ 8 .

There is a degree of subjectivity to this assessment and adding to the complexity is that many women with significant hirsutism would currently or previously have used treatment for their hirsutism such as creams, shaving, waxing, electrolysis or medication. Single site assessment of central hirsutism of the chin or lower abdomen have been used and are sensitive but have poor specificity in larger populations (Knochenhauer et al. 2000). They have an advantage in that they are less personally invasive. The best discrimination between a control population and a hirsute population has been found using the sum of the scores for four regions: upper lip, chin, abdomen and thighs with good inter-observer agreement. However, this has not been used in many PCOS studies (Derksen et al. 1993).

Self-reported hirsutism and oligomenorrhoea has also been found to be a good predictor of PCOS in mothers and sisters of women with PCOS, with sensitivities of 100% and 79% and specificities of 93% and 97% respectively (Kahsar-Miller &

Azziz 2003). This study was performed with women in the USA; the majority were non-Hispanic, white women and just under a third were African American. The results may not be applicable to all populations and may be different if applied to women without family members affected by PCOS.

The reported presence of unwanted hair has been found to be a good predictor of PCOS. Studies of large groups of women with hirsutism seeking medical care found 50–80% had PCOS (Azziz et al. 2004a, Carmina 1998, Glinborg et al. 2004, Unluhizarci et al. 2004). Even in women without objective evidence of hirsutism self-reported hirsutism can be significant. In 228 women without clinical hirsutism (FG score <5) who reported minimal unwanted hair growth, 54% had evidence of an androgen excess disorder and of these, 50% were due to PCOS (Souter et al. 2004). The data available suggests the presence of acne and alopecia are not as predictive of androgen excess. However hirsutism is frequently accompanied by normal plasma androgen levels (Azziz et al. 2004a, Carmina et al. 2006b, Moran et al. 1994). Idiopathic hirsutism is more common in some populations and conversely hirsutism is not always present in women with biochemical androgen excess. There are ethnic and individual differences in target tissue sensitivity to circulating androgens and intracellular androgens, such as the response of 5 α reductase. Low 5 α reductase activity, the pattern common in Asian populations, despite being associated with marked androgen excess may not be associated with hirsutism (Carmina 1992).

1.4.1.3.2 Biochemical Hyperandrogenism

There are a number of androgens produced endogenously. In decreasing order of potency they are: dihydrotestosterone (DHT), testosterone, androstenedione, dehydroepiandrosterone (DHEA) and DHEAS. The adrenal gland is responsible for the almost all of DHEA and DHEAS and 50% of androstenedione (Azziz et al. 2006). The ovary is responsible for 50% androstenedione and 75% of circulating testosterone. DHT circulates in negligible quantities and results primarily from 5 α reductase conversion of testosterone.

There are a number of factors that contribute to difficulties in assigning normal androgen values in women: levels change with age, with hyperandrogenaemia normalizing as a woman approaches her 40's ; there may be ethnic differences; there

is a wide range of normal; different assays are used in different laboratories and there is interassay variability.

Measurements of androgen excess have included androstenedione, DHEAS and testosterone. Some studies use a cut-off level of what are deemed “normal” values, while others use the 95th centile in a given population, recognising the difficulties in assigning a normal cut-off point that is applicable to all women. Clinically, laboratories supply normal ranges for androgens. In research, normal values may be taken from clinical laboratories; alternatively androgens from a general population of women can be used to establish normal values. An assignment of normal from a population may include using the 95th centile as the upper limit of normal. In a general population where the diagnosis of PCOS may be as high as 10% this is already more than a cut-off point at the 95th centile would allow. An alternative is to use the 95th centile of androgens from women who do not have any overt clinical hyperandrogenism or menstrual dysfunction. Determining the best androgen to use for the assessment of hyperandrogenaemia in assessing health risk factors and for diagnosing PCOS has been the subject of debate for many years.

DHEAS is almost exclusively produced by the adrenal gland so is not ideal for assessing hyperandrogenism due to PCOS, even though some 20–30% of women with PCOS will have supranormal values of DHEAS. There are few studies assessing the suitability of androstenedione in diagnosis of PCOS.

Testosterone is the androgen of most importance in women’s health. Circulating total testosterone has been shown to be the best hormonal correlate of polycystic ovaries and hyperandrogenic anovulation (Azziz et al. 2008). Many prefer the free testosterone or free androgen index as it reflects the amount of bioavailable hormone and accounts for the suppressive effect of insulin and obesity on SHBG.

This can be measured in a number of ways – the most reliable being equilibrium dialysis or liquid chromatography-mass spectrometry. At present, these are not practical for clinical use. Other methods for assessing free testosterone include: the free androgen index (FAI: Total testosterone x100/SHBG), direct radioimmuno assay

(RIA – which has been found unreliable and is not validated for use in women), and equations based on the law of mass action (the Vermeulen equation) giving a free testosterone result. Apart from the direct RIA these all depend on accurate measurements of SHBG and total testosterone. Free testosterone or the FAI, have been shown to be the most reliable and clinically useful substitutes for mass spectrometry and equilibrium dialysis as measures of PCOS (Azziz et al. 2008, Hahn et al. 2007, Miller et al. 2004).

1.4.1.3.3 Factors affecting androgens in women

A number of factors affect androgen production and metabolism including age, obesity, smoking, alcohol intake, and potentially stress, birth weight and ethnicity. These will be discussed briefly as they become important in populations with an excess of risk for high androgens and in the determination of normal values for androgens in women.

Androgens decline with age, whilst age has been found to have varying effects on SHBG (Davison et al. 2005, Pollard et al. 2006, Winters et al. 2000).

Androgen production and metabolism in women is altered with obesity resulting in higher serum levels with increasing BMI and waist circumference (Sowers et al. 2001). Reduction in SHBG is associated with an increased clearance of the SHBG-bound steroids: testosterone, dihydrotestosterone and androstenediol. However this increased clearance is compensated for by an increase in production rates. Obesity also leads to an increase in production and clearance rates of the non-SHBG-bound steroids, androstenedione and DHEA (Kurtz et al. 1987, von Schoultz & Carlström 1989). Central obesity may result in even more severe abnormalities as there is a greater decline in SHBG and higher testosterone production rates compared to women with peripheral obesity (Pasquali et al 1990, Tchernof & Després 2000, Kirschner et al 1990).

Lifestyle factors such as smoking, alcohol consumption and physical activity have been assessed for their effect on androgens. Daily alcohol consumption has been reported to increase both total and free testosterone in pre-menopausal women who are light-moderate drinkers and has been reported to have variable effects in

postmenopausal women (Cigolini et al. 1996, Gavalier & Van Thiel 1992, Sierksma et al. 2004, Sowers et al. 2001). It is possible that the effects of alcohol may vary with age and amount of alcohol ingested.

Smoking has also been reported in some, though not all, studies to increase androgens (Barbieri & Gargiulo 2004, Pollard et al. 2006, Randolph et al. 2003, Sowers et al. 2001).

The reported effect of physical activity on female androgen levels varies and there appears to be a number of confounding effects: the age of the woman, whether the exercise is acute; the degree of exertion; whether it is aerobic or strength training and whether the woman normally is trained or sedentary (Enea et al. 2008).

Stress potentially can affect testosterone levels in females, though results are variable. A study assessing women in a Health Maintenance Organisation found that there was a positive linear relationship between stress and testosterone (King et al. 2005). However, in female students the effect of stress on testosterone varied (Kunstmann & Christiansen 2004).

Some studies indicate there may be ethnic differences in androgens and SHBG in women. Little is known about Indigenous women; however other ethnic groups have been studied. Chinese American women between the ages of 42 and 52 years have been reported to have lower SHBG and higher FAI than white women of the same age after adjustment for BMI (Sutton-Tyrrell et al. 2005). Similarly, South Asian women living in the UK have been found to have higher free testosterone and lower SHBG than European women (Reed et al. 1993). In contrast, African American women have been found to have lower total and free testosterone and, in most studies, also higher SHBG than white women after adjusting for age and BMI (Lamon-Fava et al 2005, Randolph et al. 2003, Spencer et al. 2007, Sutton-Tyrrell et al. 2005). There may be a genetic basis to ethnic differences in androgens but lifestyle and intrauterine and early life factors have also been postulated to play a role in altering hormone production (Pollard et al. 2006).

1.4.1.4 Oligomenorrhoea/Anovulation

There is little consensus on the definition of anovulation/oligomenorrhoea. Some studies have relied on biochemical evidence of anovulation, whilst others assume that if a woman has irregular cycles she is most likely anovulatory. The definition of 'irregular' can vary between: cycles greater than 35 days, less than 6–8 cycles per year, cycles less than 24 days and some simply use cycle irregularity. Overt menstrual dysfunction with oligo-amenorrhoea has been found in 60–85% of women with PCOS. The remainder may have eumenorrhoea and less frequently polymenorrhoea (Azziz et al. 2004a, Conway et al. 1989). A prospective study of 400 unselected women presenting for an employment physical assessment found that of the women diagnosed with PCOS (NIH criteria) 40% had regular cycles but experienced oligoanovulation (Azziz et al. 2004b). Therefore the best clinical means of assessment in someone who is not oligomenorrhoeic is to perform consecutive luteal phase progesterones to confirm anovulation. However, this is not practical in epidemiological studies. Furthermore, the menstrual dysfunction of PCOS ameliorates with age, possibly because of the natural decline in androgens (Winters et al. 2000).

1.4.1.5 AMH

Anti-Mullerian hormone (AMH) plays a role in folliculogenesis in females. It is present in very small amounts from postnatal life until puberty. During puberty levels rise and then have a progressive decline during the reproductive years becoming undetectable at menopause (de Vet et al. 2002, La Marca et al. 2005, Lee et al. 1996, Tremellen et al. 2005).

AMH can be measured in serum though the levels are much lower than measurements in an individual follicular fluid. Serum levels of AMH appear to have minimal fluctuation throughout the menstrual cycle (Hehenkamp et al. 2006, La Marca et al. 2006). Ultrasound has shown the number of small antral follicles correlate closely with serum AMH levels (Wang et al. 2007). It is therefore not surprising that serum AMH is increased in women with polycystic ovaries and PCOS (Pellatt et al. 2007, Pigny et al. 2003, Pigny et al. 2006, Wachs et al. 2007, Wang et al. 2007). The magnitude of the increase in AMH with PCO in age-matched women may correlate with insulin resistance, serum androstenedione, testosterone and pre-antral follicle

numbers (Wang et al. 2007). BMI is not significantly related to AMH levels in women with PCOS. The oral contraceptive pill decreases the number of follicles but does not consistently change AMH in controls or women with PCOS (Somunkiran et al. 2007).

1.4.2 Definitions of the Polycystic Ovary Syndrome

There are four main definitions of PCOS used in recent times, which involve one or more of the following key features:

- Polycystic ovaries on ultrasound
- Hyperandrogenism (biochemical and/or clinical)
- Anovulation

There continues to be controversy about the definition as some clinicians and researchers believe hyperandrogenism is the most important component and others who believe it is important to treat it as a condition with a spectrum of severity and therefore include ultrasound in the criteria.

1.4.2.1 The European Model

The European literature has traditionally diagnosed PCOS as polycystic ovaries plus one of hyperandrogenism or anovulation, thereby emphasising the role of polycystic ovaries in the syndrome. Whilst polycystic ovaries are common in women with PCOS according to the NIH criteria, up to 30% of normal cycling women with normal androgens have polycystic ovaries on ultrasound (Polson et al. 1988). Women who are on the oral contraceptive pill and women with other conditions such as hypothalamic amenorrhoea and hyperprolactinaemia can also have PCO on ultrasound (Ardaens et al. 1991, Futterweit et al. 1988).

Certainly there does not seem to be significant clinical implications for women with polycystic ovaries alone, except when the ovaries are hyperstimulated. Studies report that women with polycystic ovaries and regular cycles do not seem to have a reduction in fertility (Clayton et al. 1992, Hassan & Killick 2003). Studies vary on whether androgens are higher or similar in asymptomatic women with PCO compared

with women with normal ovaries but they remain within normal ranges (Adams et al. 2004, Clayton et al. 1992, Koivunen et al. 1999).

1.4.2.2 The United States model: NIH-NICHD⁶

The National Institutes of Health – National Institute of Child Health and Human Development (NIH-NIHCD) conference in 1990 identified PCOS as hyperandrogenic anovulation and this is the definition used predominantly by clinicians and researchers in the USA and South America (Zawadzki JK, 1992). The definition requires oligomenorrhoea and clinical and/or biochemical hyperandrogenaemia. The consensus was that PCO which can be seen across a spectrum of normal, PCOS and other conditions is not essential for diagnosis. This definition is the one that has been used in most of the prevalence studies. It has the advantage of being much easier to assess in large numbers of women because ultrasound equipment, ultrasound expertise and additional private space are not required. In addition, in obese women it can be difficult to visualize the ovaries transabdominally, and transvaginal scanning is more invasive and therefore less likely to be performed.

1.4.2.3 ESHRE/ASRM Rotterdam consensus⁷

At the ESHRE/ASRM PCOS symposium in the Netherlands 2004, many leading experts in PCOS produced a consensus statement for the diagnosis of PCOS to try and reconcile the differences across the world. The consensus diagnostic criteria are that a woman must have two of the following features for a diagnosis of PCOS: oligomenorrhoea, PCO appearance of the ovaries on ultrasound and hyperandrogenism (The Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group 2004). This requires caution because of conditions such as hypothalamic amenorrhoea. It does however mark a change from the absolute importance of PCO alone in diagnosis.

⁶ Will be referred to as NIH for this thesis

⁷ This will be referred to as the Rotterdam definition through this thesis

1.4.2.4 Androgen Excess Society and PCOS Society (AES-PCOS) definition

The Androgen Excess and PCOS societies determined that PCOS is above all a hyperandrogenic disorder and that this is an essential component of the diagnosis. In addition to this there need to be evidence of ovarian dysfunction which can be oligo-anovulation and/or polycystic ovaries. Similarly to the other diagnostic criteria, they also recommended the exclusion of other androgen excess or related disorders (Azziz et al. 2008).

1.4.2.5 Other

Many researchers have used all three criteria of anovulation, hyperandrogenism and polycystic ovaries on ultrasound in studying women with PCOS .This probably means they are studying women at the severe end of the spectrum of disease.

Some studies have also used a raised LH: FSH ratio but this is not commonly used now due to the issues outlined in 1.4.1.1.

All of these diagnostic criteria rely on the exclusion of other factors that may have contributed to a similar clinical picture. These include: congenital adrenal hyperplasia, Cushing's syndrome, hyperprolactinaemia, thyroid disorders, acromegaly, premature ovarian failure, obesity, virilising adrenal or ovarian neoplasm or drug related conditions (eg. anabolic androgenic steroids such as Danazol for endometriosis and some anti-epileptic drugs such as valproic acid).

As already noted, PCOS has a large spectrum of presentations and all these criteria may be valid representations of different parts of this spectrum. Some consistency is required however to enable valid comparisons between studies and to assess associated metabolic risks in PCOS. It is also important to have clear diagnostic criteria for clinicians.

1.5 Prevalence of PCOS

This discussion will include a review of the prevalence of PCOS and of polycystic ovaries only on either ultrasound or histology.

1.5.1 PCOS and Prevalence

The prevalence of PCOS is generally reported at between 4–8 % regardless of ethnicity. This is based largely on three large unselected prospective studies in Spain, the USA and the Greek island of Lesbos, which utilised the NIH/NICHD criteria for diagnosis of polycystic ovary syndrome. The majority of women assessed in these studies were Caucasian, apart from the study in the USA which included African American women (AA). There are some smaller studies in women of Australian Aboriginal and Mexican American ethnicity that are suggestive of a higher prevalence (Tables 1.3–1.7).

Table 1.3 Prevalence of PCOS in Southern Europe

Authors	Diamanti – Kandarakis et al. 1999	Asuncion et al. 2000
Diagnostic Criterion	NIH/NIHCD*	NIH/NIHCD
Study Population	Respondents to ads for free medical check Lesbos, Greece	Consecutive blood donors Spain
Participant Numbers	N=192	N=154
Age	17–45 years	18–45 years
Exclusion Criteria	Menopausal, medication affecting hormonal or metabolic studies	Menopausal symptoms Hormonal Contraception
Ethnicity	All Greek	All Spanish Caucasian
Participation	Unknown	94%
Prevalence	6.78% (10.42% #)	6.5%

* Oligomenorrhoea and hyperandrogenaemia, not including hirsutism

If include women with hirsutism and oligomenorrhoea

Table 1.4 Prevalence of PCOS in the UK and Finland

Authors	Michelmore et al 1999	Taponen et al. 2003
Diagnostic Criterion	NIH/NIHCD	NIH/NIHCD*
Study Population	Respondents to ads at two universities or invitations from GP lists, UK.	Women from a population based bith cohort of 1966
Participant Numbers	N=230	N=2007
Age	18–25 years	All 31 years
Exclusion Criteria	None reported	Pregnancy, hormonal contraceptive use
Ethnicity	Mostly Caucasian (97%)	Northern Finland
Participation	16% and 11% from general practices. Universities unknown	67.1% of those eligible and still living in Northern Finland
Prevalence	7.4%	10.3% [#]

* Self-reported symptoms of oligomenorrhoea and hirsutism

[#]70.0% of 185 women assessed further with ultrasound had polycystic ovaries

Table 1.5 Prevalence of PCOS in the USA

Authors	Knochenauer et al. 1998	Azziz et al 2004	Goodarzi et al 2005
Diagnostic Criterion	NIH/NIHCD	NIH/NIHCD	NIH/NIHCD*
Study Population	University pre-employment medical, USA	University pre-employment medical, USA	Participants in screening for IR as one parent had CVD, USA
Participant Numbers	N=369	N=608	N=156
Age	18–45 yrs	18–45 yrs	34±8.6
Exclusion Criteria	None reported	Pregnancy, menopause, previous hysterectomy, bilateral oophorectomy	None reported
Ethnicity	African American (n=148) White (n=221)	African American (n=223) White (n=166)	Mexican American
Participation	75.1% of those approached	66% of those approached and eligible	100%
Prevalence	In all women= 4.0% AA = 3.4% White = 4.7%	In all women= 6.6% AA = 8.0% White= 4.8%	13%

* Self-reported symptoms of oligomenorrhoea and hirsutism

Table 1.6 Prevalence of PCOS in Australia

Authors	Davis et al. 2002	March et al. 2009
Diagnostic Criterion	NIH/NIHCD	NIH/NIHCD Rotterdam AES
Study Population	Respondents from two communities (WA and Victoria) to offer of women's health check.	Retrospective birth cohort from Adelaide (QEH)* 1973–1975 traced around 30 years of age
Participant Numbers	N=38	N=728
Age	18+ years	27–34 years
Exclusion Criteria	Pregnancy Unable to give informed consent	Death, disability, living outside metropolitan Adelaide
Ethnicity	All Australian Aboriginal	94% European (of these 29% were of Mediterranean ethnicity)
Participation	Unknown	52.9%
Prevalence	18.0%	NIH 8.7% \pm 2.0% Rotterdam 11.9% \pm 2.4% AES 10.2% \pm 2.2%

* Queen Elizabeth Women's Hospital, Adelaide

Table 1.7 Prevalence of PCOS in Asia

Authors	Kumarapelli et al 2008	Vutyavanich et al. 2007
Diagnostic Criterion	NIH/NIHCD	NIH/NIHCD
Study Population	Cross sectional community survey with cluster sampling	Recruited from gynaecology and infertility clinics
Participant Numbers	N=3030	N=1095
Age	15–39 years	18–40 years
Exclusion Criteria	Pregnancy, childbirth < 1.5 years, HRT* <12 months, Current OCP or injectable progestagen in 18 months	Menopause, drugs or hormones in last 3 months that would interfere with hormones, hysterectomy/ bilateral oophorectomy, malignancy, urgent medical attention required or communication difficulties
Ethnicity	All Sri Lankan	All Thai
Participation	96.1%	Unknown
Prevalence	6.3%	5.5%

*HRT = hormone replacement therapy

The study in Spain was of 154 women presenting for blood donation, based on the NIH/NICHHD criteria, and they found a prevalence of 6.5% (Asunción et al. 2000).

Hyperandrogenaemia was determined as total testosterone and DHEAS and/or FAI above the 95th centile of eumenorrhoeic participants without acne or hirsutism or were taking any exogenous hormones. Clinical hyperandrogenism was classified as a FG score >8, women receiving hormonal treatment for hirsutism, acne persistent in the third decade of life or androgenic alopecia. The acne and alopecia were not scored which leaves room for subjectivity in assessment and, as noted earlier, there are other explanations for these conditions. Of these women, there were nine who did not have bloods taken. Thirty one women were taking the oral contraceptive pill (OCP), including three with hirsutism and regular cycles. These women were excluded. Depending on the number of women using the OCP solely for contraception and those using it for other reasons (such as to regulate menstrual cycles, treat hirsutism) this may have led to an underestimation of PCOS. Bias was minimised by approaching consecutive women donating blood. However, blood donors themselves are a selected sample.

Diamanti-Kandarakis et al. on the Greek island of Lesbos offered a free medical check to women in the community. 192 women who presented and were eligible were assessed for PCOS. They reported a prevalence of PCOS of 6.8% based on biochemical hyperandrogenaemia (FT>95th centile in eumenorrhoeic, non-hirsute (FG<6 participants) and oligomenorrhoea (Diamanti-Kandarakis et al. 1999).

Additionally, based on the NIH criterion which includes clinical hyperandrogenism (used by the other prevalence studies), an additional seven women with a normal free testosterone (FT) but clinical hirsutism would have been diagnosed with PCOS, giving a prevalence of 10.4%, higher than the other studies. However, there was a high proportion of women in this group with hirsutism (29%), possibly due to ethnicity or that women with problems were more likely to present for screening. Recruitment was designed to avoid selective sampling with advertisements through local radio, television and newspapers for free medical checks with an endocrinologist, without specific reference to PCOS or other conditions. Potentially, women who perceived themselves to have or be at risk of illness may have been more

likely to respond. Current use of hormonal contraceptives or other drugs were exclusion criteria.

Knochenauer et al. assessed 369 women presenting for pre-employment medicals in the University of Alabama, south east USA (Knochenauer et al. 1998). They also used the NIH/NICHD criteria and found a prevalence of PCOS of 4.7% in the white women and 3.4% in the black women. This was not a statistically significant difference. Biochemical hyperandrogenism was defined as total or free testosterone, androstenedione and/or DHEAS >95th centile in regularly cycling women without hirsutism or receiving hormonal treatment while hirsutism was based on a FG score >6.

Out of the 369 women screened, 277 (75.1%) consented to complete evaluation and there was no difference in the groups between these women and those who declined in mean BMI or ethnicity. The women who did not participate were slightly older and had a lower mean FG score. Seventy-nine women in the study were taking the OCP and did not have their sera assayed. Two thirds of these were using the OCP for contraception. They were still included in the study and therefore it is possible that the prevalence was underestimated. Conversely it is possible that the estimation may have been too high if the 25% of women who refused to participate beyond the initial screening did so because they were well without any problems. Bias in this study is limited by the fact that workers across a range of occupations were screened and the university is the largest employer in the city and the third largest in the state.

Azziz et al. conducted a similar assessment of a further 608 women presenting at the University of Alabama for employment physicals in the two years following that of Knochenauer et al. (Azziz et al. 2004b). They found a slightly higher prevalence of PCOS at 6.6% (8.0% black women and 4.8% white women) probably because they included women who had not completed their evaluation and these were excluded in the former study.

Michelmores et al. 1999 in the UK assessed 230 women, predominantly Caucasian, between the ages of 18–25 and found a prevalence of PCOS of 7.4% using the NIH criteria, though they used a definition of menstrual irregularity which was very broad.

They also included women who were using hormonal contraception which was nearly half of the participants. This may have affected results in terms of recall of cycles and the assessment of ovaries on ultrasound. They did however also find a higher than previously reported prevalence of PCO in this group of 33% (Michelmore et al. 1999). There is a possibility of bias in that whilst women did not know the detailed rationale for the study, they were aware that a pelvic ultrasound would be performed and that it was a woman's health study. Women with problems may therefore have been more likely to attend.

A study assessing PCOS based on self-reported symptoms in 156 Mexican American women found a high prevalence of 13% (Goodarzi et al. 2005). Whilst the authors argue self-report has been shown to be a reliable means of assessing PCOS based on work by Kahsar-Miller, there is obviously room for misdiagnosis (Kahsar-Miller & Azziz 2003). Further, these women were daughters who all had one parent with cardiovascular disease, which may have led to a bias in favour of PCOS.

A large community based survey on a retrospective birth cohort in South Australia found a prevalence of PCOS $8.7\% \pm 2.0\%$ by the NIH criteria, $11.9\% \pm 2.4\%$ by the Rotterdam criteria and $10.2\% \pm 2.2\%$ by the AES criteria (March et al 2009). Attempts were made to trace all women who were born at the Queen Adelaide hospital Adelaide between 1973 -1975 (n=2199). Excluding those who were deceased, disabled or lived outside metropolitan Adelaide, 1375 women were eligible to participate and were invited for interview. A final total of 33% of the original cohort (n=728) women participated in the interviews where they asked to self-report any symptoms of hirsutism and oligomenorrhoea (n=277). Those with a self-reported modified FG score ≥ 8 and/or oligomenorrhoea were then asked to have a clinical examination, including a pelvic ultrasound and blood tests. Just over half (56%) had an ultrasound and/or bloods. Whilst the prevalence figures were not dissimilar to that of the previous studies there were 122 women who declined a clinical assessment who may have had PCOS. Of the 108 women who had an ultrasound, 38.0% had polycystic ovaries and applying this figure to those women who were eligible for but declined a clinical assessment, the prevalence by the Rotterdam criteria was increased to 17.8% (15.0, 20.6) and by AES 12.0% (9.7, 14.4).

It is possible the estimated prevalence by the NIH criteria was underestimated as there were a small number of women among the 122 who declined an examination but may have been diagnosed with PCOS if they had a raised free testosterone.

Underestimation may also have occurred as women currently using hormonal contraception were included and this is known to decrease free testosterone. Recall bias of cycle regulation and frequency may also have occurred in women who had prior hysterectomy or been using hormonal contraception for long periods of time.

One small study of Indigenous women in Australia was undertaken as the authors had noted that hirsutism was common in these women and this, combined with obesity and rising prevalence of type 2 diabetes, led them to hypothesise that PCOS may be more common in this group of women. Women who took part were from the West Kimberley region of Western Australia and south west Victoria. They found that of 38 women, ten of them had oligomenorrhoea. Of these six had facial hirsutism⁸ and one woman had a raised FAI. The three non-hirsute women did not have blood taken. Based on these results, using the NIH criteria 18.4% of women in the group had a diagnosis of PCOS (Davis et al. 2002).

There is obviously the potential for bias here in that women who perceived they had a problem might have been more likely to present to clinics run by visitors to the communities. The prevalence of hirsutism in the community generally is not known, and some of these women may have had idiopathic hirsutism. However, as noted earlier, up to 78 % of women with hirsutism may have PCOS (Azziz et al. 2004a, Carmina 1998, Glinborg et al. 2004, Unluhizarci et al. 2004). This study was only small but the higher prevalence of PCOS is consistent with high rates of diabetes and central obesity in the Australian Indigenous population and highlights the need for more work to be done in this area.

⁸ facial hirsutism only assessed due to lack of a private space for Ferriman Galwey score

In summary, the differences in prevalence across studies are undoubtedly related to lack of consistency in study design and diagnostic criteria for PCOS. However, it does appear that certain populations are at particularly high risk of PCOS.

1.5.2 PCO and Prevalence

Polycystic ovaries as an isolated finding on ultrasound have been found in 20 to 25% of European, non-Indigenous Australian and New Zealand women of reproductive age, slightly lower at 10.0–17.0% in Greece and Scandanavia and in a high 52% of South Asian women living in the UK. Prevalence varies due to different populations studied, selection criteria and age of women studied as polycystic ovary appearances change with age (Alsamarai et al. 2009). Polycystic ovaries on ultrasound alone are not diagnostic of PCOS and it is important to be aware of individual radiologists criteria for classification of PCO due to the possible pitfalls of diagnosis discussed in 1.4.1.2. The details of these studies are listed in Table 1.8 to Table 1.10 (Borgfeldt & Andolf 1999, Botsis et al. 1995, Clayton et al. 1992, Farquhar et al. 1994, Lowe et al. 2005, Polson et al. 1988, Rodin et al. 1998).

Table 1.8 Prevalence Studies and PCO in the United Kingdom

Authors	Polson et al 1998	Clayton et al 1992	Rodin et al 1998
Population	Volunteers and clinical and secretarial staff London hospital	Volunteers born between 1952-1969 recruited by random postal invitation from a general practice list in Harrow, UK	Women from general practice list and translating service in UK, women from diabetes unit
Number	N=257	N=190	N= 212
Age (years)	18–36	18–36	18–40
Exclusion criteria	No prior treatment for infertility, hirsutism or menstrual disturbance	Current pregnancy	Menopause, hysterectomy or previous ovarian surgery
Response rate	Unknown	17.8%	100%
Ethnicity	Not reported	91% Caucasian, 4.3% Afro/Caribbean, 2.9% Asian, remainder “other”	South Asian
Percentage with PCO	22% (95% CI ,17–27%)	22% (95% CI 16–28)	52%

Table 1.9 PCO prevalence in mainland Europe

Authors	Botsis et al. 1995	Koivunen et al. 1999	Borgfeldt et al. 1999
Population	Women attending a gynaecology clinic for routine Pap smear from 1989–1993 in Greece	Healthy volunteers (Finland), no details as to recruitment methodology	Recruited from population register 1996 in Lund (Sweden)
Number	N=1078	N=189	N=335
Participant Age (years)	17–40	20–45	25–40
Exclusion criteria	None reported	Current medication, pregnancy or IUCD	Mental incapacity, pregnancy, moved away from study location, previous oophorectomy or current gynaecological or surgical care.
Response rate	Not reported	Not reported	72%
Ethnicity	Not reported	Not reported	Not reported
Percentage with PCO	17.0%	14.2%	10.2% (95% CI± 4.2%)*

* not using hormonal contraceptives

Table 1.10 PCO prevalence in Australia and New Zealand

Authors	Farquhar et al. 1994	Lowe et al. 2005
Population	Electoral roll, New Zealand	Retrospective review of Australian women who had ultrasound following infertility treatment due to azoospermia
Number	N=183	N=100
Participant Age (years)	18-45	Not reported
Exclusion criteria	Hysterectomy or current pregnancy	None reported
Response rate	16%	N/A
Ethnicity	95% European descent, 2.2% Maori, 1.6% Pacific Islander, 1.1% Asian	Not reported
Percentage with PCO	21%	23%

1.6 Pathophysiology of PCOS

1.6.1 The ovulatory cycle

The ovulatory cycle is a complex system primarily regulated by FSH, LH, oestradiol, inhibin and progesterone transmitting signals between the ovary and the hypothalamic-pituitary axis. Initially, at menses, negative feedback from the corpus luteum products (oestradiol, progesterone and inhibin) to the pituitary results in the critical initial rise in FSH. Later in the cycle the positive feedback relationship between oestradiol and LH is the ovulatory stimulus. Within the ovary, insulin like growth factor (IGF), inhibin and activin modify follicular receptor response necessary for growth and function. Disruption in the cycle can be due to an abnormality in one of the various roles for any one of these substances or an inability to respond to signals.

Anovulation, menstrual cycle dysfunction, infertility and hyperandrogenism are key features of PCOS and to understand the development of these features it is necessary to understand the normal menstrual cycle and ovulation. This section therefore will discuss the normal ovulatory process and what changes can disrupt ovarian function.

Primordial germ cells begin developing in the early weeks of pregnancy and from 6–8 weeks gestation there is rapid multiplication of the germ cells which result in a maximum number of oocytes at 16–20 weeks gestation (Gondos et al. 1971, Himmelstein-Braw et al. 1976). Primordial ovarian follicle development begins mid-gestation in the fetus and is completed just after birth. Each primordial follicle consists of an oocyte surrounded by granulosa cells and they begin to grow and undergo atresia from infancy through to the early phases of menopause, independent of other physiological circumstances such as anovulation and pregnancy (Speroff L & Fritz MA 2005b, Zeleznik 2004).

There are a number of primordial follicles which have developed to pre-ovulatory status at any one time in the ovary. Cohorts of follicles begin growing independent of hormonal stimulation and will reach pre-ovulatory status after around 85 days at which point they are 2–5 mm in diameter and can be identified on ultrasound. Usually in young women this cohort numbers from 3–11 follicles per ovary (Gougeon 1996, Pache et al. 1990).

Once at the pre-ovulatory stage they will be recruited by FSH or undergo atresia. Therefore the sustained increase of FSH at the beginning of the menstrual cycle is critical (Oktay et al. 1998).

Follicular development, in response to FSH, begins with an increase in oocyte size, a change in cell shape of the granulosa cells to cuboidal and the development of gap junctions between the granulosa cells and the oocyte, which allow communication between the two.

With proliferation of the cuboidal granulosa cells the primordial follicle becomes a primary follicle. This has a basal lamina that separates the granulosa cells from the stroma. The stroma is comprised of an inner (theca interna) and outer (theca externa) layer (Speroff L & Fritz MA 2005b).

Acceleration of growth leads the follicle into the pre-antral stage. Granulosa cells have FSH receptors and FSH stimulates further granulosa cell growth and proliferation as well as the production of oestrogen by granulosa cells, through aromatisation of androgens (via P450aromatase) (Yong et al. 1992).

With growth, under the combined influence of FSH and follicle produced oestrogen, there is an accompanying increase in follicular fluid that coalesces to form a cavity as the follicle progresses to the antral stage. Oestrogen and FSH continue to dominate in the follicle and are essential for granulosa proliferation and a healthy oocyte (Andersen 1993).

As the follicle develops the theca cells develop LH receptors and the enzymes required for steroidogenesis, including 17 α -hydroxylase/C₁₇₋₂₀ lyase cytochrome P450 (CYP17), CYP11A and 3 β hydroxysteroid dehydrogenase. LH stimulates the expression of these enzymes, the theca LH receptors and the steroidogenesis acute regulatory protein (StAR) that facilitates the transport of the steroid substrate, cholesterol, into the cell mitochondria for steroidogenesis. Together LH and these enzymes regulate the synthesis of androgens from cholesterol in the theca cell (Magoffin 2005). The androgens then diffuse across to the granulosa cells where they are metabolised to oestrogen.

The dominant follicle is the one that is selected to ovulate from the cohort. The dominant follicle will be the one with the highest micro-oestrogenic environment which in turn has led to the most granulosa cell proliferation. The rise in oestrogen from this follicle has a negative feedback on pituitary FSH production resulting in a drop in FSH. The fall in FSH leads to the demise of all but the dominant follicle; the remaining follicles experience a decline in FSH dependent oestrogen production, an increase in androgens and cell atresia. The dominant follicle is protected from these events as it is more sensitive to the effects of FSH so it can continue aromatisation and oestrogen production even with lower FSH. This increased sensitivity is due to: larger numbers of granulosa cells and therefore FSH receptors; increased follicular oestrogen and other autocrine factors which enhance local FSH action; increased vasculature enabling increased delivery of gonadotropins; and the acquisition of LH receptors on the granulosa cells that enable the cells to respond to both FSH and LH (Zelevnik et al. 1981).

Whilst oestradiol has a negative feedback on FSH it has a positive feedback effect on pituitary LH production. Oestradiol production accelerates in the dominant follicle and peak levels are reached triggering a LH surge and subsequent ovulation. Following this oestrogen levels drop and progesterone levels rise.

The corpus luteum is comprised of combined granulosa cells and theca lutein cells from the ovulatory follicle. Progesterone production increases after ovulation and is produced by the corpus luteum. Down-regulation of the CYP17 theca lutein cell enzyme by the LH surge transforms theca cells to produce progesterone instead of androgens (Magoffin 2005). This is facilitated by intense vascularisation of the cells, mediated by vascular endothelial growth factor (VEGF), which allows cholesterol to be delivered in sufficient amounts as a substrate for the increasing progesterone production (McClure et al. 1994). The corpus luteum will subsequently regress unless pregnancy occurs.

Meanwhile, as the non-dominant follicles undergo atresia, the theca cells return to their origin as a component of the stroma. They retain their ability to respond to LH with P450 activity and androgen production. The resultant stromal increase causes a rise in mid-cycle plasma androgens (androstenedione and testosterone). This rise may help to enhance atresia in the non-dominant follicles.

Other factors involved in the ovarian follicular development include:

Peptides

- **Activin:** produced by both the pituitary gland and ovarian granulosa cell augments FSH secretion and may suppress the stimulatory action of LH and IGF-1 on steroidogenesis (Corrigan et al. 1991, Hillier et al. 1991, Sawetawan et al. 1996).
- **Inhibin:** secreted by the granulosa in response to FSH and suppresses FSH production assisting in the development of the ovulatory follicle (Robertson et al. 2009). Facilitates LH stimulation of androgen synthesis in the ovary and blocks the actions of activin (Bilezikjian et al. 1996).
- **Follistatin:** secreted by pituitary and suppresses FSH synthesis and secretion. Production stimulated by activin but also blocks the effects of activin. Also produced by granulosa cells in response to FSH (Robertson 1992)
- **AMH:** produced by granulosa cells in small pre-antral and antral follicles (4–6mm) that have been recruited from the primordial follicles but have not yet been selected for dominance (Visser & Themmen 2005). The role is unclear but in mice it has been shown to decrease initial follicle recruitment from the primordial follicle stage. It may also affect the sensitivity of the follicle to FSH thereby playing a role in the selection of the dominant follicle (Visser & Themmen 2005).

Growth Factors

- **Insulin like Growth Factors I and II (IGF I and IGF II):** these are structurally and functionally similar to insulin. Both IGF- I and IGF-II action can bind by the type I IGF receptor, which is structurally similar to the insulin receptor and their actions are mediated by a group of binding proteins (IGFBP) (Roberts & Echterkamp 2003, Spicer & Aad 2007). IGF-I is produced in theca cells, IGF- II is produced in granulosa, thecal and luteinised granulosa cells (el-Roeiy et al. 1993, Mason et al. 1996). Studies with humans and animals have shown IGFs to be key intraovarian regulators of follicular growth, selection, atresia and steroidogenesis, with many of their actions mediated through enhancement of gonadotropin action (Kwintkiewicz & Giudice 2009).

1.6.2 Androgens, LH, insulin and the ovulatory cycle

The ovulatory process therefore requires a delicate balance of the growth factors in the follicle throughout the cycle. In the early part of the cycle, FSH and oestrogen are essential in order to promote growth of the follicle and provide a nurturing environment for the oocyte. A predominantly androgenic environment, early exposure to LH or no exposure to FSH will disrupt this balance and subsequently the ovulatory process. Hyperinsulinaemia can also disrupt the process as will be discussed below. Increased androgens, an imbalance in the FSH, LH ratio and high insulin are all factors common in PCOS and help explain why anovulation is a frequent feature of PCOS.

Androgens

The role of androgens in oocyte development and ovulation is complex. In the pre-antral follicle, in low concentrations, androgens serve as the essential substrate for oestrogen production through FSH induced aromatisation. In contrast, a high androgen concentration early in the cycle, whatever the cause, leads to the granulosa cells favouring production of 5 α -reduced androgens rather than oestrogens. These androgens cannot be readily converted to oestrogen and, in fact, inhibit further aromatase activity and prevent LH receptor formation leading to follicular atresia. Therefore excessive local androgens can prevent normal cycling and ovulation (Speroff L & Fritz MA 2005a).

Elevated plasma free testosterone levels also affect the ovulatory process by altering hypothalamic-pituitary function either directly or through aromatisation.

Factors that can be responsible for raised ovarian androgens include: PCOS, obesity, exogenous androgens, non-classic 21 hydroxylase adrenal hyperplasia (NCAH), androgen secreting tumours.

Insulin

There is a high prevalence of insulin resistance and hyperinsulinaemia in women with PCOS. Hyperinsulinaemia can interfere with the ovulatory cycle through a number of mechanisms:

- Direct stimulation of steroidogenesis in the ovary. Insulin binds to insulin receptors on the ovary and activates a signaling system that stimulates testosterone and androstenedione production (Nestler et al. 1998).
- Indirectly increases plasma androgens. Hyperinsulinaemia inhibits the hepatic synthesis of SHBG thereby increasing the bioavailability of both androgen and oestrogen (Plymate et al. 1988).
- Decreases insulin growth factor binding protein 1 (IGFBP-1) synthesis in the liver and ovary and upregulates IGF-1 binding. This leads to an increase in free IGF-1 and IGF-2 in the ovary, thereby increasing ovarian androgen production (De Leo et al. 2000).

LH

An increase in pulse amplitude and frequency of LH accompanied by a decrease in FSH is often seen in PCOS and leads to an increase in the LH: FSH ratio. This change is due to an increased pulse frequency of GnRH (gonadotrophin releasing hormone) with increased LH and decreased FSH. Factors mediating this may be increased insulin, oestradiol, androstenedione and other androgens (Blank et al. 2006, Doi 2008). LH is not normally present in follicular fluid until midcycle and if elevated in the plasma and follicle before this time inhibits granulosa cell proliferation and causes intra-follicular androgens to rise, thereby preventing the development of the micro-oestrogenic environment necessary for development of a mature follicle. The higher LH also continues to stimulate androgen production by theca cells as the follicle undergoes atresia.

1.6.3 The development of the Polycystic Ovary

The effects of hyperinsulinaemia, raised androgens and disrupted gonadotrophin cycles, particularly with an increase in LH, result in the lack of development of a dominant follicle and anovulation. The hormonal picture seen is one of an increase in pulse frequency and amplitude of LH, a decrease in FSH, raised ovarian androgen production and increased oestrone from peripheral conversion of ovarian androstenedione. FSH is present in sufficient amounts to stimulate new follicular growth but is not sufficient to enable growth to maturation and ovulation. The high androgen environment also prevents follicle maturation through the block of aromatisation. Whilst these follicles will not achieve maturation they may persist in the ovary for some months in the form on

follicular cysts, usually around 2–10mm in size. They are surrounded by hyperplastic theca cells, which are often luteinised in response to high LH levels. These follicles continue to produce androgens and, when they undergo atresia and rejoin the stroma, secrete increased amounts of androstenedione and testosterone in response to the high LH.

The polycystic ovary therefore has the following characteristics, which may be seen on morphology or ultrasound:

- An increase in number of growing and atretic follicles, without a regular recruitment of a dominant follicle resulting in the appearance of numerous follicular cysts, usually between 2–10 mm.
- An increase in the stroma due to hyperplasia of theca cells and increased number of follicles developing and undergoing atresia.
- An increase in surface area of the ovary.

1.6.4 The development of PCOS

The development of PCOS is almost certainly multifactorial, involving a variable combination of ovarian and adrenal hyperandrogenism, hyperinsulinism and insulin resistance, obesity, and alterations in gonadotrophin secretion.

Whether there is one primary abnormality or several resulting in the above combination of abnormalities remains unclear. There are a number of theories proposed to explain the combination of abnormalities:

- i) A primary ovarian defect leading to local increased androgen production.
- ii) Increased adrenal androgen production.
- iii) A primary gonadotrophin abnormality leading to increased LH pulse frequency and amplitude.
- iv) Insulin resistance and hyperinsulinaemia

1.6.4.1 Ovarian defect

Women with PCOS have increased formation of androstenedione and 17α -hydroxyprogesterone in response to LH due to abnormal enzymatic regulation of steroidogenesis (Rosenfield et al. 1990, Rosenfield et al. 1994). There is also evidence that the ovarian steroidogenic activity in PCOS is an intrinsic abnormality and not solely related to LH stimulation, as in vitro studies show increased androgen production is a stable steroidogenic phenotype of PCOS in cells propagated in long term culture (Nelson et al. 1999, Nelson et al. 2001). This is supported by other in vitro data demonstrating that the theca cells of polycystic ovaries have both increased basal and LH-induced androstenedione production compared with cells from normal ovaries (Gilling-Smith et al. 1994).

Androgen synthesis in the ovary is regulated by the enzyme activity of P450c17, which has both 17α -hydroxylase and $17,20$ lyase activity and both are required for androstenedione production. This is then converted by 17β hydroxysteroid dehydrogenase (17β HSD) to form testosterone or is aromatised to form oestrone. The activity of the P450c17 cytochrome has been shown to be increased in PCOS theca cells compared with controls (Nelson et al. 2001). The high levels of androstenedione produced by the theca cells favour metabolism to testosterone and 5α reduced androgens rather than oestrone, as discussed in 1.6.2. How this disturbance in expression and activity of steroidogenic enzymes occurs is unclear. No underlying genetic basis has been identified to date.

Another possible abnormality is in phosphorylation of the ovarian insulin receptor. This affects androgen production in the ovary as serine phosphorylation induces the $17,20$ lyase enzymatic activities stimulating androgen production. 50% of women with PCOS have an abnormally high phosphorylation. Serine phosphorylation also inhibits insulin receptor signaling, and this could account for some of the insulin resistance that is seen in PCOS (Ehrmann et al. 1992).

1.6.4.2 Adrenal defect

The ovary and adrenal cortex share the bulk of androgen biosynthesis as discussed in 1.4.1.3.2. The enzymes involved are similar in both glands although the ovarian androgen synthesis is largely controlled by LH and in the adrenal by adrenocorticotrophic hormone (ACTH). Increased adrenal androgen production has been found in one quarter to one third of women with PCOS (Kumar et al. 2005). This may occur due to genetic traits, secondary to ovarian hormonal secretion, or due to increased cortisol metabolism (Chin et al. 2000, Moran & Azziz 2001, Stewart et al. 1990, Vassiliadi et al. 2009). Increased cortisol metabolism would lead to compensatory increase in ACTH and therefore increased androgen production. It has been proposed that increased cortisol metabolism may be due to increased 5α -reductase activity, which also mediates the effects of testosterone in the skin, perhaps also explaining the hirsutism seen in PCOS. Lower cortisol is also possibly mediated through 11β HSD activity and this in turn may be associated with insulin resistance and hyperinsulinaemia (Rodin et al. 1994, Vassiliadi et al. 2009).

1.6.4.3 Gonadotropin dysfunction

The characteristic picture seen in many women with PCOS is increased LH and decreased FSH. Women with PCOS have increased LH pulse frequency and amplitude and a greater response to GnRH compared to normal women (Rebar et al. 1976). This may reflect, in part, increased GnRH activity and an increased responsiveness of LH to GnRH stimulation (Hall et al. 1998, Hoff et al. 1979). Whilst the mechanism for this increase is unclear it would seem that these changes in GnRH and LH production are influenced by other factors.

The ovarian hormones, oestrogen and progesterone, may affect GnRH and LH secretion. Oestrogen has been shown to have a positive feedback with an increase in LH response to GnRH as well as an effect on GnRH pulse frequency itself, and women with PCOS have chronic elevated oestrone (Smith et al. 1984, Waldstreicher et al. 1988). Conversely, progesterone, which is lower in PCOS due to the lack of ovulation, has a suppressive effect on LH release. In addition, this effect is more pronounced in normal women than in those with PCOS (Daniels & Berga 1997, Pastor et al. 1998).

Increased androgens have been shown to be associated with increased LH levels and increased response of LH to GnRH in women with congenital adrenal hyperplasia and in women with PCOS. Treatment which lowers or inactivates androgens normalises the LH response (Barnes et al. 1994, Eagleson et al. 2000). However, other studies do not support a direct role for androgens in control of gonadotrophin secretion (Dunaif 1986, Serafini et al. 1986).

There is some evidence that insulin may contribute to regulation of LH secretion in PCOS. Some studies have shown that increased insulin leads to increased LH, and conversely, decreasing insulin levels with insulin lowering drugs decreases androgens and LH levels. However, other studies have reported no effect of increasing or decreasing insulin on LH production (Mehta et al. 2005, Patel et al. 2003). Most of these studies, however, have assessed obese women with PCOS, and hyperinsulinaemia is positively correlated with BMI and LH inversely correlated with obesity, which contributes to the difficulty of interpreting these results.

1.6.4.4 Insulin resistance/ hyperinsulinaemia

Hyperinsulinaemia is considered one of the main instigators for the development of PCOS. There is a strong correlation between insulin resistance and hyperandrogenism and most of the evidence supports the proposal that hyperinsulinaemia leads to hyperandrogenism rather than the other way around.

- in vitro, insulin stimulates thecal cell androgen production
- insulin sensitisers and loss of weight not only decrease insulin but may also decrease androgens
- administering a GnRH analogue decreases ovarian androgens but does not cause a corresponding decrease in insulin
- genetic links have been shown between insulin resistance (IR) and hyperandrogenism

Hyperinsulinaemia develops either due to increased peripheral target tissue resistance, reduced hepatic clearance of insulin or increased pancreatic sensitivity. Studies utilising the euglycaemic clamp technique suggest that the hyperinsulinaemia in hyperandrogenic

women is due to peripheral insulin resistance and a reduced sensitivity of hepatic glucose production to insulin suppression in obese women with PCOS (Bremer & Miller 2008, Dunaif et al. 1992).

The increased peripheral resistance may arise from defects in insulin receptors – decreased binding or a post-receptor defect. Currently research with PCOS supports the proposal that a post receptor function is responsible for insulin resistance in PCOS (Ciaraldi et al. 1992). Insulin normally binds to the insulin receptor and induces a conformational change that activates tyrosine autophosphorylation initiating insulin signaling.

In PCOS, one mechanism of insulin resistance is that serine instead of tyrosine phosphorylation of the insulin receptor occurs reducing signal transmission for glucose transport (Dunaif et al. 1995, Li et al. 2002, Mor et al. 2004). It is possible that in some women with PCOS a similar mechanism may occur at the level of the adrenal gland or ovary P450c17 enzyme (Pasquali et al. 2007b). Serine phosphorylation has been shown to be a regulator of P450c17's enzymatic activity, increasing the 17, 20 lyase component and thereby increasing androgen production (Auchus et al. 1998, Dunaif et al. 1995)

Initially, in response to target tissue resistance, there is compensatory hyperinsulinaemia. This excess insulin causes adverse effects in other, less traditionally responsive tissues, such as the skin and vasculature. Clinically these effects can result in hypertension and an increased risk of coronary artery disease. Paradoxically, the ovary remains sensitive to insulin. It is proposed that this occurs because whilst there is a change in the metabolic response to insulin, the mitogenic function remains unchanged, thereby allowing ongoing steroidogenesis in response to insulin (Courbold et al 2006, Diamanti-Kandarakis E et al 2008, Dunaif 2006).

Generally it has been accepted that over time, the B cells of the pancreas lose their ability to compensate, insulin levels decline and the result is impaired glucose tolerance and Type 2 diabetes mellitus. Some studies have shown that there may be B cell dysfunction early in PCOS though studies disagree as to whether this is observed in all women with

PCOS or only those who are obese (Dunaif & Finegood 1996, Ehrmann et al. 1995, Morales et al. 1996, O'Meara et al. 1993).

Insulin resistance and B cell dysfunction are therefore common in PCOS and probably intrinsically linked with mechanisms common to all women with PCOS with effects modified by obesity.

There are probably a number of contributing factors to the development of insulin resistance in women with PCOS. These include genetic and developmental origins with birth weight disorders being associated with insulin resistance, obesity and type 2 diabetes in adult life (Hofman et al. 2004, Stocker et al. 2005). Alterations in birthweight have also been implicated in the development of PCOS in some, though not all studies (Cresswell et al. 1997, Laitinen et al. 2003, Sadrzadeh et al. 2003). Further, as noted in 1.7.2.1.2, depending on the gestational age, antenatal exposure of the fetus to androgens can lead to PCOS and insulin resistance and obesity in animals.

1.7 PCOS and Risk Factors

1.7.1 Inheritance

1.7.1.1 Family History

Familial clustering of PCOS has suggested a heritable component, although results are inconsistent in their assessment of inheritance. Difficulties associated with family studies include the heterogeneity of the PCOS phenotype, the lack of clear features that can identify a male phenotype of PCOS, and the amelioration of symptoms with age, meaning assessment of mothers of women with PCOS who are often postmenopausal relies on recall. This makes formal segregation analysis as well as genetic linkage studies more difficult (Legro et al. 1998). Additionally the association of infertility and PCOS leads to a paucity of large pedigrees for linkage analysis.

Despite these difficulties, most studies have found the components of PCOS and insulin resistance to be more common in affected families (Diamanti-Kandarakis et al. 2004, Govind et al. 1999, Hague et al. 1988, Jahanfar et al. 1997, Kahsar-Miller et al. 2001,

Legro et al. 1998, Lunde et al. 1989). The pattern of inheritance is unclear although is possibly dominant inheritance with low penetrance and variable expressivity affected by environmental and epigenetic factors (Kahsar-Miller et al. 2001, Legro et al. 1998).

1.7.1.2 Genetics

The evidence of familial aggregation of PCOS, supports a genetic contribution to the aetiology of PCOS. There is no single gene that has been shown to cause PCOS. There are instead a number of genes that show altered patterns of expression. The approach to date has been largely based on candidate genes. This involves evaluating genetic markers (single nucleotide polymorphisms (SNPs) or microsatellites) of genes hypothesised to play a role in PCOS and determining their association with phenotypes in families or populations. Genes encoding for androgen production, insulin resistance, regulation of the hypothalamic-pituitary-ovarian axis and weight and energy regulation have been the main focus of research.

Difficulties in establishing genetic contributions include small study numbers, the heterogeneity of the PCOS phenotype and the varying diagnostic criteria, the lack of a reliable phenotype for prepubertal girls, menopausal women and men, lack of appropriate controls, only one or two variants genotyped in each gene indicating incomplete coverage of candidate genes and the uncertain role of the environment and epigenetics (Dasgupta & Reddy 2008, Diamanti-Kandarakis & Piperi 2005, Goodarzi & Azziz 2006).

Future research would benefit from large study numbers, genome wide scans and cDNA technology which may help ascertain the functional aspect of candidate genes (Dasgupta & Reddy 2008)

1.7.2 Environmental Factors

1.7.2.1 Fetal Programming of PCOS

1.7.2.1.1 Birth Weight and PCOS

The “thrifty phenotype” hypothesis suggests that fetal nutritional deprivation during critical periods of development forces adaptive survival strategies, which restrict fetal

growth and entail a resetting of the normal course of metabolic, physiological and anatomical development. These adaptations become detrimental if the organism encounters over nutrition in later life leading to an increased risk of obesity, insulin resistance, diabetes and cardiovascular disease (Barker 2007, Sayer et al. 2004). Excessive childhood growth further compounds these effects (Forsen et al. 2000, Ong 2006). The adaptive mechanisms may involve altered fat deposition, adipocyte function, programming of appetite and metabolism (Taylor & Poston 2007).

Impaired fetal growth has been linked with associated features of PCOS in adults compared to adults who had a normal birth weight, and may be due to an effect on insulin resistance (Ibanez et al. 1998, Ibanez et al. 1999b, Ibanez et al. 2008, Laitinen et al. 2003, Pandolfi et al. 2008).

Indigenous babies have twice the rate of low birth weight compared to non-Indigenous Australians as noted in 1.2.4.2. This, coupled with rising adult obesity may contribute to an increased risk of PCOS in Indigenous women.

1.7.2.1.2 Androgen exposure in utero and PCOS

Animal studies have found that exposure to excess androgens early in gestation antenatally can affect multiple organs as they are developing, and program the exposed animals to develop signs consistent with PCOS, diminished oocyte quality, metabolic defects such as insulin resistance and abdominal obesity, and general health disorders such as low birth weight with associated heart, kidney and adrenal enlargement (Abbott et al. 2009, Demissie et al. 2008, Foecking et al. 2005, Manikkam et al. 2006, Manikkam et al. 2008, Padmanabhan et al. 2006, Sullivan & Moenter 2004, Abbott et al. 2002, Abbott et al. 2005, Zhou et al. 2005).

Hyperandrogenism may be induced in the human female fetus by genetic and/or environmental factors. The androgens may come from the following sources: hyperandrogenic fetal ovaries, the fetal adrenal cortex, from the circulation of a hyperandrogenaemic mother (eg. maternal PCOS, congenital adrenal hyperplasia) or possibly through fetal ovarian hyperplasia due to maternal hyperinsulinaemia. However, in the majority of circumstances, these would not lead to supraphysiological doses in the

fetus as, in humans, placental aromatase protects the fetus from excess androgens by converting androgens to oestrogens. Consistent with this, human studies to date do not support the theory that antenatal androgens in normal doses have any significant adverse effect on the fetus.

1.7.2.1.3 Maternal stress

1.7.2.2 Obesity

Obesity is generally defined by BMI. This is a figure derived from an individual's weight (kg)/ height (m) squared. A BMI between 25–29.9 kg/m² is deemed overweight, whilst a BMI of >30 kg/m² is obese.

The reproductive, metabolic manifestations and complications of PCOS are exacerbated by obesity, and weight gain is probably a factor that triggers the phenotypic expressions of PCOS in women who are susceptible (Norman et al. 2002). Therefore obese women with PCOS are less likely to achieve pregnancy spontaneously or with medical assistance, are more likely to miscarry, have a higher prevalence of fetal abnormality and suffer from pregnancy complications. They also have an increased risk of abnormalities of glucose metabolism (Legro 2001, Norman et al. 2001).

Obesity varies worldwide depending on the population being studied but is a rapidly increasing problem globally. In the USA, 35.3% of adult women are obese and the problem is greatest in black and Mexican American women (Ogden C. et al. 2007). Obesity has risen across Europe but not uniformly; the prevalence of obesity in adult women ranges from as low as 5.9% in Norway to a high of 35.6% in Albania (WHO 2007). The prevalence of obesity in the Asia Pacific region also varies greatly. Australia has one of the highest prevalences with 22.2% obese compared with 10.8% in Thailand, 3.4% in Japan and down to a low of 0.6% in India (Asia Pacific Cohort Studies Collaboration 2007, Dunstan 2009).

Obesity in women with PCOS tends to be related to obesity prevalence of their parent population. Obesity was present in 42% of women diagnosed with PCOS in an unselected population from Alabama, USA (Azziz et al. 2004b). Other prevalence studies have found

lower mean BMI ranging from 25–28 kg/m² in women from Spain, Greece and Finland (Asunción et al. 2000, Diamanti-Kandarakis et al. 1999, Taponen et al. 2003). A survey of women presenting to outpatients in Thailand found those with PCOS had a mean BMI of 23 kg/m² – which is within the normal range for populations of European origin, but probably “overweight” in that population (Vutyavanich et al. 2007).

A comparison of women with PCOS from Italy, Japan and the USA found that the BMI was similar in the women from Italy and the USA but was significantly lower in the Japanese women (Carmina E 1992). Within the United States, a study in Boston of the effect of ethnicity on the phenotype of PCOS found the highest BMI in the African American women at 36.3±7.9 kg/m² followed by the Hispanic and Caucasian women, with the lowest BMI in the Asian women at 26.3 ± 5.9 kg/m² – paralleling the different obesity prevalences found among the different ethnic groups of adult females in the USA (Welt et al. 2006).

1.7.2.2.1 Central Obesity

Relative to peripheral obesity, an abdominal or central pattern of fat deposition is associated with increased risk of insulin resistance, diabetes, dyslipidaemia, hypertension and atherosclerosis (Després 2006, Salehi et al. 2004). There are two major components comprising central obesity, visceral and subcutaneous fat. Visceral fat may confer more adverse metabolic effects such as increased insulin resistance and dyslipidaemia and raised CRP but this relationship may vary according to ethnicity (Cossrow & Falkner 2004, Després 2006, Jensen 2006) .

Whilst BMI at the population level is simple and a good measure of overall excess adipose tissue, on an individual level it cannot discriminate between high overall fatness and high muscle mass and does not indicate the location of the excess fat deposition. To assess central obesity other measures including waist circumference (WC), waist height ratio (WHtR) and waist hip ratio (WHR) are employed. Waist circumference is relatively easy to measure, but assumes that people with the same waist circumference have the same health risks regardless of height or body build. Generally it has a high correlation with BMI (Ford et al. 2003, Ohlson et al. 1985, Wei et al. 1997). In ethnically

homogeneous populations, WC is the best clinical measure to predict visceral adiposity on CT scan, however at the individual level (and in ethnically heterogeneous populations) this association is not consistent after adjusting for age, BMI, sex or ethnicity (Hill et al. 1999, Molarius et al. 1999, Molarius & Seidell 1998). WHtR takes into account the distribution of body fat in the abdominal region with an adjustment for height and is easy to perform in low resource settings. WHR demonstrates the ratio of central to peripheral adiposity, which is an important distinction as some studies have suggested an accumulation of fat in the legs, as seen with a high hip circumference, has a protective effect with lower glucose and triglycerides and higher HDL-C (Snijder et al. 2004b, Snijder et al. 2004a). However WHR has been shown to be the most susceptible of the above obesity indices to measurement errors (Lee et al. 2008).

Most, but not all, studies indicate that central obesity is higher in women with PCOS consistent with the increased metabolic risks (Barber et al. 2008). Some, though not all, studies suggest this is significant in lean women with PCOS compared to controls (Carmina et al. 2007, Good et al. 1999, Kirchengast & Huber 2001, Svendsen et al. 2008). Two studies reported that increased central obesity associated with PCOS only applied to the normal and overweight women and not to those who were obese (Carmina et al. 2007, Svendsen et al. 2008).

The mechanism of the adverse effect of visceral fat may occur through increased release of free fatty acids (FFA) into the portal circulation leading to hepatic insulin resistance. Increased gluteal or femoral fat may have a protective effect because adipocytes in these areas are less sensitive to lipolytic stimuli. They are therefore less likely to take up FFA from the circulation and, when they do, are less likely to release them. Therefore gluteal fat here may lower the exposure of some organs such as the liver and pancreas from excessive FFA.

It is difficult to know whether central obesity increases the risk of PCOS or whether PCOS itself leads to increased central obesity. Central obesity has significant effects on a number of reproductive and other hormones leading to higher insulin, lower SHBG and increased total and free androgens; all important components of PCOS. Conversely, testosterone may cause accumulation of visceral adipose tissue (Pasquali 2006).

Some populations with excess central adiposity and/or decreased peripheral adiposity/lean mass may therefore be at higher risk of insulin resistance, diabetes, cardiovascular disease and PCOS and their associated complications. These groups include Australian Aborigines, South Asian Indians, Malay, Chinese, Indonesians and Filipina American and Japanese American women (Araneta et al. 2002, Deurenberg et al. 2002, Deurenberg-Yap et al. 2002, Ko et al. 2001, Piers et al. 2003).

1.8 Health Consequences of PCOS

1.8.1 Insulin resistance (IR)

Insulin is important in stimulating glucose transport, carbohydrate and lipid storage and cell growth and division. It may be one of the contributors to the causes of PCOS as discussed or it may arise as a consequence of PCOS.

Screening for insulin resistance is difficult. The euglycaemic–hyperinsulinaemic clamp test is the gold standard but is labour intensive, requires multiple blood sampling and is impractical for screening large numbers of people. Alternatives include the homeostasis model assessment (HOMA), the insulin tolerance test and the frequently sampled intravenous glucose tolerance test. The HOMA uses a formula of a ratio of fasting glucose (mmol/l)/insulin (mU/l) x 22.5) and has a reasonable correlation with the euglycaemic clamp. It is practical for clinical use and large epidemiological studies.

Insulin resistance has been reported in women with PCOS, accompanied by compensatory hyperinsulinaemia and contributes to the hyperandrogenaemia of PCOS. The prevalence of insulin resistance varies depending on the assessment of insulin resistance and the PCOS diagnostic criterion used, but is present in about 50% of women with PCOS. Insulin resistance is increased in the presence of higher androgens and/or oligomenorrhoea and in women from ethnic populations with increased risk such as South Asian and Mexican American women (Carmina et al. 2005, Dewailly et al. 2006, Kauffman et al. 2002, Norman et al. 1995).

A number of groups have found increased insulin resistance and hyperinsulinaemia or decreased insulin sensitivity index in women with PCOS compared with controls across

the spectrum of BMI (Chang et al. 1983, Dunaif et al. 1992, Dunaif & Finegood 1996, Jialal et al. 1987, Morales et al. 1996, Norman et al. 1995, Park 2001, Park 2007, Toprak et al 2001). Even after controlling for fat distribution two small studies also found increased insulin resistance in women with PCOS compared with controls (Barber et al. 2008, Carmina et al. 2007). However, not all agree insulin resistance is increased in lean women with PCOS (Dale et al. 1992, Herbert et al. 1990, Holte et al. 1994b, Morin-Papunen et al. 2000, Ovesen et al. 1993, Vrbikova et al. 2004). Conversely, some have found no difference between obese women with PCOS and controls. Potential reasons for the different findings may relate to the small numbers used in most studies, definition of PCOS used, the method of determination of insulin resistance, variations in central adiposity and family history of diabetes.

It is generally agreed however, that obesity has a synergistic effect on the insulin resistance of PCOS. Obese women with PCOS tend to have more severe insulin resistance and B cell dysfunction than lean women with PCOS (Morales et al. 1996).

There have been few studies that have compared risk of insulin resistance in PCOS across populations. Insulin resistance and diabetes are higher in Mexican Americans than Caucasian Americans and this was reflected in a study in the USA which found a higher prevalence of insulin resistance in Mexican American women with PCOS (73.1%) compared with Caucasian American women with PCOS (43.8%) (Cowie et al. 2009, Kauffman et al. 2002). However, there was no difference in the women with a BMI < 24 kg/m² (Kauffman et al. 2002). Similarly, in a comparison of Caribbean Hispanic women and non Hispanic white women with PCOS in the USA compared with controls matched for ethnicity, age and body composition there was an additional detrimental effect of Caribbean Hispanic ethnicity on insulin sensitivity (Dunaif & Sorbara 1993). In contrast to these findings, as already noted, Carmina et al. compared Hispanic women from the USA, Japanese and Italian women, all with PCOS. Obesity was less common in the group from Japan and they had lower fasting insulin levels. However, insulin resistance, as defined by the insulin tolerance test, was similar and occurred in 70% of all participants (Carmina et al. 1992). A further study of Italian women compared to non Hispanic white women in the USA found the American women to be more insulin resistant, which was

largely due to more significant obesity and possibly a higher saturated fat diet (Carmina et al. 2003).

Women of South Asian heritage are known to have higher risks of insulin resistance, diabetes and metabolic syndrome than their counterparts of Caucasian heritage. Two studies have compared women with PCOS of South Asian background with women from a Caucasian background, one in Australia/ South Africa and one in the UK. Norman et al. compared obese and non-obese women with PCOS and non-PCOS from Australian Caucasian and South African Indian backgrounds (Norman et al. 1995). They found that in the women with PCOS, hyperinsulinaemia was more common in the South African Indian women than the Australian women although the effect was less marked in obese women. Similarly, in non-obese controls insulin resistance was higher in the South African Indian women (Norman et al. 1995). A comparison of South Asian and Caucasian women in England also found that despite similar BMI and WHR that the insulin resistance of the South Asian women with PCOS was higher than that of the Caucasian women with PCOS and was associated with a lower SHBG. However there was no difference between the two control groups (Wijeyaratne et al. 2002).

1.8.2 Diabetes

1.8.2.1 Prevalence of IGT and Type 2 diabetes in PCOS

The insulin resistance, B cell dysfunction and obesity observed in PCOS, leads to an increased risk of affected women developing impaired glucose tolerance or type 2 diabetes mellitus. IGT is a known risk factor for developing diabetes and is usually asymptomatic. Therefore the identification of IGT through screening may be an important tool in preventing or delaying long term problems such as diabetes and associated health complications such as renal and cardiovascular disease.

The evidence supporting increased risk of diabetes and IGT in women with PCOS comes from epidemiological studies, prevalence of IGT and diabetes in women with PCOS and prevalence of impaired glucose metabolism in PCOS compared with controls. Evidence is mixed as to the significance of PCOS and diabetes in women of normal BMI.

There are reports of increased risk of diabetes with oligomenorrhoea, thus indirectly supporting the notion of increased risk of diabetes with PCOS. The Nurses Health Study in the USA, a large prospective study followed 101,073 predominantly Caucasian women over eight years found type 2 diabetes was increased by 2–2.5 fold in women with menstrual cycles greater than 40 days apart. This difference decreased but remained significant after adjusting for BMI (Solomon et al. 2001). Similarly, a cross sectional study of 695 Pima Indian women aged between 18 and 44 years found the prevalence of type 2 diabetes in women with a BMI $<30.0 \text{ kg/m}^2$ was 37% in women with oligomenorrhoea (cycles >3 months apart) compared with 13% of those with regular cycles (Roumain J 1998). However in women with a BMI $>30.0 \text{ kg/m}^2$ diabetes was high regardless of cycle characteristics. In contrast, a study of 874 white college graduates in the USA who prospectively kept menstrual diaries for five years and were reassessed for diabetes 56 years later did not find an association between long cycle length (42 days) and diabetes (Cooper et al. 2000). Differences may reflect different cycle lengths designated “oligomenorrhoea/long”, different populations assessed, and the different timing of the studies as diabetes has been rising in the USA since Cooper et al. originally recruited in 1934, to Solomon et al. 1989 and Roumain et al. in the early 1990’s.

Four longitudinal studies of women with PCOS, three prospective and one retrospective, have shown an increase in diabetes with PCOS compared either to controls or general population figures, although the contribution independent of obesity remains unclear. The diagnosis of PCOS was different in each study contributing to difficulties in determining the generalised applicability of findings.

Norwegian women with PCOS⁹ (n=149) were assessed 15–25 years after open ovarian wedge resection and found to have an increase in standardized incidence ratio of 6.1 (2.2–1.3) for diabetes. However, the researchers could not exclude that obesity, rather than PCOS, was the major contributing factor to diabetes (Lunde & Tanbo 2007).

⁹ Polycystic ovaries on histology following ovarian wedge resection plus one of oligomenorrhoea, hirsutism, obesity or infertility

Another small prospective study in Sweden following women who had ovarian wedge resection (n=33) found an increase in diabetes (15%) compared with 2.3% in age matched controls but there was no adjustment for obesity (Dahlgren et al. 1992).

A retrospective review of 319 women diagnosed with PCOS¹⁰ before 1979 and 1060 controls found an increased odds ratio of having diabetes in those with PCOS (OR 2.8 (95% CI 1.5–5.5, p=0.03) which became insignificant after adjusting for BMI (OR 2.2 95% CI 0.9–5.2, p=0.08) (Wild et al. 2000b).

In the Netherlands, Elting et al. 2001, conducted phone interviews with 346 women previously diagnosed with PCOS¹¹ and compared the proportion of reported diabetes with national figures for women in the same age groups. A higher proportion of women with PCOS had diabetes compared to those in the same age group, especially in those 45–54 years (Elting et al. 2001). However, when stratified by BMI category, there was no increase in diabetes with PCOS in the obese group.

Prevalence of abnormal glucose metabolism has been assessed in a number of cross sectional studies of women with PCOS and found to be higher than nationally comparable figures in Australian, Italian, Chinese, Thai and American (USA) women with prevalence of diabetes in PCOS being 4.0%, 2.5% ,1.9%, 17.7% and 7.5–10% respectively (Table 1.11)(Chen et al. 2006, Dabadghao et al. 2007, Ehrmann et al. 1999, Gambineri et al. 2004, Weerakiet et al. 2001). Importantly, these have shown significant risk of diabetes and IGT in young women <40 years (Ehrmann et al. 1999, Weerakiet et al. 2001).

Additionally, a few cross sectional studies have compared women with PCOS and controls for abnormal glucose metabolism. The largest compared 254 women aged 14–44 years with PCOS in both an urban and a rural location with 80 rural controls in the USA (Legro et al. 1999). They found that abnormalities of glucose metabolism were more prevalent in women with PCOS and that even slim women with PCOS had IGT and

¹⁰ diagnosed with definite or possible PCOS by histological or ultrasound diagnosis plus evidence of ovarian dysfunction

¹¹ Oligo-/amenorrhoea and increased LH in the presence of normal FSH.

diabetes. The urban group (n=144) was ethnically diverse, although the single largest group was Caucasian at 47%, and the rural group (n=110) was all Caucasian. Just over a third of the women with PCOS (38.6%) had an abnormal oral glucose tolerance test (OGTT); 31.1% had IGT and 7.5 % had Type 2 diabetes mellitus. This was markedly higher than the controls of whom 14% had IGT and none diabetes mellitus (1985 WHO). In contrast to other studies, a significant proportion of slim women with PCOS (BMI<27kg/m²) had an abnormal OGTT; 1.5% had diabetes and 10.3% had IGT. There was no difference between PCOS women from the two different locations.

The factors most predictive of risk of glucose intolerance have been found to be: PCOS, age, obesity and family history of diabetes (Ehrmann et al. 1999, Legro et al. 1999, Weerakiet et al. 2001).

Table 1.11 Prevalence of impaired glucose tolerance (IGT) and diabetes mellitus (DM) in women with PCOS

Author	PCOS diagnostic criteria	Participants	Age (years)	BMI (kg/m ²)	% with diabetes (n)	% with IGT (n)
Dabadghao et al 2007	Rotterdam	Australian Recruited from reproductive endocrine clinic in Adelaide Ethnicity not recorded N=372	30.3 + 5.6	35.1+ 8.0	4.0 (15)	15.6 (58)
Chen et al. 2006	Rotterdam	Chinese women from gynaecology outpatients at major hospital N=102	24.3 + 6.0	21.7 + 4.3	1.9 (2.0)	20.5 (21)
Weerakiet et al. 2001	Presence of all three Rotterdam criteria	Thai	28.2 + 6.2	27.2 + 5.9	17.7 (14)	22.8 (18)
Gambineri et al. 2004	Presence of all three Rotterdam criteria	Women attending endocrine unit Italy, n=121	14.0–37.0	*	2.5 (3)	15.7 (9)
Ehrmann et al. 1999	Oligomenorrhoea plus hirsutism/hyperandrogenaemia/infertility/acne	Women attending medicine, paediatric, gynaecology or endocrinology clinics who were > 2 years post menarche, Chicago. Ethnicity not specified.	13.5– 40.0	*	10.0 (12)	35.0 (43)
Legro et al. 1999	NIH	Recruited from private practices and advertisements in two locations, USA. Ethnicity mostly non-Hispanic white 47% (Pennsylvania) and 96% (Mt.Sinai) Total PCOS, n=254				
		Pennsylvania, n=144	28.0 ± 6.0	35.9 ± 8.0	7.3 (11)	30.0 (46)
		Mt.Sinai, n=110 Controls (Pennsylvania) , n=80	27.0 + 5.0 30.0 + 7.0	29.9 + 8.1 32.7 + 8.8	7.6 (8) 0	31.9 (33) 14.0 (11)

*not specified for the study group as a whole

Importantly, studies in adolescents have shown that the risk of diabetes and IGT in PCOS may be very high even at an early age although this would appear to be only in those who were overweight or obese (Arslanian et al. 2001, Lewy et al. 2001, Palmert et al. 2002, Silfen et al. 2003). Obesity may therefore be more significant than PCOS – an hypothesis supported by the findings of Silfen et al. 2003 and Nur et al. 2009 of no difference in glucose intolerance between obese girls with and without PCOS (Nur et al. 2009, Silfen et al. 2003).

1.8.2.2 Progression to diabetes in women with PCOS

The proportion of subjects in the general population who progress from IGT to type 2 diabetes varies from 13–52%, with a rate of progression of 1–6%/year. Of the risk factors studied to date, the single most important predictor for the subsequent progression is the baseline glucose concentration. Other less consistent factors include BMI, age and serum insulin (Edelstein et al 1997, Petersen et al 2005, Unwin et al 2002).

The rate of progression has been shown to vary with ethnicity: one study with South Asian Indians in South Africa found 50.4% of subjects progressed to type 2 diabetes over four years, one of the highest recorded in the literature (Motala et al 1993). Two other populations with both high prevalence of diabetes and high risk of progression from IGT to diabetes are Pima Indians, 31% over 1.6–11yr (rate 5–6%/yr) and Nauruans 26% over six years (rate 4%/year) (King et al 1984, Saad et al 1988).

The conversion from IGT to type 2 diabetes is accelerated 5–10 fold in PCOS. Factors reported to accelerate this are higher levels of glucose, family history, obesity, lower stimulated insulin levels and ethnicity (Ehrmann et al. 1999, Legro et al. 2005, Norman et al. 2001).

1.8.2.3 The risk of PCOS among women with type 2 DM

Conn et al. 2000 documented the prevalence of PCO in 38 women with type 2 diabetes. In a cross sectional study of 38 women they found 82% of women had PCO. Of these, 31% had hirsutism and/or 26% menstrual disturbances (Conn et al. 2000).

A retrospective review of medical records reported the prevalence of PCOS in women with Type 2 diabetes mellitus to be 26.7% (Peppard et al. 2001).

Whilst these are small, uncontrolled studies and Conn's study was of PCO only, they suggest there may be basis for the hypothesis that the risk of PCOS is increased in women with type 2 DM.

1.8.2.4 Family history of diabetes in women with PCOS

Women with PCOS are more likely to have a family history of diabetes, and abnormalities of glucose tolerance are probably increased in first degree relatives of women with PCOS (Ehrmann et al.1999, Ehrmann et al.2005, Legro et al.1999).

IGT and type 2 DM have been found to be present in more mothers and fathers of women with PCOS (40–58%) than controls (15%) (Yildiz et al. 2003, Yilmaz et al. 2005). Comparing mothers and sisters of women with PCOS and normal glucose tolerance to mothers and sisters of controls has shown the PCOS relatives had a higher WHR, mean testosterone and insulin resistance, and 8% and 16% of these respectively were identified as having PCOS themselves (Yildiz et al. 2003). Additionally, family history of diabetes is itself a risk for developing diabetes and insulin resistance; those with a family history or even current diabetes were included in the PCOS relatives, but excluded from the controls (Yildiz et al. 2003, Yilmaz et al. 2005). Kaushal et al. confirmed the importance of family history of diabetes when assessing brothers of women with PCO, the risk of insulin resistance was higher in those with a family history of diabetes than those with no family history of diabetes (Kaushal et al. 2004).

Baillargeon assessed brothers of women with PCOS and controls excluding those with diabetes from both groups and not discriminating by family history of diabetes, although this was still higher in the brothers of PCOS women. There was a higher proportion of IGT in the brothers of women with PCOS at 18.0% compared to none in the controls ($p=0.05$). The brothers of women with PCOS had a 38% decrease in insulin sensitivity associated with decreased tolerance to glucose and higher post stimulated insulin. These effects were mainly seen in the obese brothers

(BMI>26.5kg/m²) implying a synergistic effect of heredity and acquired obesity (Baillargeon & Carpentier 2007).

Other studies have shown decreased disposition index, lower insulin sensitivity index and abnormalities of insulin secretion and action in first degree relatives of those with PCOS (Legro et al. 2002, Sam et al. 2008).

1.8.2.5 Gestational Diabetes

Gestational diabetes mellitus (GDM) develops in about 5–9% of pregnancies, though it does vary with ethnicity, obesity, maternal age and socioeconomic status (Anna et al. 2008, Serlin & Lash 2009). Indigenous Australian women or those born in Asia, Africa, Middle East have a higher risk than Caucasian women in Australia (Anna et al. 2008, Ishak & Petocz 2003). GDM is associated with increased risks of poorer short term outcomes including: pre-eclampsia, macrosomia with subsequent delivery implications for mother and baby such as shoulder dystocia with brachial nerve palsy, increased risk of caesarean delivery and neonatal hypoglycaemia (Ben-Haroush et al. 2009, Serlin & Lash 2009, Yogeve et al. 2009, Yogeve & Visser 2009). The resultant exposure to hyperglycaemia in utero may also play a role in the development of insulin resistance, metabolic syndrome, obesity and diabetes in the offspring through childhood and adult life (Boney et al. 2005, Pettit et al. 1993, Vohr et al. 1999, Vohr & Boney 2008, Yogeve & Visser 2009).

Women with PCOS have been shown to be insulin resistant both before and during pregnancy and pregnancy itself induces insulin resistance. Therefore it would be anticipated that women with PCOS may be more at risk for GDM than other women.

In studies with PCOS and GDM difficulties arise in assessing the literature as varying definitions of PCOS and GDM are used. In assessing the link between these metabolic syndromes there have been two main approaches: identifying women who have had GDM and assessing them post partum for PCO/PCOS and identifying women who have PCOS and assessing GDM in pregnancy. Most of the research to date in PCOS and GDM has been with Caucasian women and the majority has been retrospective.

Results are conflicting with some supporting a higher risk of GDM in women with PCOS whereas others do not (Anttila et al. 1998, Holte et al. 1998, Kousta et al. 2000, Koivunen et al. 2001, Mikola et al. 2001, Radon et al. 1999, Vollenhoven et al. 2000, Wortsman et al. 1991). Apart from the issues of heterogeneity in the diagnosis of PCOS and GDM other potential confounders include: variation in baseline risk such as the inclusion of women with known diabetes, obesity, multiple pregnancy, maternal age and parity.

Two meta-analyses have found increased odds ratio for GDM in PCOS of 2.89 (95% confidence interval 1.68–4.98) and 2.94 (95% confidence interval 1.70–5.08) (Boomsma et al. 2006, Toulis et al. 2009). Toulis et al. however reported that further study needs to be done to validate this finding due to the significant heterogeneity of the studies and dependence of outcome on study type. Boomsma reported co-existing obesity may be important.

1.8.2.5.1 Screening for glucose intolerance in PCOS

Lifestyle and/or pharmacological intervention can delay or prevent progression to type 2 diabetes and long term consequences of diabetes. Given the higher risk of glucose intolerance in women with PCOS, screening is recommended for diabetes and IGT – although the guidelines vary.

The most specific and sensitive method for diagnosing IGT and diabetes mellitus, particularly in PCOS, is the oral glucose tolerance test. This is time consuming and it has been argued impractical to screen all women with PCOS. Some guidelines have therefore recommended screening women with the most risk; those who are obese, have a family history of diabetes, a history of GDM and increasing age. The Androgen Excess Society statement argues that an OGTT should be offered to all women with PCOS (Salley et al. 2007). Repeat screening should occur around every 2 years unless there additional risk factors and annually if a woman has IGT.

1.8.3 The Metabolic Syndrome

The prevalence of metabolic syndrome in women with PCOS varies depending on the age and ethnicity of the population studied, but has been reported across different

populations to be higher in women with PCOS than in women without PCOS in a number of studies (Apridonidze et al. 2005, Barber et al. 2007, Cheung et al. 2008, Coviello et al. 2006, Ehrmann et al. 2006, Glueck et al. 2003, Soares et al. 2008).

Four of the larger studies in the USA have reported that metabolic syndrome was present in 33.4%–47.3% of women with PCOS (Apridonidze et al. 2005, Dokras et al. 2005, Glueck et al. 2003). However, the women studied were predominantly obese and whilst it is known that age and obesity affect metabolic syndrome these were not accounted for in all studies. Dokras et al. reported that when stratified into obese and non-obese, there was no significant difference in prevalence of metabolic syndrome (Dokras et al. 2005).

Conflicting results are seen in studies of adolescent girls. One study with PCOS age-matched to girls from NHANES III, were 4.5 times more likely to have metabolic syndrome even after adjusting for BMI (Coviello et al. 2006). However, a study of adolescents with PCOS, who were all overweight or obese, compared with BMI matched controls, found a high proportion had metabolic syndrome regardless of PCOS status (Rossi et al. 2008).

Studies of European women have found a lower prevalence of metabolic syndrome in women with PCOS perhaps due to lower obesity, 8.0%–16.0% in PCOS compared to 0–2.4% depending on criteria used (Carmina et al. 2006a, Vrbíková et al. 2005, Vural et al. 2005). Metabolic syndrome however, still remained higher in women with PCOS (Carmina et al. 2006a, Vrbíková et al. 2005, Vural et al. 2005).

In Australia, Cussons et al. found a prevalence of metabolic syndrome of 33–40% in women with PCOS that was increased independent of obesity only in those with a BMI > 30 kg/m² and was related to free androgen index (Cussons et al. 2008).

Factors affecting varying results include different exclusion criteria for factors that may influence metabolic syndrome diagnosis such as diabetes and current hormonal treatment. The definition of metabolic syndrome and PCOS that are used will also influence findings. Those with hyperandrogenism appear to have a higher prevalence

of metabolic syndrome compared to controls and women with polycystic ovaries and no hyperandrogenism (Barber et al. 2007, Carmina et al. 2006a).

It would appear that the prevalence of metabolic syndrome is increased in women with PCOS, although a significant effect is the influence of increasing obesity.

1.8.4 Cardiovascular disease (CVD)

Cardiovascular disease remains one of the leading causes of death in women. Many of the risk factors particularly those of hyperinsulinaemia, dyslipidaemia, glucose intolerance, diabetes, hypertension and central obesity are increased in women with PCOS. High androgens and low SHBG have also been shown to increase CVD risk in both pre- and post- menopausal women (Sutton-Tyrrell et al. 2005).

There are limited studies to date suggesting that women with PCOS have subclinical evidence of premature CVD. However, the long term outcomes studies examining the prevalence of CVD among women with PCOS have failed to demonstrate a significantly increased risk of cardiovascular death. Possible explanations for this discrepancy include the long lag time between the diagnosis of PCOS in the reproductive years and that of cardiovascular events later in life, leading to data inaccuracy. Further, women who seek treatment for their symptoms may ameliorate their risk factors. There is also the pervasive problem of heterogeneity of diagnosis of PCOS.

1.8.4.1 Cardiovascular events and PCOS

Pierpoint et al. conducted a study of mortality of 786 women in the UK with PCOS who were followed up after an interval, on average of 30 years, from their diagnosis (Pierpoint et al. 1998). The women were sourced from hospital records across the UK and classified as definite PCOS (histological PCO with clinical evidence of ovarian dysfunction) or probable PCOS (macroscopic appearance of PCO eg. at laparoscopy and clinical ovarian dysfunction or diagnosis by an experienced clinician). They compared death rates in these women with the expected rates as calculated from the National mortality rates and did not find any increase in cardiovascular related deaths, though there was an increase in diabetes related complications. Whilst this may have

been due to the fact that a large number had been treated with wedge resection of the ovary at the time of diagnosis this would not be expected to show long term benefits.

A subset of 319 women from this group who also had at least one feature of acne, hirsutism, oligomenorrhoea or infertility was subsequently assessed and compared to 1060 matched controls. Again they found an increased prevalence of several cardiovascular risk factors: diabetes ($P=0.002$), hypertension ($P=0.04$), hypercholesterolaemia ($P<0.001$), hypertriglyceridaemia ($P=0.02$) and increased waist:hip ratio ($P=0.004$) in the women with PCOS but no excess cardiovascular mortality or morbidity.

The increase in cardiovascular risk factors was contributed to by obesity and, after adjustment for BMI, the only risk factors that still had a significantly greater odds ratio in those with PCOS were hypercholesterolaemia and cerebrovascular disease (Wild et al. 2000b).

A cross sectional evaluation of 143 women in New Zealand under 60 years of age who presented for coronary angiography for assessment of chest pain or valvular disease found polycystic ovaries in 42% of the women. Polycystic ovaries were associated with: hirsutism; higher testosterone, triglyceride and C peptide levels; and lower HDL-C. By multivariate logistic regression analysis, the extent of coronary artery disease was found to be independently associated with the presence of polycystic ovaries. The odds ratio for more than 50% arterial stenosis was 1.7 (95% CI 1.1–2.3) for women with PCO compared with women with normal ovaries (Birdsall et al. 1997).

1.8.4.2 Cardiovascular events and chronic anovulation

Two large epidemiological studies have shown an increase in cardiovascular disease in anovulatory women. The first was a large Dutch breast cancer screening study, which found a greater incidence of anovulatory cycles in women who later developed cardiovascular disease (Gorgels et al, 1997). The Nurses Health Study in the USA following just over 80,000 women found that, compared with women reporting regular cycles, those with irregular cycles had an increased risk for both non-fatal and

fatal coronary heart disease adjusted relative risk 1.53 (95% CI 1.24–1.90) (Solomon et al. 2002).

1.8.4.3 Cardiovascular risk factors and PCOS

Insulin resistance, diabetes and metabolic syndrome in PCOS have all been discussed. Cross sectional studies also generally support that PCOS is also associated with dyslipidaemia, the most common abnormality being low HDL-C. Other abnormalities seen include hypertriglyceridaemia and increased small LDL-C particles (Holte et al. 1994a, Wild et al. 2000a, Yildirim et al. 2003). Other less traditional risk factors for cardiovascular disease are increased including CRP and plasminogen activator inhibitor -1(PAI-I).

A smaller longitudinal prospective study of a nested group of women from the North Finland birth cohort at age 31 years found women with self reported symptoms of PCOS (hirsutism and/or oligomenorrhoea, n=518) had lower HDL-C, higher triglycerides, WHR and CRP compared to controls (n=1036). Much of this effect was mediated by obesity; when stratified by BMI, obese women with PCOS symptoms had a significantly lower HDL-C than controls and overweight women with PCOS symptoms had a greater WHR but the other differences were no longer significant (Taponen et al. 2004).

1.8.4.4 Early markers of cardiovascular disease

Many studies have used different methods to assess aspects of cardiovascular risk in women with PCOS. Non-invasive surrogate markers of vascular disease are used extensively in studies as markers of early vascular disease. Intimal media thickness (IMT) is an established marker for early atherosclerotic disease that is predictive of future cardiac events.

Talbott et al. 2000 used women previously recruited from practice records of a reproductive endocrinology clinic and community controls seven years earlier who were then greater than 30 years of age to assess IMT and other cardiovascular risk factors. One hundred and twenty five Caucasian women with PCOS and 142 Caucasian community controls participated. In the total sample there was no

difference in the two groups but in the women >45 years old, PCOS was a significant predictor of carotid IMT (P= 0.003). This would be understandable as metabolic abnormalities have a long lag time to cause measurable physical effects. In this same subgroup of women BMI, LDL-C also both exerted an independent effect on IMT. Obesity (WHR) and log insulin however did eliminate the effect of PCOS as an independent predictor of IMT in this subgroup of women. It would seem then that in part the association between PCOS and IMT in women >45 yr is due to central obesity and hyperinsulinaemia.

Obesity and hyperinsulinaemia have both been found to be related to increased IMT in earlier studies. It is also possible that other related factors such as plasminogen activator inhibitor-1 (PAI-1), CRP and tumour necrosis factor may have a role (Talbot et al. 2000).

An observational study in Australia examined carotid IMT along with other assessments in 80 women with PCOS. It was found that DHEAS was inversely related to IMT suggesting that it may have a protective effect in early atherosclerotic disease (Meyer et al. 2005). This effect of DHEAS had been suggested previously by Bernini et al. who showed that IMT was negatively correlated with DHEAS and Slowinska et al. who found that women with coronary artery stenosis had lower DHEAS than healthy controls (Bernini et al. 1999, Bernini et al. 2001, Slowinska-Szrednicka et al. 1995). In contrast, a large study (n=900) of postmenopausal women found that higher levels of DHEAS were associated with several major cardiovascular risk factors. However there was no association with fatal cardiovascular events (Barrett-Connor et al 1995).

1.9 Summary and rationale for this thesis

The focus of this thesis is the burden of disease PCOS and associated complications may impose on Aboriginal and Torres Strait Islander women. PCOS is a common endocrinological problem for all pre-menopausal women with reproductive and metabolic implications. The prevalence and complications can vary with ethnicity as already described whether this is due to genetic factors or, more likely, socioeconomic and environmental influences related to ethnicity and disadvantage.

It is proposed PCOS may pose more significant problems for Australian Indigenous women for the following reasons:

- health professionals report significant problems due to PCOS in these women
- the pilot study by Davis et al. 2008 of 35 Aboriginal women which found 18% had PCOS (Davis et al. 2002).
- rising obesity and central obesity in the Indigenous population (Piers et al. 2003)
- high frequency of diabetes and related glucose abnormalities in Indigenous people at a young age (DeCourten M et al. 1998, O'Dea et al 2008).
- significant infertility in Indigenous women despite overall higher fertility rates compared with non-Indigenous Australians (Kildea and Bowden 2000, Menon A & Coppola A 2009, Australian Bureau of Statistics & Australian Institute of Health and Welfare 2008).
- low birth weight and other adverse intra-uterine factors that increase the risk of adult disease including insulin resistance and diabetes (Leeds et al 2007).

Azziz et al. estimated that the diagnosis and management of PCOS in the USA conservatively accounts for over \$4 billion annually. Around 40% of this cost is due to the increased prevalence of diabetes, 30% to menstrual dysfunction, 12% to hirsutism and 12% to infertility treatment (Azziz et al. 2005). Using the same prevalence figures and proportion of women with associated fertility and metabolic abnormalities as Azziz, Teede estimated the cost of PCOS to Australian women of reproductive age is around \$40 million annually. Therefore there are economic as well as policy and practice imperatives to examine the possibility of a higher frequency of PCOS in Indigenous women.

The aim of this thesis therefore is to assess the frequency of PCOS, its components and associated metabolic variables in a group of Australian Indigenous women from an urban area in the Top End of the Northern Territory.

Chapter 2 Methods

2.1 Participants

This study was conducted as part of the National Health and Medical Research Council (NHMRC)-funded DRUID study (Diabetes and Related conditions in Urban Indigenous people in the Darwin region) and methods have been published (Cunningham et al. 2006). The following are eligibility criteria for the DRUID study:

- Participants identify as Aboriginal and/or Torres Strait Islander
- age ≥ 15 years
- resided within the Yilli Rreung Aboriginal and Torres Strait Islander (ATSI) region for at least the last 6 months
- living in a private dwelling (ineligible if living in an institution such as boarding house, boarding school, nursing home or correctional facility).

Recruitment was through advertisements in the community, recruitment through the local Aboriginal controlled Health service (Danila Dilba) in Darwin and local high schools, personal advocacy of the project by members of the Indigenous Steering Committee and members of the project team, and a mail out.

Informed consent was obtained by project workers after participants had read a pamphlet and viewed a video explaining the study.

Of 467 women who participated in DRUID in the specified age group, 15–44 years. 6 were pregnant and excluded, leaving 461 eligible to participate. Of these, 456 women consented to take part in the study, 424 women completed the Women's reproductive health questionnaire and 393 completed both questionnaire and had bloods taken. The remainder had either bloods taken *or* answered the questionnaire.

2.1.1 Population Representation

There was an excellent response from women in DRUID who agreed to participate in the Women's Reproductive Health questionnaire of 92% (424 of 461 eligible women), those who agreed to an assessment of reproductive hormones including

androgens of 91% (419 of 461), and 85% (393 of 461) who both answered the questionnaire and had a hormonal assessment.

The Aboriginal and/or Torres Strait Islander female population of the Yilli Rreung ATSIC region aged 15-44 years living in private dwellings in 2004 was estimated according to Australian Bureau of Statistics Data from the 2001 Census as 6113. The estimated numbers by age group were 1191 between 15-24 years, 1025 between 25-24 years and 846 between 35 and 44 years. Based on these figures, 12% of women 15–24 years, 13% of 35–34 years and 17.0% of 35–44 years participated in this women’s reproductive health study. It is not possible to comment on the representativeness of the sample. Therefore the frequencies reported are not population prevalence but represent frequency within the cohort studied. .

2.2 Ethics

Ethics approval for the project was given by the combined Human Research Ethics Committee (HREC) of NT Department of Health and Community Services and Menzies School of Health Research (MSHR), Darwin. The Aboriginal Subcommittee of the HREC has veto rights for applications concerning Aboriginal participants. The studies reported in this thesis are covered by two different ethics approvals granted by the combined HREC of NT Department of Health and Community Services and Menzies School of Health Research: The DRUID study: Diabetes and related conditions in urban Indigenous people in the Darwin region- Stages 1 & 2 (02/65) and Polycystic Ovary Syndrome in Indigenous Women (03/44).

2.3 Screening

The following information was obtained from the baseline screening for both population groups: lifestyle questionnaire, Women’s reproductive health questionnaire , anthropometry, blood pressure, fasting blood glucose, total cholesterol, triglycerides, HDL-C, LDL-C, HbA1c, insulin, homocysteine, hs-CRP, DHEAS, SHBG, AMH, testosterone, calculated free testosterone, free androgen index (FAI) and urine albumin to creatinine ratio (urine ACR). Any woman thought to have possible PCOS based on the diagnostic criteria in Section 2.3.7 had further

investigations including: thyroid stimulating hormone (TSH), prolactin and 17-hydroxyprogesterone (17-OHP).

2.3.1 Lifestyle and Women's Reproductive Health Questionnaire

The DRUID study involved three questionnaires. Questionnaire 1 included questions on medical history and lifestyle factors such as smoking and alcohol intake. There were also questions in relation to socioeconomic indicators including home ownership, education, private health insurance, employment and income. The other two questionnaires concerned psychosocial health (Questionnaire 2) and Women's reproductive health. The women's questionnaire included questions about: menarche, menstrual cycle regularity, hormonal contraceptive use, pregnancies, infertility, hysterectomy and menopause. The Women's reproductive health questionnaire is attached Appendix 4. The other questionnaires are not attached in their entirety due to their length. Appendix 3 contains the questions from Questionnaire 1 and Questionnaire 2 that were used for the analyses presented in this thesis.

2.3.2 Anthropometry

Participants wore light clothing with footwear removed. Body weight was recorded to the nearest 0.1kg (using a digital portable scale Seca Deutschland, Hamburg, Germany) and height to the nearest 0.1cm using a wall mounted stadiometer. Body mass index (BMI) was expressed as weight in kg divided by height in metres squared (kg/m^2). Waist and hip circumference were measured to the nearest 0.1cm with participants standing erect with arms at their sides and feet together. The waist measurement was performed by firstly marking the lowest rib margin with a felt tip pen on the skin, then palpating and marking the iliac crest in the midaxillary line. The point midway between the lowest rib margin and iliac crest was then used as the site of measurement of waist circumference, performed using a non-stretch tape measure in a horizontal plane at the end of expiration. The hip circumference was measured at the point yielding the maximum circumference over the buttocks with the tape in a horizontal plane over very light clothing. Waist and hip measurements were each taken three times and a mean value calculated. The waist-hip ratio (WHR) was calculated as mean waist circumference divided by mean hip circumference.

2.3.3 Blood Pressure

Blood pressure and heart rate were measured in the sitting position after 5 minutes rest using an automated Welch Allyn “Spot Vital Signs” (Welch Allyn Medical Products, Skaneateles Falls, NY, USA). Three blood pressure measurements were performed each one minute apart. Heart rate was recorded on the second measurement only. Systolic and diastolic blood pressure were each calculated as the mean of the second and third measurements. Hypertension is defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg.

2.3.4 Fasting blood sample

The DRUID study was conducted in Darwin or surrounding areas. Venipuncture was performed after an overnight fast and blood was drawn directly into multiple vacutainers containing EDTA, fluoride, lithium heparin and plain (serum). After a fasting blood sample, a 75gm oral glucose tolerance test (OGTT) was performed on all eligible participants except : those who were previously diagnosed with diabetes and reported being on diabetes medication; those who were pregnant and those who did not give consent. Participants were classified as having diabetes if they met any of the following criteria: (1) fasting plasma glucose (FPG) ≥ 7.0 mmol/L; (2) 2-h plasma glucose (2hPG) ≥ 11.1 mmol/L; or (3) previously diagnosed as having diabetes and currently taking tablets and/or insulin for diabetes. Among participants who were not currently taking tablets and/or insulin for diabetes, impaired glucose tolerance (IGT) was considered present if FPG < 7.0 mmol/L and 2hPG > 7.8 and < 11.1 mmol/L; impaired fasting glucose (IFG) was considered present if FPG was ≥ 6.1 and < 7.0 mmol/L, and 2hPG was less than 7.8 mmol/L. Tubes were kept on ice from immediately after blood was taken (except serum tubes were left at room temperature for 10 minutes to allow blood to clot) and were centrifuged after one hour. They were then stored at Menzies in a Revco freezer (Kendro Laboratory Products, Ashville, NC, USA) at MSHR at -80°C . Frozen samples were transported by courier on dry ice to Adelaide for analysis.

Samples were analysed at The Clinical Trials Laboratory, Flinders Medical Centre, Bedford Park, South Australia, in a laboratory accredited in the U.S CDC Lipid Standardisation Program. Plasma glucose and lipids were measured by standard

automated colorimetric methods using commercial reagents on a Hitachi 917 analyser; HbA1c was assayed using cation exchange high performance liquid chromatography (HPLC) on a Pharmacia MonoS column; plasma insulin was measured by immunoassay on an Abbott AxSYM; plasma homocysteine assayed by HPLC after conversion to a fluorescent derivative with ABDF (7-Fluoro-2,1,3-benzoxadiazole-4-sulfonamide); CRP was assayed by immunoturbidimetry on a Hitachi 917 analyser at The Clinical Trials Laboratory, Flinders Medical Centre as above.

SHBG, testosterone, FAI, free testosterone, 17-hydroxyprogesterone, prolactin and thyroid stimulating hormone (TSH) analysis was performed by Dr. Alan Gilmore at Repromed Medical Laboratory (RML) Adelaide and AMH and DHEAS were performed by Mr. Brenton Bennett at the same institute. Samples for SHBG estimation were analysed with an IRMA assay, Orion Diagnostica.

Immunoradiometric Assay Cat No. 68563; testosterone was analysed with a DSL – 4100 RIA (Radioimmunoassay) kit, DSL; AMH levels were measured using a commercially available AMH Enzyme Immunoassay (Beckman Coulter, Marseille, France) and DHEAS using the DHEAS RIA kit (cat. No. DSL-2700) from Diagnostic System Laboratories.

The free androgen index (FAI) was calculated by $FAI = \frac{TT \text{ (nmol/L)}}{SHBG \text{ (nmol/L)}} \times 10$ and the free testosterone was calculated with the Vermeulen equation, assuming a serum albumin concentration of 43g/L (Vermeulen et al. 1999).

Women who had a free androgen index greater than 5.4 and oligomenorrhoea had further analysis of their samples, also at RML, for 17-hydroxyprogesterone, DHEAS, TSH and prolactin to exclude other causes of hyperandrogenaemia and oligomenorrhoea, apart from PCOS. The value of FAI at 5.4 was chosen as the determining level at the study inception as this was the upper limit of normal for the RML laboratory and at the beginning of the study was the only reference range available. Samples for 17-OH Progesterone estimation were analysed with a DSL – 6800 RIA (Radioimmunoassay) kit, DSL. TSH and Prolactin were analysed on the Centaur Advia Automated Chemiluminescence system. All intra-assay and inter-assay coefficients were less than 10%.

The HOMA method was used to calculate a measure of insulin resistance using the results of fasting glucose and insulin. HOMA-IR score of insulin resistance = (fasting glucose, mmol/L x fasting insulin, mU/L)/ 22.5.

High total cholesterol is ≥ 5.5 mmol/L, high triglycerides is ≥ 2 mmol/L and low HDL cholesterol is ≤ 1.0 mmol/L

2.3.5 Medical history and use of medications

For the DRUID study, information on history of medical conditions was self-reported by participants. Current use of medications was also self-reported.

2.3.6 Menstrual cycle assessment

Assessing cycle regularity from the reproductive health questionnaire involved some classification subsequent to the answers as follows (Appendix 4 and Figure 2.1):

1. Women who reported irregular cycles but cycle length between 24–34 days have been termed “regular.”
2. Those that reported regular cycles but cycle length greater than 35 days have been classified as irregular.
3. Those that reported don’t know to whether cycles are regular or not but
 - i) reported cycle length greater than 35 days are coded as irregular,
 - ii) reported cycle length as <24 days are coded as regular
 - iii) reported cycle length as 24–34 days are coded as regular
 - iv) reported cycle length as don’t know/varies are coded as “don’t know”
4. Regardless of what a woman answered to cycles if she was currently using exogenous hormones (including the oral contraceptive pill, depo-provera, implanon or hormone replacement therapy) or was menopausal or had a hysterectomy classification was “not definable.” When assessing factors affecting menstrual cycles such as obesity and smoking these women were excluded from analysis.

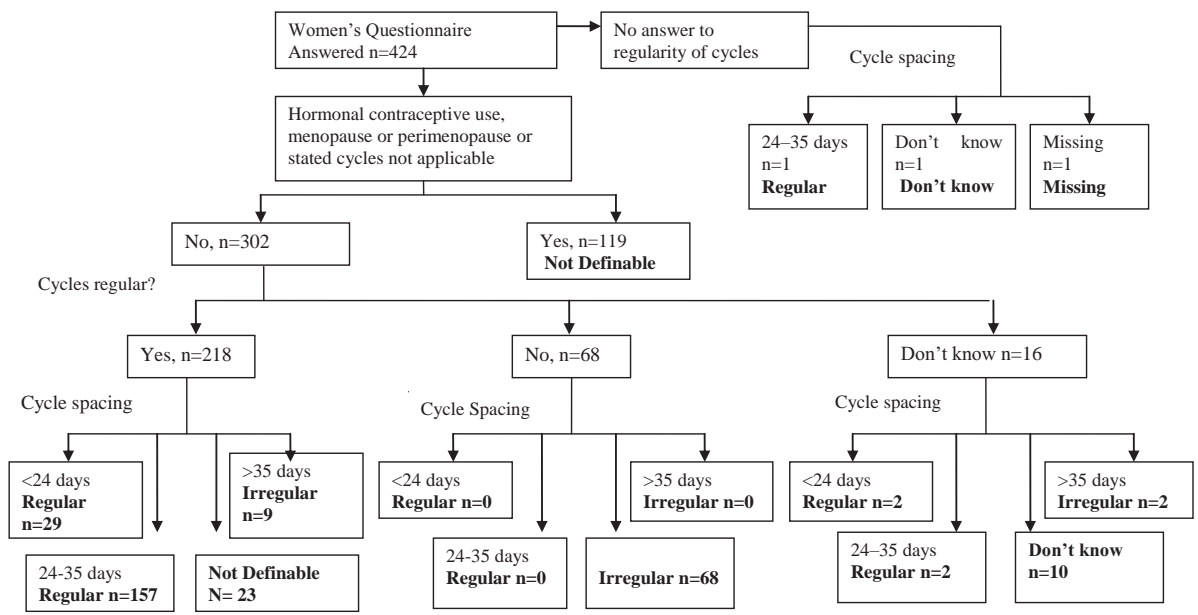
2.3.7 Diagnosis of PCOS

Diagnosis of PCOS was based on the NIH/NICHD criteria. This criteria determines PCOS as chronic anovulation and hyperandrogenism (biochemical or clinical) as discussed in Section 1.4.2.2. This classification was chosen because it is the one most

widely used in previous prevalence studies. Furthermore it would have been extremely difficult to perform pelvic ultrasounds to assess ovarian morphology in these settings. A private space for scans to be performed would have been extremely difficult to locate, the recruitment was prolonged, unpredictable and sporadic and in many of the obese women a transvaginal scan would have been required which would have been logistically difficult and probably not acceptable to most women.

However, an assessment of PCOS using the ESHRE/ASRM Rotterdam consensus was also made. This was modified in that AMH was used as a surrogate marker for polycystic ovaries, instead of ultrasound.

Figure 2.1 Classification of menstrual cycles: regular, irregular, “don’t know”, not definable.



2.3.7.1 Oligomenorrhoea/Anovulation

Those women classified as having “irregular cycles” were assumed to be anovulatory for the purposes of the diagnosis of PCOS by the NIH criteria. This was not confirmed with luteal phase progesterone measurements as this was not practically possible.

2.3.7.2 Hyperandrogenaemia

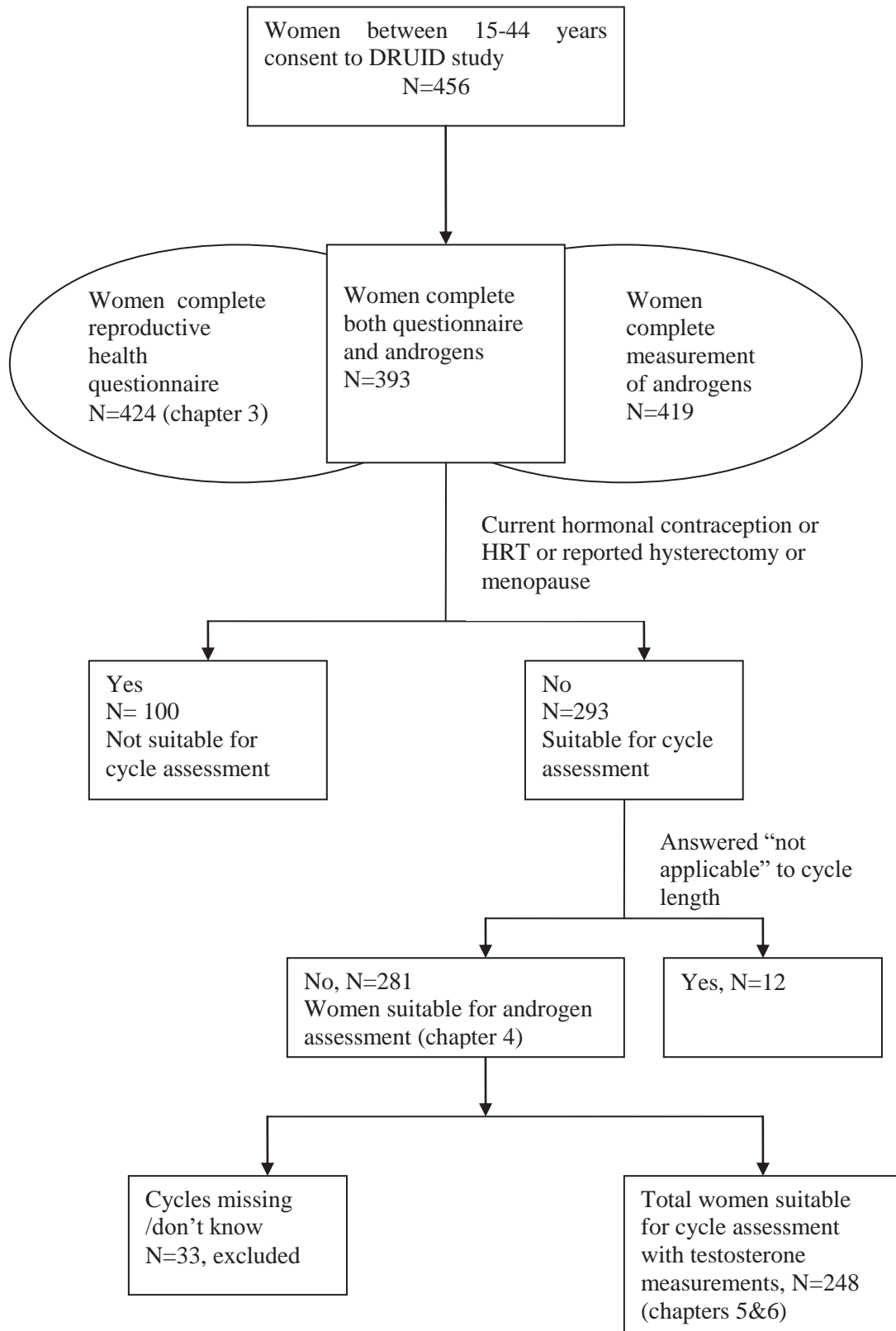
Initially in order to determine which women should have further testing for possible PCOS the upper limit of normal for the laboratory of $\text{FAI} > 5.4$ was used as the determining level. For the purposes of diagnosing PCOS, the 95th centile for free testosterone (34.2 pmol/L) of a group of Caucasian women without PCOS assessed at the same laboratory was used as described in section 4.2.

Hirsutism was not used to diagnose hyperandrogenism as it was not possible in the settings available to assess any more than facial hair due to privacy issues. Due to the prolonged time of recruitment it was also difficult to have consistency with hirsutism assessors and it was therefore decided to use only biochemical hyperandrogenism in the diagnosis of PCOS.

2.3.7.3 Polycystic Ovaries

As discussed in section 1.4.1.5 AMH has been shown to correlate with the number of antral follicles seen on ultrasound in women with polycystic ovaries. In this study, as it was impractical to perform ultrasound on all women AMH was used instead. A value greater than 23.0 ng/mL was deemed to be consistent with polycystic ovaries and this was based on findings from a study of AMH and pre-antral follicles on ultrasound in women presenting for fertility screening at Repromed, Adelaide (Poprzecny 2007).

Figure 2.2 Selection of Women for Assessment of PCOS



2.4 Statistical Analysis

Collected data was de-identified and entered into a Microsoft Access database. Statistical analyses were performed using Intercooled Stata 8.0 (Stata Corporation, Texas, USA). Analyses for the following summary statistics were performed: frequencies, means and standard deviations. Variables whose distribution was not normal were log transformed for analysis and summary statistics presented as geometric mean and 95% confidence interval. Data are presented as mean (SD) and groups compared using unpaired t test. Statistical significance was accepted at $P < 0.05$. A two-tailed χ^2 test was used to compare categorical variables. Regression was used to determine associations between multiple independent variables.

Data used for analysis and discussion varies throughout the thesis (Figure 2.2)

Chapter 3 Reproductive health characteristics: $n=424$, all participants who answered the women's reproductive health questionnaire

Chapter 4 Androgens: $n=393$, all women with androgen measurements who answered the women's reproductive health questionnaire, subgroup of pre-menopausal women not on exogenous hormones ($n=281$) and finally comparing those with regular and irregular cycles ($n=248$).

Chapter 5 PCOS, Chapter 6 PCOS and the Metabolic Syndrome: $n=248$, all women eligible for assessment of PCOS.

Chapter 3 Reproductive Health Characteristics

3.1 Introduction

Indigenous women are known to experience disproportionately poor health in many aspects of their lives, including their reproduction. Much of this poor health status is related to socioeconomic disadvantage and associated health behaviours.

Knowledge of contemporary reproductive issues for Indigenous women is sparse and what is available is fragmented. The aim of this study was to explore self reported features of reproduction including menstrual cycles, contraception, parity, and age of first childbirth in a group of urban Indigenous women in Darwin. The effects of factors known to influence these features including education, socioeconomic status (SES), smoking and obesity will be assessed.

3.2 Methods

The methods used are described in detail in the Chapter 2. Women who participated in a health examination as part of the DRUID study were asked to complete a women's health questionnaire and consent to reproductive hormone testing on the blood samples taken.

A total of 461 DRUID participants were females between 15–44 years old. Of these 456 women consented to take part in the Women's Health Study and of those 424 completed the women's reproductive health questionnaire (Appendix 4). Of those who completed the questionnaire 411 had anthropometry measures and 409 had blood taken. Information will be presented on the 424 who completed the women's health questionnaire including socioeconomic characteristics, anthropometry, menstrual cycle characteristics, hormonal contraceptive use and fertility. Biochemical data on androgens, lipids, markers of inflammation, diabetes and polycystic ovary syndrome are presented in Chapters 4–7.

Statistical analyses were performed using Stata version 8.0 (Stata Corporation, Texas, USA). Frequencies and median values with inter-quartile ranges were used to assess variables and groups are compared using Mann Whitney rank sum two sample test for continuous variables and a two-tailed Chi² test for categorical variables. Statistical

significance was accepted at $P < 0.05$. For variables of interest whose distribution was not normal, log transformation was used for analysis. Simple and multiple logistic regressions were used to determine associations between cycle irregularity and other factors and between hormonal contraceptive use and other factors.

3.3 Results

3.3.1 Socioeconomic and anthropometric characteristics of participants

Key socioeconomic and anthropometric characteristics are presented in Table 3.1. They show that, among women in the sample, a minority had private health insurance (19%), lived in a privately owned home (40.3%) and had post school qualifications including certificates, trade qualifications or university degree (31.6%). Smoking was common and increased with age. Regardless of how it was measured, obesity was also common and increased with age (Table 3.1).

Table 3.1 Socioeconomic and Anthropometric characteristics of participants

	Age Groups (years)			
	15–24 (n=143) %	25–34 (n=136) %	35–44 (n=145) %	Total (n=424) %
Has private health insurance	10.5	19.9	27.6	19
Living in house owned or being purchased by occupier	34.3	33.8	52.4	40.3
Full time work	22.4	53.7	57.2	44.3
Gross Weekly Household Income (\$)				
1–399	34.3	20.6	18.6	24.5
400–799	16.8	31.6	16.2	24.8
800+	8.4	34.6	45.5	29.5
not stated	40.6	13.2	9.7	21.2
Highest Qualifications				
Current school student	35.9	n/a	n/a	
No formal qualifications	9.1	15.4	11.7	12.0
Year 10	18.9	24.3	22.8	22.6
Year 12	12.6	23.5	18.6	18.4
Trade, certificate, diploma	11.9	29.4	27.6	23.1
University or higher	2.1	6.6	16.6	8.5
Smoking Status				
Current smokers	43.6	47.0	58.2	49.2
Former smokers	19.3	22.4	19.2	20.6
Anthropometry				
BMI*				
Overweight (25–29.9 kg/m ²)	18.9	29.3	29.3	25.8
Obese (≥30kg/m ²)	24.6	34.6	37.1	32.0
WC#				
≥ 80cm	47.4	77.3	77.5	67.5
WHR**				
Obese (0.80)	60.9	76.3	81.9	73.1

*BMI= Body Mass Index (kg/m²) # Waist circumference** Waist: Hip ratio

Note: some values are missing or “declined to answer” and the following numbers apply. Private health insurance (N=379), Gross weekly household income (n=314), smoking status (n=415), BMI (n=411), Waist circumference (n=403), WHR (n=402)

3.3.2 Reproductive Characteristics

The majority of women (84%) said they had experienced menarche by 16 years of age. Given the relatively young age of this group of women, not surprisingly, only 11 women had had hysterectomies. Thirteen women (2.6%) reported being menopausal or going through menopause and five were currently using hormone replacement therapy.

Of the women who were pre-menopausal, without hysterectomy or current hormonal contraceptive use, 70.7% were classified as having regular menstrual cycles (Table 3.2).

Table 3.2 Self-reported Reproductive Characteristics of Participants (n=424)

All Women	Percent (n=424)
Regular menstrual cycles	70.7 *
Ever use of hormonal contraception	68.9
Oral contraceptive pill (OCP)	59.6
Implanon/depo provera (progestagens)	27
Previous pregnancy	67.9
Previous parity	66.5
Self-reported infertility	
Difficulty getting pregnant only	8.4
Repeated miscarriages only	5.3
Both infertility and miscarriage	3.1
Hysterectomy	2.6
Menopause/perimenopause	3.2
Women who have had a child [#]	Median [‡] (n=282)
Number of children	2.0 (2.0–3.0)
Age at first child	20.0 (18.0–24.0)

*Of n=270 women with classifiable cycles

[#] Note that of the 282 women who had children 16 responded “don’t know to age first child” and eleven didn’t respond to the subsequent questions about number of children and age first birth

[‡]Median with interquartile range

3.3.2.1 Children

Of the 282 women who reported a previous pregnancy, 26 (9.2%) did not report having any children. This was reported across the age groups though was highest in those aged 15–24 years at 26.5%, compared with 6–7% of those in the older age groups. Just under half of these women reported experiencing recurrent miscarriage (n=5), infertility (n=3) or both miscarriage and infertility (n=3). Whilst miscarriage may have been the reason it is not possible to be sure of the reasons why they or the remaining 14 women who had a pregnancy did not have children. Other possibilities include: termination of pregnancy, adoption, children living with others or perhaps no current living children. Certainly there have been issues in the past identified with relying on reports of own children for fertility estimates, as it is more common for children of young Aboriginal mothers to live with aunts or grandmothers, and of this sample twelve women (46.2%) reported a pregnancy as a teenager but no current children (Gray 1989).

Among mothers, the median number of children was 2.0 with a range of 1.0 to 9.0 and the median age at first birth was 20.0 years with a range of 10.0 to 38.0 years. The majority of mothers (87.7%) had their first child before 29 years of age.

A substantial minority of mothers (39.5%) had their first child before 20 years. Of those who had their first child as a teenager, the majority (82.5%) were currently 25 years or older. The majority of teenage births were in girls ≥ 16 years but there were 8 (7.8%) first births in girls of 15 years or younger.

Table 3.3 Children by mother’s age group (years)

	15–24	25–34	35–44	Total
All women (n)	141	136	132	424
Percent (%) with children	17.5	79.4	85.5	60.6
Women with ≥ 1 child (n)	25	108	124	257
Median age first child	18 (17–20)	20 (18–24)	21 (18–26)	20 (18–24)
Number of children	1 (1–2)	2 (2–3)	3 (2–4)	2 (2–3)
Percent (%) first child <20 years	69.2	39.1	33.6	39.5

3.3.2.2 Hormonal Contraceptive use

Around one fifth of women reported current hormonal contraceptive use. There were 28 women who reported menopause, perimenopause or hysterectomy. If these women are excluded 22% of women were currently using hormonal contraception.

Two thirds of the participants had used a form of hormonal contraceptive at some time in their life with the oral contraceptive pill being more popular than the long acting injections of Depo-provera or the insertion of an Implanon rod. Of the women who had used hormonal contraception at some time 25% had used both the oral contraceptive pill and Implanon or Depo-provera at different times. Of the women who had used the oral contraceptive pill, 37% took it for greater than 5 years whereas only 5% of women used Implanon or Depo-provera for this time frame and 77% of women had discontinued the injectable forms of contraception within 2 years from commencement.

Age was a significant factor in current hormonal contraceptive use. The 25–34 year old age group was most likely to be using the OCP, Depo-provera or Implanon and, at the time of the study, the age group greater than 35 years least likely to. The greatest effect however was in Implanon/ Depo-provera use rather than oral contraceptive use (See Table 3.4). This may be because Depo-provera puts on weight and Implanon was a relatively new contraceptive at the time of the study¹² (ARTG 2008, TGA 2007).

¹² Implanon was approved for use by the Therapeutic Goods Authority in 1999 and registered on the Australian register of Therapeutic goods in 2000.

Table 3.4 Contraceptive use and age group (years)*

	Age Groups			Total
	15–24 n=143 (%)	25–34 n=136 (%)	35–44 n=145 (%)	
Oral Contraceptive				
Current	17 (11.9)	20 (14.7)	13 (9.0)	50 (11.8)
Former	38 (26.6)	68 (50.0)	96 (66.2)	202 (47.6)
Never	84 (58.7)	46 (33.8)	36 (24.8)	166 (39.2)
Implanon/Depo Provera				
Current	12 (8.4)	22 (16.2)	3 (2.1)	37 (8.7)
Former	20 (14.0)	26 (19.1)	25 (17.2)	71 (16.7)
Never	106 (74.1)	84 (61.8)	111 (76.6)	301 (71.0)
Any hormonal contraceptive				
Current	29 (20.3)	42 (31)	16 (11.0)	87 (20.5)
Former	44 (30.8)	62 (45.6)	96 (66.2)	202 (47.6)
Never	68 (47.6)	32 (23.5)	33 (22.8)	87 (21)

* Note that oral contraceptive use and Implanon/Depo-Provera use are not mutually exclusive

3.3.2.2.1 Factors affecting current hormonal contraceptive use

Factors that have been demonstrated to affect contraceptive use and fertility such as age, education, employment and other indicators of economic status were assessed.

The oldest age group (35–44) were least likely to be currently using hormonal contraceptives and the 25–34 year olds were most likely to currently use hormonal contraceptives. Those living in a home owned or being purchased and those in full-time work were also more likely to currently use hormonal contraceptives.

Multivariate regression with age showed that these factors remained significant (Table 3.5).

Table 3.5 Relative odds of contraceptive use by socioeconomic indicators

	Unadjusted Model			Age Adjusted Model		
	OR	95% CI	p	OR	95%CI	p
Age Group						
15–24	1.97	(1.02,3.82)	0.001	–		
25–34	3.5	(1.85,6.61)	0.04	–		
35–44	1.00			–		
Full Time Work						
No	1.00			1.00		
Yes	1.76	(1.1,2.84)	0.02	1.94	(1.16,3.25)	0.01
House owned or being purchased by occupants						
No	1.00			1.00		
Yes	1.71	(1.1,2.75)	0.03	2.08	(1.27,3.43)	0.004
Previous pregnancies						
No	1.00			1.00		
Yes	1.36	(0.81, 2.3)	0.25	1.36	(0.81,2.3)	0.25
Private health insurance						
No	1.00			1.00		
Yes	1.47	(0.83, 2.58)	0.19	1.7	(0.94,3.08)	0.08
Educational Qualifications						
No formal qualifications	1.00			1.00		
Year 10 or still at school	0.98	(0.43, 2.26)	0.97	1.02	(0.43,2.41)	0.97
Year 12, trade, certificate or university	1.45	(0.66, 3.18)	0.36	1.58	(0.71, 3.52)	0.27

3.3.2.3 Infertility

Our questionnaire did not specify a time required for a definition of infertility; we asked women whether they had experienced “difficulty in becoming pregnant,” had recurrent miscarriage or both. Responding to questions about infertility, 35 women (8.4%) reported “difficulty becoming pregnant”, 22 (5.2%) reported recurrent miscarriage and 13 women (3.1%) both. Estimates of population levels of infertility are generally based on women aged 20–44 years. If only the women in this age group (n=240) are considered the numbers remain the same but the percent increases due to

the lower denominator; therefore, 35 (14.6%) reported “difficulty becoming pregnant,” 22 (6.9%) reported recurrent miscarriage and 13 women (4.1%). It is important to note that of the women over 35 years 14.5% remain childless.

Assessing the 35 women who reported infertility only, 17 (48.6%) reported having had investigations performed. It is not possible to know whether those who did not have investigations did not seek medical advice, or received medical advice that their problem did not require investigation, or required investigation but did not proceed.

Of the 17 women who had investigations, the majority (15) had treatment and five had more than one form of treatment. Four were recommended a lifestyle change with diet and exercise, six had treatment with tablets (which one woman reported was for PCOS) and five had treatment with injections or IVF. The median age of those women who reported infertility only was 35 years (20–44).

It is not possible to ascertain from our questionnaire whether the women with difficulty achieving a pregnancy had secondary infertility or whether their children were as a result of treatment. Of the women who had in vitro fertilisation (IVF) or injections, three of them had their first child in their late 30’s suggesting primary infertility. There were four women over 35 years who had not received treatment and had only one child as a teenager suggesting secondary infertility. Of the 12 nulliparous women ≥ 30 years who reported infertility only three reported any investigations or treatment.

3.3.2.4 Menstrual cycles

Women who were currently using hormonal contraception, hormone replacement therapy or had a hysterectomy were excluded from cycle assessment. Of the remaining 309 women 25.9% had irregular cycles. Of the 270 women who had menstrual cycles able to be classified as irregular or regular as described in the methods section, 29.3% had irregular cycles.

3.3.2.4.1 Factors affecting regularity of menstrual cycles

Table 3.6 Menstrual Cycle Regularity and Age

Cycle Regularity	Age Groups			Total n=270(%)
	15–24 years n=90(%)	25–34 years n=84(%)	35–44 N=96(%)	
No	30 (33.3)	24 (28.6)	25 (26.0)	79 (29.3)
Yes	60 (66.7)	60 (71.4)	71 (74.0)	191 (70.7)

Frequency, percentage in brackets

There was little difference in the percentage of women with regular cycles by age. There was also minimal difference in the proportion of women whose cycles were deemed “not definable due to exogenous hormone use, menopause or hysterectomy”, although the reasons for this varied with age. The majority of these “non definable” cycles were due to current hormonal contraceptive use in the women aged 15–34 years and due to hysterectomy, menopause, perimenopause and/or hormone replacement therapy in women 35–44 years. Just over half of those whose cycles were not definable due to their question responses were teenagers.

Current and former smoking was common among participants (Table 3.1) but smoking was not significantly associated with irregular cycles (Table 3.7). There was also no difference in the cycle length between smokers and non smokers.

Increasing BMI and waist circumference were associated with a small increased chance of having irregular cycles (BMI Odds ratio 1.1 95%CI 1.01, 1.08 p=0.006) and WC OR 1.02 95% CI 1.01-1.03, p=0.05) but WHR was not. The relationship with irregular cycles for BMI persisted after adjusting for age and smoking, although the effect became insignificant for WC (p=0.06) (Table 3.7).

Table 3.7 Relationship of cycle irregularity to measures of obesity and age and smoking (n=270)

	Unadjusted Model			Age Adjusted Model			Adjusted for smoking and age		
	OR	95% CI	p value	OR	95%CI	p value	OR	95%CI	p value
Age	0.99	(0.97,1.02)	0.64	–			–		
Smokers									
Never	1.00			1.00			–		
Former	1.96	(0.90,4.24)	0.09	2.06	(0.94,4.52)	0.07	–		
Current	1.28	(0.69, 2.38)	0.43	1.35	(0.71,2.54)	0.36	–		
BMI	1.05	(1.01, 1.08)	0.006	1.05	(1.02, 1.08)	0.003	1.05	(1.02, 1.08)	0.01
Waist circumference	1.02	(1.002, 1.03)	0.05	1.02	(1.002, 1.03)	0.03	1.01	(0.999, 1.03)	0.06
WHR	0.82	(0.05, 12.9)	0.89	0.99	(0.06, 16.4)	0.99	0.88	(0.05, 14.9)	0.93

3.4 Discussion

There is little known about reproductive health in Indigenous women and this chapter reports on reproductive issues in a group of urban Indigenous women and the socioeconomic and health behaviour factors such as obesity and smoking that are associated with reproduction.

A high proportion of these women reported experiencing irregular cycles. There was a low median age of first childbirth and, for a large proportion, this occurred as a teenager. Infertility was around the same as for national figures at 10.5% (infertility only) for women greater than 20 years and the majority of these women did not have investigations or treatment. Hormonal contraceptive use was low and was significantly associated with age and indicators of socioeconomic status.

Overall this group of women experience economic disadvantage as determined by the socioeconomic indicators of: housing, private health insurance, full time work and household income. Obesity is a significant health issue across all ages, though it only had a modest effect on cycle regularity, and the number of women smoking is high.

This sample of urban Indigenous women is not necessarily representative of other Indigenous urban groups but the findings for obesity, smoking, employment and education are similar to reported national figures. For example, the National Aboriginal and Torres Strait Islander Health Survey (NATSIHS) 2004–2005 found that 52% of urban Indigenous women were current smokers, 20% were former smokers and 28% had never smoked (Australian Bureau of Statistics 2006). Within our sample 49.6% were current smokers, 20.2% former and 30.2% had never smoked. There is an established association between poor socioeconomic status and high rates of smoking in Indigenous people (Thomas et al. 2008).

NATSIHS 2004–2005 also found high proportions of women were overweight or obese and increased with age, though less than the women in this study. Overweight and obesity in women from NATSIHS was 29% in the women 18–24 year age group and increased with age to a peak of 61% in the 55 and over age group (Australian Bureau of Statistics 2006). This compares to 43.4% of women 15–24 and 66% of women 35–44 in DRUID. The small differences may be due to the reliance on self-

reporting of BMI in NATSHIS and their data included both urban and remote Indigenous women

The 2006 Census found that 20.6% of all households in Darwin were low income (less than \$650.00 per week), 54.6% of dwellings were being purchased/ were owned (Australian Bureau of Statistics 2008c). We cannot compare the housing and income figures directly as they are assessed in different ways but 43.1% of participants in this study lived in a house owned or being bought and 43.1% lived in a household where the income was less than \$600.00 per week. This is despite the fact that it is common for Indigenous households to have a large number of residents, which would increase the total income.

Menstrual Cycles

Of the 424 women who answered questions on menstrual cycles, 19% of all women reported irregular cycles (cycles > 35 days). If those women whose cycles were “not definable” due to questionnaire response, exogenous hormone use, menopause or hysterectomy or answered “don’t know” then 29% of women had irregular cycles. Large longitudinal studies in women with natural cycles have found cycle irregularity of around 20% (Treloar et al. 1967, Vollman 1956, Vollman 1977). March et al. in their PCOS prevalence study in South Australia found 23.8% of women had irregular cycles including women currently using contraception and with a more inclusive definition of “irregularity” (March W.A et al. 2009). Others documenting cycles in women in general population based studies report cycle irregularity of 8–11% (Colditz et al. 1997, Rowland et al. 2002, Solomon et al. 2001). In the more recent population studies many women were using hormonal contraception and studies have varied as to how this is managed. There is the potential for recall bias among these women who try to recount their natural cycles and there is the problem that some women will have started hormonal contraceptives as they have problems with such issues as irregular periods (possibly due to PCOS), acne or hair growth, symptoms related to PCOS. Therefore there may be some bias if these women were excluded from analysis. One of the studies above that excluded women with exogenous hormone use (Rowland et al) had a lower proportion of women with irregular cycles in comparison with this study (Rowland et al. 2002).

There are several potential reasons for the higher prevalence of irregular cycles in this study: the age of the women screened; prevalence of overweight and obesity in the women; ethnicity; and the fact that self-reported cycles may be unreliable.

Rowland et al. and Solomon et al. assessed women between the ages of 21–40 years and 20–35 years respectively which could potentially account for some of the differences. Increased cycle irregularity at the extremes of the reproductive age span have been reported and our study incorporated these extremes (Burger 1996, Ferrell et al. 2006, Johannes & Crawford 1999, O'Connor et al. 2001, Treloar et al. 1967). Whilst we did not find any effect of age this was probably because cycles were assessed in terms of categories not actual days. Women reported if their cycles were regular or irregular and length was reported as either: less than 24 days, 24–35 days or greater than 35 days.

Overweight and obesity have been reported to increase the chance of changes in cycle length and regularity in both Western and non-Western women which is consistent with the observations in this study (Castillo-Martinez et al. 2003, Hartz et al. 1979, Jensen et al. 1999, Williams 2006). There was a higher proportion of overweight or obese women in our study compared to the participants in Rowland et al. and Solomon et al which may account for the higher reported irregularity.

Smoking has been associated with variation in menstrual cycle patterns as discussed in 1.2.4.1. Smoking was not found to be a factor affecting cycles in the present study. This may have been because so many of the women in the study group overall were current smokers or that other factors, such as obesity, neutralised the effects of smoking on cycles. Previous studies by Windham and Liu recruited women who were more likely to be ovulating whereas in the present study there is, potentially, a large number of women who are anovulatory based on irregular cycles perhaps altering the effects of smoking (Liu et al. 2004, Windham et al. 1999, Windham et al. 2005). The categories of menstrual cycles used in this questionnaire would not detect subtle changes in cycle lengths and we are also unable to break down cycle lengths into follicular and luteal phases to assess any changes in these. Unlike BMI, which is easily measured and able to detect small effects, there was no quantifiable measure of smoking ascertained in this study. Perhaps better quantified smoking exposure and a larger number of participants would have shown an effect of smoking on cycles.

Finally, it is also possible that women may not have answered the reproductive health questionnaire correctly for many reasons, including embarrassment. However women tend to under report rather over report menstrual cycle regularity when cycles are compared to calendar records (Smith-DiJulio et al. 2005, Treloar et al. 1967) and the candidates own clinical experience suggests that this tendency to underestimate cycle irregularity also occurs among Indigenous women in the area where this study took place. A small number of women who consented to be interviewed in detail by the candidate (n=16) some months after their participation in the study. Of these, 14 (81.3%) had answered questions on cycle regularity correctly and two incorrectly. The findings for the two women who answered incorrectly were: one had reported regular cycles which were in fact irregular and one woman reported her cycles “not applicable”. The woman who reported cycles “not applicable” did so because she had been diagnosed previously with PCOS and the woman who reported regular cycles did not say why she reported this; in fact, she experienced irregular cycles and androgen levels were high, consistent with PCOS. Although these numbers only represent 3.8% of those who answered the women’s reproductive questionnaire they do not appear to indicate over-reporting of irregular cycles.

Hormonal Contraception

Around 20% of our participants were currently using hormonal contraception. NATSHIS 2004–5 found that in urban Indigenous women, aged between 18–45 in a sexual partnership and not trying to conceive, 18% were currently using the oral contraceptive pill, 26% condoms and 11% depoprovera or implanon. The lower figures in this sample are consistent with the number of prescriptions issued in the Northern Territory for all women for hormonal contraception compared with national figures (Jones C et al. 2005). The figures in this study are probably lower for a number of reasons: it is not possible to know how many of the women in the study thought they were at risk of pregnancy; how many women were actively trying to conceive; or how many women were using permanent (tubal ligation/vasectomy) or barrier (condom) methods. Certainly in the age group over 35 years old it is quite possible that women or their partners who have completed their families may have had sterilisation procedures. It is also possible that the figures are lower in this group of women because we included girls aged from 15 years who may be less likely to be using contraception if they are not sexually active. However, other studies with young

Indigenous people in WA and North Queensland have reported nearly half had started sexual activity under 16 years (Larkins et al. 2007, Zubrick et al. 2005).

Factors associated with being sexually active in these teenagers included age, having left school, alcohol and/or marijuana use, (Zubrick et al. 2005) idolised vision of the role of parenting, perceived increasing sexual activity of peers, and alcohol use (Larkins et al. 2007). Teenagers in the Queensland study were also less likely to be sexually active if they had personal plans or if their parents expected them to go on to tertiary education (Larkins et al. 2007)

Research across Australia from Queensland, South Australia and the Northern Territory suggests that Aboriginal women are concerned about young girls having children and short intervals between children and expressed an interest in contraception (Jones et al. 2005). Little is known about Indigenous women's knowledge of contraception or attitudes to use of contraception. There is a suggestion from this study that socioeconomic factors such as home ownership and full time employment are associated with greater hormonal contraceptive use. In Indigenous youth in Townsville, QLD, factors that impacted on contraceptive use were: "shame" in talking to health providers and "shame" discussing it with a sexual partner; for a young woman to be prepared with a condom was to risk her reputation as it implied pre-meditated sex; a lack of knowledge about contraception and STI's; a lack of access to hormonal contraception; getting carried away and having unplanned sex; boys dislike of condoms ; and desired pregnancy (Larkins et al. 2007). Some of these factors have been reported before and they certainly need to be explored further in urban Indigenous youth who experience high teenage pregnancy, violence and STIs.

Fertility

Consistent with national figures for Indigenous women, this sample reported an earlier age of first childbirth with a significant minority in women under 20 years old (39.5%). National figures from 2001–2004 found that around half of first time Aboriginal mothers were under 20 years old and that the mean age for first time Indigenous mothers was lower at 20.6 years than for non-Indigenous first time mothers of 27.7 years. Younger age at birth and higher parity in Indigenous women is associated with living in a remote location and with socioeconomic disadvantage. The

mean age of birth decreased across the spectrum from major cities to inner regional areas through to outer regional, remote and very remote (Leeds et al. 2007). Area of residence and socioeconomic status may have been additional factors in early age of first birth in this sample of women; Darwin is classified as an Australian Standard Geographical Classification (ASGC) inner regional area and a significant proportion of women (43.1%) were from low income households (<\$600.00/week).

Teenage pregnancy has been a concern among Indigenous people for some time with worry that it may be the result of children being more sexually promiscuous, or as a result of an assault. It also leads to an increased number of single parents, social disadvantage for the mother and child, more grandmothers or other family members bringing up babies, and an increasing number of neglected children (Jones et al. 2005, Wild & Anderson 2007).

Infertility

The prevalence of infertility among Indigenous women has been reported as high as 26% in an Indigenous community in Arnhem land (Kildea & Bowden 2000).

The figures from our study of urban Indigenous women are lower but were consistent with international figures that suggest overall infertility (of 12 months) is around 9% in both developed and developing countries. Factors such as sexually transmitted infections are more important in developing countries but are generally balanced by age-related factors and obesity in developed countries. There are some nations however with very high rates of sexually transmitted infections where infertility is higher (Boivin et al. 2007).

One of the limits of the current study is that duration of infertility was not determined. However 50% of the women who reported infertility were over 30 years of age and had one or no children. Given the tendency to have children at a young age this would suggest that they would have long standing infertility. A study of remote Indigenous women in Arnhem Land found that infertility was under-reported in response to a questionnaire as women often don't consider they have a problem if they already have one or more children. They may also adopt a child given them on behalf of relatives (Gray 1989) (candidates personal experience).

Seeking treatment for infertility is generally low world wide at just over half of infertile couples in both developed countries and developing countries, consistent with our figures of 52.6% (Bunting & Boivin 2007). This would seem surprising given that around 95% of people in parenting surveys report they would like to have children at some time in their adult lives (Lampic et al. 2006). Reasons for this may vary across countries. In developing countries it may be due to a lack of health care resources, regulations prohibiting treatment or high cost of assisted reproductive care. Whilst assisted reproductive technology may be subsidised in developed countries, there still may be significant financial costs to the couple. The costs and personal factors contribute to decisions not to pursue treatment. An online survey by Bunting et al. was carried out on 426 women predominantly from the USA and UK, recruited from a website targeted at couples in the early stages of trying to conceive (Bunting & Boivin 2007). This survey found that the women in the survey who already had infertility of one year duration and didn't seek treatment were influenced by the following factors: the threat of "discovery" (concern about being labelled or diagnosed and the effect this would have on relationships); a difference in treatment beliefs such as success rates; ease of access to treatment; and knowledge about how to get help. In Darwin, there is access to GP's, specialists and assisted reproductive technology. However, these processes may take some time due to a shortage of doctors and financial considerations may affect help seeking as there is a paucity of bulk billing by GPs, and no bulk billing for Assisted Reproductive Technology (ART). Little is known about personal factors influencing the decision making of Indigenous couples to seek advice about infertility.

3.5 Conclusion

Indigenous women generally have poorer health than non-Indigenous women in terms of their general and metabolic health, and also in reproductive health. This should be addressed as a priority. There is a need to explore Indigenous women's attitudes towards early motherhood and contraceptive use. Evidence from other studies suggests that higher educational attainment, full time participation in the work force and other socioeconomic indicators such as home ownership may be related to higher hormonal contraceptive use and later age at first childbirth. This highlights the importance of encouraging girls to engage more fully in the education and employment sectors.

Factors such as obesity and smoking are potentially preventable and important factors in reproductive, general metabolic and cardiac health and strategies are needed to develop preventive programs targeted at Indigenous women.

Evidence is accumulating that the intrauterine environment and early childhood factors influence adult disease. Factors such as teenage pregnancy, diabetes in pregnancy, obesity and smoking all contribute towards poorer outcomes for babies and infants. Strategies targeted at improved maternal and child health and which help Indigenous women with their own health will also benefit the next generation.

Finally, having children is very important to women and their communities and there should be further work done to explore the reasons for infertility in Indigenous women. Particularly important is the prevention of problems such as obesity and sexually transmitted infections, prompt treatment of sexually transmitted infections and the exploration of knowledge and attitudes to reproduction and reproductive health care.

Chapter 4 Androgens

4.1 Introduction

There are a number of different androgens produced endogenously in women and issues of reliable measurement are discussed in detail in Section 1.4.1.3.2.

Testosterone is the androgen of most importance in many aspects of women's health and it is the free biologically active component that is of interest. This increases with an increase in total testosterone or a decrease in sex hormone binding globulin.

Currently, the most reliable and clinically accessible measurements of free testosterone are the calculated free androgen index or the calculated free testosterone.

Of particular interest in women is that an increase in androgens and decrease in sex hormone binding globulin (SHBG) are linked to anovulation and polycystic ovary syndrome in pre-menopausal women and an increase in diabetes and cardiovascular risk factors in both pre and post-menopausal women (Ding et al. 2006, Sutton-Tyrrell et al. 2005). The cardiovascular risk factors include central obesity, increased triglycerides and decreased HDL-C levels. These effects have been shown in women of many ethnic groups (Sutton-Tyrrell et al. 2005).

Hyperandrogenaemia refers to levels of circulating androgens that are above normal. Measurements of androgen excess have included androstenedione, DHEAS and testosterone, either bound or unbound (free). Clinically, laboratories supply normal ranges for androgens. These may be determined in a number of ways as discussed in section 1.4.1.3.2. Determining the best androgen to use for the assessment of hyperandrogenaemia in assessing health risk factors and for diagnosing PCOS has been the subject of debate for many years.

In this thesis free testosterone will be used as the androgen determining hyperandrogenaemia as this or the FAI, have been shown in some studies to be the better androgens to use as a measure of PCOS (Azziz et al. 2006, Hahn et al. 2007).

As discussed in section 1.4.1.4, there are a number of factors that contribute to difficulties in assigning normal androgen values in women: levels change with age with hyperandrogenaemia normalising as a woman approaches her 40s; there may be varying

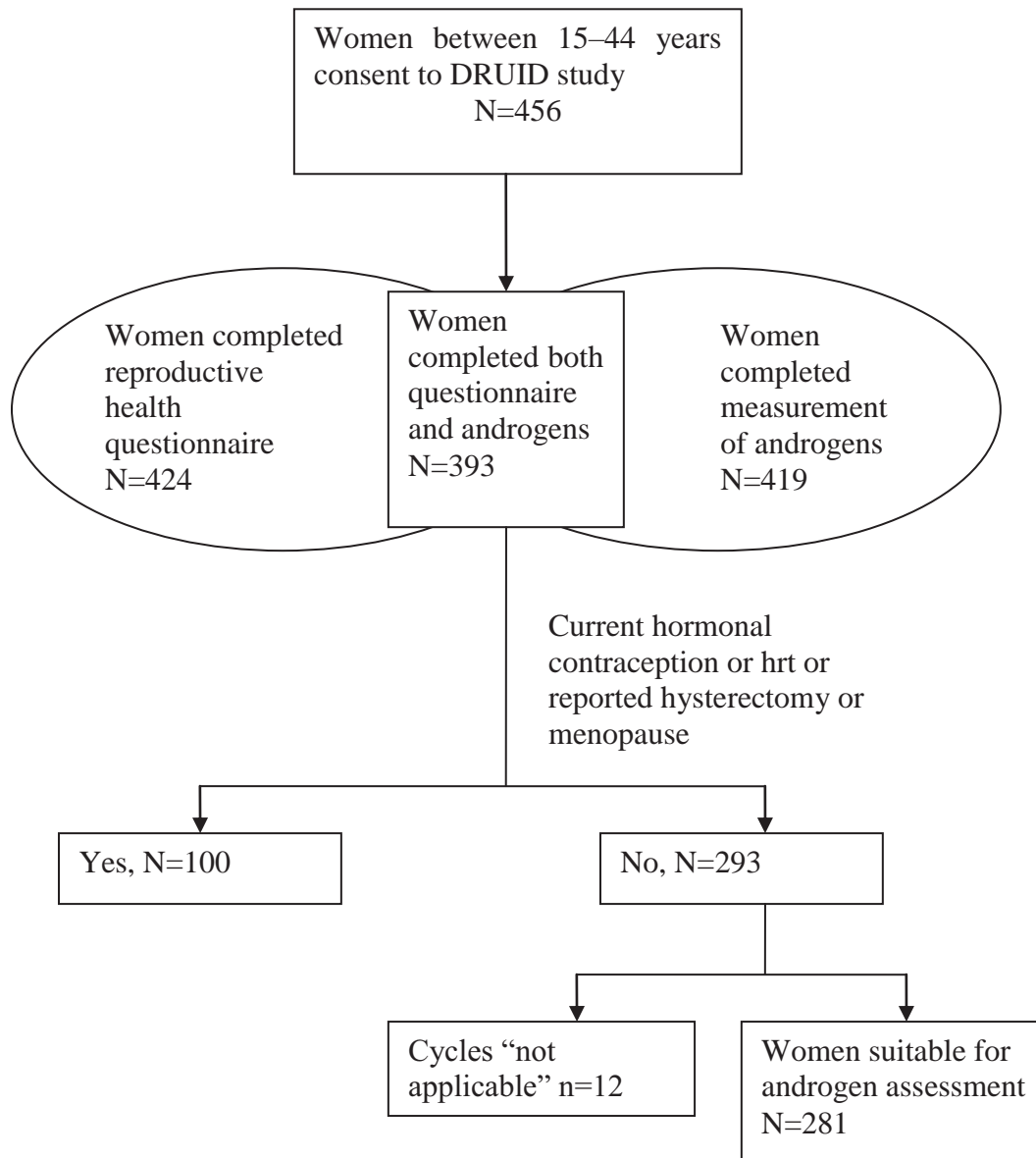
prevalence of obesity; there may be ethnic differences; there is a wide range of normal; different assays are used in different institutions and there is interassay variability. Further, other factors such as smoking and alcohol intake may affect androgens.

This chapter will explore the variations of androgens in Indigenous women as there are no normal values established for Indigenous women. Initially a subgroup of women from DRUID with regular cycles, no hirsutism and no acne were matched for age and BMI with a Caucasian group of women with regular cycles and no PCOS. An assessment and comparison of androgens between these two groups will assist in determining what value of free testosterone or free androgen index should be assigned as “hyperandrogenaemia.” The effects of age, obesity and other factors such as insulin on androgens and SHBG will be explored in pre-menopausal women who are not using hormonal contraception. Finally, cycle regularity, hormonal contraception and androgens will be assessed.

4.2 Methods

As described in the methods section, 424 women completed the women’s health questionnaire, 419 had bloods taken for androgen assessment and 393 women completed both questionnaire and had bloods taken for androgens. Of these, 81 women reported current use hormonal contraception and 19 women current hormone replacement therapy, previous hysterectomy or menopause. Further in response to cycle regularity, twelve women responded “not applicable”. Some specified reasons such as current hormonal treatment for endometriosis and others did not. These groups of women were excluded from analysis in the first part of the chapter as we were interested in pre-menopausal women who were not affected by exogenous factors such as hormonal treatment, n=281 (see Figure 4.1).

Figure 4.1 Selection of Participants for assessment of androgens



In determining normal values for testosterone in our study group, the calculated free testosterone of a reference group was used from a group of women who had their sera analysed in the same laboratory in Adelaide with the same method and with the same kit (DSL 4100). This group of 67 Caucasian women aged between 26 and 42 had regular cycles with cycle length 21–35 days apart, no evidence of polycystic ovaries on ultrasound, no evidence of congenital adrenal hyperplasia, Cushing’s syndrome or androgen producing tumours. The mean BMI of the group was 26.6 ± 5.7 (minimum 17.5, maximum 32.0). Biochemical values for this group of women above the 95th centile were considered abnormal.

A subset of women has been drawn from our study group who reported regular periods, no hirsutism and no acne and were matched in range and mean values to the Caucasian group for age and BMI (N=40). This group had a mean BMI of 25.0 ± 3.2 (minimum 19.5, maximum 31.0).

Details of the methods employed in serum testing, anthropometry and women's questionnaire are described in Chapter 2.

Collected data was de-identified and entered into a Microsoft Access database. Statistical analyses were performed using Intercooled Stata 8.0 (Stata Corporation, Texas, USA). Analyses for the following summary statistics were performed: frequencies, means and standard deviations. Variables whose distribution was not normal were log transformed for analysis and summary statistics presented as median with interquartile values and ranksum used to compare groups. Data are presented as mean (SD) and groups compared using unpaired t test. Statistical significance was accepted at $P < 0.05$. A two-tailed Chi^2 test was used to compare categorical variables. Regression was used to determine associations between multiple independent variables. Breusch-Pagan / Cook-Weisberg test for heteroskedasticity was used to check the validity of the regression models and the leverage and residuals assessed to determine if any individual results were significantly affecting the model outcomes. If individuals did have substantial leverage affecting the models these are reported in the text.

Backward stepwise regression analysis was used to enable assessment of how variables predict the dependent variable while adjusting for a number of other possible predictor values. For example, the predictor effect of SHBG on free testosterone while adjusting for BMI. The predictor with the highest p value is dropped from the model, the model is refitted and then the decision is made whether to eliminate any further predictors. This process is repeated until all variables that remain have coefficients that are significantly different from zero at the maximum p level determined (in this chapter set at $p = 0.06$ due to the small numbers).

4.3 Results

There are various factors that may affect androgens and SHBG such as age and body size or, that are in turn, influenced by the androgens themselves including menstrual cycles and hirsutism. Results will be reported in relation to these factors.

4.3.1 Androgens in women from two populations with normal cycles

Androgens are compared between the Caucasian control group of non- PCOS women from Adelaide and a subset of women from the DRUID study who reported regular cycles, were in the same age group and the same BMI limits as those from Adelaide. None of these women reported hirsutism or acne. While there was no difference between the two groups in SHBG or DHEAS, testosterone ($p=0.0001$), FT ($p=0.004$) and FAI ($p=0.03$) were significantly higher (Table 4.1). There is no further information on the Adelaide women to assess for differences in measures of central obesity, which is known to be associated with increased testosterone.

Table 4.1 Androgens and SHBG in a group of non-Indigenous women in Adelaide with regular cycles and normal pelvic ultrasound compared with a subset of Indigenous women with regular cycles, no hirsutism or acne and matched for age and BMI from the DRUID study

	Percentiles																		P* value
	Mean		Median		SD		5		10		25		75		90		95		
	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	
Testosterone (nmol/L)	1.0	1.5	1.0	1.4	0.3	0.6	0.6	0.7	0.7	0.8	0.8	1.0	1.2	1.9	1.5	2.3	1.7	2.6	0.0001
FT (pmol/l)	17.2	25.2	16.0	22.8	7.8	15.0	7.1	6.9	8.5	10.5	11.9	14.0	20.7	30.1	29.1	52.0	34.2	60.3	0.004
SHBG (mmol/l)	43.5	45.2	40.0	40.2	19.6	23.0	18.3	13.4	21.8	18.2	28.7	29.5	51.1	58.7	72.2	74.3	85.0	104.8	0.79
FAI	3.0	4.7	2.6	3.7	1.9	4.2	0.9	0.8	1.2	1.5	1.8	1.9	3.8	5.2	5.6	11.9	6.5	17.7	0.03
DHEAS (umol/l)	3.5	3.7	3.5	3.6	1.4	2.0	1.4	0.6	1.8	1.6	2.4	2.5	4.4	4.4	5.4	6.4	6.2	7.5	0.98

A: non-Indigenous Adelaide women

D: DRUID subset of women with regular cycles, no hyperandrogenism matched for BMI and age to Adelaide women

*P= comparison of median values in both groups by Wilcoxon Mann Whitney test

4.3.2 Age and Androgens in women in the DRUID study suitable for androgen assessment

All androgens were significantly lower in older women. Whilst there was a trend to increasing SHBG with age this was not significant. There was a decrease in 27.8% of median free testosterone between the youngest and oldest groups ($p=0.008$), 12.7% in total testosterone ($p=0.0001$), 31.9% in FAI ($p=0.003$), 34.0% DHEAS ($p=0.0001$) and a non significant increase of 21.0% of SHBG ($p=0.08$) (Table 4.2).

Table 4.2 Androgens and SHBG in the women from DRUID suitable for androgen assessment (n=281) by age

Age group (years)	15–24	25–34	35–44	Total	P#
N	101	81	99	281	
BMI* Kg/m ²	23.6 (20.1,30.7)*	29.0 (23.8,32.5)*	27.1 (22.7,33.2)	26.5 (21.9,32.5)*	0.02
Total testosterone Mmol/l	1.9 (1.5 – 2.5)	1.8 (1.2 – 2.5)	1.5 (1.2 – 1.9)	1.8 (1.3–2.2)	0.0001
SHBG Mmol/l	27.4 (18.4–40.0)	27.8 (19.8–46.6)	31.4 (23.1–46.9)	28.9 (20.0–46.0)	0.08
Free testosterone Pmol/l	37.1 (26.2–51.8)	31.5 (21.8–55.8)	26.8 (18.4–40.3)	31.5 (21.0–49.5)	0.008
Free androgen Index	6.9 (4.0–11.6)	5.9 (3.5–11.4)	4.7 (2.7–7.7)	5.6 (3.5–5.5)	0.003
DHEAS* Mmol/l	4.7 (3.4–6.5)	3.9 (3.0– 5.7)*	3.1 (2.2–4.4)*	3.8 (2.7–5.5)	0.0001
	*n=98, 83	*n=80, 74	* n= 97	* n=275, 260	

#p values based on Wilcoxon Mann Whitney ranksum of median values between age group 15–24 and age group 35–44

4.3.3 Obesity and Androgens in women from DRUID suitable for androgen assessment

Women, who were overweight or obese by any measure, had higher total and free testosterone and free androgen index and lower sex hormone binding globulin. Regression analysis showed free testosterone increased significantly with all measures of obesity (BMI $p=0.001$, WHR $p=0.001$ and waist circumference $p=0.001$). The results were similar between FAI and total testosterone and these same measures of obesity; higher levels with increasing obesity. DHEAS did not change significantly with obesity by any measure. SHBG, as expected, decreased significantly with increasing BMI ($p=0.001$), WHR ($p=0.001$) and waist circumference ($p=0.001$) (Figure 4.2–4.5).

Figure 4.2 Total testosterone by BMI category in all women in DRUID suitable for androgen assessment

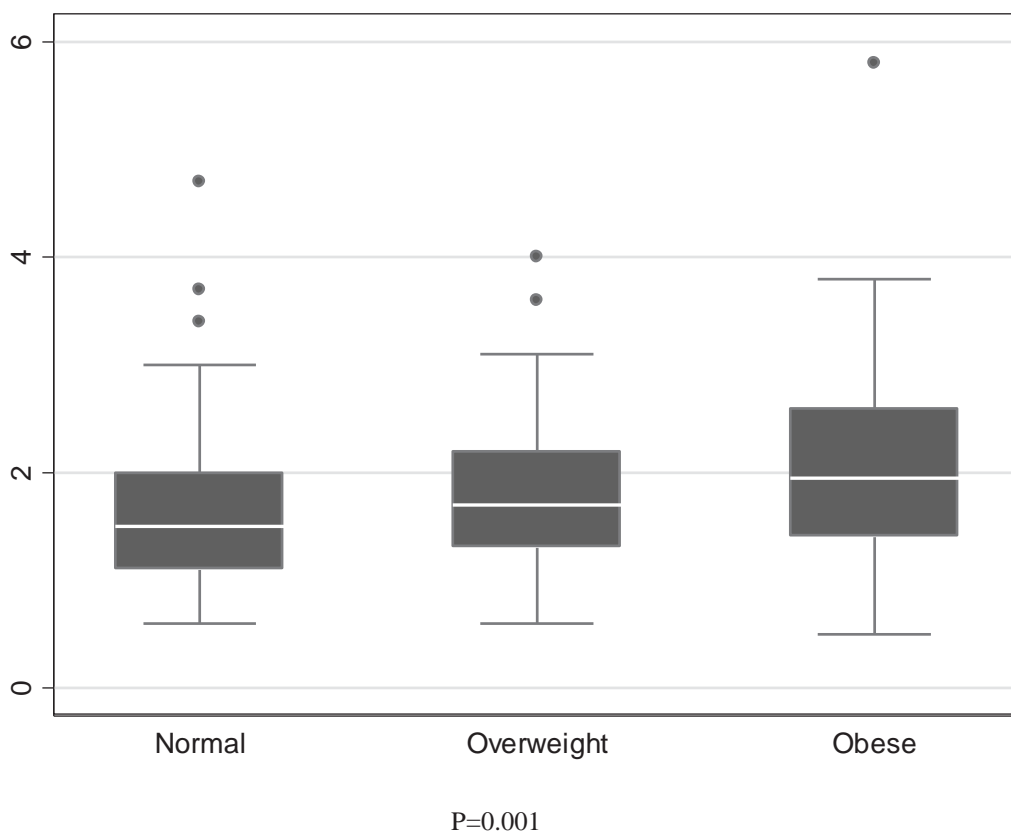
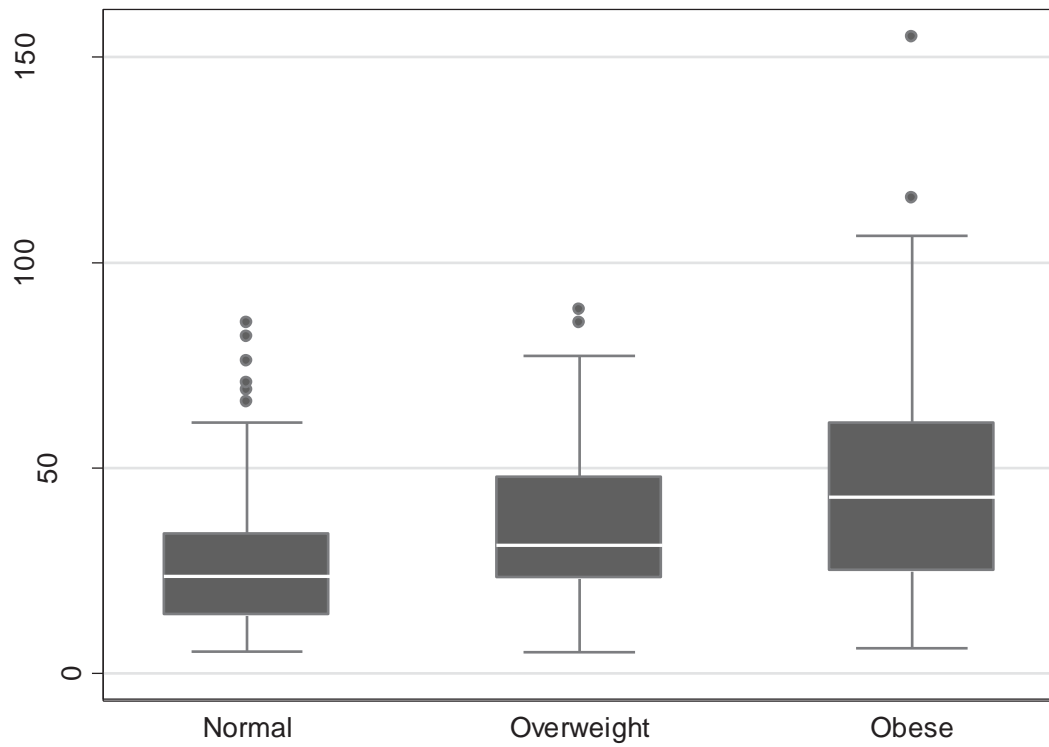
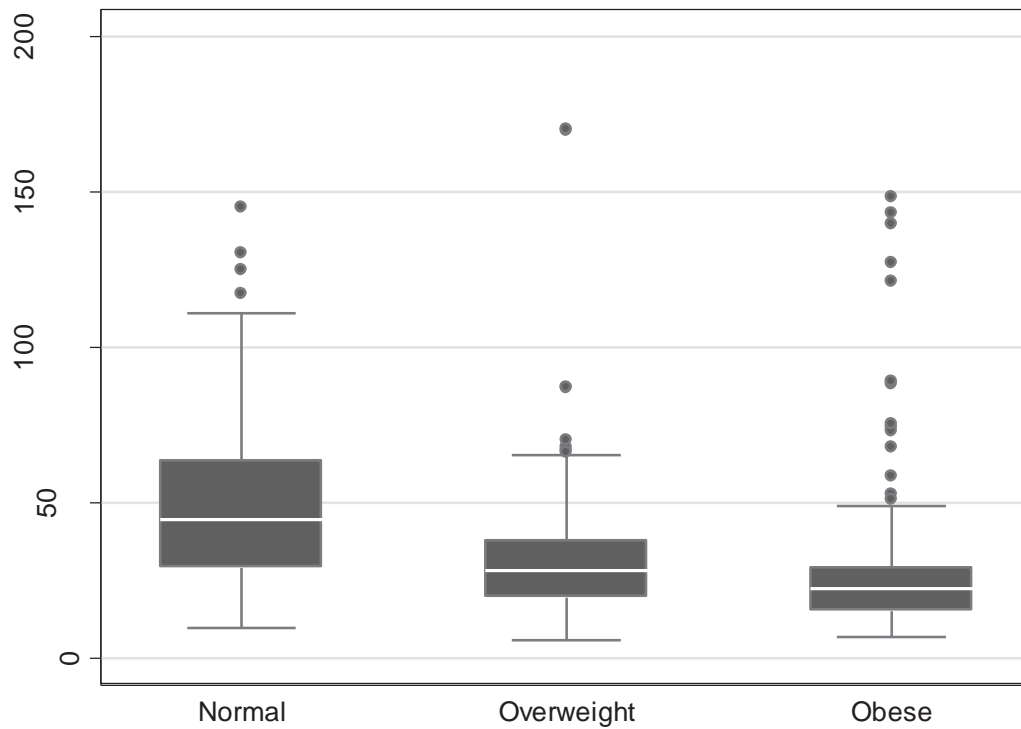


Figure 4.3 Free testosterone by BMI category in women from DRUID suitable for androgen assessment



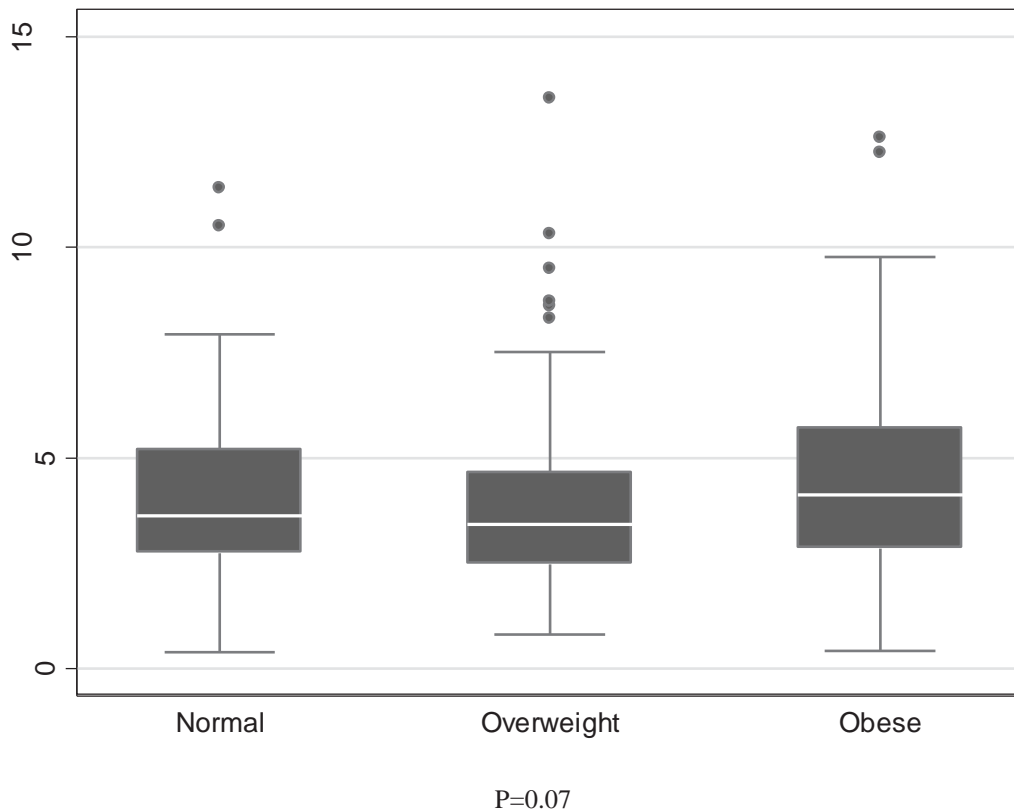
P=0.001

Figure 4.4 SHBG by BMI category in women from DUID suitable fro androgen assessment



P=0.001

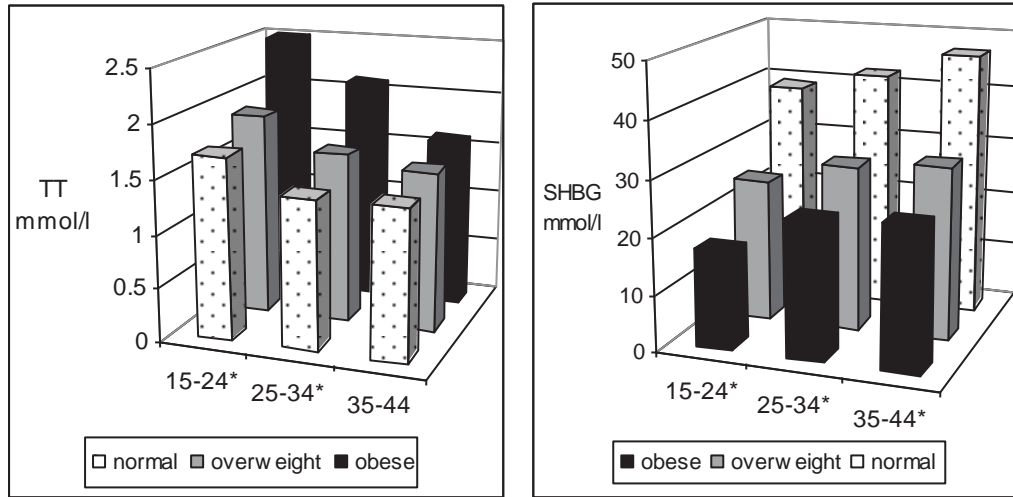
Figure 4.5 DHEAS by BMI category in women from DRUID suitable for androgen assessment



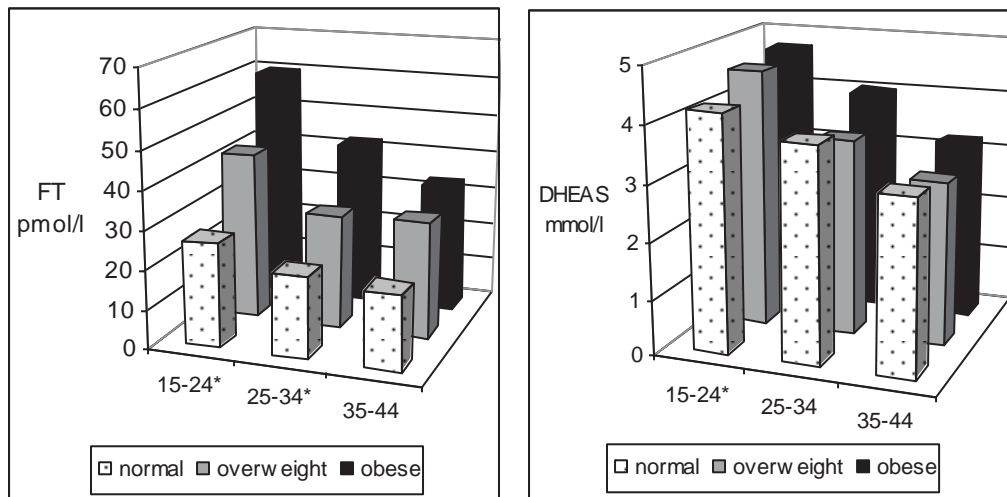
4.3.4 Obesity, Age and Androgens in women from DRUID suitable for androgen assessment

All the androgens in this group of women decrease with age and all, apart from DHEAS, increase with obesity which itself increases with age. If the effect of age and obesity together on androgens are assessed it is apparent that in younger women (15–24 and 24–35 years) there are significant differences in total and free testosterone by BMI category. Similarly in women in 15–24 age group there is a significant difference in DHEAS by BMI category. This impact of BMI on androgens was not significant in the older women (35–44 years). Whilst there was a non significant trend for SHBG to be higher in older women, increasing body size was significantly associated with lower SHBG. The difference in SHBG between normal weight and obese women remains significant across all age groups (Figure 4.6).

Figure 4.6 Relationship between BMI category, Age groups and Androgens/SHBG in women from DRUID suitable for androgen assessment



*p<0.05 between normal BMI and obese BMI category



*p<0.05 between normal BMI and obese BMI category

Possible explanations for the lack of significance of obesity on testosterone measures in the older women include

- Higher median BMI or WHR in the younger women within each BMI categories
- High insulin in the young women
- Higher smoking prevalence in the young women
- Higher proportion of irregular cycles in the young women

These are assessed below.

There was no significant difference in BMI between the age groups as a whole or when compared by BMI category and WHR was higher in the 35–44 years age group women compared to the 15–24 years group in the normal and overweight BMI categories (Table 4.3). Higher obesity in the younger women therefore is not an explanation for the significant influence of obesity on testosterone in the younger women and not in the older women.

High insulin levels can increase androgens and the median fasting insulin was high in all age groups of women assessable for androgens (Table 4.3). However the median insulin was similar across the ages and in fact, in those with a normal BMI was lower in older women. At present there is no evidence that insulin has a greater effect on androgens in younger women.

Smoking has been reported in some studies to increase androgens and younger women were more likely to smoke. However there was no association in these women between smoking status (current, former, never) and testosterone (total or free), FAI, SHBG or DHEAS. It is possible any effect on androgens could vary with the amount smoked but we did not have that data available and are unable to assess a dose related response.

Irregular cycles potentially could account for higher androgens in young obese women but women in the young age group had the same proportion with irregular cycles as did the women in the older age groups whether they had a normal BMI or were obese.

Finally it may be that age is more influential than obesity on androgen production leading to the attenuation of obesity effects in older women. This would appear to be supported by the multivariate regression analysis in 4.3.5.

Table 4.3 Fasting insulin by age group and BMI in all assessable women

	15–24 N=99	25–34 N=79	35–44 N=97	P*
Fasting insulin (IU/l)				
All	9.0 (6.0,14.0)	9.0 (5.0, 15.0)	8.0 (5.0,15.0)	0.13
Normal BMI	8.0 (6.0, 11.0)	6.0 (4.0, 8.5)	5.0 (4.0, 7.5)	0.001
Overweight BMI	9.0 (7.0,17.0)	8.0 (8.0, 12.0)	9.0 (6.0, 15.0)	0.71
Obese BMI	16.0 (12.0,22.0)	20.0 (10.0,24.0)	16.0 (8.0, 21.0)	0.52
BMI (kg/m²)				
All	23.6(20.1, 30.7)	28.9(23.8,32.5)	27.1(22.7,33.2)	
Normal BMI	20.1(18.6, 22.4)	23.0(18.8,23.8)	21.8(19.6,23.7)	0.08
Overweight BMI	27.9(26.0,28.5)	28.5(26.3, 29.3)	27.5(26.6,28.6)	0.99
Obese BMI	35.2(31.9,39.7)	35.0(31.1,41.6)	35.5(33.2,40.0)	0.38
WHR				
All	0.81(0.78,00.86)	0.85(0.80,0.92)	0.87(0.82,0.92)	
Normal BMI	0.79(0.77,0.82)	0.80(0.77,0.85)	0.82(0.79,0.88)	0.004
Overweight BMI	0.84(0.77,0.93)	0.86(0.84,0.90)	0.90(0.85,0.92)	0.009
Obese BMI	0.88(0.83,0.93)	0.91(0.84,0.96)	0.89(0.83,0.98)	0.43
Testosterone (mmol/l)				
All	1.9 (1.5, 2.5)	1.8 (1.2, 2.5)	1.5 (1.2, 1.9)	0.0001
Normal BMI	1.7 (1.2, 2.0)	1.5 (1.0, 2.1)	1.4 (1.0, 1.9)	0.04
Overweight BMI	2.1 (1.1, 2.6)	1.8 (1.3, 2.4)	1.7 (1.2, 2.0)	0.02
Obese BMI	2.5 (2.0, 2.8)	2.3 (1.4, 3.1)	1.5 (1.3, 2.0)	0.0001
SHBG (mmol/l)				
All	27.4 (18.4, 40.0)	27.8(19.8, 46.6)	31.4(23.1, 46.9)	0.08
Normal BMI	36.2 (27.6, 64.3)	41.2(28.5, 63.4)	46.6(23.3, 55.5)	0.20
Overweight BMI	25.0 (17.3, 28.4)	27.7(19.7, 46.6)	30.1(22.1, 38.0)	0.08
Obese BMI	17.3 (11.9,22.9)	22.3(16.2, 27.7)	24.3(18.2, 27.3)	0.01

*p value for the difference between the 15–24 and 35–44 age groups

4.3.5 Androgens and other variables

Backward stepwise multivariate analysis of androgens and SHBG in the premenopausal women not taking exogenous hormones was performed with the following variables assessed as predictor variables: age, BMI, waist circumference (WC), waist hip ratio (WHR), DHEAS, AMH, CRP, homocysteine, fibrinogen, fasting glucose and insulin, HOMA, 2 hour glucose and insulin, smoking and irregular cycles. Additionally for SHBG, testosterone measures and DHEAS were included as predictor variables, for testosterone measures SHBG was included as a predictor variable and for DHEAS testosterone measures and SHBG were included as predictor variables. The final models for total and free testosterone include significant interactions in a negative direction with age and SHBG, and positively with DHEAS and triglycerides.

SHBG is found to be significantly negatively associated with BMI and free testosterone and positively associated with HDL-C. DHEAS was negatively associated with age and HOMA and positively associated with free testosterone (Table 4.4).

Table 4.4 Backward stepwise multivariate regression analysis of androgens and SHBG and predictors.

Dependent Variable	Constant	R2	Predictor variable	B coefficient	Standard Error	95% CI		P value
Total Testosterone*	1.07	0.31	Age	-0.007	0.002	-0.01	-0.002	0.0001
			SHBG*	-0.18	0.04	-0.25	-0.09	0.0001
			TG*	0.15	0.05	0.05	0.24	0.001
			DHEAS	0.06	0.01	0.04	0.08	0.0001
SHBG*	5.75	0.66	BMI	-0.01	0.003	-0.02	-0.004	0.001
			Free Testosterone*	-0.67	0.04	-0.74	-0.60	0.0001
			HDL-C	0.19	0.07	0.05	0.32	0.006
Free Testosterone	5.95	0.71	Age	-0.01	0.002	-0.01	-0.001	0.009
			SHBG*	-0.76	0.04	-0.84	-0.69	0.0001
			DHEAS	0.06	0.01	0.04	0.08	0.0001
			Triglyceride*	0.14	0.05	0.05	0.24	0.004
DHEAS	0.84	0.27	Age	-0.05	0.01	-0.08	-0.02	0.0001
			HOMA*	-0.48	0.15	-0.77	-0.20	0.001
			FT*	1.56	0.20	1.16	1.95	0.0001

* log transformed for analysis

4.3.6 Androgens in women from DRUID according to response to menstrual cycle question in women's reproductive health questionnaire

The women included for androgen analysis in the next section are only those with regular or irregular cycles or currently using hormonal contraception (groups a, b and c)

- a Regular cycles (n=174)
- b Irregular cycles (n=74)
- c Those currently using hormonal contraception (OCP, Depo-provera or Implanon, n=81)

Those excluded from further androgen analysis in the remainder of the chapter are those from the following three groups:

- d. Those who responded "don't know" or were missing to the question on cycles regularity (n=33)
- e. Those using HRT, experienced menopause or had a hysterectomy (n=19)
- f. Those who responded "not applicable" to the questions about cycles regularity (n=12)

Table 4.5 Women grouped by cycle regularity responses: age, androgens and clinical symptoms

	Regular cycles	Irregular cycles	Hormonal Contraception
N	174	74	81
Age (years)	32 (22, 38)	30 (21, 37)	28.0 (22, 32)
BMI (kg/m ²)	25.9 ^a (22.4,30.7)	29.7 ^{a b} (23.3, 35.5)	26.0 ^b (22.7, 30.1)
WC (cm)	88.3 (76.5, 98.6)	94.7 ^b (76.1, 108.0)	88.3 ^b (74.9, 98.1)
WHR	0.85 (0.80, 0.90)	0.84 (0.78, 0.93)	0.83 (0.79, 0.88)
Testosterone (nmol/l)	1.7 ^a (1.2, 2.1)	2.0 ^{a b} (1.5, 2.7)	1.4 ^{a b} (1.0, 1.9)
SHBG (mmol/l)	30.1 ^a (21.2, 46.6)	25.5 ^{ab} (17.3, 40.1)	38.0 ^{ab} (24.3, 69.3)
FT (pmol/l)	29.2 ^a (18.9, 45.2)	41.8 ^{ab} (24.1, 57.5)	22.3 ^{ab} (12.1, 35.5)
FAI	5.1 ^a (3.2, 8.7)	8.0 ^{ab} (14.7)	3.6 ^{ab} (1.6, 7.3)
DHEAS (mmol/l)	3.7 (2.5, 5.4)	4.2 ^b (3.1, 5.8)	3.7 ^b (2.8, 4.6)
AMH (ng/ml)	18.1 ^a (10.1, 29.3)	26.6 ^{ab} (13.4, 41.4)	18.2 ^b (12.1, 33.0)
Fasting insulin mU/L	8.0 ^a (5.0, 13.0)	11.0 ^a (6.0, 20.0)	8.0 (6.0, 15.0)
% with hirsutism	38.0	47.2	32.1
% with acne	15.7	15.3	8.6
% with infertility	4.7	17.8	2.5

Results are presented as median values with interquartile ranges

^ap<0.05 between regular cycles and marked group

^bp<0.05 between irregular cycles and marked group

Androgens

The women with irregular cycles had the highest median free testosterone and FAI and the lowest sex hormone binding globulin and these were significantly different to the results from women with regular cycles or currently using contraception. AMH was also highest in those with irregular cycles.

Hormonal contraception is known to increase SHBG and therefore decrease free testosterone, regulate menstrual cycles and ameliorate hirsutism. In light of these influences and the fact that it would be difficult to ascertain cycle regularity prior to the commencement of contraception the decision was made to exclude these women from androgen analysis. Our data confirmed current hormonal contraceptive users had lower free testosterone than the other groups. They also had a lower BMI than those with irregular cycles and less reports of hirsutism than those with irregular cycles although

this was not significant ($p=0.06$). It is not possible to ascertain whether the smaller proportion of women with hirsutism predates the hormonal contraceptive use or whether the smaller numbers are a result of improvement of hirsutism due to the contraception.

There was no difference in DHEAS between any of the groups.

Hirsutism

There was a high proportion of women who reported hirsutism in this study. Reports were highest in those with irregular cycles at 47.2%, followed by those with regular cycles at 38.0% and finally those using hormonal contraception at 32.1%. Of these 79.4%, 55.4% and 61.5% treated their hirsutism with depilatory creams, shaving, waxing or medication.

Infertility

The highest proportion of women reporting infertility was in those with irregular cycles at 17% with much smaller proportions in those with regular cycles (4.7%) and those currently using hormonal contraception (2.5%).

4.4 Discussion

In this group of Indigenous women androgens fell with age and increased with obesity as has been observed in other populations. However in this group the effect of obesity weakened with age. This meant that in older women there was less difference in all measures of testosterone between those classified as overweight or obese compared with women in the normal BMI range.

Age related declines in androgens have also been documented by other studies in Australia and the USA (Davison et al. 2005, Spencer et al. 2007, Winters et al. 2000).

There have been other studies confirming testosterone increases with obesity. Spencer et al. comparing African American women and white women found the differences in androgens between the groups to be more marked in younger women, after controlling for BMI, HOMA and WHR (Spencer et al. 2007). It is likely that the drivers of

androgen production, or the response to drivers such as increased insulin, are more pronounced in young women.

The testosterone levels in the women in this study were high across the whole study group. Even the women with regular cycles and no clinical hyperandrogenism had higher testosterone measures compared with a group of non-Indigenous women without PCOS whose blood samples were assessed in the same laboratory with the same assay. In looking for explanations of this there are a number of possibilities:

- High central obesity in this study group
- The young age of this study group
- Ethnic differences
- Environmental factors: diet, intrauterine environment, smoking, stress
- Sampling bias

In order to address some of these issues, a comparison was made of androgens between a subgroup from our study sample with regular menstrual cycles and no hirsutism matched for BMI and age with the Caucasian reference group. The Indigenous women still had higher testosterone, free testosterone and free androgen index whilst there was no difference in SHBG or DHEAS.

This may reflect ethnic differences as studies have shown that premenopausal African American women have lower levels of total and free testosterone and androstenedione than white American women after controlling for age, BMI and central obesity (Lamon-Fava et al. 2005, Spencer et al. 2007). However it is quite possible there are other factors such as central obesity, hyperinsulinaemia and environmental factors that are the reasons for these differences and we were not able to assess these in the non-Indigenous group.

The two groups were matched for BMI, but it was not possible to match for central obesity as there were no reported measures in the Caucasian reference group. Central obesity has been reported as having a more pronounced effect on androgens than general obesity and in this DRUID subgroup there was significant central obesity with 45% of those with a normal BMI having a waist circumference >80 cms and 62% a WHR >0.80. Potentially, the DRUID subgroup was likely to have more centrally obesity than the Caucasians which may account for the higher total and free

testosterone. This would not be an unexpected finding as greater central obesity in Aboriginal women compared with Australian women of European ancestry of the same BMI has been reported before (Piers et al. 2003). However if the DRUID women had more significant central obesity than the non-Indigenous women it would also have been anticipated that SHBG would be lower in the DRUID group, which was not the case. Central obesity by WHR was also not found to have a significant association with testosterone measurements in the total group of DRUID women assessable for androgen analysis by backward regression analysis. However this is probably because it was present in the majority of women regardless of BMI category.

Insulin, one of the drivers of androgens production, was high in this study group. Whilst insulin levels are not available for the non-Indigenous reference group, Maple-Brown et al. assessed Indigenous women from the DRUID study and compared them to non-Indigenous women in Darwin and found the Indigenous women had significantly higher fasting insulin levels. It is known that insulin may directly affect ovarian testosterone production in addition to decreasing SHBG and increasing testosterone bioavailability (Poretsky et al. 1999). It may be this effect is more pronounced in young women. However, neither fasting insulin nor the HOMA index, was found to be a significant predictor variable for SHBG or free testosterone in backward multivariate analysis. This may have been because the median fasting insulin was high across all women.

Other environmental factors that may affect testosterone production directly or indirectly through propensity to increased central fat include psychosocial stress, smoking and diet.

Indigenous people have been shown to experience a greater degree of stress in their lives and stress can increase central obesity and indirectly therefore increase androgens. The mechanism for stress increasing central adiposity may occur through activation of the hypothalamic–pituitary-adrenal axis with increased cortisol and visceral fat tissue (VAT). Stress may also decrease clearance of triglycerides which can inhibit the ability of leptin to decrease appetite.

Diet may play a role in androgens: excess carbohydrate intake has been shown to affect insulin levels and therefore androgen levels. Fructose and sucrose have also been

shown to decrease human hepatic SHBG expression independent of insulin. Therefore a high monosaccharide diet leading to low SHBG independent of insulin can lead to increased free testosterone in women. High sucrose diets fed to rats may also affect androgen production through increasing visceral fat and muscle insulin resistance. Other evidence for this is that low carbohydrate diets or low glycaemic load diets may lead to a preferential loss of central fat.

Smoking and irregular cycles have been found to be significant influences on androgens in some studies. They were not found to be important factors in androgens in this group of women. It may not be possible to show an effect of smoking as so many women smoked and we did not ascertain a quantitative value of cigarettes smoked.

In the design of this study there are some issues possibly affecting the androgen levels we were not able to assess

- Oestradiol, LH and FSH were not measured in this group of women and may have had an influence on SHBG and testosterone we cannot measure.
- This study was designed to screen for diabetes and may have led to more women at high risk to present for screening.

A high proportion of participants reported hirsutism and irregular cycles. Excess hirsutism may be due to: ethnic differences with higher testosterone production, the high proportion of women with obesity, or ethnic differences in skin 5 α -reductase activity. Similarly, 25.3% of pre-menopausal women not using hormonal contraception who had androgens measured reported irregular cycles. Whilst this may reflect bias in sampling it may also reflect high obesity in urban Indigenous women.

4.5 Conclusion

- Testosterone, free testosterone and free androgen index are higher in a subgroup of DRUID participants compared with the no-Indigenous reference group. There was no difference in SHBG or DHEAS. This may reflect a difference in production or metabolism, may be a result of high central obesity, hyperinsulinemia or perhaps reflects other aetiologies.
- Apparent decrease in total and free testosterone and DHEAS with age. A nonsignificant increase in SHBG with age.

- An increase in testosterone and FT with obesity and a decrease in SHBG
- No effect of obesity in women greater than 35 years.
- Central obesity is not always associated with high testosterone

This study population had a high proportion of young women with a high frequency of obesity and high fasting insulin levels. A large proportion reported hirsutism. Whilst the testosterone and FT levels were higher than those in a group of non-Indigenous women of similar age and BMI, it cannot be determined that these are due to ethnic differences necessitating an assignment of differing values of “normal.” For the purposes of this thesis the classification of hyperandrogenaemia will be a free testosterone of greater than 34.2, which is greater than or equal to the 95th centile for free testosterone in the group of non-Indigenous women in Adelaide, SA.

More work needs to be done to ascertain if there are indeed ethnic differences in androgens between Indigenous and non-Indigenous women, to determine whether factors such as diet and central obesity are important drivers of androgen and SHBG production, metabolism and clearance and to assess whether these are influences on androgens that have a more significant effect in young obese women.

Chapter 5 Prevalence of PCOS in this study group

5.1 Introduction

Most of the large PCOS prevalence studies have used a diagnosis of PCOS derived from the 1990 NIH/NICHHD conference which requires menstrual disorders (anovulation), hyperandrogenism (clinical and/or biochemical) and exclusion of other related disorders (Zawadzki et al 1992). These studies are hindered by the poor reproducibility and accuracy of testosterone assays, the variability of hair growth in different ethnic populations, the subjective nature of hirsutism assessment and the impossibility of assessing hirsutism in women who are currently treating this problem.

The ASRM/ESHRE consensus statement and the AES/PCOS consensus also include polycystic ovaries on ultrasound as a diagnostic criteria (Azziz et al. 2008, Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004, The Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group 2004). However, polycystic ovaries can be found in up to 20% of normal women and can be difficult to distinguish from other conditions such as multifollicular ovaries due to hypogonadism (Farquhar et al. 1994). Ultrasound data may be subjective and varies between observers. Further, in screening large numbers of women it is impractical to assess each woman with ultrasound.

Folliculogenesis (as discussed in 1.5.1) is controlled by many factors including the ovarian produced Anti Mullerian hormone (AMH). This is increased in women with PCOS and PCO and ultrasound has shown the number of small antral follicles correlate closely with serum AMH levels (Pigny et al. 2006). The magnitude of the increase in AMH with PCO in age-matched women has been reported to correlate with insulin resistance, serum androstenedione, testosterone and pre-antral follicle numbers (Wang et al. 2007). Serum levels of AMH appear to have minimal fluctuation throughout the menstrual cycle although they do decline with age (de Vet et al. 2002, Hehenkamp et al. 2006, La Marca et al. 2006 Lee et al. 1996, Tremellen et al. 2005). It may therefore be feasible to use AMH as a surrogate marker for PCO on ultrasound.

The aim of this study was to assess the prevalence of PCOS by the NIH criteria in this group of urban Indigenous women and to assess the prevalence of PCOS by the

Rotterdam criteria using AMH as a surrogate for ovarian ultrasound. The women with PCOS-NIH will then be compared with a control group of women with regular cycles and no hyperandrogenism.

5.2 Methods

5.2.1 General Methods

The methods for data collection are described in detail in Chapter 2.

All the women who took part were asked to complete a reproductive health questionnaire, to have their blood pressure and anthropometry measures taken and to have a blood sample taken for the diagnosis of PCOS and assessment of associated metabolic complications.

Of the 456 women who either completed a questionnaire or had bloods taken as part of the Women's Health survey, 393 completed the questionnaire and had bloods taken for androgen assessment. From this cohort, women were excluded from assessment of PCOS if they were menopausal, had hysterectomies or were using hormone replacement therapy (n=22), or were current hormonal contraceptive users (n=81). They were also excluded if they reported "not applicable" to the question on cycle regularity (n=9) or if it was not possible to classify their cycle regularity from their responses (n=33). A cohort of 248 women was therefore eligible for assessment of the presence of PCOS (Figure 2.2). This is the denominator used when referring to the prevalence of PCOS in this study.

This study was commenced before the Rotterdam Consensus and our clinical decision was that the NIH/NICHHD criteria was the most suitable and practical for our study circumstances and would then be comparable with previous prevalence studies. In the assessment of hyperandrogenism we used biochemical results only as we were unable to have consistent assessors of hirsutism over the prolonged course of the study.

It is difficult to determine hyperandrogenaemia and in this study a value of free testosterone >34.2 pmol/l. This free testosterone value was the 95th centile of a reference group of non-Indigenous group of women in Adelaide without PCOS (discussed in chapter 4).

We also assessed women from this group using a variation of the Rotterdam consensus criteria, replacing polycystic ovaries on ultrasound with AMH. The AMH level identified is 23.0 ng/ml. We have used this level following an assessment of AMH and pre-antral follicles on ultrasound in women presenting for fertility screening at Repromed, Adelaide (Poprzeczny 2007).

5.2.2 PCOS diagnosis

The diagnosis of PCOS was made according to the two main criteria; the National Institutes of Health (NIH) and a modified Rotterdam consensus. To be classified as PCOS according to the NIH criteria women had to have hyperandrogenaemia and anovulation. For the modified Rotterdam they required 2 components present of anovulation, hyperandrogenaemia and PCO (as determined by AMH in our study). The individual determinants of the diagnostic criteria are described below:

1. Anovulation

In this study, menstrual cycles classified as irregular were assumed to be anovulatory as were regular cycles greater than 35 days (Figure 2.1). We did not confirm ovulation in the luteal phase with serum progesterone or urinary progesterone as this was logistically impractical. Women were screened opportunistically at any time of the cycle. In order to obtain samples for progesterone assessment in the luteal phase it would have been necessary to contact women, identify the timing of the luteal phase and have them return at that time for a serum or urine sample. This was not practical for many reasons: a significant number of women did not have a telephone contact, it would have required a dedicated Indigenous female project worker to make the contact and in most instances we would have had to provide transport for the women. Even if we had been able to provide an Indigenous assistant and transport our experience would suggest the response rate and attendance would have been low.

2. Hyperandrogenism

This was determined by biochemistry only. For reasons discussed in chapter 4 this was determined by free testosterone level at 34.2pmol/l.

3 Polycystic ovaries (PCO)

Women were classified as having PCO if they had a high AMH as a surrogate marker for PCO on ultrasound. The cut off value for AMH used was 23.0 ng/ml as discussed in 5.2.1

4 Exclusion of other aetiologies

Women who were thought to possibly have PCOS based on the two criteria above had their serum further tested for 17-OHP, TSH and prolactin. If there were abnormalities in these they were determined not to have PCOS.

Abnormal thyroid function and hyperprolactinaemia can cause oligomenorrhoea. Abnormal values for prolactin in this study were $>900\text{mIU/ml}$ and for TSH $< 0.4\text{ mIU/L}$ or TSH $>4.7\text{ mIU/L}$. There were two women who had a borderline raised TSH (TSH= 5.0mIU/L) and another woman who had a decreased TSH (TSH= 0.2mIU/L). No women eligible for assessment of PCOS had a prolactin greater than 900 IU/L . There were four women who did not have a TSH or prolactin measurement performed due to lack of serum. These were excluded from eligibility of assessment of PCOS.

A high 17-OHP can be an indicator of late onset congenital adrenal hyperplasia, which presents with symptoms very similar to PCOS. It is difficult to assign a screening value for 17-OHP as it varies throughout the menstrual cycle and different studies use different values, generally between $6\text{--}10\text{nmol/l}$ in the follicular phase of the cycle. A progesterone level greater than $>12\text{nmol/l}$ can confirm if that woman is in the luteal phase of her cycle when 17-OHP is higher and she can be recalled in the follicular phase for retesting. If the progesterone level is normal it can be assumed the woman is in the follicular phase of the cycle and she would be excluded from a diagnosis of PCOS. As already noted in section 5.2.3.1 follow up in this study was not provided. Therefore a value of 17-OHP of 10nmol/l was used as an exclusion value for exclusion from a diagnosis of PCOS, without further assessment of stage of cycle, to exclude women who potentially may have had congenital adrenal hyperplasia.

Four women with a 17-OHP $>10\text{nmol/l}$ were excluded.

5.2.3 Classification of Controls

Control groups were created out of our study sample to compare with the women diagnosed with PCOS. Whilst we assumed women with cycles <24 days were ovulatory and therefore excluded them from a diagnosis of PCOS, a greater number of these women may be anovulatory compared to those with cycles between 24–35 days. Therefore women with cycles <24 days were determined ineligible to be controls.

Women who reported hirsutism were not classified with PCOS as it was a subjective measure but these women were excluded from the controls as it is possible they may have had PCOS.

PCOS Controls (n=41)

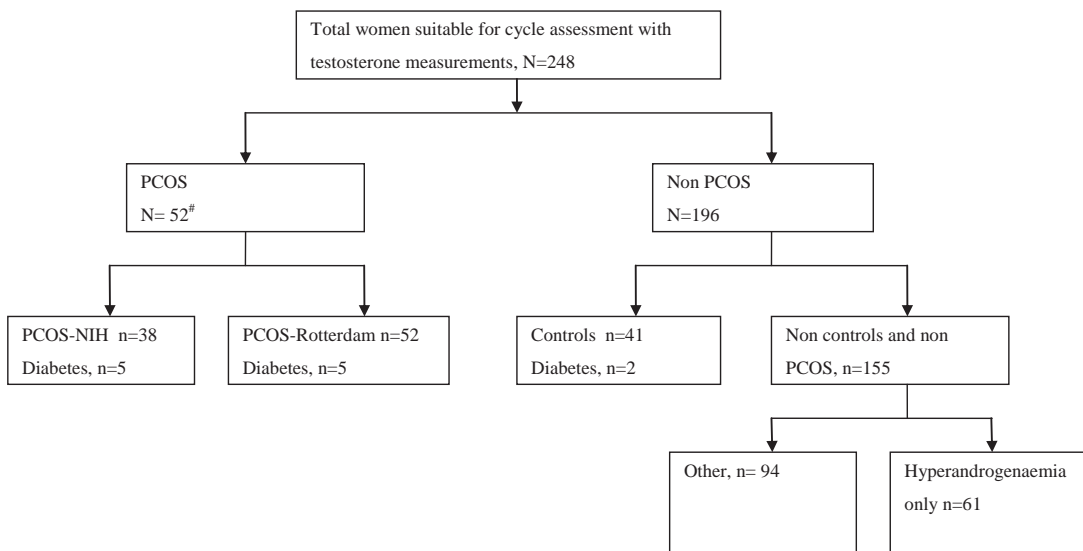
- reported regular 24–35 day menstrual cycles
- $FT \leq 34.2 \text{ nmol/l}$
- $AMH < 23.0 \text{ ng/ml}$
- did not report hirsutism or acne

5.2.4 Classification of hyperandrogenaemia and regular cycles

Of the 248 women assessable for PCOS, 61 women were found to have regular cycles and hyperandrogenaemia ($FT > 32.4 \text{ pmol/L}$). These women are also compared to those with PCOS.

Figure 5.1 demonstrates these classifications.

Figure 5.1 Classification of PCOS, Controls and other



#Note all PCOS-NIH also have PCOS-R

5.2.5 Statistical Analysis

Collected data was de-identified and entered into a Microsoft Access database. Statistical analyses were performed using Intercooled Stata 8.0 (Stata Corporation, Texas, USA). Analyses for the following summary statistics were performed: frequencies, means and standard deviations. Data are presented as mean (SD) and groups compared using unpaired t test. Variables whose distribution was not normal were log transformed for analysis and summary statistics presented as median with interquartile values and Wilcoxon rank sum used to compare groups. Statistical significance was accepted at $P < 0.05$. A two-tailed Chi^2 test was used to compare categorical variables. Regression was used to determine associations between multiple independent variables

5.3 Results

5.3.1 PCOS Prevalence

Of the 248 women eligible for PCOS assessment, 38 had PCOS according to the NIH criteria (a prevalence of 15.3%) and 52 according to the modified Rotterdam criteria (a prevalence of 21.3%). The prevalence of PCOS increased with obesity and decreased with age. It was most marked in those with an obese BMI rather than those with central obesity by waist circumference (WC) or waist hip ratio (WHR).

Figure 5.2 Prevalence of PCOS by Age group (10 years)

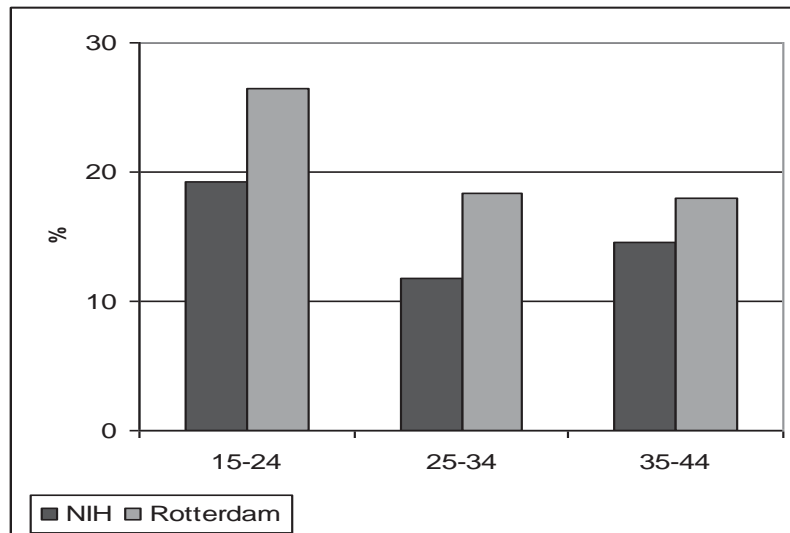
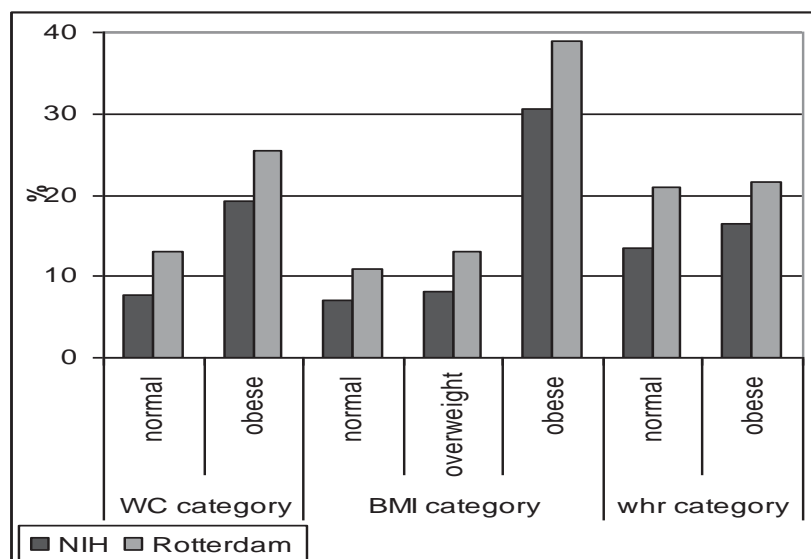


Figure 5.3 Prevalence of PCOS by Obesity Category



WC category: normal <88cm, obese ≥88cm

BMI category: normal ≤24.9 kg/m², overweight ≥25.0 kg/m² and ≤ 29.9kg/m², obese ≥30.0kg/m²

WHR: normal<0.8, obese ≥ 0.8

5.3.2 Components of PCOS

Among the 248 women assessable for PCOS, it was common to have hyperandrogenaemia by free testosterone (43.4%) and/or high AMH (41.9%). A

smaller proportion (29.1%) experienced irregular cycles. Of these, PCOS accounted for the majority of oligomenorrhoea (60.8%), a third of hyperandrogenaemia (33%) and 32.7% of those with high AMH (PCO).

Of those women who had two or more components of PCOS present, the majority with hyperandrogenaemia and oligomenorrhoea \pm high AMH were accounted for by those women with PCOS.

PCOS is responsible for:

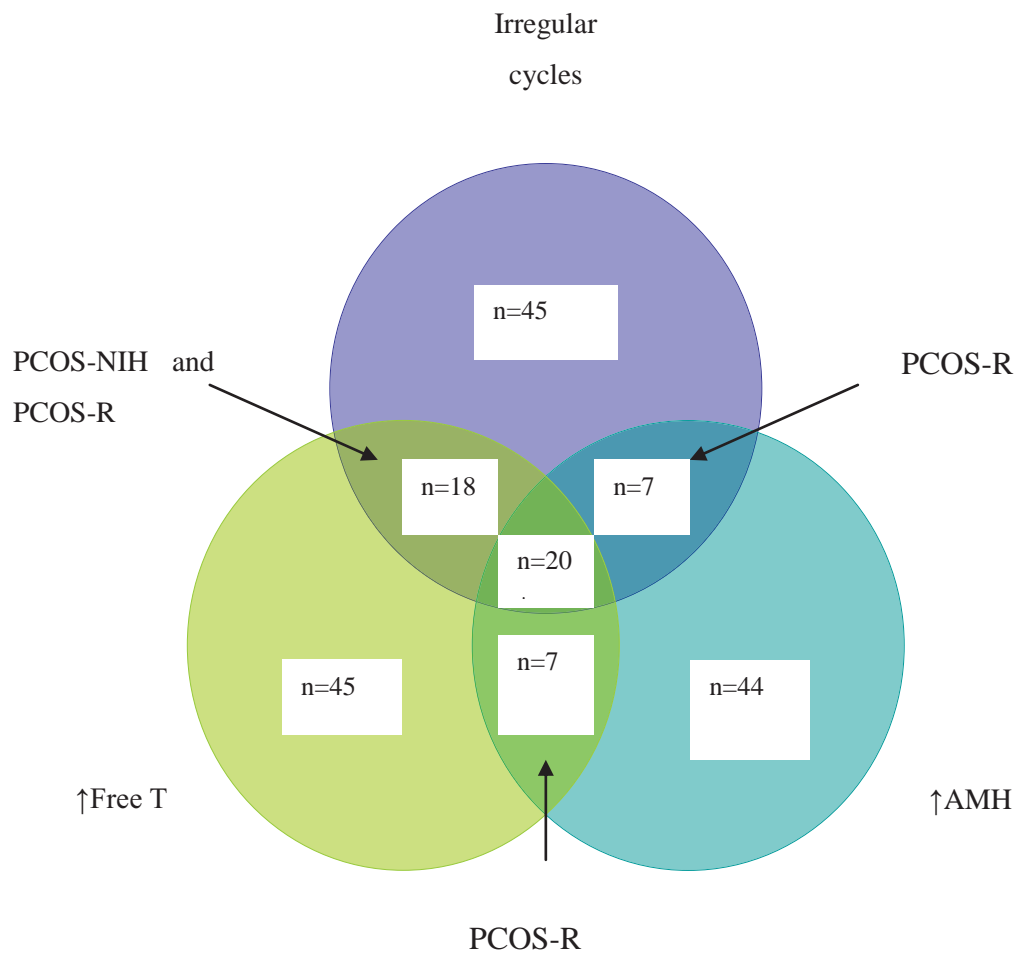
- 81% of those with oligomenorrhoea and high FT
- 21.0% of those with a high AMH and high FT
- 50.0% of those with oligomenorrhoea and high AMH
- 79.0% of those with a high AMH, high FT and oligomenorrhoea

PCOS may have been responsible for a greater number of those with a high AMH and oligomenorrhoea/high FT, but the study protocol was developed before the Rotterdam consensus definition, and we did not collect sufficient information necessary for diagnosis of PCOS in some women.

5.3.2.1 Components in those diagnosed with PCOS

The women diagnosed with PCOS by the Rotterdam criteria comprised: 18 women with oligomenorrhoea and raised free testosterone (NIH criteria); seven women with raised AMH and irregular cycles but normal FT; seven women with raised FT and raised AMH but normal cycles; and 20 women with a raised AMH, a raised FT and irregular cycles (see Figure 5.4). In total, of those who had a diagnosis of PCOS by NIH 52.6% also had a raised AMH and therefore had the full triad of characteristics of the Rotterdam criteria. There were a further four women with PCOS by NIH who did not have an AMH measurement so may or may not have had raised values.

Figure 5.4 Components of PCOS and frequency in women classified with PCOS-NIH or PCOS-R



There were no significant differences between PCOS by the NIH criteria or the Rotterdam consensus in metabolic, anthropometric variables (table 5.1). However, as AMH, has not been conclusively shown to be a reliable substitute for pelvic ultrasound, the analysis for the remainder of this chapter will use the data from the group of women diagnosed by the NIH criteria only.

Table 5.1 PCOS by NIH and Rotterdam criteria and associated anthropometric, reproductive and metabolic characteristics (no significant differences found)

	PCOS NIH		PCOS Rotterdam	
N	38		52	
Age (years)	31.5	(21, 36)	28.5	21.0, 35.0
Infertility*	19.4%	(n=7)	16.4%	(8)
Recurrent Miscarriage	2.8%	(n=1)	4.1%	(2)
Infertility and Miscarriage	2.8%	(n=1)	4.1%	(2)
BMI (kg/m ²)	33.4	(27.7, 39.7)	32.8	(26.5, 39.9)
Waist Circumference (cm)	102.5	(92.5, 119.4)	102.0	(84.4, 118.8)
Waist hip Ratio	0.86	(0.82, 0.96)	0.85	(0.80, 0.96)
Mean SBP(mmHg)	112.3	(105.0, 118.5)	113.0	(105.0, 121.0)
DBP (mmHg)	73.8	(67.0, 80.0)	74.0	(67.0, 80.0)
HDL (mmol/l)	1.1	(0.9, 1.2)	1.1	(0.9, 1.2)
LDL (mmol/l)	2.75	(2.45, 3.70)	2.9	(2.5, 3.6)
Total cholesterol(mmol/L)	4.6	(4.1, 5.5)	4.7	(4.2,5.5)
Triglycerides (mmol/L)	1.4	(0.9, 2.1)	1.3	(0.9, 2.0)
AMH (ng/ml)	29.9	(11.1, 44.9)	31.1	(16.8, 45.3)
Testosterone (mmol/L)	2.3	(1.9, 3.0)	2.1	(1.9, 3.0)
SHBG (mmol/L)	19.4	(13.7, 25.4)	19.9	(14.1, 27.4)
Free Testosterone(mmol/L)	50.9	(41.0, 78.5)	49.1	(38.1, 76.9)
FAI	11.2	(8.1,18)	10.2	(7.1, 17.5)
DHEAS (umol/L)	4.62	(3.38, 6.2)	4.6	(3.4, 5.8)
hsCRP(mg/L)	5.4	(2.0, 10.0)	4.3	(1.6, 10.0)
Homocysteine	7.9	(6.6, 9.1)	7.8	(6.5, 9.1)
Fasting glucose (mmol/L)	5.1	(4.8, 5.7)	5.0	(4.7, 5.6)
Excluding diabetics	5.0	(4.7, 5.4)	4.9	(4.7, 5.4)
Fasting Insulin (mU/L)	16	(8.0, 19.0)	15	(8.0, 20.0)
Excluding diabetics	14.5	(8.0, 19.0)	13.0	(8.0, 20.0)
HOMA	3.7	(2.0, 4.95)	3.6	(1.8, 5.0)
Excluding diabetics	2.9	(1.7, 4.7)	2.7	(1.7, 4.7)
2 hour glucose (mmol/L)	6.7	(5.3, 8.6)	6.4	(5.2, 7.9)
Excluding diabetics	6.6	(5.3, 7.7)	6.3	(5.2, 7.5)
2 hour insulin (mU/L)	91.0	(48.0, 128.0)	88.5	(48.0, 126.0)
Excluding diabetics	91.5	(48.0, 129.0)	89.0	(48.0, 127.0)

No significant differences (p<0.05) found between the two groups in any variables

5.3.3 Characteristics of women with PCOS-NIH compared to Controls

There was no difference in age between the women with PCOS-NIH and the controls but the women with PCOS-NIH were more obese by all measures of BMI, waist circumference and waist: hip ratio. However, if assessing the proportion of women with PCOS-NIH in each obesity category, a significantly greater proportion of women who are obese by BMI have PCOS-NIH whereas there is no difference in the women who are obese by WHR (Figures 5.5–5.7).

Figure 5.5 Proportion of women in each BMI category (kg/m^2) who are in PCOS-NIH or control group, $p=0.001$

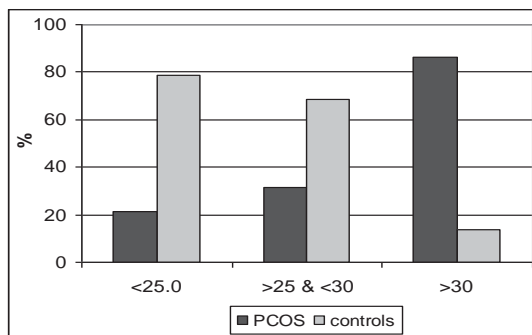


Figure 5.6 Proportion of women in each Waist circumference category (cm) who are in PCOS-NIH or control group, $p=0.01$

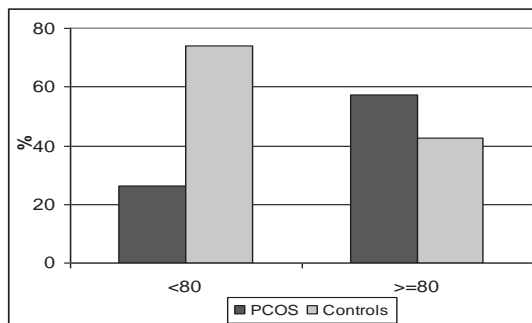
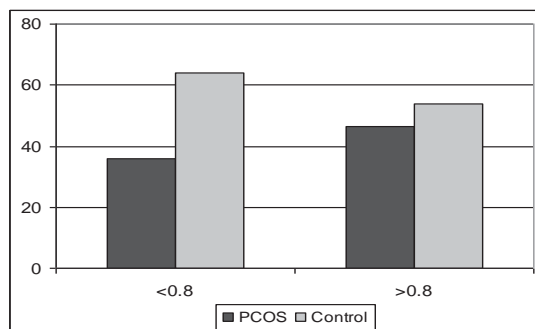


Figure 5.7 Proportion of women in each WHR category who are in PCOS-NIH or control group, $p=0.17$



Further if assessed by BMI category there is no significant difference in measures of central obesity between those with PCOS and controls (Figures 5.8 and 5.9). This may be of relevance when assessing metabolic variables as abnormalities are often related to central obesity.

Figure 5.8 PCOS and Controls, proportion of women with high WHR (>0.80) in each BMI category (normal, overweight or obese)

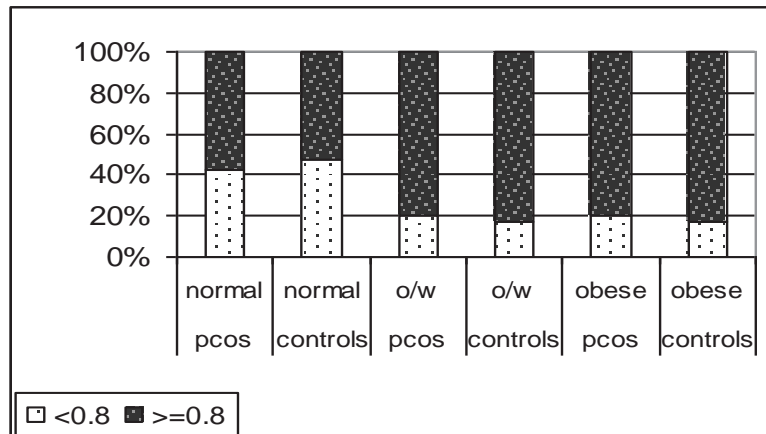
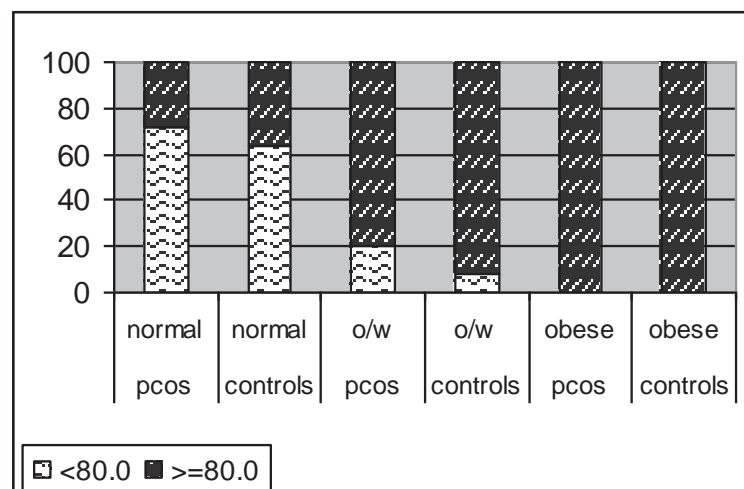


Figure 5.9 PCOS and Controls, proportion of women with high WC (≥ 80 cm) in each BMI category (normal, overweight or obese)



A higher proportion of women with PCOS reported infertility 21.6% (n=8) compared with 10.0% (n=4) of controls, though this was not statistically significant. The women with PCOS had significantly higher systolic and diastolic blood pressure, triglycerides, two hour glucose levels, fasting and two hour insulin levels and AMH. All measures of testosterone were higher in the women with PCOS. This was expected as women classified with PCOS by the definition used had a free testosterone >34.2nmol/L and the controls by definition had a free testosterone ≤

34.2nmol/L. Sex hormone binding globulin and HDL-C were lower in the women with PCOS (Table 5.2).

There were more women with diabetes in the PCOS group (13.2%, n=5) than the controls (4.9%, n=2) but this was not significant (p=0.19). However, if impaired fasting glucose, impaired glucose tolerance or diabetes, are considered together as a group there are more women with impairment of glucose metabolism in PCOS (n=14, 36.8%) than controls (n=4, 9.8 %), p=0.004. Importantly, even if women with diabetes are removed from the analyses all differences in biochemistry, anthropometry and blood pressure remain significant apart from the waist–hip ratio (see Appendix 5).

Table 5.2 Anthropometric and metabolic characteristics of women with PCOS-NIH and controls

	PCOS NIH criteria		Controls		P
N	38		41		
Age (years)	31.5	(21.0, 36.0)	34.0	(27.0, 40.0)	0.15
BMI (kg/m ²)*	33.4	(27.7,39.7)	23.8	(21.2,27.1)	0.0001
Waist Circumference (cm)*	102.5	(92.5,119.4)	83.3	(73.0,92.9)	0.0001
Waist hip Ratio*	0.86	(0.82,0.96)	0.83	(0.79,0.90)	0.04
Mean SBP(mmHg)	112.3	(105.0,118.5)	107.0	(100.0,111.5)	0.02
DBP (mmHg)	73.8	(67.0, 80.0)	69.5	(65.0, 72.5)	0.02
HDL (mmol/L)*	1.1	(0.9, 1.2)	1.2	(1.0, 1.4)	0.02
LDL (mmol/L)*	2.8	(2.5, 3.7)	2.9	(2.4, 3.4)	0.93
Total cholesterol(mmol/L)	4.6	(4.1, 5.5)	4.8	(4.0, 5.5)	0.62
Triglycerides (mmol/L)*	1.4	(0.9, 2.1)	1.1	(0.9, 1.6)	0.21
AMH(ng/ml)*	29.9	(11.1, 44.9)	11.2	(7.2, 16.6)	0.0002
Testosterone (nmol/L)*	2.25	(1.9, 3.0)	1.3	(1.1, 1.6) ^c	0.0001
SHBG (mmol/L)	19.4	(13.7, 25.4)	42.4	(31.4,66.2)	0.0001
Free Testosterone(mmol/L)	50.9	(41.0,78.5)	21.0	(13.7,26.7)	0.0001
FAI	11.2	(8.1,18)	3.3	(1.9, 4.1)	0.0001
DHEAS (umol/L)*	4.6	(3.4, 6.2)	3.2	(1.9, 4.8)	0.004
CRP (mg/L)	5.4	(2.0, 10.0)	1.9	(0.8, 3.0)	0.003
Homocysteine	7.9	(6.6, 9.1)	8.5	(6.9, 10.2)	0.13
Fasting glucose (mmol/L)*	5.1	(4.8, 5.7)	4.9	(4.7, 5.5)	0.39
Diabetes excluded	5.0	(4.7, 5.4)	4.9	(4.7, 5.4)	0.81
Fasting Insulin (mU/L)*	16	(8.0,19.0)	7.0	(5.0, 11.0)	0.0004
Diabetes excluded	14.5	(8.0,19.0)	7.0	(5.0,10.0)	0.0004
HOMA*	3.7	(2.0, 5.0)	1.5	(1.0, 2.5)	0.0005
Diabetes excluded	2.9	(1.7, 4.7)	1.5	(1.0, 2.3)	0.001
2 hour glucose (mmol/L)*	6.7	(5.3, 8.6)	5.5	(4.6, 6.5)	0.01
Diabetes excluded	6.6	(5.3, 7.7)	5.4	(4.6, 6.5)	0.005
2 hour insulin (mU/L)*	91.0	(48.0, 128.0)	34.0	(21.0, 68.0)	0.00001
Diabetes excluded	91.5	(48.0, 129.0)	30.0	(21.0, 61.0)	0.00001

*n is incomplete and the following apply

PCOS: n=37 for BMI, WC, WHR, HDL-C, TC, hsCRP, fasting glucose and insulin

n=36 for LDL-C, triglycerides

n=35 2 hour glucose and insulin

n=34 AMH, DHEAS

Controls: n=40 WC, WHR, DHEAS

n=39 HOMA, fasting and 2 hour glucose and insulin, LDL-C, triglycerides

n=37 HOMA, fasting glucose and insulin with diabetes excluded

Obesity, by all measures, is significantly greater in the women with PCOS than the controls and this alone can affect issues such as fertility and metabolic variables. To attempt to assess the effect of obesity, women were stratified into categories of either normal BMI ($\leq 24.99\text{kg/m}^2$) or overweight and obese ($\text{BMI} \geq 25.0\text{ kg/m}^2$) from both the PCOS and control groups (table 5.3).

Table 5.3 Reproductive, anthropometric and metabolic characteristics of women with PCOS-NIH and controls by BMI category. Normal BMI <25kg/m², Overweight and Obese BMI ≥ 25kg/m². Values are median with interquartile range

	PCOS -NIH Normal Weight		Controls Normal Weight		PCOS-NIH Overweight/Obese		Controls Overweight/Obese	
N	7		26		30		15	
Age (years)	18	(15, 33) ^{a,d}	33.0	(18.0, 39.0) ^a	33.0	(23.0, 38.0) ^d	35	(32,40)
Infertility*	0		7.7%	(n=2)	23.3%	(n=7)	0	
BMI(kg/m2) [#]	22.7	(19.2, 23.9) ^{b,f}	22.3	(20.7, 23.8) ^f	35.8	(32.6, 41.5) ^{b,f}	28.3	(26.1,31.1) ^f
Waist Circumference (cm) ^{ψ #}	76.1	(67.4, 81.6) ^{b,f}	76.4	(70.8, 84.0) ^f	107.2	(99.6, 121.7) ^{b,f}	95.2	(90.8, 103.1) ^f
Waist hip Ratio ^{ψ #}	0.85	(0.76, 0.93) ^f	0.80	(0.76, 0.87) ^e	0.88	(0.76, 0.99) ^f	0.90	(0.83, 0.91) ^e
SBP (mmHg)	106.5	(109.5, 115.5)	106.0	(101.0, 110.5)	115.0	(105.0, 121.5)	107.0	(98.5, 115.5)
DBP (mmHg)	72.0	(67.0, 82.5)	68.5	(65.0, 73.5)	75.5	(68.5, 80.0)	70.0	(65.0,72.5)
HDL (mmol/L)*	1.4	(1.0,1.4) ^e	1.3	(1.1, 1.4) ^d	1.0	(0.9, 1.1) ^e	1.1	(0.9, 1.2) ^d
LDL (mmol/L) ^{ψ *}	2.6	(2.5, 2.8)	3.1	(2.5, 3.4)	2.9	(2.5, 3.8)	2.9	(2.1, 3.8)
Total cholesterol(mmol/L)*	4.4	(3.9, 4.8)	4.8	(4.1, 5.3)	4.7	(4.2, 5.6)	5.0	(3.9, 5.7)
Triglycerides (mmol/L) ^{ψ *}	0.9	(0.7, 1.0) ^f	1.0	(0.7, 1.2) ^e	1.4	(1.0, 2.0) ^f	1.6	(1.2, 2.3) ^e
AMH(ng/ml) ^{† ψ *}	41.1	(30.1, 41.4) ^a	11.5	(8.3, 16.6) ^a	24.9	(10.5, 45.1) ^a	10.0	(4.3, 17.2) ^a
Testosterone (nmol/L)	1.9	(1.9, 2.2) ^b	1.3	(0.9, 1.7) ^b	2.5	(2.0, 3.1) ^c	1.4	(1.2, 1.5) ^c
SHBG (mmol/L)	32.3	(25.4, 38.2) ^{b,e}	45.8	(39.2, 69.2) ^b	17.5	(12.6, 24.3) ^{c,e}	36.7	(26.0, 66.2) ^c
Free Testosterone(mmol/L)	36.8	(35.9,39.8) ^{c,e}	16.6	(11.5, 25.2) ^c	57.2	(48.1, 79.3) ^{c,e}	25.4	(16.6,28.5) ^c
FAI	6.6	(5.9, 7.5) ^{c,d}	2.3	(1.7, 3.9) ^c	14.1	(10.0, 23.3) ^{c,d}	3.9	(2.2, 5.2) ^c

	PCOS -NIH Normal Weight		Controls Normal Weight		PCOS-NIH Overweight/Obese		Controls Overweight/Obese	
DHEAS (umol/L) [¶] ^ψ *	3.9	(3.4, 5.2)	3.2	(2.2, 4.3)	5.0	(3.3, 6.2) ^a	2.8	(1.6, 5.1) ^a
CRP (mg/L)	1.6	(0.6, 2.6) ^e	1.5	(0.6, 2.5) ^e	6.6	(2.3, 12.7) ^e	3.0	(2.0, 7.7) ^e
Homocysteine	7.7	(6.6, 9.6)	8.6	(6.9, 10.6)	7.9	(6.8, 9.1)	8.4	(6.6, 9.7)
Fasting glucose (mmol/L) [¶]	5.0	(4.9, 5.1)	4.8	(4.7, 5.4) ^e	5.2	(4.7, 6.0)	5.3	(4.9, 5.9) ^e
Diabetes excluded	5.0	(4.9, 5.1)	4.8	(4.7, 5.4) ^e	4.9	(4.7, 5.5)	5.2	(4.9, 5.7) ^e
Fasting Insulin (mU/L) [¶]	6.0	(4.0, 10.0) ^e	5.0	(4.5, 7.5) ^e	17.0	(11.0, 20.0) ^{a,e}	11.0	(7.0, 16.0) ^{a,e}
Diabetes excluded	6.0	(4.0, 10.0) ^e	5.0	(4.5, 7.5) ^e	17.0	(11.0, 19.0) ^{b,e}	10.0	(7.0, 14.0) ^{b,e}
HOMA [¶]	1.4	(0.9, 2.0) ^e	1.1	(1.0, 1.8) ^e	4.1	(2.4, 5.0) ^e	2.4	(1.5, 4.7) ^e
Diabetes excluded	1.4	(0.9, 2.0) ^e	1.1	(1.0, 1.8) ^e	3.7	(2.3, 4.7) ^e	2.1	(1.5, 3.5) ^e
2 hour glucose (mmol/L) ^{¶*}	5.0	(4.5, 6.1) ^d	5.2	(4.5, 6.4)	7.3	(6.1, 9.1) ^d	5.9	(5.3, 6.6)
Diabetes excluded	5.3	(4.5, 6.1) ^d	5.2	(4.5, 6.4)	7.0	(5.9, 8.0) ^d	5.9	(5.3, 6.6)
2 hour insulin (mU/L) ^{¶*}	35.0	(27.0, 47.0) ^e	28.5	(19.0, 58.0)	96.5	(58.5, 48.0) ^{b,e}	56.0	(24.0, 83.0) ^b
Diabetes excluded	34.0	(27.0, 47.0) ^e	28.5	(19.0, 58.0)	103.0	(86.0, 406.0) ^{b,e}	42.0	(24.0, 68.0) ^b

Incomplete data

[#]Controls normal BMI: n=25 WC and WHR; n=24 triglycerides, LDL-C, fasting and 2 hour glucose and insulin and HOMA

[¶]PCOS normal weight: DHEAS, AMH n=5; fasting insulin and glucose, homa, 2 hour insulin and glucose n=6.

^ψ Controls overweight and obese: DHEAS, AMH n=37; TG, LDL-C, WC and WHR n=36

*PCOS overweight and obese: 2 hour glucose and 2 hour insulin, DHEAS, AMH n=28; total cholesterol, LDL-C and HDL-C, triglycerides n=29

P values assessed by Wilcoxon Mann Whitney rank sum test of medians:

^a p ≤ 0.05 PCOS compared with control group · ^b p ≤ 0.01 PCOS compared with control group ^c p ≤ 0.0001 PCOS compared with control group

^d p ≤ 0.05 overweight/ obese PCOS compared to normal BMI PCOS; overweight or obese controls compared to normal BMI controls

^e p ≤ 0.01 overweight/ obese PCOS compared to normal BMI PCOS; overweight or obese controls compared to normal BMI controls

^f p ≤ 0.0001 overweight/ obese PCOS compared to normal BMI PCOS; overweight or obese controls compared to normal BMI controls

Normal BMI: PCOS compared with Controls (Table 5.3 and Table 5.4)

In the women with a normal BMI, those with PCOS-NIH were younger but there was no difference in any measures of general or central obesity between those with PCOS-NIH and controls. As would be expected, there was a lower SHBG and higher androgens and AMH but no difference in any metabolic variables. This may reflect the reality of little difference between the groups or may be a result of an insufficient small sample size to show any differences as there were only seven women with PCOS who had a normal BMI.

Overweight and Obese BMI: PCOS compared with controls (Table 5.3 and Table 5.4)

Compared to controls, the overweight and obese women with PCOS-NIH had a higher BMI and higher waist circumference, though there was no difference in the waist hip ratio. They reported more infertility and, as expected by definition, had a lower SHBG and higher testosterone, FT, FAI and AMH. Two hour insulin was significantly higher in PCOS-NIH. The two hour glucose and fasting insulin (after exclusion of diabetics) was higher in PCOS though this was not statistically significant ($p=0.06$). Importantly, there were no differences in other metabolic parameters such as HDL-C, triglycerides, blood pressure or CRP between those with PCOS-NIH and controls in this group.

Table 5.4 PCOS compared with Controls by BMI category; normal BMI (<25kg/m²) and overweight or obese BMI (≥ 25kg/m²)

	BMI <25.0kg/m ² : PCOS compared with Controls	BMI ≥25.0 kg/m ² : PCOS compared with Controls
Age	↓	–
BMI	–	↑
WC	–	↑
AMH	↑	↑
Testosterone	↑	↑
SHBG	↓	↓
FT	↑	↑
FAI	↑	↑
DHEAS	–	↑
2 hour insulin	–	↑

Controls: Overweight and obese compared with normal BMI (Table 5.3 and Table 5.5)

The overweight and obese controls were similar in age to those with a normal BMI. They had greater central obesity by waist circumference and waist hip ratio and higher CRP, triglycerides, fasting glucose, insulin and HOMA index. There was a trend for lower HDL-C and higher free testosterone and FAI but these were not statistically significant (p=0.06).

PCOS-NIH: Overweight and obese compared with normal BMI (Table 5.3 and Table 5.5)

The overweight and obese women with PCOS-NIH were older with a greater waist circumference though, interestingly, they had a similar waist hip ratio to those with a normal BMI. Significant differences in lipids were also observed with lower HDL-C and higher triglycerides in those who were overweight/ obese. SHBG was lower and all measures of free and total testosterone higher as were CRP and most of the measurements of insulin and glucose. The differences between those with a high BMI and a normal BMI were much more pronounced in women with PCOS-NIH than controls. This may be due to the extremely high BMI in the overweight /obese PCOS-NIH women or it may reflect a compounding effect of increasing BMI on PCOS-NIH. Given the lack of significant difference in most metabolic parameters between women with PCOS-NIH and controls who are all overweight/obese it would appear the former may be more likely.

Table 5.5 PCOS: obese and overweight BMI ($\geq 25\text{kg/m}^2$) compared with normal BMI ($< 25\text{kg/m}^2$)

Controls: obese and overweight BMI compared to normal BMI

	PCOS: overweight and obese compared with normal BMI	Controls: overweight and obese compared with normal BMI
Age	↑	–
BMI	↑	↑
WC	↑	↑
WHR	–	↑
HDL-C	↓	–
Triglycerides	↑	↑
Testosterone	↑	–
SHBG	↓	–
FT	↑	–
FAI	↑	–
CRP	↑	↑
Fasting insulin	↑	↑
2 hour insulin	↑	–
HOMA	↑	↑
Fasting glucose	–	↑
2 hour glucose	↑	–

Univariate analysis of PCOS and the metabolic and anthropometric variables shows a significant positive association between PCOS and the following: BMI, waist circumference, WHR, DBP, AMH, testosterone, free testosterone, FAI, DHEAS, hsCRP, 2 hour glucose, fasting and 2 hour insulin, and HOMA. There was still a significant association between all these and PCOS after excluding diabetics. A significant inverse association was present between PCOS and HDL and SHBG (Table 5.6).

Table 5.6 Univariate logistic regression analysis of variables with PCOS as a Predictor variable.

Dependent variables	R²	Constant	B coefficient	Standard Error	95% CI for B		P value
BMI (kg/m ²) *	0.27	3.19	0.31	0.06	0.19	0.42	0.001
Waist Circumference (cm) *	0.21	4.43	0.20	0.04	0.11	0.28	0.001
Waist hip Ratio	0.05	-0.18	0.06	0.02	0.002	0.11	0.04
SBP (mmHg)	0.02	109.2	3.37	2.81	-2.22	8.96	0.23
DBP (mmHg)	0.05	70.02	4.25	2.04	-0.19	8.31	0.04
HDL-C (mmol/L) *	0.06	0.17	-0.12	0.06	-0.23	-0.01	0.03
LDL-C (mmol/L)	0.003	2.97	0.09	0.18	-0.28	0.48	0.65
Total cholesterol(mmol/L)	0.000	4.9	-0.01	0.21	-0.42	0.39	0.95
Triglycerides (mmol/L) *	0.03	0.19	0.16	0.11	-0.06	0.38	0.14
AMH(ng/ml) *	0.12	2.22	0.76	0.23	0.29	1.22	0.002
Testosterone (nmol/L) *	0.53	0.25	0.61	0.06	0.48	0.74	0.001
SHBG (mmol/L) *	0.50	3.82	-0.89	0.10	-1.09	-0.68	0.001
DHEAS (umol/L)	0.08	3.67	1.21	0.49	0.23	2.19	0.02
hsCRP* (mg/L)	0.11	0.62	0.84	0.28	0.28	1.40	0.004
Homocysteine*	0.05	2.22	-0.15	0.07	-0.29	0.003	0.05
Fasting glucose(mmol/L)	0.04	5.14	0.72	0.41	-0.10	1.55	0.08
Excluding diabetics	0.001	5.04	0.04	0.13	-0.21	0.29	0.77
Fasting Insulin (mU/L) *	0.14	2.07	0.51	0.14	0.22	0.80	0.001
Excluding diabetics*	0.17	2.01	0.53	0.14	0.24	0.82	0.001
HOMA *	0.15	0.58	0.59	0.17	0.26	0.93	0.001
Excluding diabetics*	0.15	0.50	0.53	0.16	0.22	0.85	0.001
2 hour glucose (mmol/L) *	0.08	1.72	0.20	0.07	0.05	0.35	0.01
Excluding diabetics*	0.19	1.71	0.16	0.06	0.03	0.28	0.01
2 hour insulin (mU/L) *	0.22	3.62	0.81	0.18	0.46	1.18	0.001
Excluding diabetics	0.27	3.55	0.91	0.12	0.55	1.27	0.001

* log transformed for analysis

The following backward stepwise multivariate analysis of the total women who have PCOS or are controls will assess the effect of age, BMI, WHR and the presence of PCOS, on a number of metabolic variables. There was no significant interaction between WHR and BMI in these models. As the numbers are small predictor variables with a p value of <0.08 were included. This multivariate analysis finds that PCOS-NIH remains a significant predictor variable for the following only: systolic and diastolic blood pressure, AMH, SHBG and 2 hour insulin (Table 5.7). The remaining metabolic variables are more influenced by obesity (both BMI and WHR) than PCOS itself.

Table 5.7 Backward stepwise multivariate regression with PCOS, Age, BMI and WHR as predictor variables.

Dependent Variable	R2	Predictor variable	B Coefficient	SE	95% CI		P value
SBP (mmHg)	0.12	PCOS	4.75	2.72	-0.67	10.2	0.08
		Age	0.43	0.15	0.13	0.72	0.005
DBP (mmHg)	0.17	PCOS	5.40	1.94	1.53	9.27	0.007
		Age	0.36	0.11	0.15	0.57	0.001
HDL* (mmol/L)	0.14	BMI	-0.01	0.003	-0.01	-0.001	0.01
		WHR	-0.48	0.23	-0.94	0.01	0.04
Triglycerides (mmol/L)	0.18	WHR	1.71	0.43	0.86	2.55	0.000
AMH (ng/ml)	0.32	PCOS	0.58	0.21	0.16	0.99	0.008
		Age	-0.05	0.01	-0.08	-0.03	0.000
SHBG (mmol/L)	0.56	PCOS	-0.72	0.11	-0.94	-0.49	0.000
		BMI	-0.01	0.01	-0.02	0.000	0.05
		WHR	-1.05	0.44	-1.93	-0.16	0.02
DHEAS (umol/L)	0.12	Age	-0.05	0.03	-0.10	0.004	0.07
		PCOS	1.09	0.49	0.12	2.07	0.03
hsCRP (mg/L)	0.36	BMI	0.07	0.01	0.04	0.10	0.000
		WHR	2.20	1.10	0.02	4.38	0.05
HOMA*	0.41	BMI	0.04	0.01	0.03	0.06	0.000
		WHR	1.67	0.62	0.43	2.90	0.009
Fasting insulin (mU/L)	0.36	BMI	0.03	0.01	0.02	0.05	0.001
		WHR	1.06	0.55	-0.03	2.15	0.06
Fasting glucose (mmol/L)	0.16	BMI	0.04	0.02	0.004	0.09	0.03
		WHR	4.39	1.78	0.85	7.92	0.02
HOMA	0.41	BMI	0.04	0.01	0.03	0.06	0.000
		WHR	1.67	0.62	0.43	2.90	0.009
2 hour insulin (mU/L)	0.33	PCOS	0.51	0.19	0.12	0.89	0.01
		BMI	0.02	0.01	-0.001	0.04	0.06
		WHR	2.04	0.75	0.53	3.64	0.009
2 hour glucose (mmol/L)	0.25	BMI	0.01	0.004	0.004	0.02	0.003
		WHR	0.92	0.31	0.31	1.54	0.004

* log transformed for this analysis

5.3.4 PCOS compared to women with hyperandrogenaemia only

Hyperandrogenaemia may play a role in metabolic abnormalities and to assess this the women with PCOS-NIH have been compared with women who had regular cycles and hyperandrogenaemia (high FT>34.2nmol/L). IGT, IFG and diabetes together were more common in the women with PCOS-NIH at 36.8% compared to 19.7% although this did not reach statistical significance (p=0.06). There were few differences between those with hyperandrogenaemia and PCOS-NIH: SBP, DBP, fasting and 2 hour insulin and HOMA were all higher in women with PCOS. Two hour glucose was higher in PCOS but this was not significant once women with diabetes were excluded. However, once again, whilst there was no difference in age, there were differences in BMI and waist circumference between PCOS and the “hyperandrogenaemia only” group which may explain these differences (Table 5.8). If only the women who are obese by BMI (BMI \geq 30.0 kg/m² are assessed the only differences seen are: the women with PCOS are older, have higher systolic and diastolic blood pressure, HOMA-IR and 2 hour glucose (Table 5.9). When the women with diabetes are removed then the differences in HOMA and glucose are no longer significant although 2 hour insulin was then higher in PCOS. The differences in blood pressure disappeared after adjustment for age in regression analysis (p=0.10 for systolic blood pressure and p=0.13 for diastolic blood pressure). It would appear then that if current diabetes, obesity and age are taken into account that women with PCOS have similar risks to women with hyperandrogenaemia and regular cycles.

Table 5.8 Anthropometric, Reproductive and metabolic characteristics of women with PCOS and women with hyperandrogenaemia and regular cycles

	PCOS-NIH		Hyperandrogenaemia Regular cycles		P value
N	38		61		
Age (years)	31.5	(21, 36)	28.0	(18.0, 34.0)	0.38
Infertility	19.4%	(n=7)	3.6%	(n=2)	ns
Recurrent Miscarriage	2.8%	(n=1)	10.7%	(n=6)	ns
Infertility and Miscarriage	2.8%	(n=1)	1.8%	(n=1)	ns
BMI (kg/m ²) *	33.4	(27.7, 39.7)	28.6	(23.7, 32.1)	0.003
Waist Circumference (cm) *	102.5	(92.5, 119.4)	90.6	(80.6, 100.7)	0.005
Waist hip Ratio *	0.86	(0.82, 0.96)	0.86	(0.82, 0.91)	0.48
Mean SBP(mmHg)	112.3	(105.0, 118.5)	105.5	(102.0, 113.0)	0.009
DBP (mmHg)	73.8	(67.0, 80.0)	67.0	(63.5, 76.0)	0.008
HDL (mmol/l) *	1.1	(0.9, 1.2)	1.2	(0.9, 1.3)	0.15
LDL (mmol/l) *	2.75	(2.45, 3.70)	2.95	(2.4, 3.4)	0.97
Total cholesterol(mmol/l)	4.6	(4.1, 5.5)	4.6	(4.2, 5.4)	0.98
Triglycerides (mmol/l) *	1.4	(0.9, 2.1)	1.2	(1.0, 1.8)	0.24
AMH(ng/ml) *	29.9	(11.1, 44.9)	20.6	(11.1, 37.1)	0.39
Testosterone (mmol/l)	2.25	(1.9, 3.0)	2.2	(1.9, 2.6)	0.34
SHBG (mmol/l)	19.4	(13.7, 25.4)	20.3	(17.4, 27.2)	0.20
Free Testosterone(mmol/l)	50.9	(41.0, 78.5)	49.7	(42.5, 60.4)	0.29
FAI	11.2	(8.1,18)	10.1	(7.8, 14.0)	0.18
DHEAS (umol/l) *	4.62	(3.38, 6.2)	5.2	(3.4, 6.4)	0.56
hsCRP(mg/L) *	5.4	(2.0, 10.0)	3.9	(2.0, 8.8)	0.42
Homocysteine	7.9	(6.6, 9.1)	8.5	(7.4, 9.7)	0.09
Fasting glucose (mmol/L) *	5.1	(4.8, 5.7)	5.0	(4.7, 5.3)	0.17
Diabetes excluded	5.0	(4.7, 5.4)	4.9	(4.7, 5.2)	0.63
Fasting Insulin (mU/L) *	16.0	(8.0, 19.0)	10.0	(6.0, 14.5)	0.007
Diabetes excluded	14.5	(8.0, 19.0)	10.0	(6.0, 14.0)	0.02
HOMA	3.7	(2.0, 5.0)	2.2	(1.4, 3.3)	0.004
Diabetes excluded	2.9	(1.7, 4.7)	2.1	(1.4, 3.3)	0.03
2 hour glucose (mmol/L) *	6.7	(5.3, 8.6)	6.0	(4.9, 6.7)	0.04
Diabetes excluded	6.6	(5.3, 7.7)	6.0	(4.9, 6.7)	0.11
2 hour insulin (mU/L) *	91.0	(48.0, 128.0)	51.5	(34.5, 88.5)	0.02
Diabetes excluded	91.5	(48.0, 129.0)	51.0	(33.0, 89.0)	0.007

*incomplete data.

PCOS: n=37 for BMI, WC, WHR, HDL-C, TC, hsCRP, fasting glucose and insulin
n=36 for LDL-C, triglycerides
n=35 2 hour glucose and insulin
n=34 AMH, DHEAS

Hyperandrogenaemia: n=56 for WC, WHR
n= 57 for 2 hour insulin and glucose
n= 59 for BMI and DHEAS
n=60 for LDL-C, triglycerides, AMH, fasting measures glucose insulin and HOMA-IR

Table 5.9 Anthropometric, Reproductive and metabolic characteristics of women with BMI ≥ 30.0 kg/m² and either PCOS or hyperandrogenaemia with regular cycles

	PCOS NIH		Hyperandrogenaemia Regular cycles		P value
N	25		21		
Age (years)	34.0	(24.0, 36.0)	26	(17, 30)	0.02
Infertility	24.0%	(n=6)	4.8%	(n=1)	ns
Recurrent Miscarriage	4.0%	(n=1)	14.3%	(n=3)	ns
Infertility and Miscarriage	-		-		-
BMI (kg/m ²) *	36.9	(33.4, 42.7)	34.4	(31.4, 37.3)	0.09
Waist Circumference (cm) *	113.4	(102.0, 122.1)	104.6	(98.9, 111.8)	0.09
Waist hip Ratio *	0.88	(0.83, 0.99)	0.89	(0.84, 0.92)	0.16
SBP (mmHg)	116.5	(105.0, 121.5)	107.0	(104.5, 112.0)	0.02
DBP (mmHg)	78.5	(69.0, 80.0)	70.0	(63.5, 75.5)	0.01
HDL (mmol/L) *	1.0	(0.9, 1.1)	1.2	(0.9, 1.3)	0.27
LDL (mmol/L) *	2.9	(2.5, 3.7)	2.9	(2.4, 3.3)	0.66
Total cholesterol(mmol/L) *	4.7	(4.2, 5.5)	4.5	(4.0, 5.1)	0.44
Triglycerides (mmol/L) *	1.5	(1.0, 2.1)	1.1	(1.0, 1.9)	0.15
AMH (ng/ml) *	23.2	(11.1, 44.9)	22.2	(13.0, 39.6)	0.96
Testosterone (mmol/L)	2.7	(2.0, 3.1)	2.5	(2.1, 2.6)	0.51
SHBG (mmol/L)	17.2	(11.9, 24.3)	19.5	(15.9, 27.2)	0.10
Free Testosterone(mmol/L)	57.5	(47.1, 79.3)	52.9	(47.8, 62.4)	0.08
FAI	14.8	(10.0, 23.3)	11.4	(8.7, 14.7)	0.18
DHEAS (umol/l) *	4.9	(3.0, 6.0)	4.2	(3.5, 8.4)	0.59
hsCRP(mg/L)	7.2	(3.9, 13.8)	7.4	(3.2, 14.9)	0.97
Homocysteine	8.1	(7.0, 9.1)	7.8	(7.1, 9.7)	0.60
Fasting glucose (mmol/L) *	5.2	(4.8, 6.0)	4.9	(4.7, 5.4)	0.11
Diabetes excluded	5.0	(4.7, 5.6)	4.0	(4.7, 5.4)	0.87
Fasting Insulin (mU/L) *	18.0	(12.0, 21.0)	13.5	(10.0, 16.5)	0.22
Diabetes excluded	17.5	(11.5, 20.5)	13.5	(10.0, 16.5)	0.16
HOMA *	4.3	(3.1, 5.4)	3.0	(2.1, 4.1)	0.05
Diabetes excluded	3.9	(2.5, 4.8)	3.0	(2.1, 4.1)	0.22
2 hour glucose (mmol/L) *	7.5	(6.5, 9.1)	6.2	(5.6, 6.4)	0.02
Diabetes excluded	7.1	(6.0, 8.3)	6.2	(5.6, 6.4)	0.09
2 hour insulin (mU/L) *	92.0	(56.0, 166.0)	83.0	(63.0, 93.0)	0.11
Diabetes excluded	97.5	(71.0, 168.0)	83.0	(63.0, 93.0)	0.05

* Incomplete data

PCOS-NIH: n=24 for HDL-C, LDL-C, total cholesterol, triglycerides, AMH, DHEAS, 2 hour measures of glucose and insulin.

Hyperandrogenaemia: n=20 for WC, WHR, LDL-C, triglycerides, AMH, DHEAS, glucose and insulin measures

5.4 Discussion

The main findings in this section were that there was a high prevalence of PCOS that increased significantly with BMI and that metabolic variables all showed a worse profile in those with PCOS. However, the majority of these metabolic differences appear to be largely due to the significant obesity associated with PCOS. In contrast to other studies, BMI was the best indicator of body fat and associated metabolic abnormalities. This is because central fat deposition was overwhelmingly the normal pattern of fat distribution in this study group.

Applying the NIH criteria to this study group and utilising only a raised free testosterone as the determinant of hyperandrogenism there is a prevalence of PCOS of 15.3% in 248 women. If a modified version of the Rotterdam criteria is applied utilising raised free testosterone as the determinant of hyperandrogenism and raised AMH as a surrogate for an ultrasound diagnosis of PCO there is a prevalence of 26.6% in 248 women. This prevalence increased with obesity and in those women with a BMI ≥ 30 kg/m² the prevalence was 29.9% for PCOS-NIH and 37.9% for those with PCOS-R.

The prevalence of PCOS in this study group is higher than has been reported in other studies using the NIH criteria (Asunción et al. 2000, Diamanti-Kandarakis et al. 1999, Knochenhauer et al. 1998). It is important to note there are factors within the study that may have led to **both** underestimation or overestimation of the prevalence of PCOS.

Factors that may have underestimated the prevalence of PCOS include

- Hirsutism was not included as a diagnostic criteria and the prevalence would have been higher if it had been included. This was a criteria in two of the large prevalence studies by Knochenhauer et al. and Asuncion et al. with lower prevalence (Asunción et al. 2000, Knochenhauer et al. 1998). Studies of

women presenting with hirsutism have found that, even if the Ferriman Galwey score was low, up to 82% had PCOS (Azziz et al. 2004a, Carmina et al. 2006b, Souter et al. 2004). The prevalence of hirsutism has been reported at 2.8–15% in predominantly Caucasian populations and it could be argued that the prevalence of hirsutism in this study at 38% is abnormally high compared to these (Asunción et al. 2000, Knochenhauer et al. 1998). This may have been due to hirsutism being self-reported but 64% also reported physical or medical treatment for their hirsutism suggesting it was a significant cosmetic problem for these women. Hirsutism also varies with ethnicity and was present in 29% of women in the PCOS prevalence study in Greece, although this may also have been partly due to selection bias (Diamanti-Kandarakis et al. 1999). Anecdotally, hirsutism is common in Indigenous women though there is little data to confirm this. Consistent with the present study, Davis et al. found a prevalence of hirsutism of 39.5% in Indigenous women although numbers were small (Davis et al. 2002).

- The definition we used for hyperandrogenaemia only included free testosterone. The other studies all used a combination of androgens which would have increased the prevalence in this study.
- Exclusion of women currently using hormonal contraception. This may have led to a bias in either direction depending on the reason women were using the contraception. If they were using it to control cycles then it would have underestimated the prevalence of PCOS.
- If it had been possible to follow up the four women with an elevated 17-OHP we may have found it was a transient elevation associated with the luteal phase and these women may have been diagnosed with PCOS.

Factors that may have led to an overestimation of PCOS

- This study was not a population based sample and had a low response rate from the community. The DRUID female participants did however have an age distribution similar to that of the estimated regional female population. Based on the estimated population numbers, this study screened 12% of women 15–24 years, 13% of 35–34 years and 17.0% of 35–44 years (2.1.1). DRUID was also a study focused on diabetes which may have led to bias in our sampling, but only 8.5% of the total women who self-presented for screening at DRUID and agreed to take part in the Women's Reproductive Health survey had

diabetes (previously diagnosed or diagnosed by the DRUID study) and these were not overly represented in the PCOS group.

- For similar reasons women with obesity, a family history of diabetes or other risk factors may have been more likely to present for screening. There was a high proportion of women in this study who were overweight or obese, with central obesity as measured by waist circumference and WHR. However many studies have shown obesity to be more prevalent in Indigenous compared to non-Indigenous Australians and central obesity to be more prominent than in those of European background at the same BMI (Piers et al. 2003). It is therefore possible that the higher indicators of obesity found in this group is typical of Indigenous people in Darwin.
- If we had chosen a lower screening cut off measure, for example 6 nmol/l, for 17-OHP to exclude late onset congenital adrenal hyperplasia then the prevalence would have been slightly lower but still high at 11.3% for PCOS-NIH.
- Other studies have used the 95th centile of androgen of regularly cycling women within their sample without hirsutism as the determining value of hyperandrogenaemia for diagnosis of PCOS. Instead we used a cut off from a non-Indigenous group of women without PCOS in Adelaide whose blood was measured in the same laboratory with the same assay. This may have led to an overestimation of PCOS, but as discussed in Chapter 4, the androgens in this study sample were probably high due to significant central obesity and other factors and not necessarily due to ethnic differences.

There were no significant differences between the two different groups of women diagnosed with PCOS by the NIH or the Rotterdam criteria. Studies have shown that women with a diagnosis of PCOS based on PCO on ultrasound and irregular cycles or hyperandrogenaemia with and regular cycles may not have the same metabolic abnormalities as those with PCOS-NIH but are usually more abnormal than controls, although this may be related to obesity, particularly central obesity (Moran and Teede 2009). There were fourteen women in the modified Rotterdam that did not have either oligomenorrhoea or hyperandrogenaemia but still experienced significant central obesity, which perhaps explains the similar metabolic findings in the two PCOS definition groups.

The median measures of obesity, blood pressure, androgens, SHBG, HDL-C, triglycerides, fasting insulin, insulin resistance (HOMA), 2 hour glucose and insulin and

hsCRP were all higher in the women with PCOS. This was largely explained by the higher BMI and central obesity in the women with PCOS compared with controls.

There was a higher prevalence of diabetes and IGT in the women with PCOS by the NIH criteria and of IGT by the Rotterdam criteria. There was also significantly higher fasting insulin, insulin resistance (HOMA), CRP, triglycerides, systolic and diastolic blood pressure, all measures of androgens, SHBG and 2 hour insulin and glucose in both groups of women with PCOS compared to their respective controls. However there was no difference in diabetes or IGT and most of the metabolic variables once stratified by BMI category. There were significant differences between the obese and non-obese controls and obese and non-obese PCOS-NIH. Multivariate analysis also showed that obesity (BMI and WHR) was a more significant factor for most metabolic variables than PCOS. Further, comparing obese women with PCOS-NIH and obese women with hyperandrogenaemia and regular cycles revealed few differences once those with diabetes were excluded.

These results are different to a number of studies that show a difference in metabolic characteristics due to PCOS even after accounting for obesity. It may be that because this group of women is so abnormal in terms of their health that differences normally seen are attenuated. The women in this study have very high obesity and in particular central obesity, even with a normal BMI. The total study group had significant abnormalities in metabolic indicators such as high CRP, fasting insulin and low HDL. This is consistent with observations of Indigenous women as a population where aspects of the metabolic syndrome are evident in Indigenous women even when lean (Shemesh et al. 2007, Shemesh et al. 2008)

5.5 Conclusion

There was a high prevalence of PCOS in this group of women and this increased with increasing obesity as defined by BMI. Therefore in contrast to other studies, in this group BMI was a better predictor of PCOS than central obesity. This is probably because, unlike other populations, a central pattern of fat distribution was present in the majority of women.

Whilst there were more metabolic abnormalities in the women with PCOS, when differences in BMI between the controls and those with PCOS were accounted for, the only factors that remain significantly associated with PCOS are blood pressure, AMH,

SHBG, androgens and 2 hour insulin. Similarly, comparing women with regular cycles and hyperandrogenaemia to those with PCOS, found lower measures of blood pressure, insulin and insulin resistance and but the driver for this may have been the lower obesity rather than PCOS itself as when only obese women from these groups without diabetes were compared there were no differences. The prevalence of PCOS-NIH increased with obesity by BMI and the metabolic abnormalities increased in obese women with PCOS compared to those who were overweight or lean. It may be that there is a propensity to increase weight with PCOS and that the accumulation of body fat exacerbates the metabolic abnormalities seen with PCOS.

Due to the high prevalence in this group it is important to increase the awareness of both diagnosis and best practice management of PCOS among health providers. It would also be interesting to further explore the apparent role of increased BMI in amplifying / unmasking the predisposition to PCOS.

At this stage AMH does not seem to add much information to a diagnosis of PCOS in Indigenous women: however, this would be worth exploring further, particularly as women in remote communities have difficulty accessing ultrasound and this would facilitate diagnosis.

Chapter 6 PCOS and the Metabolic Syndrome

6.1 Introduction

The metabolic syndrome is a constellation of risk factors including obesity, dyslipidaemia hypertension and impaired glucose regulation and/or insulin resistance and individuals with this syndrome are at increased risk of cardiovascular disease and diabetes (Alberti et al. 2009).

There are a number of definitions of the metabolic syndrome that are used clinically and in research. As discussed in Chapter 1 there have been attempts to define metabolic syndrome in a way that is acceptable and applicable internationally. The three definitions most commonly referred to are the National Cholesterol Education Program Adult Treatment Panel III (NCEP), the World Health Organisation (WHO) and the International Diabetes Federation (IDF) (Alberti et al. 2009, Grundy et al. 2005, Third report NCEP Expert Panel 2002) (Details Table 1.1).

Age, obesity, ethnicity and the presence of PCOS have all been shown to affect the risk of developing metabolic syndrome. It is reasonable to anticipate that metabolic syndrome would be common among Indigenous women with PCOS, as metabolic syndrome has been reported to be highly prevalent in Indigenous women, in women with PCOS and in women with obesity. Further a low HDL-C ($<1.0\text{mmol/l}$) has been shown to be present in $>70\%$ of Indigenous men and women from Central Australia (Shemesh et al 2008).

Within Australia, prevalence of metabolic syndrome in women in the general population as assessed by Ausdiab was 19–29% by the NCEP and IDF definitions respectively. In rural South Australian and Victorian women the figure was one third by the same definitions (28.3% NCEP and 30.1% IDF) and ethnicity was not specified. In non-Indigenous South Australian and Darwin women the prevalence by the NCEP definition was 14 and 22% respectively (Cameron et al. 2007, Janus et al. 2007, Maple-Brown 2005). Metabolic syndrome appears to occur more frequently in Indigenous women from Central Australia and the Torres Strait with a reported prevalence of 30–40% (Schutte et al. 2005). A study of Indigenous adults in the Top End of the Northern Territory reported

similar frequencies with metabolic syndrome present in 30–38% in the urban area and 46–50% from a remote area (NCEP and modified WHO definitions) (Maple-Brown 2005¹³).

Women with PCOS also have a higher prevalence of metabolic syndrome than controls, although this varies depending on age and ethnicity of the study participants (1.8.3). One study in Western Australia found metabolic syndrome to be four times higher in women with PCOS compared with age matched women from the Australian national diabetes survey, Ausdiab. Interestingly, the effect of PCOS was evident independent of obesity only in the women with a BMI > 30.0 kg/m² (Cussons et al. 2008). The influence of PCOS on metabolic syndrome over and above the impact of obesity is difficult to ascertain. Some studies do not account for the effects of obesity, others agree with Cussons et al. (2008) that PCOS remains influential after adjusting for obesity, and yet others have reported no effect of PCOS once adjusting for obesity (Apridonidze et al. 2005, Coviello et al. 2006, Dokras et al. 2005, Glueck et al. 2003). The phenotype of PCOS may also be important in relation to metabolic syndrome. Women with ovulatory PCOS and no hyperandrogenism have been found in some studies to have lower prevalence of metabolic syndrome and its components (Barber et al. 2007, Carmina et al. 2006a).

In this chapter the relationship between PCOS-NIH and metabolic syndrome in Indigenous women will be explored, beginning with the prevalence of metabolic syndrome and its components in Indigenous women with PCOS and controls. Factors influencing metabolic syndrome, including age and obesity, will be assessed.

6.2 Methods

Clinicians recognise that women with diabetes have an increased risk of cardiovascular disease and the potential value of diagnosing metabolic syndrome in women is alerting clinicians to those without diabetes who may be at risk and require monitoring and management to delay or prevent diabetes and cardiovascular disease. Participants with diabetes were therefore excluded from the assessment of metabolic syndrome in this chapter. There were 14 women with known diabetes among the participants who were eligible for assessment of PCOS. In the women with PCOS, 13.2% (n=5) had diabetes in the group defined by the NIH criteria and 9.6% (n=5) by the Rotterdam criteria. This

¹³ Note that the urban adults in the study of Maple-Brown were men and women in the DRUID study aged \geq 18 years

contrasts with controls who had 3.5% (n=2) and the differences between the groups were not significant possibly because of small numbers.

The definition of metabolic syndrome that will be used is the NCEP ATPIII (Section 1.2.2). The reasons for this include: the measures used in NCEP are routine ones performed in clinical practice, the WHO definition requires an OGTT and insulin levels among other indicators, and IDF uses ethnic-specific waist circumference cut-offs. This is difficult for Indigenous women as there is no “ideal” waist circumference determined for Aboriginal or Torres Strait Islander women and there is no mechanism to adjust for those women of mixed ethnic background. Only PCOS-NIH will be reported as there was no significant difference between PCOS-NIH and PCOS-R and AMH as yet has not been proven to be a consistently reliable substitute for ultrasound in PCOS diagnosis.

The groups of women that will be discussed are defined in detail in section 5.2 but a brief summary follows

- PCOS-NIH: women diagnosed with PCOS based on raised free testosterone and oligomenorrhoea (n=38)
- Controls: the women who have regular cycles (25–35 days), normal free testosterone and normal AMH (n=41).

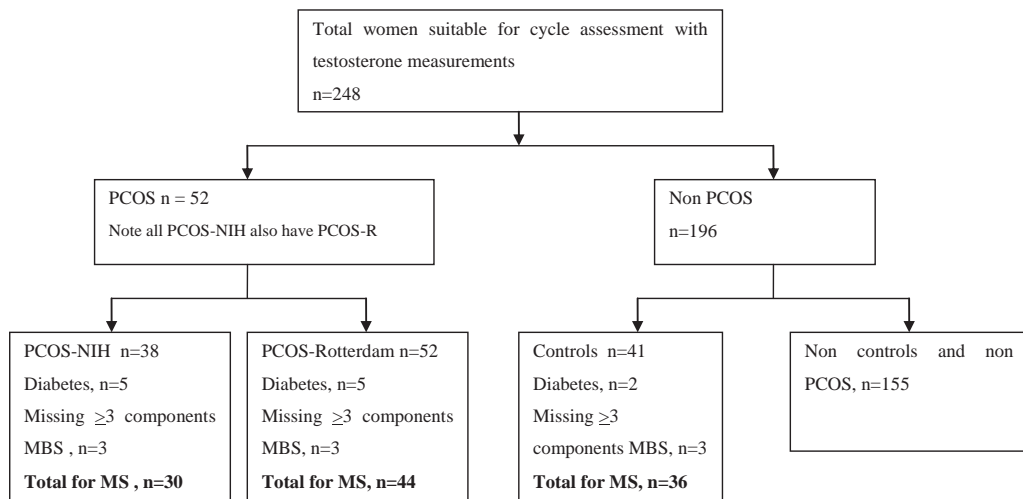
Women with diabetes were excluded as were those with missing data for one or more of the factors of the metabolic syndrome, unless they already had three or more positive factors (where a missing value would not change the classification) (see figure 6.1).

Within the total group of women (n=248), 14 had diabetes and 16 were missing components of the metabolic syndrome, leaving a sample size of n=218. The group with PCOS-NIH n=38: 8 women were excluded due to diabetes or missing data, leaving n=30.

Five Controls were excluded due to diabetes or missing data, leaving n=36.

Note that the National Cholesterol Education Program Adult Treatment Panel III will be referred to from here on as NCEP and the modified WHO classification of the metabolic syndrome will be referred to as WHO.

Figure 6.1 Women in PCOS and Control groups assessed for metabolic syndrome (MS)



6.3 Results

6.3.1 NCEP in PCOS and Controls

6.3.1.1 Prevalence of metabolic syndrome by NCEP

Metabolic syndrome by the NCEP criteria (≥ 3 components) was present in 25% of women deemed assessable. The group with the highest proportion with metabolic syndrome was the women with PCOS; 43.3% of PCOS-NIH and 39.5% of PCOS-R. There was no significant difference between PCOS-NIH and controls (Table 6.1).

Table 6.1 Number of components of the metabolic syndrome NCEP definition in women with PCOS and Controls (no diabetes)

Number of NCEP ATP III metabolic syndrome components	Controls N=36 % (n)	PCOS-NIH N=30 % (n)
0	19.4 (7)	13.3(4)
1	41.7 (15)	13.3(4)
2	13.9 (5)	30.0(9)
≥ 3	25.0 (9)	43.3(13)

Metabolic syndrome was significantly more common in obese women ($\text{BMI} > 30.0 \text{ kg/m}^2$) being present in at least half of these women in both PCOS-NIH and control categories. There was no difference between the groups in each BMI category. Interestingly, there were three women with a 'healthy BMI' ($< 25.0 \text{ kg.m}^2$) but significant central obesity ($\text{WC} > 88 \text{ cm}$ and $\text{WHR} > 0.88$) who had metabolic syndrome, consistent with previous reports that Indigenous people can have central obesity and associated complications at what is considered a 'normal BMI' for Europeans.

Table 6.2 Proportion of women with metabolic syndrome by BMI category in Controls and PCOS-NIH

	Controls N=36 % (n)	PCOS-NIH N=30 % (n)
BMI<25.0 kg/m ²	8.7 (2)	16.7 (1)
BMI ≥25.0 and <30.0 kg/m ²	55.6 (5)	20.0 (1)
BMI ≥30.0kg/m ²	66.7 (2)*	57.9 (11)*

^a p<0.05 between BMI<25.0kg/m² and BMI≥30.0kg/m² category within each group

There was an increase in frequency of metabolic syndrome with age but this was not significant and there were no differences between those with PCOS or controls .in each age group.

Table 6.3 Proportion of women with metabolic syndrome by age group in Controls and PCOS-NIH

	Controls N=36 % (n)	PCOS-NIH N=30 % (n)
15–24 years	14.3 (1)	25.0 (3)
25–34 years	15.4 (2)	57.1 (4)
35–44 years	37.5 (6)	55.6 (6)

6.3.2 Components of the metabolic syndrome by NCEP

The most frequent component of metabolic syndrome (irrespective of presence or absence of PCOS) was a low HDL-C, followed closely by a high waist circumference, then raised triglycerides, and finally elevated fasting glucose and blood pressure. The most striking and only significant differences between the groups were the higher proportions of women with PCOS-NIH with a waist circumference >88cm and HDL-C≤1.29 mmol/L (table 6.4). There were no significant differences between the groups in the proportion of women who had other components of metabolic syndrome present.

Table 6.4 Proportion of women with each component of the NCEP definition of metabolic syndrome by category of PCOS, Control

	Controls	PCOS-NIH
	% (n)	% (n)
N	50	30
BP ≥130/85mmHg	18.0 (7)	18.2 (6)
HDL-C ≤1.29mmol/L	56.4* (22)	78.1* (25)
Triglycerides ≥1.7mmol/L	18.9 (7)	29.0 (9)
Fasting glucose ≥5.6 mmol/L	14.8 (9)	18.8 (6)
WC >88cm	31.6* (12)	71.9* (23)
Metabolic syndrome present	25.0 (9)	43.3* (13)

* p<0.05 between PCOS-NIH and controls

Even in women without the metabolic syndrome the pattern was the same: HDL-C and waist circumference were the two most common abnormalities (Table 6.5). A similar proportion of women in the controls had the NCEP blood pressure component present irrespective of whether they had metabolic syndrome.

A striking majority of women in the PCOS-NIH groups had low HDL-C by NCEP definition, regardless of the presence of metabolic syndrome.

The presence of elevated triglycerides was an important marker of the presence of the metabolic syndrome: very low proportions of women without MS had high triglycerides but over 50% of those with MS had high triglycerides (Table 6.5).

Table 6.5 Proportion of women with each component of the NCEP definition of metabolic syndrome by category of PCOS and Control, (no diabetes) with and without metabolic syndrome (MS)

	Controls		PCOS-NIH	
	% (n)		% (n)	
	No MS	MS	No MS	MS
N	27	9	17	13
BP ≥130/85mmHg	14.8 (4)	33.3 (3)	5.9 (1) ^b	38.5 (5)^b
HDL-C ≤1.29mmol/L	48.2 (13) ^a	88.9 (8)^a	64.7 (11)	92.3 (12)
Triglycerides ≥1.7mmol/L	3.7 (1) ^a	66.7 (6)^a	5.9 (1) ^b	53.9 (7)^b
FG≥5.6 mmol/L	7.4 (2) ^a	55.6 (5)^a	0 ^b	38.5 (5)^b
WC >88cm	11.1 (3) ^a	100 (9)^a	52.9 (9) ^b	100 (13)^b

^a p<0.05 between Controls with and without metabolic syndrome

^b p<0.05 between PCOS-NIH with and without metabolic syndrome

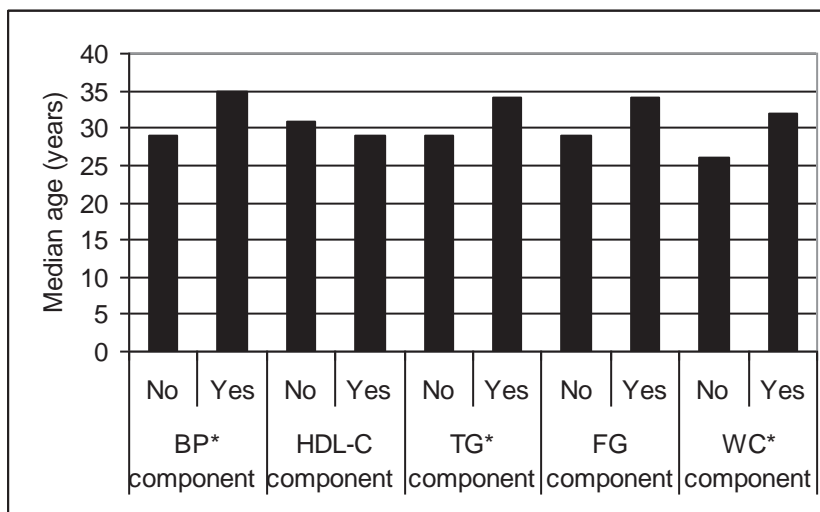
6.3.3 Determining factors of metabolic syndrome and components

6.3.3.1 Age

There was no difference in the number of components of metabolic syndrome present, except when comparing those with PCOS-NIH and no components of the metabolic syndrome to those with ≥ 3 components ($p < 0.05$). There were in fact nine women less than 25 years with the metabolic syndrome, accounting for 17.6% of those with metabolic syndrome. All these women had central obesity with the metabolic component for waist circumference and all had low HDL-C.

There were however some differences observed in the individual components of the metabolic syndrome. The women who had the triglyceride, waist circumference and blood pressure component of the metabolic syndrome present were older than those who did not. However, there was no significant difference in age between those with the HDL-C or fasting glucose (FG) component of the metabolic syndrome, indicating age was not an important factor in determining their presence or not (figure 6.2).

Figure 6.2 Median age and absence or presence of individual components of the metabolic syndrome



* denotes $p < 0.05$ difference in age between the absence (No) and presence (yes) for each individual component of the metabolic syndrome

6.3.3.2 Obesity and metabolic syndrome

Elevated BMI is a much better discriminator of severity of metabolic syndrome than WHR (figure 6.3 and 6.4). Similar results were seen for waist circumference as for BMI.

Figure 6.3 Mean BMI in Controls, PCOS-NIH, NPNC in those with zero, one, two or > three components of the metabolic syndrome

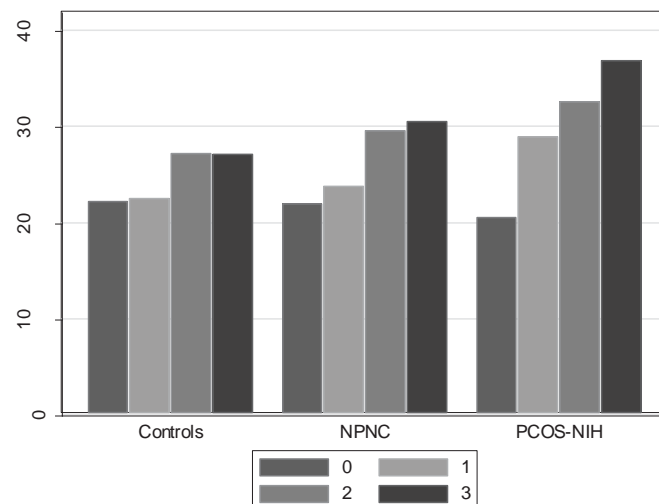
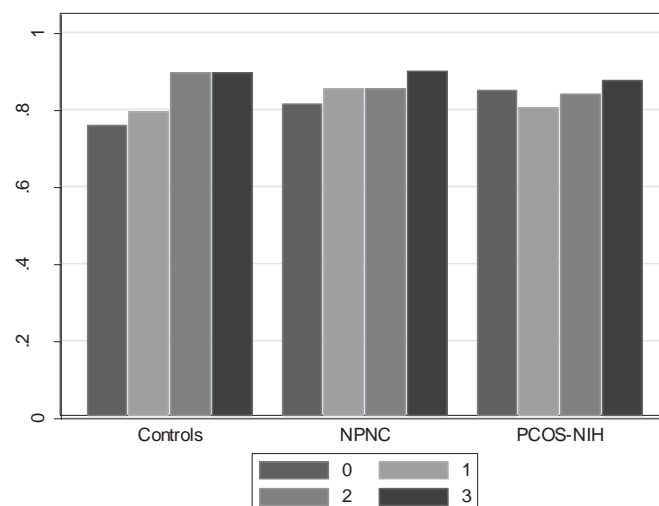
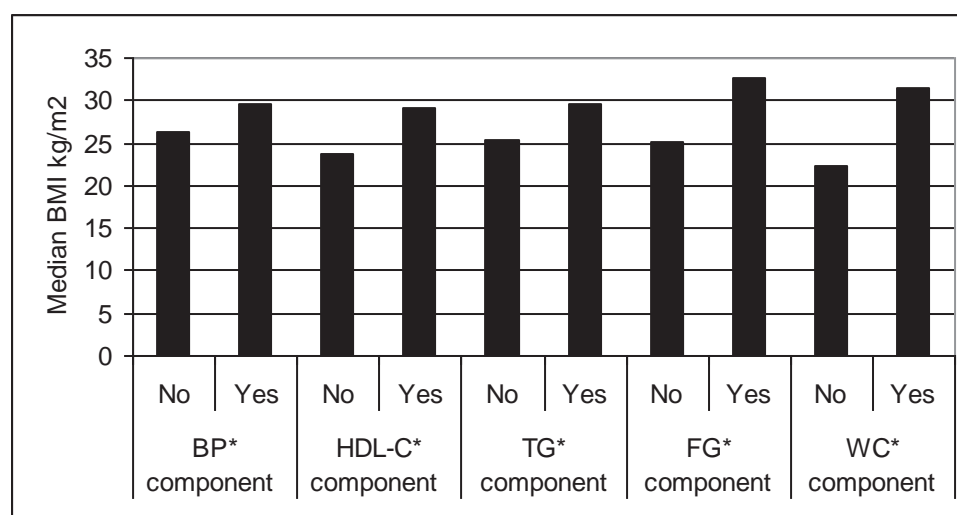


Figure 6.4 Mean WHR in Controls, PCOS-NIH, NPNC and in those with zero, one, two or > three components of the metabolic syndrome



Whilst age was not different between those with and without the HDL-C and fasting glucose components of the metabolic syndrome, BMI was higher in the presence of all components of the metabolic syndrome (figure 6.5).

Figure 6.5 Median BMI and the absence or presence of each individual component of the NCEP metabolic syndrome



*p<0.05 between BMI and the absence and presence of each individual metabolic syndrome component

6.3.4 Other factors influencing metabolic syndrome

Other factors apart from age and BMI are associated with the presence or absence of the metabolic syndrome: HOMA index of insulin resistance, SHBG, free testosterone (FT) and CRP. These all had significant associations with the metabolic syndrome by simple univariate logistic regression modelling (Table 6.6).

Table 6.6 Univariate regression analysis with NCEP as dependent variable

Predictor variable in each model	OR	SE	P	CI
Age	1.04	0.02	0.02	1.01 1.08
BMI	1.16	0.03	0.0001	1.10 1.21
HOMA	1.73	0.19	0.0001	1.40 2.15
SHBG	0.96	0.01	0.0001	0.93 0.98
FT	1.03	0.01	0.0001	1.01 1.05
CRP	1.12	0.03	0.0001	1.06 1.18

When all the above variables are entered into a multivariate logistic model, which enables adjustment for the combination of factors, only age, BMI, HOMA and SHBG are found to be significantly associated with metabolic syndrome, although the odds ratios are small.

Overall this model accounts for 31% of the variance of MS by NCEP ($r^2=0.31$) (Table 6.7).

Table 6.7 Multivariate backward stepwise logistic regression modelling with NCEP as the dependent variable and age, BMI, SHBG and HOMA as the predictor variables.

Predictor variables	OR	SE	P	CI
Age	1.04	0.02	0.05	0.99 1.09
BMI	1.09	0.03	0.005	1.02 1.15
SHBG	0.96	0.01	0.005	0.94 0.99
HOMA	1.27	0.13	0.02	1.03 1.55

6.4 Major Findings

- Metabolic syndrome was present in one in four women (25.5%).
- Metabolic syndrome was highest in those with PCOS-NIH (40.0%) and although this difference was not significantly different to controls this may have been due to small numbers.
- The components of metabolic syndrome most frequently present were HDL-C \leq 1.29 mmol/L and a waist circumference $>$ 88cm. These were most frequent in women with PCOS-NIH.
- HDL-C \leq 1.29 mmol/L was commonly present in women with PCOS-NIH regardless of the presence of metabolic syndrome whereas triglycerides were a better discriminator of metabolic syndrome
- The median age of women was higher in those with the blood pressure, fasting glucose and triglyceride components of metabolic syndrome.
- The median BMI of women was higher in those with any component of the metabolic syndrome when compared to those without. However metabolic syndrome was still present in some women in the ‘normal BMI’ range.
- Whilst elevated free testosterone was associated with the presence of metabolic syndrome in univariate logistic regression, after adjusting for age, BMI, and SHBG there was no significant association between free testosterone and metabolic syndrome.

6.5 Discussion

Overall, a quarter of the Indigenous women had metabolic syndrome by NCEP, which is higher than the frequency found in adult women from the general population in South Australia (14.4%), similar to that found in rural Victorian and South Australian women at

19% and non-Indigenous women in Darwin at 20.0%. It was lower than the prevalence in other Australian Indigenous women (Central Australian Aboriginal women 41.5% and Torres Strait Islander women 43.7%); possibly because of the younger age and/or greater ethnic heterogeneity of the women in this study and women with diabetes were excluded from assessment of metabolic syndrome. The proportion of women with metabolic syndrome in this total group had women with diabetes been included was 29.8%

The two most common components of metabolic syndrome in this group, high waist circumference and a low HDL-C, were similar to Torres Strait Islander and Central Australian women (Schutte et al. 2005).

HDL-C has been shown previously to be low across ages in Aboriginal and Torres Strait Islander women. This was also the case in this study, despite the young age of participants, low HDL-C was present in a majority of women across age groups. However, both this current study and Schutte et al. found the blood pressure, triglyceride and waist circumference components of metabolic syndrome to be more frequent in the older age groups. This may explain why high triglycerides, hypertension and high waist circumference were less frequent in the present study.

Studies assessing metabolic syndrome in other populations have reported that women with PCOS have a higher prevalence of metabolic syndrome when compared to healthy controls (Dokras et al. 2005, Glueck et al. 2003). Glueck compared 138 women with PCOS-NIH with a sample from the NHANES III study and found age adjusted prevalence of metabolic syndrome by NCEP to be 46.4% and 22.8% respectively, although they did not adjust for obesity. Small subject numbers may have limited the statistical power of the current study. As already noted, central obesity was common in all the groups in this study which may have attenuated the differences.

Similar to this study, Glueck et al. found that the women with PCOS were more likely to have the components of central obesity (WC >88cm) and a low HDL-C (<1.29mmol/L) than other women. However, they also found high blood pressure to be more commonly present in PCOS-NIH which was not seen in this study. That may be because hypertension is not as commonly a burden in young Indigenous people as dyslipidaemia and central obesity.

Finally, although metabolic syndrome was present in a high proportion of women with PCOS this may again have been due to the higher overall obesity in these women.

6.6 Conclusion

Whilst BMI was higher in women with metabolic syndrome, women with a normal BMI also had metabolic syndrome. This is less common in other populations and is probably a consequence of the presence of significant central obesity at lower BMI in Aboriginal women. This concurs with other studies suggesting that the “normal BMI” range should be lower in Indigenous women (Piers et al. 2003).

Metabolic syndrome was highly prevalent in the women with PCOS – almost double that of controls. That it was not statistically significant may have been due to the small numbers. Once adjusted for obesity, age and SHBG the difference in metabolic syndrome between controls and PCOS was not due to hyperandrogenaemia.

Central obesity and a low HDL-C were the most common components present in this group of women. Therefore efforts should be concentrated on preventing central obesity at a young age and modifying risk factors for low HDL-C.

Chapter 7 Conclusion

I will discuss the main features of this thesis under the following headings

- Reproductive health
- Androgens
- PCOS

7.1 Reproductive health

Reproductive health encompassing factors such as obesity, smoking, cycle regularity, contraceptive use and infertility are not well documented in Indigenous women. Valuable information was gained through the women's reproductive health questionnaire.

7.1.1 Menstrual cycles and fertility

Oligomenorrhoea in this group of women was high at 26%. Contributing factors may have included young women near menarche who are more likely to have amenorrhoea and a high proportion of women who were overweight or obese, were current smokers or had hyperandrogenaemia.

Self-reported infertility was similar to that of national figures but lower than reported in a remote community in the Northern Territory (Kildea & Bowden 2000). Women did not readily seek treatment and the reason for this are unknown, although may be related to cost and accessibility to treatment and this should be explored further. Health authorities need to develop programs relevant for Indigenous women relating to contraception and fertility.

7.1.2 Contraceptive use

Whilst there is a wealth of information on poor pregnancy outcomes in Indigenous women there is little known about Indigenous people's knowledge of and views on many aspects of pre-conception health that are so important in improving birth outcomes.

There is little data around Indigenous women's contraceptive use. Around 20% of participants in this study were currently using hormonal contraception, which is lower than national figures for Indigenous urban women (Australian Bureau of Statistics 2006). However, this study did not determine if women who were not using hormonal

contraception were trying to conceive or had accessed permanent methods of contraception. Women aged 25–34 years had the highest proportion of hormonal contraceptive use at 31.0%.

Teenage pregnancy was high in this cohort with 39.5 % having their first child in their teenage years and one fifth of these were ≤ 17 years. Aboriginal women have expressed their concern about young girls having children, the short intervals between births and they often bring young girls in to discuss contraception (Jones C et al. 2005)(personal experience). Information is scarce about Indigenous women's knowledge of contraception or attitudes to use of contraception. Larkins et al. in Queensland found young Aboriginal people lacked knowledge about contraception, were “shamed” to discuss contraception with health providers or sexual partners, did not wish to appear promiscuous and lacked access to health care (Larkins et al. 2007).

There is a suggestion from this study that socioeconomic factors such as home ownership and full time employment are associated with greater hormonal contraceptive use. This is consistent with International data, National data for all Australian women and for Indigenous women in other parts of Australia. It suggests that education of young women is important in delaying age of childbirth.

.It is important that we expand our knowledge around factors that impact on young women's reproductive choices in order to address early teenage pregnancy and poor birth outcomes.

7.2 Androgens

Measures of total and free testosterone were high in this group of Indigenous women, and a subset with regular cycles had higher testosterone levels when compared to a group of non-Indigenous women without PCOS from Adelaide. There was however no difference in SHBG or DHEAS. Whilst the groups were matched for BMI and age, central obesity measures were not available for the Adelaide group.

Factors contributing to hyperandrogenaemia such as obesity, smoking and high insulin were significant problems in this study group who had androgens assessed. Just over half were overweight or obese by BMI and central fat deposition was the normal pattern with 73.4% having a $WHR \geq 0.8$. Whilst this was more common in those who were overweight

or obese, the majority with a normal BMI (53.4%) also had a WHR ≥ 0.8 . Central obesity has been reported as having a more pronounced effect on androgens than general obesity and the DRUID subgroup was likely to have more central obesity than the Adelaide Caucasian reference group as greater central obesity in Aboriginal women compared with Australian women of European ancestry of the same BMI has been reported previously (Piers et al. 2003). However it would be anticipated that this would also lead to a lower SHBG in the DRUID women which was not the case. Central obesity by WHR was also not found to have a significant association with testosterone measurements in the total group of DRUID women assessable for androgen analysis by backward regression analysis. However this is probably because it was present in the majority of women regardless of BMI category.

An interesting observation in the total group of women assessed for androgens was that total and free testosterone fell with age and increased with obesity as observed in other populations. However in this group the effect of obesity weakened with age. This meant that in older women there was less difference in all measures of testosterone between those classified as overweight or obese compared with women in the normal BMI range.

Age related declines in androgens have also been documented by other studies in Australia and the USA (Davison et al. 2005, Spencer et al. 2007, Winters et al. 2000).

Other studies also confirm testosterone increases with obesity. However the decreased influence of obesity on testosterone in older women has not been previously reported. It is unclear what this is due to although it is possible that the drivers of androgen production, or the response to drivers such as increased insulin and smoking, are more pronounced in young women. It may be this effect is more pronounced in young women.

Fasting insulin was high across age groups with 26.8% of women aged 15–24 years with a fasting insulin ≥ 14 and 17.9% a HOMA-IR >4.5 . This increased with age and in those 35–44 years 23.7% had a raised fasting insulin and 31.1% a raised HOMA-IR. It is known that insulin may directly affect ovarian testosterone production in addition to decreasing SHBG and increasing testosterone bioavailability (Poretsky et al. 1999). Again, neither fasting insulin nor the HOMA index, was found to be a significant predictor variable for SHBG or free testosterone in backward multivariate analysis. This may have been because the median fasting insulin was high across all women.

Current smokers comprised 42.8%–59.5% of women and whilst this study did not find any association with androgens or cycles and smoking the effect may have been dose related and we were not able to assess this. In women with PCOS, smoking has been found to be associated with hyperandrogenaemia (Cupisti et al. 2009).

It is possible the differing total and free testosterone findings may reflect ethnic differences as studies have shown that premenopausal African American women have lower levels of total and free testosterone and androstenedione than white American women after controlling for age, BMI and central obesity (Lamon-Fava et al. 2005, Spencer et al. 2007). However it is quite possible there are other factors such as central obesity, hyperinsulinaemia and antenatal and environmental factors that are the reasons for these differences and we were not able to assess these in the non-Indigenous group. Further research would be useful to ascertain the effect of these factors on androgens and determine the reason for the differences observed in the two groups of Indigenous and non-Indigenous women. It is clear that new and improved assays for androgens are central to adequate diagnosis of PCOS in Indigenous women.

7.3 PCOS

7.3.1 Prevalence

The proportion of women with PCOS in this study was higher than that reported in other studies at 15.3% by the NIH criteria and increased significantly with obesity being present in 29.9% of those women with a BMI ≥ 30.0 kg/m². As discussed in section 5.5 there are a number of factors that may have led to both an overestimation and an underestimation of PCOS in this study group. The high proportion with PCOS in the overweight and obese group is similar to that found in a Spanish study of overweight and obese women (28.3%) (Alvarez-Blasco et al. 2006). The high proportion of women with PCOS cannot be entirely attributed to overall obesity as the mean BMI (28.1 kg/m²) of the women assessable for PCOS was similar to that of other prevalence studies, apart from that of Thai women. However, it is possible that the overwhelming central deposition of fat may play a role as the mean WHR in all women assessable for PCOS was high at 0.86 ± 0.1 .

Insulin resistance is strongly related to central obesity and was postulated to be the reason for the high prevalence of PCOS (13.0%) in Mexican American women where the mean HOMA values for the total group assessed was not dissimilar to the total group assessed

for PCOS in this study (4.1 ± 2.9 vs 3.3 ± 4.6). Central obesity was not reported for the Mexican American women although previous studies report preferential central fat distribution in Mexican Americans. The median insulin levels in this study group of Indigenous women assessable for PCOS was high at 9.0 $\mu\text{u/l}$ (6.0, 15.0) although no other prevalence studies report insulin levels for their total study group for comparison.

7.3.2 Metabolic factors and abnormalities of glucose regulation

A high (36.8%) proportion of women with PCOS had IGT or diabetes which was significantly higher than the controls while insulin resistance as measured by HOMA-IR was greater in women with PCOS.

Metabolic syndrome was higher in the women with PCOS but this was not statistically significant possibly because of the small numbers of women. Metabolic syndrome increased with obesity but, surprisingly, did not increase significantly with age in women with PCOS or controls. The most frequent components of metabolic syndrome present were the same in all categories: a high waist circumference and low HDL-C and this applied to women with and without metabolic syndrome. Importantly these still occurred in women with a “normal” BMI range.

7.4 Conclusion

A number of issues have been confirmed or identified in this study which warrant further attention. These are: high early teenage pregnancy, significant infertility (at least comparable to national figures), high testosterone measures and a high prevalence of PCOS with significant metabolic features along with IGT and diabetes. Women with these metabolic or glucose complications were often young and a small number were not obese. There are implications not only for the individual women but developmental effects on the fetus in utero and the effects may prove to have significant intergenerational consequences.

There is potentially an enormous health in a prevalence of PCOS this high; as discussed, Teede et al. estimated with a prevalence of 4–8% the costs nationally are already around \$40 million dollars.

In the first instance, awareness of a high prevalence of PCOS in Indigenous women should be promoted among health care providers. Additionally, it is important that whilst the

majority of women with metabolic or glucose abnormalities were overweight or obese and ≥ 35 years, a significant minority are younger with normal BMI. This would suggest that screening should occur for all these women with PCOS for dyslipidaemia and IGT/diabetes. Community awareness also needs to be a priority, for women with PCOS and potentially other family members for associated diabetes.

The prevention and delay of onset of obesity and PCOS is important and efforts should occur before child bearing. Health promotion and education should begin in early youth and could encompass a number of the issues raised above to promote broad reproductive health.

Future areas of research are required particularly with young women to explore; knowledge and attitudes to family planning and other aspects of reproductive health, the best ways to provide education and health services to Indigenous women in these areas, the potential of youth health checks to identify problems early, the use of medication in young women with PCOS to prevent future metabolic complications, and a comparison of androgens in Indigenous and non-Indigenous women.

Appendix 1 Mortality rates of women

Mortality rate per 10,000 and rate ratio of top 20 leading causes, Indigenous Australian women and total Australian women populations, 2003

NOTE:
This table is included on page 200
of the print copy of the thesis held in
the University of Adelaide Library.

^a Age standardised to the total Indigenous population,2003

^b Indigenous Australian to total Australian rate ratio

Adapted from: Vos et al. The burden of disease and injury in Aboriginal and Torres Strait Islander peoples, 2003. Brisbane: School of Population Health, The University of Queensland

Appendix 2 Birth weight and PCOS

	Population	Numbers	Birthweight (grams)	Age (years)	Exclusion criteria	Findings where difference between groups was P<0.05
Ibanez et al. 2002	Adolescent girls recruited from healthy relatives of hospital staff and from paediatric endocrine clinic	25 term SGA* 24 term controls (AGA [#])	2300 ± 0.1 3300 ± 0.1	15.4 ± 0.3 15.6 ± 0.2	Chromosomal, infectious or syndromatic cause of LBW**, FG score ≥8, thyroid dysfunction, Cushing syndrome, hyperprolactinaemia, OCP use, personal or family history of diabetes	LBW had higher fasting insulin, testosterone, androstenedione and DHEAS, lower SHBG and had higher proportion of anovulatory girls, 40% vs 4%
Ibanez et al. 1999	Adolescent girls recruited from Barcelona hospital after discharge for minor illness					
Jaquet et al 1999	French Recruited from population data base of birth records	130 LBW at term 150 term controls	2550 ± 334 3410 ± 216			Higher insulin and lower SHBG in LBW women
Pandolfi et al. 2008	Italian Recruited from hospital birth records	29 LBW <2500gm 26 Term Controls >3000gms	2093 ± 414 3259 ± 311			LBW women had lower insulin sensitivity, higher FAI and a higher proportion of irregular cycles and PCOS (40% vs 5.7%)

* SGA, small for gestational age < 2 standard deviations (SD)

[#] AGA, appropriate for gestational age (between -1 SD and +1 SD)

** LBW, low birth weight

Appendix 3 DRUID Questionnaires

Only the relevant excerpts from DRUID Questionnaire 1 are included here.

Section 1. Medical History

3. Have you ever been told by a doctor or other health professional that you have high blood pressure or hypertension?
4. Are you currently taking tablets for high blood pressure?
5. Are you currently taking tablets to lower your cholesterol/triglycerides?

Section 2. Diabetes

The next section is about diabetes.

1. Have you ever been told by a doctor or other health professional that you have diabetes or high sugar levels in your blood or urine?

- 0 = No (go to Q16)
- 1 = Yes, diabetes
- 2 = Yes, high sugar levels (go to Q15)
- 8 = Don't know (go to Q16)

2. What type of diabetes were you told you had?

- 1 = Type 1 (Insulin-dependent diabetes mellitus)
- 2 = Type 2 (Non-Insulin dependent diabetes mellitus)
- 3 = Gestational diabetes (Diabetes during pregnancy)
- 4 = Diabetes – type unknown
- 5 = Other type (specify)

3. Do you still have diabetes?

- 0 = No (go to Q15)
- 1 = Yes
- 8 = Don't know/not sure

4. Are you currently taking any medication for your diabetes?

- 0 = No (go to Q7)
- 1 = Yes

Section 3. Smoking

- | | | |
|---|----|-----|
| 1. Do you currently smoke tobacco/cigarettes? | No | Yes |
| 2. Have you ever smoked? | No | Yes |

Section 4. Health Insurance

The next questions are about health insurance. Having access to health insurance can affect the level of health care people seek or receive, and this can affect health.

1. Do you have private health insurance?

- 0 = No
- 1 = Yes, hospital cover only
- 2 = Yes, extras cover only
- 3 = Yes, both hospital and extras cover
- 4 = Yes, but not sure what type of cover
- 8 = Don't know

Section 10. Household

1. I am now going to ask you about the place you are now living in. Is it ...?

- 1 = Fully owned or being purchased by you or someone in the household
- 2 = Being rented
- 3 = Being occupied rent-free
- 4 = Other, specify:(_____)

Section 12. Employment

I'm now going to ask you some questions about work.

1. Which of the following describes your current employment status?

For each item, use the following:

- 0 = No

1 = Yes

8 = Don't know/not sure

- a = Working full-time (not CDEP)
- b = Working part-time (not CDEP)
- c = CDEP
- d = Casual work
- e = Home duties
- f = Full-time student
- g = Part-time student
- h = Retired
- i = Not working (but not retired)
- j = Permanently unable to work / ill
- k = Other, specify:(_____)

Section 13. Income

3. Looking at the card in front of you, which number best describes your total household income before tax (gross income)?

Note: If participant is sharing a household with someone who is not a partner and they live independently then record the participant's income only.

- 1 = \$1,500 or more per week, \$3000 per fortnight (\$78,000 or more per year)
- 2 = \$800 – \$1,499 per week, \$1600–\$2900 per f/night (\$41,600 – \$77,999 per year)
- 3 = \$600 – \$799 per week, \$1200–\$1599 per fortnight (\$31,200 – \$41,599 per year)
- 4 = \$400 – \$599 per week, \$800–\$1199 per fortnight (\$20,800 – \$31,199 per year)
- 5 = \$200 – \$399 per week, \$400–\$799 per fortnight (\$10,400 – \$20,799 per year)
- 6 = \$80 – \$199 per week, \$160–\$399 per fortnight (\$4,160 – \$10,399 per year)
- 7 = \$1 – \$79 per week, \$2–\$159 per fortnight (\$52 – \$4,159 per year)
- 8 = Don't know or declined to answer (*Do not read this option*)

Appendix 4 Women's Reproductive Health Questionnaire

Women's Reproductive Health

The next questions are about women's business. This includes topics such as menstruation, pregnancy, infertility, hysterectomy, menopause, and hormone replacement therapy. We are asking these questions because there are things that affect your risk of diabetes, heart disease and other conditions. If you don't wish to answer any of the questions, please tell me.

Section 1. Menstrual history

1. How old were you when you had your first period?

- 1 = Less than 10
- 2 = 10
- 3 = 11
- 4 = 12
- 5 = 13
- 6 = 14
- 7 = 15 or older
- 8 = Don't remember/not sure

2. Were your periods regular as a teenager?

- 0 = No
- 1 = Yes
- 2 = Still a teenager
- 8 = Don't know

3. Are your periods regular now?

- 0 = No (go to Q5)
- 1 = Yes
- 2 = Not applicable, please specify: _____ (go to Section 2)
- 8 = Don't know

4. About how often do you get them now?

- 1 = Less than 24 days
- 2 = 24–34 days
- 3 = 35 days or more
- 8 = Don't know/varies

5. About how many days do they usually last?

- 1 = Less than 4 days
- 2 = 4–5 days
- 3 = 6–7 days
- 4 = More than 7 days
- 8 = Don't know/varies

Section 2. Contraceptive use

1. Have you ever taken the oral contraceptive pill?

- 0 = No (go to Q4)
- 1 = Yes
- 8 = Don't know (go to Q4)

2. For how long altogether have you taken the oral contraceptive pill? (Please estimate the total of all periods of use.)

- 1 = Less than 6 months
- 2 = Between 6 months to 2 years
- 3 = Between 2 to 5 years
- 4 = Between 5 to 10 years
- 5 = 10 years or more
- 8 = Don't know

3. Are you currently taking the oral contraceptive pill?

- 0 = No
- 1 = Yes

4. Have you ever used any other hormonal form of contraception, such as implants (e.g. Implanon) or injections (e.g. Depo-Provera)?

- 0 = No (go to Section 3)
- 1 = Yes
- 8 = Don't know (go to Section 3)

5. For how long altogether have you used other hormonal forms of contraception, such as implants or injections? (Please estimate the total of all periods of use.)

- 1 = Less than 6 months
- 2 = Between 6 months to 2 years
- 3 = Between 2 to 5 years
- 4 = Between 5 to 10 years
- 5 = More than 10 years
- 8 = Don't know

6. Are you currently using other hormonal forms of contraception, such as implants or injections?

- 0 = No
- 1 = Yes

Section 3. Pregnancy

1. Have you ever been pregnant?

- 0 = No (go to Section 4)
- 1 = Yes

2. What age were you when you were first pregnant?

years 98 = Don't know/can't remember

3. How many children have you had?

number

4. What age were you when you first child was born?

years 98 = Don't know/can't remember

5. At any time when you were pregnant, did a doctor or other health professional tell you that you had diabetes or high sugar levels in your blood or urine?

- 0 = No
- 1 = Yes, diabetes
- 2 = Yes, high sugar levels
- 8 = Don't know/can't remember

Section 4. Infertility

1. Have you ever had any difficulties getting pregnant or had repeated miscarriages?

- 0 = No (go to Q4)
- 1 = Yes, difficulty getting pregnant
- 2 = Yes, repeated miscarriages
- 3 = Yes, both
- 8 = Don't know (go to Q4)

2. Have you ever had any tests or treatment for this?

- 0 = No (go to Q4)
- 1 = Yes
- 8 = Don't know/can't remember (go to Q4)

3. What kinds of tests or treatment did you have? (Mark all that apply.)

For each item, use the following:

0 = No tests/treatment

1 = Yes, had tests/treatment

8 = Don't know/not sure

- a. Diagnostic tests, such as blood tests, x-rays or ultrasound
- b. Weight loss, diet or other lifestyle change
- c. Tablets
 - c.1 If tablets = 'Yes', were they for PCOS?
- d. Injections or IVF
- e. Other (specify) _____

4. Do you have problems with acne?

- 0 = No (go to Q6)
- 1 = Yes

5. Have you ever used any treatment for this, such as creams or pills?

- 0 = No
- 1 = Yes
- 8 = Don't know/can't remember

6. Do you have hair on your chin, chest or tummy?

- 0 = No (go to Section 5)
- 1 = Yes

7. Have you ever used any treatment for this, such as removing the hair (by shaving, waxing or plucking), lightening it (using bleaches or creams), or taking tablets?

- 0 = No
- 1 = Yes
- 8 = Don't know/can't remember

Section 5. Hysterectomy

1. Have you had a hysterectomy, which is an operation to remove the uterus?

- 0 = No (go to Section 6)
- 1 = Yes
- 8 = Don't know (go to Section 6)

2. What age were you when you had the hysterectomy?

<input type="text"/>	<input type="text"/>	years
----------------------	----------------------	-------

98 = Don't know/can't remember

3. Were the ovaries removed as well?

- 0 = No
- 1 = Yes, only one ovary removed
- 2 = Yes, both ovaries removed
- 8 = Don't know

Section 6. Menopause

The next few questions are about menopause, or what some women refer to as the "change of life". Menopause is when periods have stopped for more than 12 months and is often accompanied by symptoms such as hot flushes, irritability and palpitations.

1. Have you gone through or are you now going through menopause?

- 0 = No (go to Section 7)
- 1 = Yes, have gone through menopause
- 2 = Yes, now going through menopause
- 8 = Don't know/not sure (go to Section 7)

2. At what age did symptoms of the menopause begin?

years 98 = Don't know/can't remember

Section 7. Hormone replacement therapy

1. Other than for birth control, has your doctor ever prescribed estrogen pills or hormone replacement therapy (HRT) for you?

- 0 = No (go to Section 8)
- 1 = Yes
- 8 = Don't know (go to Section 8)

2. Are you currently taking estrogen pills or hormone replacement therapy?

- 0 = No
- 1 = Yes

*That brings us to the end of the questions.
Thank you very much for your time. (direct to next station)*

Section 8. About the interview – Interviewer to complete

1. Was the interview obtained?

- 1 = Fully in English
- 2 = Partly in language other than English
- 3 = Fully in language other than English

2. Was the interview obtained?

- 1 = Easily
- 2 = With difficulty

3. Was the interview obtained by PROXY?

- 0 = No
- 1 = Yes, due to English language difficulties
- 2 = Yes, other reasons – Specify _____

4. Was anyone else present?

- 0 = No
- 1 = Yes, specify relationship: _____

5. Did you get through all the questions?

- 0 = No (note what was missed in the comments section of the Data Record Form)
- 1 = Yes

Appendix 5 Characteristics of PCOS and Controls, no diabetes

	PCOS NIH criteria		Controls		P
N	33		39		
Age (years)	31.0	(21.0, 36.0)	34.0	(27.0, 40.0)	0.13
BMI (kg/m2)*	32.8	(26.6,37.1)	23.8	(21.1,27.1)	0.0001
Waist Circumference (cm)*	99.9	(85.4,113.6)	82.1	(73.0,92.4)	0.0001
Waist hip Ratio*	0.85	(0.79,0.94)	0.82	(0.79,0.90)	0.09
Mean SBP(mmHg)	113.0	(105.0,118.5)	107.0	(99.0,111.5)	0.04
DBP (mmHg)	73.5	(67.0, 80.0)	69.5	(65.0, 73.5)	0.03
HDL (mmol/L)*	1.1	(1.0, 1.2)	1.2	(1.0, 1.4)	0.03
LDL (mmol/L)*	2.8	(2.4, 3.6)	2.9	(2.4, 3.4)	0.91
Total cholesterol(mmol/L)*	4.6	(4.1, 5.5)	4.8	(4.0, 5.5)	0.47
Triglycerides (mmol/L)*	1.3	(0.9, 2.0)	1.0	(0.9, 1.5)	0.26
AMH(ng/ml)*	29.9	(9.9, 44.0)	10.9	(7.1, 14.8)	0.0006
Testosterone (nmol/L)	2.3	(1.9, 3.0)	1.3	(1.1, 1.6)	0.0001
SHBG (mmol/L)	20.5	(14.1, 26.3)	44.7	(36.7,67.9)	0.0001
Free Testosterone(mmol/L)	50.5	(41.0,74.2)	20.8	(12.1,26.4)	0.0001
FAI	10.9	(7.7, 15.7)	2.8	(1.8, 4.1)	0.0001
DHEAS (umol/L)*	4.8	(3.4, 6.2)	3.2	(1.9, 4.5)	0.005
CRP (mg/L)	3.9	(1.8, 9.6)	1.8	(0.8, 2.8)	0.01
Homocysteine	7.8	(6.8, 8.9)	8.4	(6.8, 10.4)	0.16
Fasting glucose (mmol/L)*	5.0	(4.7, 5.4)	4.9	(4.7, 5.4)	0.81
Fasting Insulin (mU/L)*	14.5	(8.0,19.0)	7.0	(5.0,10.0)	0.0004
HOMA*	3.7	(2.0, 4.95)	1.5	(1.0, 2.5)	0.0005
2 hour glucose (mmol/L)*	6.6	(5.3, 7.7)	5.4	(4.6, 6.5)	0.005
2 hour insulin (mU/L)*	91.5	(48.0, 129.0)	30.0	(21.0, 61.0)	0.00001

*incomplete data.

PCOS: n=32 for BMI, WC, WHR, HDL-C, TC, hsCRP, fasting glucose and insulin, homa-ir, 2 hour glucose and insulin
n=31 for LDL-C, triglycerides
n=30 AMH
n=29 DHEAS

Controls: n=38 for WC, WHR, DHEAS
n= 37 for LDL-C, triglycerides, fasting glucose and insulin and 2 hour insulin and glucose

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