The role of adipokines in polycystic ovary syndrome

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UNIVERSITY OF ZAGREB

SCHOOL OF MEDICINE

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The Role of Adipokines in Polycystic Ovary Syndrome

GRADUATE THESIS

Zagreb, 2014

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This graduation paper has been done at the department of Gynaecology and Obstetrics at the Clinical Hospital Centre Zagreb under the supervision of Prof.dr.sc Dinka Pavičić Baldani and was submitted for evaluation during the academic year 2013. /2014.

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LIST OF ABBREVIATIONS

AES ....Androgen Excess Society

AgRP............. Agouti regulated protein

AMH ....Anti Mullerian Hormone

AMPK ....Adenosine monophosphate protein kinase

ASRM ....American Society for Reproductive Medicine

BMI...............Body mass index

CARP........... Cocaine-amphetamine related protein

ESHRE ....European Society for Human Reproduction and Embryology

FSH ....Follicular stimulating hormone

GDF-9 ....Growth differentiation factor-9

GnRH ....Gonadotrophin releasing hormone

IL-6 ....Interlukin-6

LH ....Luteinising hormone

NIH ....US National Institute of child Health and human development

NPY ....Neuropeptide-Y

PCOS ....Polycystic ovary syndrome

POMC ....Pro-opiomelanocortin

Pro- αC......... α-inhibin precursor proteins

TNF- α ....Tumor necrosis factor −α

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1.0 SUMMARY

Title: The role of adipokines in polycystic ovary syndrome.

Name: Maja Popović

Polycystic ovary syndrome (PCOS) is an exceedingly complex endocrine, reproductive

and metabolic disorder of a diverse and still unclear pathogenesis. PCOS affects

approximately 10% of women of reproductive age and is considered one of the most

common endocrine and reproductive disturbances in its group (1)(2). The syndrome is

clinically characterised by oligo/anovulation and infertility, hirsutism and abdominal obesity

(3).

However, the absence of clearly defined causative factors and mechanisms has

historically made research and collaboration regarding PCOS difficult. Nevertheless,

recently, European and American communities have come to a consensus regarding the

definition of PCOS. The current requirement for diagnosis of PCOS necessitates that at least

two of the following criteria are present: ultrasound evidence of polycystic ovaries,

oligo/anovulation and/or biochemical evidence of hyperandrogenism after the exclusion of

other conditions which cause the same features (4)(2).

In addition to the reproductive abnormalities, most women diagnosed with PCOS are

predisposed to obesity and insulin resistance as well as dyslipidemia and hypertension (5). In

other words, there is a high incidence of metabolic syndrome in patients with PCOS. This

encompasses the development of type 2 diabetes and cardiovascular disease. Clearly it is the

high incidence as well as the yet unknown mechanisms linking PCOS to metabolic and vascular abnormalities which make PCOS of high socioeconomic importance.

Additionally, the amelioration of these metabolic and vascular abnormalities has been shown to improve hyperandrogenism and fertility and subsequently requires more scientific elucidation (5). Recent studies have focused on the role of obesity, or more specifically the role of various adipocytes in PCOS.

It has been shown that adipocytes are secretory cells that produce a number of proteins with hormonal type functions, collectively referred to as adipokines. In this review we see that a number of studies have shown that the secretion of various adipokines is disrupted in PCOS (6)(7) and that it is considered that this disruption of adipokine secretion could be a significant predictor of metabolic and vascular disease in patients with PCOS (8)(5)(9). Current research is aimed at further elucidating this connection because the early detection of these associated diseases would allow preventative treatment and subsequently result in improved clinical outcome, and improved quality of life for patients with PCOS.

Keywords: polycystic ovary syndrome (PCOS), adipokines, anovulation, hyperandrogenism.

2.0 LITERATURE REVIEW

2.1 Defining PCOS

PCOS is described as a syndrome because it presents as a collection of signs and symptoms that often vary between affected individuals. The lack of a clearly defined causal factor and incompletely elucidated pathological mechanisms means that there is no sole diagnostic test that can be used in the diagnosis of PCOS. Despite this, years of research and investigation have singled out three symptoms which are deemed most frequent and significant to the characterization of the syndrome. These are: hyperandrogenism, oligo/anovulation and ultrasonic evidence of polycystic ovaries (4). In light of the fact that the exact functional disturbance remains of unclear aetiology, PCOS is a disease of exclusion. Namely, that all other diseases with similar clinical presentations must be ruled out before the diagnosis of PCOS can be made (10).

2.2 Diagnostic Criteria for PCOS

The syndrome was first described in 1935 by Stein and Leventhal who portrayed what seemed to be a unique gynaecological condition that later came to be known as PCOS. They first described a combination of characteristics in 7 women, including amenorrhea and polycystic ovaries, often accompanied by hirsutism and obesity (11). Since its first description, both our awareness and understanding of the syndrome has expanded. Over the years, and across different regions, the criteria for the diagnosis of PCOS have changed. The

association of metabolic disturbances such as insulin resistance, dyslipidemia, obesity and hypertension have also been proven to be good clues in the diagnosis of the syndrome (12). However, because of the highly heterogeneous clinical presentation of the syndrome and the lack of clearly understood aetiology, it is still highly debated as to which features of the syndrome comprise its diagnosis (13).

The first formal attempt to establish a clinical definition of the syndrome with diagnostic criteria was completed in 1990 by the US National Institute of child Health and human development (NIH). The NIH criteria defined PCOS as the combined presence of hyperandrogenism and oligo/anovulation with the exclusion of all other possible causes of anovulatory infertility (14). While the creation of this, first, NIH criteria was an important initial step in creating a universally accepted consensus on the clinical picture of PCOS, it was at odds with the thinking and practices in Europe which had long considered ultrasonic evidence of polycystic ovaries as key in the diagnosis of PCOS (15). It is also noteworthy that these criteria were based on a majority opinion of clinical experts at that time and not evidenced based data.

Another expert conference was jointly held by the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) in 2003 in Rotterdam, Holland, hence the name the Rotterdam Criteria. These criteria essentially expanded on the NIH criteria by adding to the list a third clinical component, the presence of polycystic ovaries. The criteria also, controversially, outlined that only two of the three criteria need to be fulfilled for diagnosis (16). It appears as though the aim behind these criteria was to stress that PCOS was primarily a syndrome of ovarian dysfunction (either oligo/anovulation or polycystic morphology) with a spectrum of clinical

presentations. As you can see in **Table 1**, it meant that, there were now four accepted

phenotypes of PCOS, one of which did not include any clinical or biochemical evidence of

hyperandrogenism (16). This was in stark contrast to the NIH criteria which outlined

hyperandrogenism to be the most important clinical component of the syndrome (12).

Table 1: The four different phenotypes of PCOS as outlined by the Rotterdam criteria:

	APPARENT PCOS	CLASSIC NIH PCOS	OVULATORY PCOS	NORMOANDROGENISM PCOS
OLIGO/ANOVULATION	+	+	-	+
HYPERANDROGENESIM	+	+	+	-
POLYCYSTIC OVARIES	+	-	+	+

The contentiousness of the Rotterdam criteria meant that it was followed by studies

which were aimed at determining if these additional phenotypes could really still fall into the

spectrum of PCOS (13). Also in response to this, the Androgen Excess Society (AES) formed

a taskforce to review the Rotterdam criteria. They concluded that there was not enough

evidence of features of PCOS, such as ovarian dysfunction and insulin resistance, in the

group of women without hyperandrogenism. Subsequently the AES proposed a new set of

diagnostic criteria where hyperandrogenism is a requirement of diagnosis as well as one of

either oligo/anovulation or polycystic ovaries (13). This taskforce therefore concluded that

while there may be a form of PCOS without overt hyperandrogenism, there is currently insufficient evidenced based medicine for this to be proven.

Currently the most widely accepted criteria for PCOS are the AES criteria. Despite this, even the AES taskforce acknowledged that the criteria for PCOS are likely to change and evolve in the future, as further research will hopefully shed a clearer light on the aetiology of the syndrome (13). On the other hand, the two extra phenotypes described in the Rotterdam criteria are still considered by some experts to be milder forms of the disease and indeed that is the case in Croatia. These experts acknowledge that these two phenotypes also have a lower likelihood than other phenotypes of developing the metabolic and vascular complications associated with the syndrome but believe that this does not mean that PCOS can be ruled out (14)(17). **Figure 1** shows a timeline of the various criteria created for PCOS. Today it is clear that we are in need of further research concerning PCOS to help the medical community come to a firm agreement for the criteria of the syndrome. Many researchers are choosing to focus on the metabolic aspects of the disease in order to shed more light on the disease aetiology and pathology. As such, this review we will focus in on metabolic players contributing to the clinical picture of PCOS.

1990 **NIH Criteria**

1. Clinical and/or biochemical signs of hyperandrogenism

+

2. Oligo/anovulation

2003 **Rotterdam Criteria**

Two out three of:

- Olig/anovulation

- Clinical and/or biochemical signs of hyperandrogenism

- Polycystic ovaries

2006 **AES Criteria**

1. Clinical and/or biochemical signs of hyperandrogenism

+

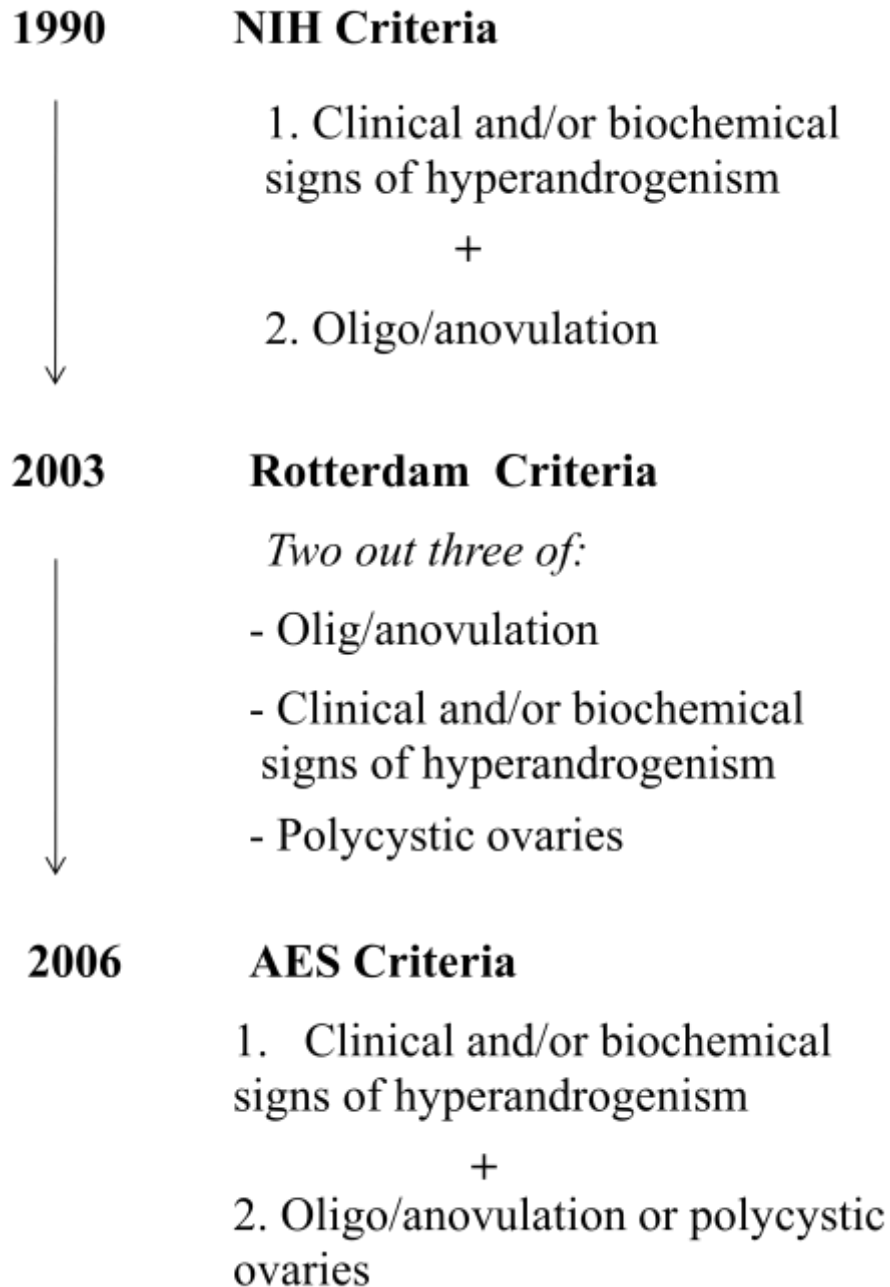2. Oligo/anovulation or polycystic ovaries

Figure 1: A timeline of changes in diagnostic criteria for PCOS from 1990 till present day 2014.

2.3 Prevalence of PCOS

While PCOS is absolutely a common syndrome among women, the exact prevalence of PCOS in the population as a whole is somewhat unclear (March 2010). This is due to several reasons, the first being because there is still no universal agreement on the criteria used in the diagnosis of PCOS, the second stems from this because without a consensus on the criteria of diagnosis it is difficult to perform population based studies. As such there are separate sets of statistics; based on the NIH criteria the prevalence of PCOS lies between 6% and 8%. According to these statistics approximately every fifteenth women in the world has the diagnosis of PCOS (18). The number of women affected by the syndrome is even higher when we base our diagnosis on the Rotterdam criteria, where prevalence is between 15% and 22% (16) (19). On the other hand if we use the AES criteria prevalence is considered to be approximately 12% (20). It is important to note that different studies report different statistics due to varied methodologies and the use of different population groups. Most of these studies did not use fully randomised methods to pick their population subsets so it is hard to say with what accuracy they predict the prevalence of PCOS in the general population (20).

Another challenge faced by the medical community when trying to determine the prevalence of PCOS is difficulty in diagnosis of the disease due to its always varied clinical presentation. Some patterns have been noted, for example, the syndrome is more frequent in adolescence than at any other age (19). Ageing combined with a reduction in number of antral follicles seems to diminish hyperandrogenism and increase the frequency of ovulation (20). It has been suggested that a more rigorous approach be taken when diagnosing the disease in adolescence and that there should be evidence of all three Rotterdam criteria

before a diagnosis is made (19). In addition to this, PCOS is more frequently seen in women who are obese, those with either type 1 or 2 diabetes and those with a family history of the disease and the knowledge of this can be used to determine groups at risk but caution should be taken not to over diagnose the syndrome (18) (16).

2.4 The clinical picture of PCOS

The clinical signs and symptoms which prompt the consideration of PCOS as a diagnosis are as varied as the presentation of the disease itself. This variability means that the process of diagnosis needs to be rigorous. It is important to choose one of the diagnostic criteria and stick to it. For example, in the Republic of Croatia specialists employ the Rotterdam Criteria. Before doing a complete diagnostic workup according to the chosen criteria we must remind ourselves that PCOS is a diagnosis of exclusion. This means that other diseases must be ruled out before the diagnosis of PCOS can be considered.

The diseases to exclude will vary slightly with the specific clinical presentation of the patient but in general the diseases to rule out are: thyroid dysfunction, hyperprolactinemia, late-onset congenital adrenal hyperplasia, ovarian hyperthecosis, Cushings syndrome, virilising tumours of the ovaries and adrenal gland, hypothalamic amenorrhea, glucocorticoid resistance, insulin resistance and iatrogenic causes (21).

The three main clinical presentations of PCOS, used in diagnosis, are outlined below. It is clear to all that the methods used in their diagnosis require improvement in accuracy and consistency if we are to have improved detection of the syndrome.

2.4.1   Clinically and/or biochemically evident hyperandrogenism

Hyperandrogenism, otherwise known as biochemical androgen excess, affects approximately 7% of women of reproductive age and the majority of these have been diagnosed with PCOS (22). The exact prevalence of hyperandrogenism in women with PCOS is not clear because there is no consensus on which androgen should be measured and which technique should be utilised. Still, the most frequently used marker of hyperandrogenism in women with PCOS is serum free testosterone (23).

The most common clinical presentation of hyperandrogenism in women with PCOS is hirsutism and it affects more than 70% of women with the syndrome (19). Hirsutism is defined as "excessive terminal hair growth that takes on a male pattern of distribution" (12). Excessive hair growth in women is measured using the Ferriman-Gallwey Scoring System which grades terminal hair growth on a scale of 0 to 4 (from none to excessive) on 9 separate anatomical sites. After the age and ethnicity of the patient has been taken into account, a score greater than or equal to 5 or sometimes 8 is considered hirsutism (24). The cut off scores are hotly debated amongst specialists. This means that the diagnosis of hirsutism using this scoring method is incredibly subjective. The inadequacy of techniques used to diagnose hirsutism adds to the difficulty of diagnosing PCOS and needs to be addressed if we are to improve our recognition of the disease.

Other less commonly seen clinical presentations of hyperandrogenism in PCOS are acne, more frequently seen in younger women and alopecia, more frequently seen in older women. Acne is another indicator of hyperandrogenism because androgens increase

sebum production and subsequently promote ideal conditions for bacterial colonization and acne formation. Alopecia can also be considered and indicator of hyperandrogenism. Scoring systems for severity of acne and alopecia are readily available. Increases in the presence and severity of acne have been noted in adolescence with PCOS but it is unclear if the prevalence of acne is increased in all women with PCOS. Conversely increases in the prevalence of alopecia in older women with PCOS have been noted but factors other than hyperandrogenism could also be considered the cause. Subsequently, acne and alopecia are rarely solely used as indicators of hyperandrogenism (12).

2.4.2   Oligo/anovulation

The reduced frequency or absence of ovulation in women with PCOS clinically manifests itself as oligoamenorrhea and amenorrhea, the reduced frequency or absence of menstruation (19). It is important to keep in mind that ovulatory dysfunction may still be present in women with PCOS who have regular menstrual cycles and ovulation is confirmed with serum progesterone levels greater than or equal to 30nmol/L during the middle of the luteal phase (Day 21 of the cycle), visualized by ultrasonography (12). Clinically it is important to keep in mind the consequences of ovulatory dysfunction in these women as they are likely to greatly impact on the quality of life of the woman. They include sub and infertility, carcinoma of the endometrium and menorrhagia (25) (26).

2.4.3   Ultrasonic evidence of polycystic ovaries

The guidelines for ultrasonographic diagnosis of polycystic ovaries were written down during the Rotterdam consensus. They define a polycystic ovary as 12 or more follicles that measure between 2 and 9mm or a total ovarian volume greater than 10mm3 (15). Concerning this method, it is important to note that these cut off values were determined using a single report and their specificity and sensitivity have not been reproduced by repeated scientific investigations. This is something that remains to be done if ultrasonography is to remain the diagnostic criteria for polycystic ovaries.

2.5 Aetiology of PCOS

The precise cause of PCOS remains as yet unknown. However, what is known is that the syndrome is, to an extent, heritable. Initial suggestions of heritability of PCOS were made as early as the 1950's with various studies examining the occurrence of the syndrome in families, including monozygotic twin studies (27). Later studies showed that indeed there is a strong genetic component involved in the development of PCOS (28). Recent studies suggest that women with a first degree relative with PCOS have a 61% chance of being affected themselves (29). Considering the diversity in the clinical presentation of this syndrome amongst women, it is clear that the heritable component of the disease is not attributable to simply one gene (28). It has long been considered that the likelihood of an individual developing PCOS can be attributed to multiple

predisposing genes with exposure to an environmental trigger such as diet or intrauterine or prepubescent hormonal exposure.

Interestingly, recent studies suggest that the disregulation in the synthesis of androgens in ovarian theca cells plays a key role in the aetiology of PCOS. A 1998 study by Abbott et al (30) found that in monkeys a hyperandrogenic fetal environment permanently up regulated adult adrenal androgen synthesis and that these adult monkeys presented with PCOS like features. In particular they exhibited hypersecretion of luteinizing hormone (LH), abnormal insulin secretion and hyperandrogenic anovulation. These results suggest that the hyperandrogenism seen in women with PCOS is potentially pre determined in-utero (31).When these studies were done in sheep the female offspring also exhibited enlarged follicular ovaries (32). These studies suggest that many of the features of PCOS, including abnormal ovaries and ovulation, hyperandrogenism and even insulin resistance, can be attributed to in-utero exposure to excess androgens. However, it is unlikely that androgen excess in the mother would be able to cause PCOS in humans because the fetus is protected by sex-hormone-binding globulin and placental metabolism which act to prevent these excess androgens reaching the fetus. It is subsequently postulated that androgen excess in the mother might be able to affect the fetus if these mechanisms were compromised. Postulated causes are placental aromatase deficiency and stress or inadequate diet (33).

It is also possible that the androgen excess comes from the fetus itself during differentiation of the fetus. It is postulated that the androgen excess could potentially come from two sources, the adrenal glands or the ovaries themselves. Mechanisms which might lead to this are unclear and would require further investigation but it is possible

that abnormal ovarian folliculogenesis may lead to an ovarian abnormality which may explain the excess androgen production by the ovaries of women with PCOS. The high serum concentrations of LH in women with PCOS result in a dysregulation of hormonal negative feedback and hypothalamic-pituitary function, which would normally provide normal cyclic changes in gonadal steroids (31)(33). This clinical feature is also seen in the monkeys of the previously mentioned experiments and the authors suggest that in-utero exposure to androgens could permanently affect this hormonal negative feedback system thereby resulting in lifelong hyperandrogenism. In an attempt to find evidence of increased androgen exposure in-utero, maternal and umbilical vein blood of newborns was tested for androgens. Raised testosterone levels were found in the umbilical vein blood of girls with PCOS compared with controls (34). This was the first evidence of a hyperandrogenic in-utero environment in PCOS babies.

While the latest research answers many questions in regards to PCOS aetiology, it also raises further questions which need to be answered in order to complete our understanding of PCOS aetiology. Considering the diversity of clinical presentations of PCOS this does not seem likely to be a simple task.

2.6 Mechanisms of pathogenesis in PCOS

The mechanisms behind the pathogenesis of PCOS are considered one of the most complicated in modern medicine. The reasons for this are partly because the syndrome remains incompletely elucidated but mostly because the syndrome itself is complex and affects a number of different biological systems and results in abnormal reproductive,

endocrine and metabolic function which can produce a spectrum of clinical and biochemical presentations.

When describing the pathogenesis of PCOS, it is difficult to know where to begin. However, the disruption in normal follicular development, and subsequently ovulation, seems like a good place to start since it has recently been implicated in the aetiology of PCOS. It would seem that both endocrine and metabolic factors contribute to anovulation in women with PCOS but the possibility of an intrinsic abnormality in folliculogenesis is also implicated.

The typical endocrine abnormalities of anovulation in PCOS are, raised serum concentrations of androgens and LH as well as slightly depressed levels of follicular stimulating hormone (FSH). It is these that contribute to the typical morphology of polycystic ovaries in PCOS. This results in two significant changes in folliculogenesis: an increase in the number of small antral follicles (in the size range of 2-8mm diameter) followed by the subsequent failure of dominant follicle selection. A simple explanation for this would be that the slightly lower FSH concentrations do not reach above a threshold required for stimulation of normal follicle maturation. Certainly induction of ovulation can be achieved in women with PCOS by treating with exogenous FSH, or anti-estrogens, both of which result in raised levels of serum FSH (35). Research into the mechanisms underlying abnormal follicle function suggests that the function of theca and granulosa cells in PCOS is abnormal. We have already mentioned that increased steroidogenic activity of theca cells contributes to this dysfunction but it is not considered the only mechanism. It was discovered that the granulose cells of small antral follicles (2-

9mm), as typically seen in women with PCOS, were prematurely responsive to LH (responsiveness to LH is normally confined to the dominant follicle) and are likely to contribute to the premature arrest of follicular growth seen in PCOS (36).

Folliculogenesis is a long and dynamic process involving many different signally factors and receptors to go from the initial cohort of follicles to the selection of a dominant follicle ready for ovulation. It means that these signalling components need to be investigated before we can have a clearer picture of what results in the abnormalities we see in the ovaries of women with PCOS. Some of the specific signalling abnormalities thought to contribute to an increased number of smaller follicles in the ovaries of women with PCOS are; a reduced expression of growth differentiation factor-9 (GDF-9) (which normally blocks secretion of androgens in the oocyte) as well as increased production of anti Mullerian Hormone (AMH) and increased serum level of α-inhibin precursor proteins (pro- αC) (which increases LH induced androgen increase) (35). Some factors which may contribute to the arrest of follicular maturation include increased levels of inhibin B and increased levels of AMH, both of which contribute to reduced levels of FSH (36). However further studies are needed to elucidate the precise effects of these specific differences seen in PCOS follicles compared with normal follicles found in ovulatory women. It is these differences which are thought to result in the loss of cyclic rhythm in women with PCOS. The key point to remember here is that the arrest in follicular development and failure of dominant follicle selection is what results in anovulation and low levels of progesterone in women with PCOS.

The low levels of progesterone in PCOS also play a large role in the pathogenesis of the syndrome. The lower than normal levels are what lead to chronically increased release of gonadotrophin releasing hormone (GnRH) which results in increased levels of LH, biochemically present in 70% of women with PCOS. This increase in LH is thought to sustain the increased production of androgens and is also thought to be the reason behind the increased incidence in spontaneous abortions typically seen in women with PCOS. In light of this and the already outlined contributors to lower levels of FSH we get a change in the FSH/LH relationship in women with PCOS which most significantly results in anovulation (36).

Insulin resistance is seen in 40-80% of women with PCOS, with the incidence being higher in obese women with PCOS (37). The precise cause of the increased susceptibility of women with PCOS to insulin resistance is as yet unclear. Obesity alone is not the cause because women with PCOS, who are in a healthy weight range, show greater incidence then the unaffected population. The aetiology of insulin resistance can neither be entirely attributed to hyperandrogenism because successful treatment with anti-androgens does not reduce the incidence of insulin resistance in women with PCOS (38). The latest research suggests that insulin resistance in PCOS can be attributed to defects in insulin receptor signalling in peripheral tissues (39).

The consequence of insulin resistance in women with PCOS is not only the obvious metabolic disturbance but insulin resistance also contributes to anovulation. Those women with PCOS who *do* have regular ovulation (the milder form outlined by the Rotterdam criteria) have been shown to be less susceptible to the development of insulin

resistance than PCOS women affected by anovulation (39). This observation made researchers suspect that insulin resistance and the resulting hyperinsulinemia contribute to the pathogenesis of anovulation in PCOS. Indeed in *in vitro* studies insulin has been shown to increase the ability of granulose cells to respond to LH, thus potentially disturbing the FSH to LH switch which triggers follicular maturation. Most scientists consider hyperinsulinemia as a second hit rather than primary cause of anovulation in PCOS and the hyperinsulinemia itself is likely due to a genetic predisposition which accompanies the PCOS phenotype (40).

2.7 Importance of adipokines in PCOS pathogenesis

Considering that the focus of this review is the role of adipokines in PCOS we will talk about them, here, in greater detail then the other contributors of PCOS pathogenesis discussed above. Approximately 30-70% of women with PCOS are overweight with a body mass index (BMI) greater than 25, which is a greater incidence than the general population. Even in women with PCOS in a healthy weight range, increased visceral fat has been documented. It is well known that obesity (adiposity) in women with PCOS significantly contributes to the clinical picture of PCOS, affecting reproductive, hormonal and metabolic systems (41). It is well known that obesity in general increases anovulation, spontaneous abortion and perinatal complications in non PCOS women as well. In particular adiposity is linked with an increased likelihood of developing insulin resistance, type two diabetes and cardiovascular diseases, all of which occur more frequently in women with PCOS and have serious health ramifications.

The role of hyperandrogenism has also been investigated in relation to the increased adiposity found in PCOS patients. It has been shown that hyperandrogenism favours abdominal adiposity and insulin resistance (31)(33). Androgens have been shown to inhibit differentiation of adipocytes subsequently affecting their function. Testosterone specifically has been shown to cause significant decreases in insulin mediated glucose uptake which suggests that hyperandrogenism can contribute to the insulin resistance seen in PCOS (42). Considering that insulin resistance and hyperinsulinemia have also been shown to increase the production of androgens, it suggests a vicious circle between insulin resistance and hyperandrogenism, which begs the question which came first.

Adipose tissue operates as an extremely specialised endocrine and paracrine organ, producing a number of adipokines which have a number of local and systemic effects on our bodies. Mostly, adipokines play a huge role in the homeostasis of the organism in regards to input and output of energy through food intake, insulin activity, metabolism of glucose and lipids, angiogenesis and regulation of blood pressure (42). Subsequently, any disturbance in usual functioning of adipokines can have disastrous effects on our health and increased adiposity leads to this disturbance and influences the development of insulin resistance, atherosclerosis and hypertension. The exact disturbance of adipokine activity in women with PCOS remains to be elucidated and is currently a hot research topic. The current theory is that in women with PCOS there is a vicious circle of events which begins with increased production of androgens in the ovaries that potentiates abdominal fat accumulation and disruption of adipocyte functioning, which leads to disturbed secretion of adipokines, leading to the development of insulin resistance and subsequently hyperinsulinemia which then goes back and

potentiates the increased androgen production by the ovaries (**Figure 2**). The fact that adipokines pose a potential link between hyperandrogenism and metabolic disturbances in PCOS is currently a hot topic of research because the exact disturbances in precise adipokine secretion and their role in the pathogenesis of PCOS are yet to be elucidated. Subsequently their exact roles are highly debatable topics, with sometimes contradictory findings. Perhaps this can be attributed to the highly heterogeneous manifestation of PCOS, which means that precise mechanisms could be better elucidated in using subcategories of phenotypes, ensuring more homogeneity. The importance on clarifying our understanding of the role of various adipokines in PCOS is vital as it could be used as a predictor of metabolic disturbances which result in the development of diabetes and cardiovascular disease in these patients. If adipokines could be used as markers to predict metabolic disturbances in these patients then preventative measures could be used to improve clinical outcomes in women with PCOS. Subsequently, the following paragraphs will review our current understanding of the role of various selected adipokines in PCOS.
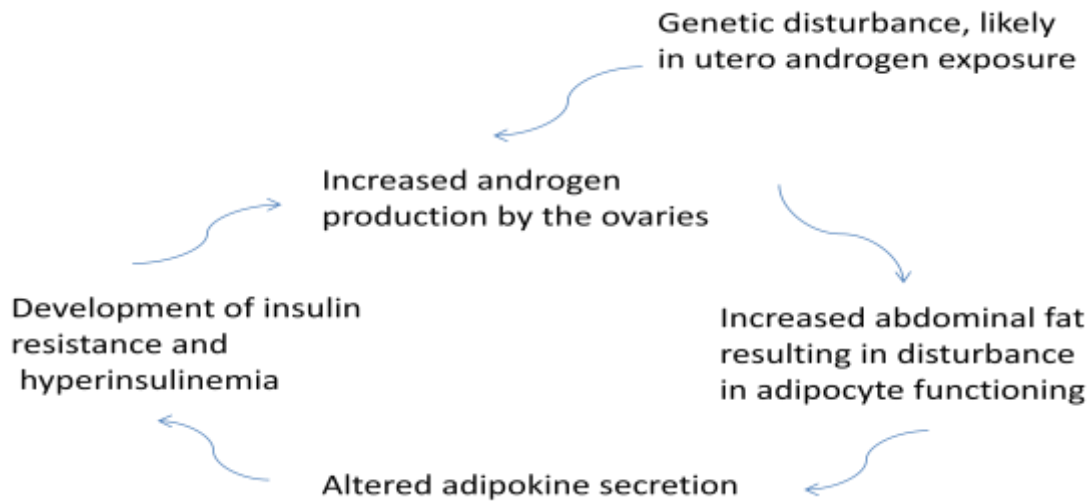
Figure 2: A simplified illustration of the current theory of the vicious circle which leads to the disruption of adipokine activity in women with PCOS. Precise mechanisms remain to be completely elucidated.

2.7.1   Leptin

Leptin is an adipokine secreted predominantly by adipocytes. It is also secreted in the stomach, placenta and by breast tissue. It is a protein which was originally cloned as the product of the *ob* gene in obese mice in the 1950's, and is now know as the *Lep* gene in humans (43). Leptin acts like a hormone, and binds to its receptor, Ob-Rb, in the hypothalamus to reduce appetite and food intake. When it binds to its receptor it reduces neuropeptide-Y (NPY) and agouti regulated protein (AgRP) activity at the same time as increasing pro-opiomelanocortin (POMC) and cocaine-amphetamine related protein (CART) neuron activity. This essentially means that it decreases orexigenic peptide synthesis at the same time as increasing anorexigenic peptide synthesis in the hypothalamus, resulting in appetite suppression (7). Leptin also has an effect on peripheral tissues where it is understood to generally have a fat metabolizing role. There is evidence that it increases lipolysis in adipose tissues, skeletal muscle and less so in the liver. Leptin also antagonises insulin action and decreases its production by pancreatic beta cells and indirectly affects glucose metabolism. The precise leptin signalling pathway in peripheral tissues is not well known but leptins ability to increase fatty acid oxidation in skeletal muscle has been shown to be due to the activation of the adenosine monophosphate protein kinase (AMPK) signalling pathway (7)(44).

Leptin expression and secretion have been shown to be increased in obese individuals and further administration of leptin in these individuals has no effect on appetite or body weight. This suggests the presence of leptin resistance probably due to saturation of leptin transport across the blood-brain barrier. However, if patients have

lipodystrophy where leptin levels are lower than normal, the administration of leptin ameliorates this condition. This is also true for rare cases of genetic leptin deficiency; dramatic weight loss is seen in these individuals upon leptin administration (7) (44). Additionally leptin receptor mutations in mice show severe insulin resistance and a predisposition to type II diabetes. Subsequent administration of leptin indirectly improves insulin resistance through reduction of fat tissue (43). All of this suggests a role for leptin, and its receptor, in insulin resistance typically accompanying obesity, particularly in PCOS.

The role of leptin in PCOS is unclear, recent studies have had conflicting results. Some studies suggest that leptin is elevated more greatly in women with PCOS when compared to women without the syndrome but in the same weight range (45)(46). Conversely other studies have found that leptin levels are not affected by PCOS but only by overall adiposity of the individual (47)(48). Regardless of this, since a majority of women with PCOS are overweight and obese, it is in their interest that the mechanism of leptin resistance in obesity be better understood, as it likely plays a role in the development of insulin resistance and hyperandrogenism in these patients.

2.7.2   Adiponectin

Adiponectin was first described in 1995 as a new serum protein primarily secreted by adipocytes. Since then it has been cloned by three more research groups and was found to be encoded by the gene *apMI* (44). Since 2001 it has become clear that adiponectin greatly affects insulin sensitivity. The c-terminal fragment of adiponectin

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was shown to reduce plasma glucose levels through fatty acid oxidation in myocytes and through paracrine action in adipocytes (49). In the liver, a full length adiponectin protein was shown to function as an insulin sensitizing agent by reducing hepatic glucose production (gluconeogenesis) and enhancing insulin action in the liver (50) (51). It is already well elucidated that adiponectin levels are significantly decreased in insulin resistance and obesity and that adiponectin secretion improves when insulin resistance and obesity improve. In vitro studies have shown that various hormones and cytokines, including catecholamines, glucocorticoids, tumor necrosis factor-α (TNF-α) and interlukin-6 (IL-6), are associated with insulin resistance and obesity (52)(53).

In addition to its effects on insulin sensitivity, adiponectin also increases fatty acid oxidation in the liver via the reduction of CD36 expression, resulting in reduced liver triglycerides (54). Adiponectin has also been shown to affect skeletal muscle fuel oxidation, also resulting in reduced triglyceride accumulation (54). Anti atherogenic properties of adiponectin have also been suggested in a study showing that adiponectin inhibited the binding of apolipoprotein B100-containing lipoproteins to vascular proteoglycans, which is an initial step in atherogenesis (43).

Unlike leptin, research results in regards to adiponectin in PCOS have shown more consistent results. They have shown that adiponectin levels are reduced in all women with PCOS regardless of their weight and that this reduction is significantly greater than in weight matched non PCOS controls (55)(1). The negative correlation was between adiponectin and both BMI and central fat mass. Insulin levels and insulin resistance have also been shown to be inversely correlated with adiponectin, even after adjusting for BMI. Interestingly this relationship with insulin resistance and adiponectin

was only seen in PCOS patients and not in control groups, suggesting a mechanism for increased insulin resistance seen in PCOS (36)(3). Thus adiponectin may be seen as a factor which contributes to obesity and independent development of insulin resistance seen in women with PCOS.

Further studies were done to discover whether there is an additional mechanism by which adiponectin contributes to metabolic symptoms in PCOS, perhaps by somehow contributing to hyperandrogenism. Interestingly, a recent *in vitro* study has shown how adiponectin inhibits LH dependant production of androgens and subsequently steroidogenesis. This is significant to our understanding of the pathogenesis of PCOS (56). On the other hand, there are conflicting studies on the relationship between testosterone and adiponectin. Some studies have shown that testosterone is not significantly or independently correlated with adiponectin levels while others have shown that it is (3)(48)(57)(58). An explanation for why some studies found no correlation is that there were heterogenous levels of androgens in the sample cohort used. Further studies are required to clearly elucidate the relationship between adiponectin and testosterone, in particular in PCOS.

While the precise physiological role of adiponectin remains to be completely understood, our current understandings show that adiponectin plays an important role in metabolic homeostasis with its insulin sensitizing potential as well as its antiatherogenic and anti-inflammatory effects. In light of the knowledge that adiponectin levels are reduced in obesity and in PCOS, regardless of weight, it is possible for us to consider using adiponectin levels in order to predict and preventatively treat patients with PCOS with a predisposition for the development of metabolic diseases.

2.7.3   Ghrelin

Ghrelin is a recently discovered peptide hormone secreted by ghrelin cells predominantly in the stomach but also in other parts of the body including the duodenum, jejunum, pancreas, kidneys and gonads. Ghrelin has important effect on energy balance, affecting food intake and subsequently regulating weight by stimulating appetite and contributing to weight gain (3). The main target site for ghrelin is found in the hypothalamus where it plays an important role in energy balance. Ghrelin levels have been shown to increase during periods of hunger and in anorexia and decrease after eating. The peptide hormone has been shown to have short acting and long acting weight regulating effects (59).Ghrelin levels were found to be lowered in obesity. It has also been associated with insulin resistance and, with its expression in pancreatic beta cells, it is postulated that ghrelin is able to inhibit insulin secretion (60).

While some studies have shown lower ghrelin levels in PCOS patients compared with weight matched controls (3), other studies have shown equal (61) or even increased levels compared with controls. Additionally there are conflicting results when looking at the relationship between ghrelin and androgen levels (61)(62)(63). PCOS is known to be a heterogenous disease with a variety of clinical presentations and it could be the heterogeneity of the PCOS patient cohorts used in these studies which contribute to mixed and conflicting results. Clearly more research needs to be done in order to elucidate the exact role ghrelin might have in PCOS. Once again one way of doing this would be to subcategorise PCOS patients, according to the Rotterdam criteria, and investigate them accordingly.

2.7.4 Resistin

Resistin was discovered concurrently in mouse adipocytes by three separate research groups in 2001. In mice, resistin is abundantly expressed and was shown to inhibit insulin signalling and glucose uptake in adipose tissue, skeletal muscle and in the liver, subsequently resulting in glucose intolerance (64)(65).

However, in humans resistin expression is low and has so far shown conflicting results. Some studies have found that it does not affect insulin sensitivity or glucose uptake (66)(67) but other studies show that resistin is associated with obesity and insulin resistance (68)(69). Although the data linking resistin to insulin regulation in humans is lacking, it has been shown to play a role in inflammation. In humans, resistin is primarily produced by mononuclear cells in plasma and its expression is regulated by the pro inflammatory cytokines TNF-α and IL-6. Conversely resistin was shown to induce the secretion of TNF-α and IL-6 in mononuclear cells and adipocytes (70)(71). Interestingly TNF-α and IL-6 have both been shown to affect insulin sensitivity. TNF-α induces insulin resistance in liver, muscle and fat tissue and its expression is increased in obesity. Its therapeutic potential in insulin resistance has already been investigated and unfortunately TNF antagonists have not resulted in improvements in insulin sensitivity (72). IL-6 has also been shown to have insulin resistance inducing effects by impairment of intracellular insulin signalling in adipocytes and hepatocytes (73). The connection between resistin and these inflammatory cytokines needs further investigation and suggests resistin may play a role in the pathogenesis of cardiovascular diseases to which inflammatory cytokines are key players.

The role of resistin in PCOS has not been investigated sufficiently and the few studies that have been done, once again, show conflicting results. Some studies have found that resistin levels are increased in PCOS independent to obesity and insulin resistance (74)(75) while other studies have found no difference between PCOS and control groups (76). Subsequently, further research is needed to improve our understanding of the role of resistin in the pathogenesis of metabolic and potentially reproductive disturbances in PCOS.

3.0 CONCLUSION

Poly cystic ovary syndrome (PCOS) is one of the most complex syndromes known to modern medicine and affects a significant part of the female population. In these individuals it affects reproductive, endocrine and metabolic functioning. It is characterised by oligo/anovulation, hyperandrogenism and polycystic ovaries. Clinical presentation is very heterogeneous, which often makes diagnosis and research of the syndrome controversial and challenging. In addition to the main three clinical findings used in the diagnosis of the syndrome, affected women frequently present with obesity and insulin resistance with higher incidence than the rest of the population. This means that women with PCOS are at increased risk of infertility, type II diabetes, hypertension and other cardiovascular diseases.

This literature review focused in on the role of adipokines in PCOS, with the aim to summarize where we currently stand in our understanding of the complexities of the syndrome. It is clear that the investigation into the role of adipokines in PCOS is a logical step as adipokines have been shown to play an important role in metabolic homeostasis. Since metabolic disturbances like insulin resistance and cardiovascular diseases are more commonly found in PCOS than in the general population, it can be said that PCOS is a good model to study the mechanism of action of adipokines. It also gives us a better opportunity to elucidate the pathogenesis of both metabolic and reproductive disturbances seen in PCOS. This review highlights four adipokines with the greatest potential involvement in PCOS, leptin, adiponectin, ghrelin and resistin. Most of them have shown some evidence of affecting insulin resistance and a disturbance in this relationship in women with PCOS compared with controls. The adipokine, adiponectin, has been shown to have the greatest

correlation to insulin resistance, in particular in PCOS patients. This adipokine has also been implicated in protecting against atherosclerosis and subsequently cardiovascular disease. The careful review of up to date research of PCOS, in particular the role of adipokines in PCOS leads us to conclude that this remains a highly interesting area of research which requires further investigation to completely elucidate all the functions of adipokines in general, and particularly their role in the pathogenesis of PCOS. In general, the inconsistency of results, commonly found regarding the role of adipokines in PCOS, can potentially be attributed to the heterogeneity of the syndrome. It would therefore be beneficial to design studies in such a way that this heterogeneity is reduced, perhaps by investigating PCOS patients in the subcategories of their phenotype group as outlined by the Rotterdam criteria. Undoubtedly further research would be of benefit to patients with PCOS because if more is known about the metabolic and reproductive disturbances from which they suffer then we are more likely to be able to find, and preventatively treat, at risk individuals and give them a better future quality of life, potentially prolonging their lives in the process.

4.0 ACKNOWLEDGEMENTS

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6.0 BIOGRAPHY

I was born in Zagreb, Croatia in 1987 and moved to Sydney Australia with my parents in 1989 where I completed my primary and high school education. In 2005 I began my Bachelor of Science at the University of Sydney and completed it in 2008 with First Class Honours. My honours thesis was in the area of Molecular biology and Genetics and was titled: Unravelling Signalling in Eye Development and Links to Blinding Disorders. It was done in collaboration with the Children's Medical Research Institute, also in Sydney, Australia. My first academic year at the University of Zagreb was 2009. Having received some subject exemptions in the first two years of Medicine due to my previous tertiary education I have been given the opportunity to complete the degree in 5, instead of the usual 6, years of study. I am currently at the end of the 6th year, my 5th year, and I am on course to graduate in July this year, 2014.

During the 9 years of my tertiary education I have kept myself busy not only with studying but also with part time jobs, some not academically related such as being a salesperson, others medically related such as mentoring lower years on the Internal wards. While English is my mother tongue, I am fluent in Croatian and am currently learning French. My biggest passions outside of Medicine are photography, travel and cycling.

My intentions are to stay in Croatia for both my internship year as well as for my specialization. The specializations that are currently most appealing to me are Paediatrics, Family Medicine, Internal Medicine and Obstetrics and Gynaecology. During my specialization my hope is to also be involved with medical research in a field that I find particularly intriguing.