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Leptin: An ostensible biomarker for polycystic ovary syndrome (PCOS)

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Abstract

An abnormally elevated androgen level in reproductive-aged women causes development of ovarian cysts, leading to ovulatory infertility, irregular menstruation, increased risk of insulin resistance, cardiovascular problems, amenorrhea, obstructive sleep apnea, cardiovascular disease, hirusitism, acne, and depression, postpartum hemorrhage, endometrial carcinoma, type 2 diabetes mellitus (T2DM), hyperglycemia, gestational diabetes mellitus (GDM), which can result into ROS formation, oxidative stress, and abdominal adiposity. The reproductive, endocrinal, metabolic and ovarian disorders are collectively known as PCOS (polycystic ovary syndrome). In about 70% of PCOS women, disrupted GnRH, FSH, LH, prolactin levels and obesity further prejudice insulin metabolism or insulin resistance. A peptide hormone Leptin, which is encoded by the obese, was found to be higher in PCOS women and now seems to be emerging as an important biomarker, as excessive leptin levels in PCOS may disrupt ovarian steroidogenesis and mature oocyte development, which could lead to ovulatory dysfunction and infertility. TNF-α and IL-6 are the inflammatory cytokines found in high concentration in PCOS women, that share close relation with leptin. Therefore, leptin, agonist and antagonist of leptin could be a novel prospective biomarker for diagnosis, prevention, treatment and cure of polycystic ovary syndrome.

KEY WORDS- PCOS (Polycystic ovary syndrome), T2DM (type 2 diabetes mellitus), GnRH (Gonadotrophin-releasing hormone), FSH (follicular stimulating hormone), LH (luteinizing hormone) and Leptin.

Introduction

The reproductive, endocrine, metabolic, and ovarian disorders collectively known as PCOS (polycystic ovary syndrome) (Gu et al., 2022). In 1935, it was 1st described by *Stein* and *Leventhal*; reported 7 cases of patients presented with amenorrhea, infertility and enlarged multicystic ovaries. An abnormally elevated male sex hormone, primarily androgen, causes about 15% of reproductive-aged women to develop ovarian cysts. The MENA (Middle East and North Africa) region had a higher YLD (Year of healthy life lost due to disability) rate of PCOS (18.7 vs. 14.7 per 100,000 populations) than the global average (Motlagh Asghari et al., 2022). The significant rise in PCOS cases could be attributed to a number of factors, including obesity, resource availability, healthcare access, aging populations, and population growth (J. Liu et al., 2021).Numerous genes, including CYP11a, CYP21, CYP17, and CYP19, which are important in PCOS, are involved in the ovarian and adrenal steroidogenesis process (J. Liu et al., 2021). Obesity, metabolic syndrome, impaired glucose tolerance, type 2 diabetes mellitus (DM-2), endometrial cancer, and nonalcoholic fatty liver disease/ nonalcoholic steatohepatitis (NAFLD/NASH), depression, obstructive sleep apnea (OSA) are various number of symptoms which linked to PCOS. The first-line treatments for PCOS symptoms, such as menstrual irregularity, hirsutism, and acne, involve lifestyle modifications in addition to combined hormonal contraceptives (CHCs) (de Melo et al., 2017). Almost 50% to 70% and 35%-80% of PCOS women have most common metabolic feature i.e. Diabetes mellitus and Insulin resistance respectively (Amisi, 2022). With a prevalence rate of about 70%, abnormal lipid metabolism is common in PCOS patients by decreasing HDL level as well as increase LDL level (Jiang et al., 2020).The prevalence of infertility in PCOS is about 70-80% (Melo et al., 2015). Hirsutism affects 4–11% of women in the general population while it is estimated to affect 65–75% of women with (Spritzer et al., 2022). PCOS and 15–25% of PCOS patients have acne (Meier, 2018) Additionally, 20% to 30% of patients exhibit an excess of adrenal androgen, which may indicate adrenal cortical hyper function cause hyperandrogenism (Yildiz & Azziz, 2007). Caucasian women with PCOS have 9% lifetime risk of endometrial carcinoma, compared to a 3% lifetime risk in women without PCOS (Shetty et al., 2023). The range of 0 to 70% is the prevalence of OSA in women with PCOS (Kahal et al., 2020).

Leptin level was found to be high in PCOS women (Peng et al., 2022). Adipose tissue secretes the peptide hormone Leptin, which is encoded by the obese (ob) gene (Y. Zhang et al., 1994). In

1994, by using molecular biology methods, Jeffrey Friedman and associates at Rockefeller University were able to identify and isolate this "obesity hormone". Halaas also demonstrated the endocrine action of the Ob protein in the *ob/ob* mouse with a reduced food intake and increased energy expenditure, they proposed that 16 kDa protein with 146 amino acid be called Leptin, derived from the Greek word *leptos,* meaning thin. Leptin has quadruple helix structure. ObR widespread in target organs such as brain tissue, heart, lungs, kidney, liver, pancreas, intestine, placenta, gonads, spleen, thymus. Leptin decreases body mass, food intake, suppresses hunger and regulate glucose (Parton et al., 2007) metabolism (Krude et al., 1998) through POMC-expressing neurons which further release the POMC (*Pro-opiomelanocortin)* cleavage product α-melanocyte stimulating hormone (α-MSH) in hypothalamic melanocortin system (Belgardt & Brüning, 2010). OBRa, OBRb, OBRc, OBRd, and OBRe are the six isoforms of ObR. OBRb demonstrating intracellular signal transmission capability. The JAK-STAT pathway is the primary signaling pathway for the LR. When leptin binds to it, the LR dimers. As a result of this dimerization, three tyrosine residues that act as docking sites for the proteins SHP2, STAT5, and STAT3 are phosphorylated by JAK2 tyrosine kinase. The transcription factor STAT 3 is in charge of mediating the main effects of leptin (Allison & Myers, 2014). Leptin acts on insulin receptor (IRS1/2) where insulin molecule bind further AKT/PKB pathway activates. AKT phosphorylate FOXO1 which stimulate steroidogenesis pathway and their genes. Along with this through central regulation of Gonadotropin-releasing hormone (GnRH) neuronal activity and secretion at the hypothalamus. Gonadotropin hormones are increased by leptin level which is essential for the activation and maintenance of normal reproductive function. (Gary J Hausman et al., 2012). However, because of the emergence of Leptin resistance, obesity (Katsiki et al., 2018) is characterized by hyperleptinemia. Leptin resistance and insulin resistance disturb the steroidogenesis pathway, increase theca cell androgen as well as overproduction of GnRH in anterior pituitary gland. This overproduction of GnRH increase LH: FSH in theca cell of ovary. This all further cause's cysts form in ovary and also lead to PCOS. That's PCOS is linked to Leptin resistance, insulin resistance, abdominal obesity, hyperandrogenism, and dysfunctional ovulation (Manneras et al., 2007) and Leptin is possible biomarker for detection of PCOS.

Egg story of Leptin

The ob gene produces the prototype adipokine leptin, which is known for its ability to suppress appetite, increase energy expenditure in the brain, and subsequently lower body weight and fat mass (Halaas et al., 1995). Adipose tissue functions as an endocrine organ secreting pro- and anti-inflammatory adipocytokine (Laclaustra et al., 2007), growth factors, enzymes, and steroid hormones (McGown et al., 2014). In 1949 at the Jackson Laboratories in Bar Harbor, Obese mice were first distinguished from littermates at 4-6 weeks of age. The gene responsible was designated *ob* (now *Lep)* (Ingalls et al., 1950). A further mutant mouse, known as db/db or Leprdb, was introduced by Coleman and colleagues after 16 years. This mouse had severe diabetic symptoms, including hyperglycemia, polyuria, and glycouria, along with obvious hyperphagia and obesity (Alves, 2013). Jeffrey Friedman and colleagues at the Rockefeller University in 1994, was able to isolate and characterize this "obesity hormone" using molecular biology techniques (Yiying Zhang et al., 1994). Halaas also demonstrated the endocrine action of the Ob protein in the *ob/ob* mouse with a reduced food intake and increased energy expenditure (Castracane & Henson, 2007). Leptin containing 16 kDa proteins with 146 amino acid secreted from brain tissue, heart, lungs, kidney, liver, pancreas, intestine, placenta, gonads, spleen, thymus and CNS. Leptin has quadruple helix structure, similar to cytokine family (IL-2, IL-6, IL-12, and IL-15). Leptin was derived from the Greek word *leptos*, meaning thin, decreases body mass and food intake and suppresses hunger.

Function of Leptin receptor in hypothalamus

White adipose tissue produces the peptide hormone leptin. On chromosome 7q31.3, the leptin gene (LEP or ob) is located (Gong et al., 1996). By attaching itself to leptin receptors (LR) on cell surfaces, leptin works. Neuronal, hepatic, pancreatic, cardiac, and perivascular intestinal tissue all have leptin receptors. The LR is a cytokine receptor with six isoforms that is a member of the glycoprotein 130 family. OBRa, OBRb, OBRc, OBRd, and OBRe are the six isoforms of ObR. OBRb demonstrating intracellular signal transmission capability. Leptin is a member of the long-chain helical cytokine family (Houseknecht & Portocarrero, 1998). It regulates energy homeostasis through its receptors, which are widely distributed throughout the body, especially in the central nervous system (CNS) of both humans and rodents. Other tissues that express Leptin mRNA include brown adipose tissue, the placenta, the ovary, skeletal muscle, the stomach (Bado et al., 1998), the pituitary gland, and the central nervous system (Bado et al., 1998) . The brain, specifically the brainstem and hypothalamus, is where Leptin primarily acts.

The ventral tegmental area and the solitary tract are the main brainstem action sites. Here, leptin regulates reward and aversion as well as satiety. Leptin primarily acts on the ventromedial, dorsomedial, ventral pre mammillary, and arcuate (ARC) nuclei in the hypothalamus, specifically the lateral hypothalamic area (Farr et al., 2015). The action of leptin on the ARC nucleus is the most well-known of them. An important component in controlling hunger and energy homeostasis is the ARC nucleus. It has both anorexigenic proopiomelanocortin-containing (POMC) neurons and orexigenic agouti-related protein/neuropeptide Y-containing (AgRP/NPY) neurons. Leptin inhibits AgRP/NPY (Shutter et al., 1997) containing neurons and stimulates POMC-containing neurons in the ARC nucleus, which results in a reduction in appetite overall (Broberger et al., 1998). Agouti gene-related protein (AGRP), an endogenous antagonist of the anorexigenic melanocortin peptides like α-melanocyte-stimulating hormone (αMSH), is coexpressed by a large number of NPY neurons in the rodent arcuate (Ollmann et al., 1997) and inhibit food (Cone, 2005) intake (Huszar et al., 1997) (Fan et al., 1997).

Leptin Signaling Pathway

Leptin controls appetite and energy homeostasis through JAK-STAT signaling pathway (Obin Kwon et al., 2016). LEPRb belongs to the cytokine receptor family of interleukin 6 (Taga & Kishimoto, 1997) (IL-6) (Baumann et al., 1996) and binds to Janus kinase 2, a cytoplasmic tyrosine kinase (JAK2) (Chen et al., 1996) Tyr985, Tyr1077, and Tyr1138 are the three tyrosine residues on which JAK2 phosphorylates (Hekerman et al., 2005) LEPRb (Banks et al., 2000) Phopho-Tyr985, -Tyr1077, and -Tyr1138 are involved in the recruitment of downstream signaling molecules with the Src homology 2 (SH2) domain to the LEPRb-JAK2 complex. This process enables JAK2 to phosphorylate these effector proteins (White et al., 1997). Cytoplasmic proteins called STAT molecules are triggered by a variety of substances, such as growth factors, cytokines, and hormones like leptin. There are seven members of the mammalian STAT family: STAT1-4, STAT5a, STAT5b, and STAT6. STAT1, STAT3, STAT5, and STAT6 are all phosphorylated by leptin; of these, STAT3 (Bates et al., 2003)and possibly STAT5 mediate the anorectic effects of leptin (Ghilardi et al., 1996). Key neuropeptides that regulate appetite, including POMC, AgRP, and NPY, are more easily transcriptionally regulated by leptin when STAT3 is phosphorylated. Prohormone convertases cleave POMC, a precursor peptide, to produce α-melanocyte stimulating hormone (α-MSH) (White et al., 1997), anorexigenic (Vaisse et al., 1996). Target genes, including the neuropeptides POMC, AgRP, and NPY, are transcriptionally modulated by STAT3 as a transcription factor in the nucleus, where it dimerizes

and translocates from (Mesaros et al., 2008) the (Mesaros et al., 2008)cytoplasm (Münzberg et al., 2003) resulting leading to reduced food intake and increased energy expenditure. Additionally, the suppressor of cytokine signaling 3 (SOCS3) is more transcriptionally active when STAT3 is phosphorylated. This creates a negative feedback loop that balances leptin signaling (Banks et al., 2000).LepRb on Tyr1077 is activated by JAK2 to phosphorylate STAT5 (Mütze et al., 2007). Leptin specifically induces the hypothalamus to express SOCS3 mRNA. Another well-known negative regulator of leptin signaling is protein tyrosine phosphatase 1B (W. Kaszubska et al., 2002) (PTP1B) (Wiweka Kaszubska et al., 2002) and *SOCS3* (Pedroso et al., 2016), which dephosphorylates JAK2 to reduce leptin-induced JAK2/STAT3 (Wunderlich et al., 2013) signaling (Dunn et al., 2005).

Along with this Leptin accomplishes its goals of decreasing food intake and raising energy expenditure by activating specific parts of the insulin-signaling cascade (Niswender et al., 2001). Insulin-receptor substrates 1/2 (IRS1/2) phosphorylation is increased by leptin through JAK2 (Kim et al., 2000)activation (Bjørbaek et al., 1997). The phosphatidylinositol 3-kinase (PI3K) is activated by the phosphorylation of both IRS1 and IRS2 (White, 1998). IRS is recruited to JAK2 by SH2B (Duan et al., 2004) adaptor protein 1 (SH2B1), and JAK2 phosphorylate IRS proteins to initiate the PI3K pathway (Li et al., 2007). PTP1B inhibits the IRS-PI3K axis by suppressing the (González-Rodríguez et al., 2010) IRS1/2 (Galic et al., 2005). Phosphatidylinositol-4, 5-bisphosphate (PIP2) is converted to phosphatidylinositol-3, 4, 5-trisphosphate (PIP3) in leptin-sensitive neurons by PI3K. AKT's PH domain causes the phosphoinositide-dependent kinase 1 (PDK1) to become active, which in turn activates the v-akt murine thymoma viral (Parikh et al., 2012)oncogene homolog 1 (AKT) (Schultze et al., 2012). This procedure reduces body fat mass by improving downstream signaling that is dependent on AKT (Schultze et al., 2012). AKT also referred to as protein kinase B (PKB), is a serine/threonine-specific protein kinase that is essential for cell migration, proliferation, metabolism, and apoptosis (Vanhaesebroeck et al., 2012). AKT localizes the forkhead box protein O1 (FoxO1) in the cytoplasm, activates the cAMP response element-binding protein (CREB), and activates the mammalian target of rapamycin (mTOR) (Sun et al., 2021). In these neurons, activated mTOR phosphorylates ribosomal protein S6 kinase beta-1 (S6K1) at Thr389 to decrease food intake, increase energy expenditure, elevate renal sympathetic nerve outflow, and guard against (Cota et al., 2008) obesity (Cota et al., 2006).

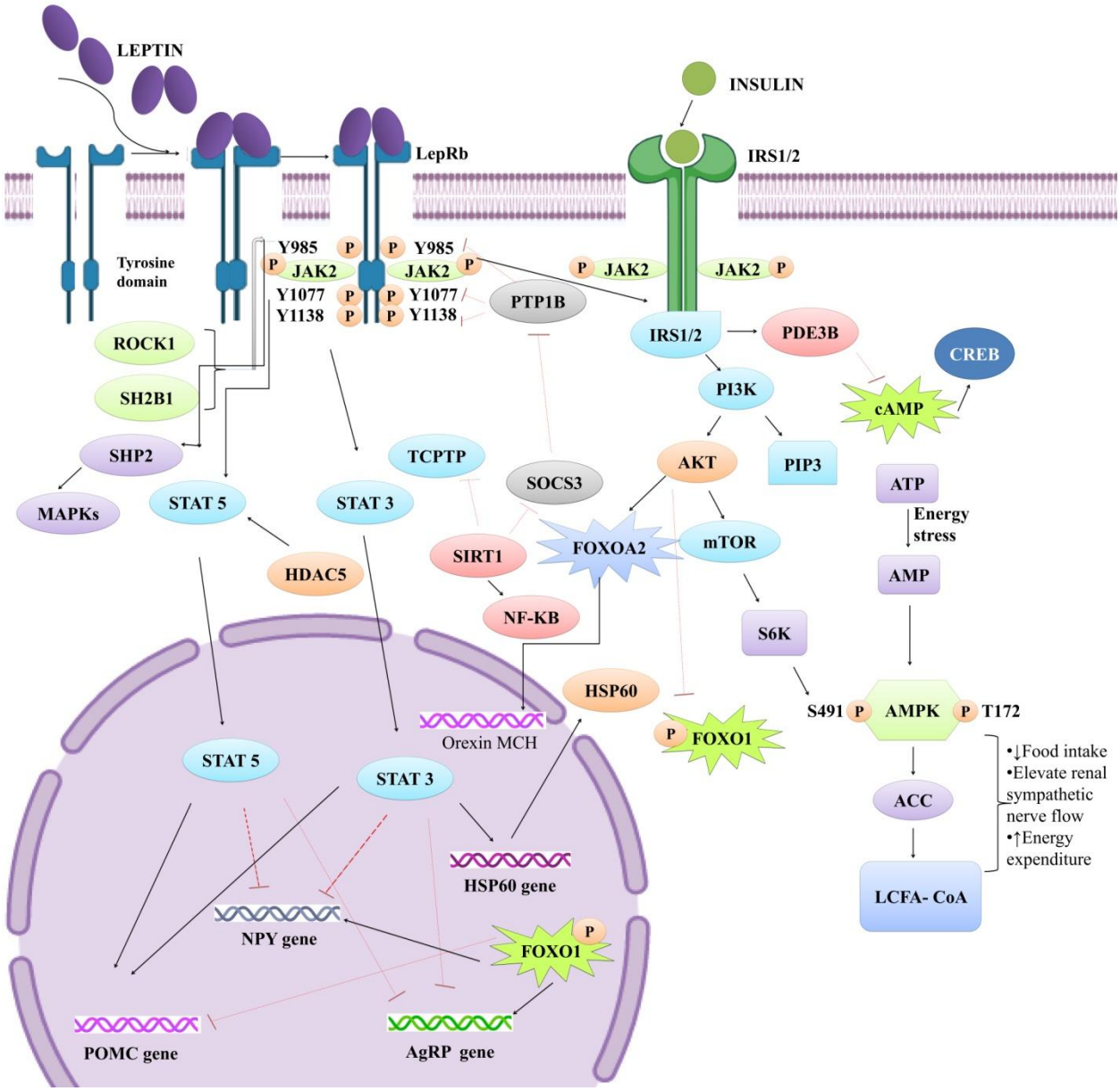
FoxO1, a transcription factor that controls the processes of adipogenesis, glycogenolysis, and gluconeogenesis by transcriptionally regulating POMC and AgRP, FoxO1, a phosphorylation

target of the PI3K-AKT axis, mediates the anorectic effects of leptin (O. Kwon et al., 2016). FoxO1 is a PI3K-AKT axis phosphorylation target that mediates leptin's anorectic effects by regulating POMC and AgRP transcriptionally (Kitamura et al., 2006). Through the PI3K-AKT signaling pathway, leptin inhibits the FoxO1-mediated transcriptional regulation of POMC, NPY, and AgRP, thereby lowering food intake (Varela & Horvath, 2012). By increasing the expression of carboxypeptidase E (Cpe) in POMC neurons, FoxO1 inhibition reduces food intake without changing energy expenditure (Plum et al., 2009). The IRS-PI3K signaling in the hypothalamic neurons activates PDE3B to lower cAMP levels and suppress CREB activity, which in turn suppresses NPY expression to cause leptin's anorexic (Zhao et al., 2002)effects (Zhao et al., 2000). Leptin suppresses AMPK (Andersson et al., 2004) activity in the ARC and PVN to lower appetite and subsequently lower body weight (Minokoshi et al., 2004). AMPK's upstream pathway in the hypothalamic leptin signaling cascades is mTOR-S6K1 signaling (Dagon et al., 2012). In the hypothalamus, AMPK-α2 subunit is phosphorylated at Ser491 by the activated S6K1, which results in decreased α2-AMPK activity. Leptin signaling is linked to the mitogen-activated protein kinases (MAPKs), specifically the extracellular regulated protein kinase 1/2 (ERK1/2). Tyr985 in the LepR intracellular domain is phosphorylated in response to leptin by JAK2, creating a docking site for the SH2-containing protein tyrosine phosphatase 2 (SHP2) (Myers, 2004). During the development of the hypothalamic feeding circuits, the SHP2-ERK1/2 pathway also demonstrates a neurotrophic role; disturbed ERK signaling hinders the development of these neuronal circuits (Bouret et al., 2004).

Serine/threonine kinase ROCK1, a crucial modulator of actin-myosin contraction and polarity in cells, is regulating the action of leptin by participating in the activation of JAK2 triggered by leptin (Huang et al., 2012). Impaired leptin sensitivity, increased food intake, decreased energy expenditure, and severe obesity are caused by the deletion of ROCK1 in either POMC or AgRP neurons (Huang et al., 2012).

In order to depolarize steroidogenic factor 1 (SF1) neurons in the VMH and preserve glucose homeostasis and energy balance, leptin activates TRPC channels (Sohn et al., 2016). In POMC neurons, AgRP neurons, and SF1-positive neurons, SIRT1 potentiates leptin signaling to decrease food intake, increase energy expenditure, and preserve glucose homeostasis (Ramadori et al., 2011). SIRT1 increases the hypothalamic leptin sensitivity by down regulating leptin signaling's negative regulators, including PTP1B, T cell protein-tyrosine phosphatase (TCPTP), and SOCS3 (Sasaki et al., 2014) SIRT1 enhances central leptin/insulin sensitivity by suppressing nuclear factor kappa-B (NF-κB) signaling (Yeung et al., 2004). Heat shock protein 60 (HSP60)

induced by leptin Insulin sensitivity and hypothalamic mitochondrial function are enhanced (Yeung et al., 2004) by leptin (Kleinridders et al., 2013). Severe obesity, elevated food intake, and decreased leptin sensitivity are caused by HDAC5 ablation in the mediobasal hypothalamus. Amplification of hypothalamic HDAC5 guards against obesity and leptin resistance brought on by high-fat diets (Kabra et al., 2016).



"Fig 1": Leptin Signaling Pathway. The PI3K–FoxO1, JAK–STAT3, and ERK pathways are activated after binding leptin binds to its receptor. In order to activate JAK–STAT, LepRb's Tyr985 and Tyr1138 are phosphorylated by activated JAK2 tyrosine kinase, which triggers the activation of STAT3/STAT5. Tyr985 that has been phosphorylated significantly attracts SHP-2

and GRB2, which activates ERK signaling. Additionally, leptin stimulates PI3K by attracting IRS proteins, which in turn causes FoxO1 to become inactive by being trapped in the cytoplasm. However, it has been observed that leptin inhibits AMPK activity. Upstream signaling, which includes LKB1 and CaMKKβ, activates AMPK. Furthermore, leptin treatment triggers the hypothalamus's mTOR/S6K signaling, which phosphorylates the AMPK α-subunit's Ser491 and reduces AMPK activity. AgRP protein related to agoutis, Forkhead box protein O1, IRS insulin receptor substrate, PTP1B protein-tyrosine phosphatase 1B, POMC proopiomelanocortin, JAK Janus kinase, and PI3K phosphatidylinositol 3-OH kinase, SOCS3 suppressor of cytokine signaling 3, and STAT signal transducer and activator of transcription.

Role of Leptin in Obesity cause PCOS

Regardless of the PCOS diagnosis, obese women are more likely to experience reproductive problems(Sam, 2007). The prevalence rate of obesity and abdominal obesity was found to be 13.85% and 50% respectively in India in which mostly women are mostly affected by these (Gupta et al., 2023). According to WHO, Globally around 1 billion individuals suffer from obesity including 650 million adults, 340 million teenagers, and 39 million children. Upto 88% PCOS women affected by obesity. In comparision to normal weight of women, obese women are more prone to experience irregular menstruation and anvolatory infertility. Body Mass Index around 24 kg/m2 is increased in reproductive aged women due to the relative risk of anovulatory infertility. The gonadal dysfunction is caused by obesity which linked with increased risk for type 2 diabetes mellitus, dyslipidaemia, hypertension, cardiovascular disorders, and even some types of cancer. By elevating free testosterone (Kiddy et al., 1990), increasing insulin resistance, decreasing sex hormone-binding globulin (Harlass et al., 1984), and among other mechanisms are impaired the ovarian function due to obesity. Both the ovaries and endometrium are affected due to obesity (Bellver et al., 2007). Obesity also disturbs the GnRH in anterior pituitary gland which produces more LH over FSH. This increased level LH cause more testosterone in women whereas less the estrogen due to decrease FSH binding its receptor (Barber et al., 2006). When comparing non-obese women with PCOS to BMI-matched control women, catecholamine-induced lipolysis within isolated visceral adipocytes increased twofold.In obese women, luteinizing hormone (LH), androstenedione, estrone, insulin, triglycerides, and very low density lipoprotein are increased as well as high density lipoprotein levels are decreased which further occur various gynecological effects by disturbing HPG axis (Parihar, 2003). Obstructive sleep

apnoea also caused due to the obesity (Barber et al., 2019). In vivo, from visceral adipocytes androgen forwards the release of non-esterified fatty acids (XU et al., 1990). Serum level of adipokines (cytokines which is secreted by adipose tissue) commonly associated by body fat mass and severity of obesity. Visfatin (one such adipokine that functions in metabolic, inflammatory, and insulin-sensitive pathways), was found to be higher in PCOS women comparison to normal women (Purwar & Nagpure, 2022). Thus, increased serum visfatin levels in PCOS may be linked to insulin resistance and metabolic dysfunction. In normal condition leptin is secreted to normalize the food intake and suppress the hunger. But leptin is over expressed at the gene level in obese people's adipose tissue. This phenomenon is called as Hyperleptinemia or Leptin resistance (Obradovic et al., 2021). Obesity is caused by IR and hyperinsulinemia, which is associated by changes in steroidogenesis and hyperandrogenemia (Dağ & Dilbaz, 2015).

Role of Leptin in PCOS associated T2DM & Insulin Resistance

T2D development, insulin resistance (IR) and ß-cell dysfunction are frequently present in women with PCOS. With an average of 16.6%, the prevalence of IGT in PCOS ranges from 4% to 35.4%; in comparison, the corresponding prevalence in the healthy peers of PCOS-affected women is between 4% and 8% (Huebschmann et al., 2019). After going through menopause, premenopausal women with PCOS cases continue to exhibit elevated ovarian androgen production and IGT (Livadas et al., 2022).These patients are at risk of developing type 2 diabetes because they typically have higher levels of insulin and fasting blood glucose (FBG), as well as insulin resistance. Patients with T2D had higher testosterone concentrations than controls, according to a meta-analysis of cross-sectional studies involving 4795 women from the general population (Ding et al., 2006). Although the pathophysiology of PCOS and abnormalities in glucose homeostasis are similar, there are notable differences between lean and obese PCOS women (Livadas et al., 2022). However, some experts advise screening for women who have at least one risk factor, such as obesity, age >40, or a family history of T2D or gestational diabetes mellitus (Wild et al., 2010).Adiposity or increased androgen levels cause T2DM to develop. Reduced SHBG levels are another aspect of PCOS that may affect the risk of diabetes. Another aspect of PCOS that may contribute to diabetes is hyperandrogenism (Jakubowski, 2005).

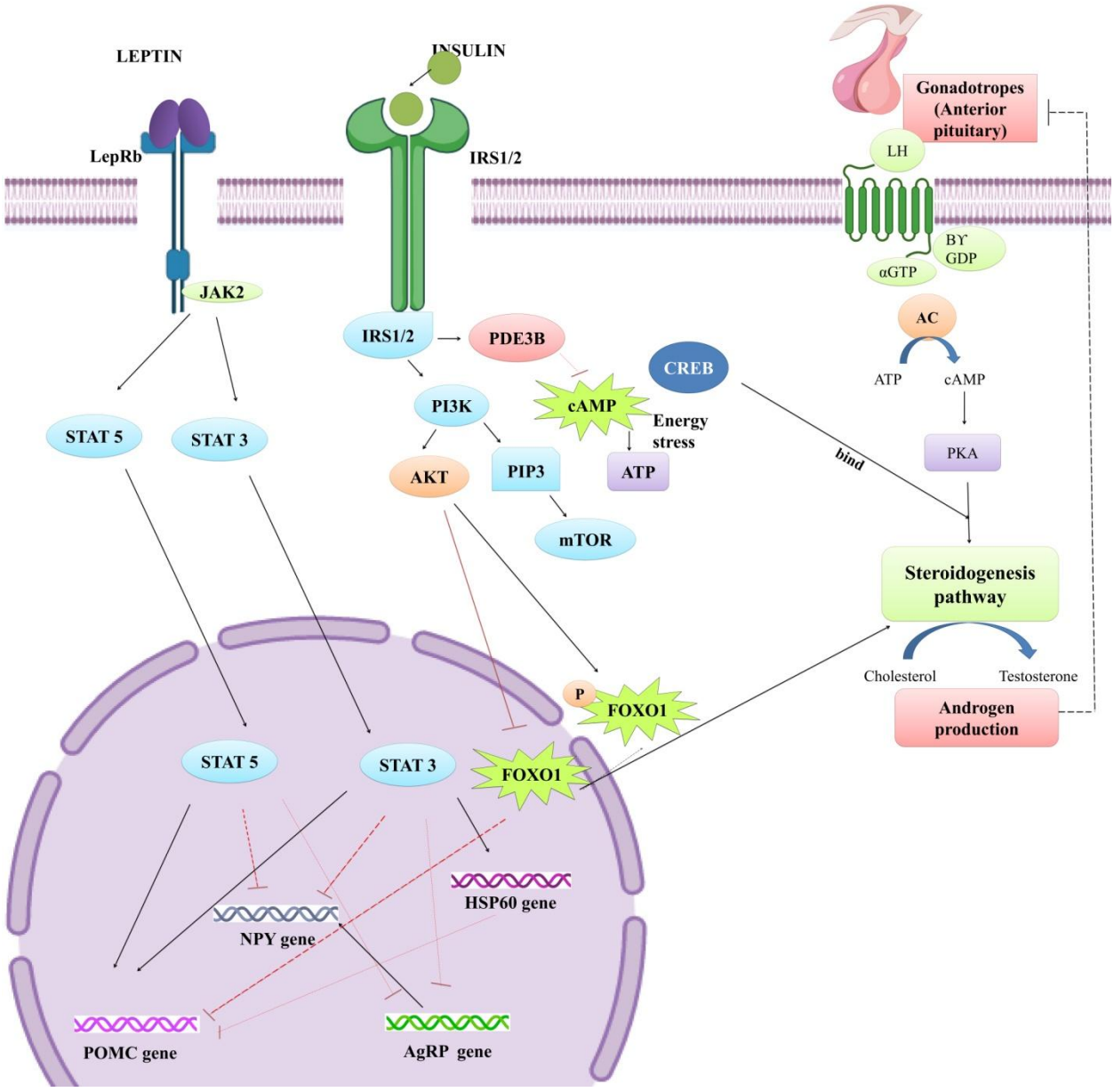
Steroidogenesis is affected by Insulin. As we know that Insulin, a peptide hormone secreted by the pancreatic beta cells in response to hyperglycemia (Fahed et al., 2022). It is sensed by

pancreatic b-cells which release insulin then binds to insulin receptor (IRs is a heterotetrameric glycoprotein composed of two ab dimmers) (De Paoli et al., 2021). In vitro the production of ovarian estrogen, androgen, and progesterone are enhanced by Insulin (Poretsky et al., 1985). One of the main pathophysiologic mechanisms underlying the emergence of PCOS clinical symptoms and other metabolic complications is insulin resistance(Rojas et al., 2014). Metabolic and cardiovascular issues arise from hyperinsulinemia. Hyperinsulinemia causes hyperandrogenemia via insulin-like growth factor-1 (IGF-1) which is secreted by human ovarian tissue along with its receptors located in the ovary. Hyperinsulinemia is feedback disruptions in the hypothalamus-hypophysis-ovary axis (HHOA) (Blank et al., 2006), which further increase the frequency of GnRH (Gonadotropin releasing hormone). Additionally, it heightens the ovarian cells' luteinizing hormone (LH)-dependent effect, leading to an increase in androgen synthesis, irregular menses, and impotence (Blank et al., 2009). The follicular development deficiency is caused due to decrease level of FSH while ovarian androgen production is stimulated by LH, contributes to hyperandrogenism. By the action of IGF-1, Testosterone production increases from theca interstitial and stromal cells(Giudice, 1992). Serum insulin level is responsible to decrease the SHBG level in obese women resulting for increasing testosterone level (Diamanti-Kandarakis & Dunaif, 1996).Both centrally and peripherally, this insulin sensitivity is increased by Leptin by decreasing adiposity and lipotoxicity. For long term insulin sensitivity is increased with the help of leptin therapy along with this reverse the type 2 diabetes and insulinemia in PCOS women(Paz- Filho et al., 2012). Mutations in LEPR that cause obesity have also been connected to the development of insulin resistance and type 2 diabetes (T2DM), which progresses slowly and frequently goes years without symptoms (Poetsch et al., 2020) .

Molecular mechanism of Leptin and insulin affect PCOS

In leptin signaling pathway, leptin binds to its activate STAT3 and STAT5 through JAK-STAT pathway (Varela & Horvath, 2012). Following phosphorylation, STAT3 and STAT5 attaches itself to the promoters of POMC and AgRP, promoting POMC expression and suppressing AgRP (Ernst et al., 2009). The signaling pathways for insulin and leptin converge at PI3K. PIP3 is synthesized from PIP2 when PI3K (Hill et al., 2008) is activated (Hill et al., 2008) by leptin (Belgardt et al., 2008). One of PDK1's downstream targets(Kim et al., 2006), AKT(Ren et al., 2012), is crucial for the control and activation of numerous proteins and transcription factors, including mTOR (Cota et al., 2006), AMPK(Minokoshi et al., 2004), and FoxO1. FoxO1 inhibits the expression of POMC (Kitamura et al., 2006). FoxO1 is exported from the nucleus after being

phosphorylated by leptin and insulin signaling. This enables STAT3 to bind to the POMC promoter and activate the expression of FoxO1. Because STAT3 can bind to the AgRP promoter and inhibit its expression, the nuclear export of FoxO1 in AgRP neurons eliminates the expression of AgRP (Morrison et al., 2005). The transcription factor FOXO1 is phosphorylated by AKt, which also causes FOXO1 to separate from DNA, exit the nucleus, and move into the cytoplasm. In both mice and humans, FOXO1 is preferentially and highly expressed in the granulosa cells of developing follicles by activating steroidogenesis pathway in ovary(Richards & Pangas, 2010) as shown in fig.2



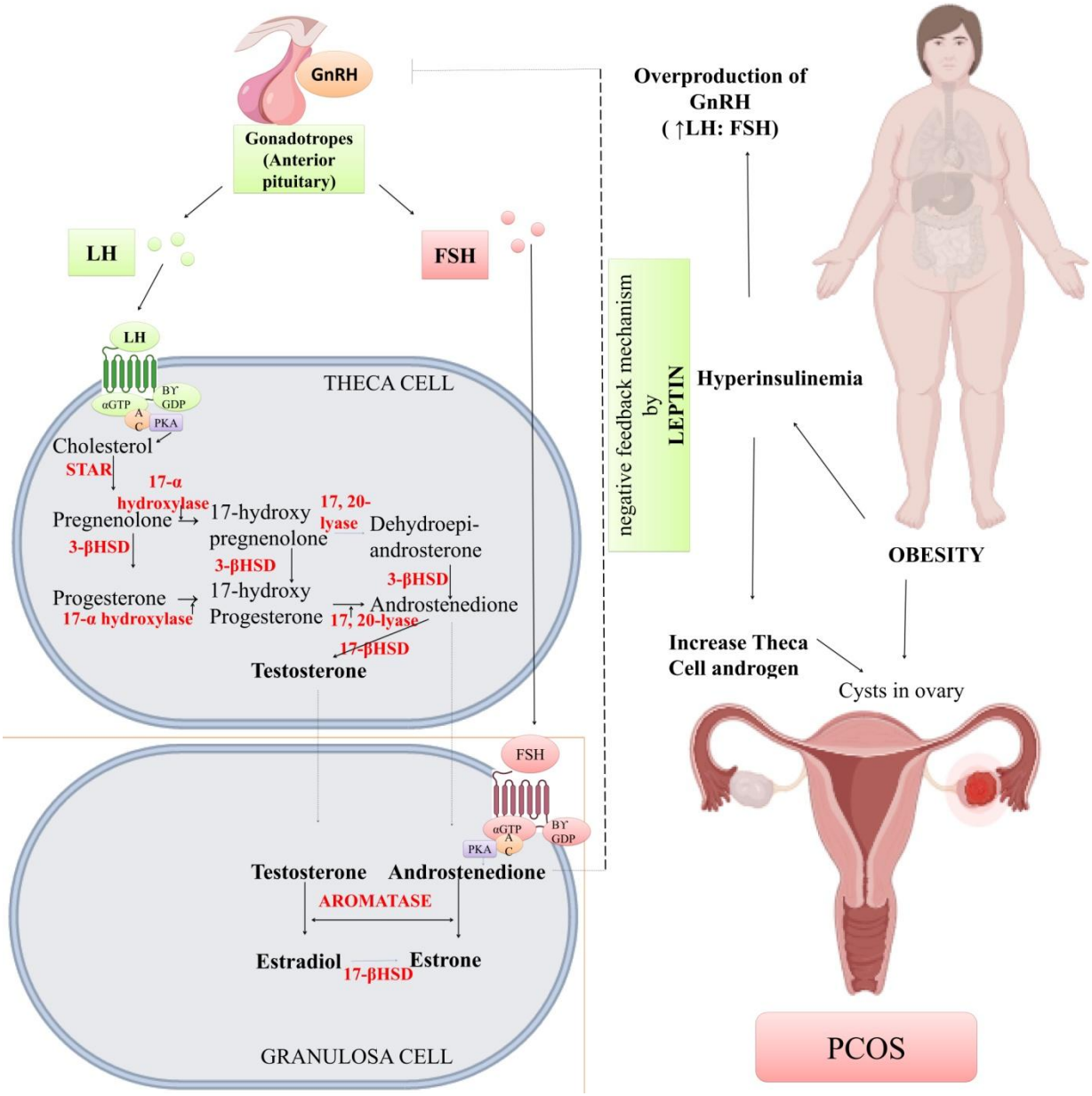
"Fig 2". Leptin Signaling disturb Steroidogenesis. Leptin activate the AKT pathway by initiation of IRS 1/2 receptor. This AKT phosphorylate FOXO1 which further disturb the

steroidogenesis pathway. In Steroidogenesis pathway Cholestrol is converted into testosterone in the presence various enzymes by binding with LH receptor. This FOXO increase LH production by trying to disbalance negative feedback inhibition.

Hormonal Changes control by Leptin

In steroidogenesis pathway, the conversion of cholesterol to testosterone is done. This androgen formation is controlled by negative inhibition in GnRH (Roozbeh et al., 2022). Gonadotropin-releasing hormone, or GnRH, occurs in the neurons of the hypothalamus and causes the downstream production of sex hormones by the gonads. It is released every 60 to 120 minutes in a rhythmic manner. . In normal condition, the medial olfactory placode is where GnRH starts. It then proceeds to the hypothalamus via the olfactory bulb. Subsequently, pulsatile GnRH is secreted into the hypophyseal portal circulation, where it travels to the anterior pituitary, its principal target. Here, it binds to the pituitary gonadotrophic cells' G-protein coupled gonadotropin-releasing hormone receptor (GnRHR). Follicle-stimulating hormone (FSH) and luteinizing hormone (LH), the two main gonadotropins, begin to signal downstream when GnRH binds to the GnRHR. These hormones LH and FSH causes 6-12 follicles to be awake & start to mature as they are going to mature. LH promotes androgen production in ovarian theca cells whereas FSH transfers androgen to estrogen in ovarian granulose cells to promote the follicles growth.The steroidogenesis pathway in which cholesterol is converted to biologically active steroid hormones by using various enzymes such as StAR, P450c17: 17α-Hydroxylase/17,20-Lyase, 3β-Hydroxysteroid dehydrogenase. When LH binds to its receptor in ovarian theca cells, the conversion of cholesterol to testosterone occurs. FSH bind to its receptor in ovarian granulose cells, testosterone is converted into estradiol as well as estrogen by using aromatase enzyme. This estrogen homone causes lining of the uterus to begin to build up. Then one of those 6-12 follicles tends to grow a little faster and becomes dominant. Then these dominant follicles moves to edge of ovary to produce an egg while other follicles are disintegrate in the next few days. After egg is released, the follicles then seals over and is called corpus luteum. This corpus luteum starts producing progesterone. This progesterone is negative feedback effect on GnRH to reduce it and maintain its frequency in a normal range. This egg then travels to the uterus, If fertization occurs, it stays in the uterus otherwise it will disintegrate after about 24 hours and women will have regular menstrual periods (Casteel & Singh, 2024). Leptin controls follicular and luteal steroidogenesis at the ovary and modifies the pituitary glands sensitivity to GnRH, which in turn controls reproductive function. By making gonadotrope cells in the pituitary gland

more sensitive to GnRH, leptin may help regulate reproduction and further increase the secretion of luteinizing hormone(G. J. Hausman et al., 2012)as shown in fig.3.



"Fig 3". Leptin causes hormonal changes. In hypothamus GnRH release two hormones LH and FSH. In ovary two cells theca and granulosa cell present.Both LH and FSH are G- Protein Couple Receptor. LH binds GPCR present in theca cell phosphorylate PKA via activation of adenylylcyclase enzyme present on theca cell membrane. Therefore, Cholestrol is converted testosterone and androstenedione by various intermediates. Testosterone and androstenedione are gone to granulosa cell. In granulosa cell FSH receptor present, also contain GPCR, bind with it

and activate adenylyl pathway to phosphorylate PKA. Further increase the androstenedione. This androstenedione and testosterone is converted into estrone and estradiol respectively. This is normal functioning of Steroidogenesis pathway and balance by negative inhibition of GnRh level. But obesity causes hyperinsulenemia as well as high level of leptin. These both are inhibit negative feed back mechanism and GnRH goes to imbalance and produce more LH in comaprision to FSH. Testosterone level increased and formed various cysts in ovary causes PCOS.

On the other hand, primary abnormalities in PCOS are the abnormal or more release of GnRH. Instead of being released in a regular cyclic manner. There are various reasons by which it is released at higher pulse frequency due to increase LH level over the FSH. We can also say that decrease the FSH level causes follicles not maturing enough to become functional and cannot ovulate. If the follicles cannot ovulate, corpus luteum is not created and without corpus luteum there is no surge in progesterone. The lack of progesterone leads to a higher pulse frequency. The follicle that does not ovulate forms multiple cysts in ovary. Along with this excessive secretion of LH level stimulate the thecal cells to produce large amount of male sex hormone androgen/ testosterone this further leads to various PCOS symptoms like hirusitism, acne, menstrual irregularity due to above hormonal disbalance and also infertility because of there is no ovulation. In this stage leptin play an important role in various reason (Miller & Auchus, 2011)such as insulin resistance, obesity as well as Oligoammerrhoea, Ammenhrhoea, Infertility, Reactive oxygen species(ROS), Inflammation etc are discussed below.

Role of leptin in PCOS associated Oligoammerrhoea / Ammenhrhoea

The term "Oligomenorrhea" refers to a woman's irregular and inconsistent menstrual blood flow (Mahjour et al., 2017)while the lack of menstruation in a woman during her reproductive years is known as amenorrhea (Klein et al., 2019). Oligomenorrhea and aberrant hormone levels, such as hyperandrogenism, hyperinsulinemia, and gonadotropin imbalance are typified a complex endocrine disorder known aspolycystic ovary syndrome (PCOS) (Harris et al., 2018). Hormonal changes in women with Oligomenorrhea, including elevated testosterone levels, a common feature of women with PCOS, increased risk of ovarian cancer (Ose et al., 2017)(Risch, 1998). Total and free testosterone levels are frequently higher in women who experience irregular

menstruation cycles (Farland et al., 2017). The primary cause of Oligomenorrhea is dysfunctions of the hypothalamus-pituitary-ovary axis, which can be influenced by a variety of factors (Check & Mitchell-Williams, 2009). Several gynecological conditions, including acne vulgaris, hirsutism, and infertility, can be brought on by Oligomenorrhea (Rostami Dovom et al., 2016). Oligomenorrhea and hyperandrogenism increased the harmful metabolic risk for metabolic syndrome (Polotsky et al., 2011). Both free and total testosterone in higher levels is frequently seen in women with irregular menstrual cycles which may have an impact on ovarian tissue. Higher levels of free testosterone and estrogen causes shorter menstrual cycles at ages 18–22 and in adulthood were linked to higher levels of early-follicular estrogens and decrease SHBG (Farland et al., 2017). Through both direct and indirect effects on the hypothalamic-pituitary-gonadal axis, leptin regulates the menstrual cycle. Ovulation, LH pulsatility, and LH levels in women whose fat mass has pathologically decreased (such as those with congenital or acquired lipodystrophy) is restored (Musso et al., 2005) by leptin (Lungu et al., 2012). Elevated pulse frequencies, mean levels of LH, ovarian volume, numbers of dominant follicles, and estradiol levels caused by leptin received (Garcia-Galiano et al., 2014) women (Welt et al., 2004). Effects of leptin on the hypothalamic-pituitary-gonadal axis ediate via intermediate neurons because GnRH neurons lack leptin receptors (Chehab, 2014). To regulate the release of GnRH, neuropeptides, such as proopiomelanocortin (POMC), neuropeptide Y (NPY), and kisspeptin are released GnRH neurons in the hypothalamus which linked with Intermediary neurons(Tena-Sempere, 2013). The anterior pituitary could directly produce LH when exposed (Kirsz et al., 2014) to leptin (Dagklis et al., 2015). In females with hypothalamic amenorrhea, recombinant leptin treatment resulted in the restoration of menstruation (Welt, 2007).

Role of Leptin in PCOS associated Infertility

The most prevalent endocrine condition affecting women, polycystic ovary syndrome, is a primary contributor to anovulatory infertility. Almost 70-80% PCOS women affected by infertility (Cunha & Póvoa, 2021). In addition to hypothalamic-pituitary dysfunction, this cyclical pathogenetic interaction between IR, hyperinsulinemia, and hyperandrogenism causes additional ovarian dysfunction, which can lead to anovulation and infertility (Escobar-Morreale, 2018). Additionally, women with PCOS may be more likely to spontaneous abortion and experience pregnancy-related issues like gestational diabetes (Rees et al., 2016). According to a

population-based study the risk of GDM and its symptoms are 2-4 folds increased in PCOS pregnant women comparision to without PCOS pregnant women (Lo et al., 2006).

Infertility causes abnormalities in folliculogenesis and steroidogenesis in PCOS women. Attenuated apoptosis, anomalies in local regulators, hyperinsulinemia, aberrant granulosa and theca cell function, and disruptions of gonadotropin secretion are among the hypothesized causes. PCOS anovulation and miscarriages both are caused by an interaction between LH, insulin, and androgen. It's possible that other variables, such as aberrant plasminogen activator inhibitor function and growth factors, result from aberrant steroidogenesis rather than being the primary cause (van der Spuy & Dyer, 2004). Infertility is caused by higher level leptin (Kamyabi & Gholamalizade, 2015). By increasing energy consumption, decreasing appetite, leptin not only controls body weight but also has a significant impact on immune, reproductive, and endocrine system regulation. Lack of leptin or its receptors not only results in obesity but also disrupts the reproductive cycle, causes hormone imbalances, and affects the immune system, hematopoietic system, and bone metabolism (Dardeno et al., 2010). Menstrual cycle is also affected by leptin directly or indirectly. Ovaries and hypothalamic-pituitary axis are also directly affected by leptin. Moreover, leptin has an indirect impact on the concentration of luteinizing hormone (LH) due to its influence on the follicle stimulating hormone (FSH)-dependent production of estradiol in animals and its function in preventing starvation-induced delay in ovulation in mice(Gogacz et al., 2001).By sending out a signal to start the hypothalamus' reproductive maturation, leptin stimulates the HPG axis.In the granulosa cells, LH-stimulated estradiol production is inhibited by leptin. The regulation of early embryo cleavage and development on reproductive functions are also affected by leptin (Moschos et al., 2002). After menopause leptin level is significantly decreased, women still tend to have higher levels of the hormone than men (Bunnell, 2021). When there is unexplained infertility, a high level of follicular fluid leptin negatively impacts reproduction. FSH-stimulated estrogen synthesis is increase through TGF-β blocking via Leptin. Leptin is useful to promoting follicular growth and maturation by supporting mechanism (e.g., augmentation of E_2 production) (Zachow et al., 1999).

Role of leptin in PCOS associated Hirusitism

A woman's abnormal amount of terminal hair distributed in a male pattern is called hirsutism, and it is the primary symptom of hyperandrogenism in women with polycystic ovary syndrome (PCOS) (Spritzer et al., 2022). As in the case of male pattern balding, terminal hairs may

"miniaturize" and become vellus hairs. During puberty by developing into terminal hair, certain vellus hair follicles respond to increased testosterone levels which are darker, longer, and more visible, turning them into sexual hair follicles (McCartney & Marshall, 2016). Higher concentration of testosterone with stand by axillary and pubic hair (Randall, 2008) and in order to achieve follicle terminalization needs greater amount of testosterone (Rittmaster & Loriaux, 1987). Free plasma testosterone is responsible for this action (Rosenfield, 1979). The first study on hair growth in Asian Indian women was published by Shah in 1957. The study evaluated 34 women, who were referred for excessive hair growth, between the ages of 15 and 41 (referred to as the "hirsute" group), and compared them to 100 women, who were between the ages of 15 and 48 but did not report having excess hair (referred to as the "non-hirsute" group) (Yildiz et al., 2010). Anagen, catagen, and telogen are three stages of the hair follicle cycle which represent the everlasting cycles of growth, involution, and rest, respectively. The most active stage of hair follicle growth is known as the anagen stage, during which time hair grows quickly and fully forms a hair shaft. Testosterone primarily promotes the transition from vellus to terminal hairs by extending the anagen stage. Longer anagen duration and successive hair cycles facilitate increase the follicular size. The follicles yield longer, thicker hair regulated by increasing testosterone level (Ceruti et al., 2018). Because of this, hirsutism is frequently linked to conditions related to androgen excess, like PCOS (Spritzer et al., 2016). Leptin level was found to higher in obese PCOS women which increased GnRH pulsatility and hyper secretion of LH.

Leptin causes Obstructive sleep apnea

The respiratory distress index, the number of apneas, hypopneas, and respiratory effort-related arousals (RERA) per hour of sleep, and the respiratory effort were used to define OSA. OSA includes sleep fragmentation, poor sleep quality, and recurrent complete (apnea) or partial (hypopnea) upper airway obstruction that results in intermittent hypoxia (repeated cycles of oxygen drops and reoxygenation) (Helvaci et al., 2017). The development of OSA is evoked by PCOS, especially in age group of prepubertal girls (Kahal et al., 2020). The high level of free androgen level or hyperandrogenism is correlated with PCOS women causes severity of OSA (Fogel et al., 2001). Elevated testosterone levels may increase the risk of developing OSA by affecting the ventilatory control mechanism and pharyngeal soft tissue deposition. This affects the pharynx's patency and makes it more collapsible when we sleep. In comparision to obese and normal weight women, obese girls with PCOS exhibited significantly lower sleep efficiency and

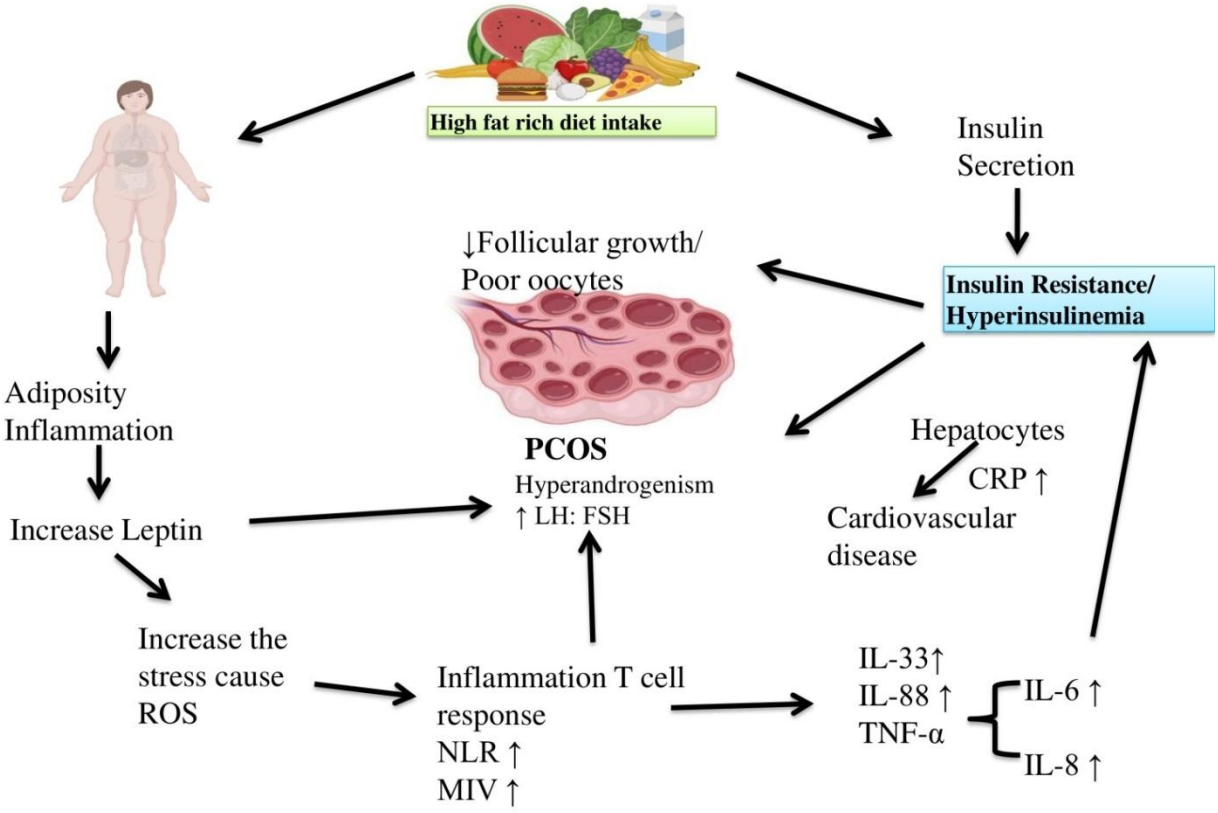
higher sleep onset latency. The risk for OSA is 9.74 times increase in PCOS women (Eckert & Malhotra, 2008). In the pathophysiology of OSA, leptin play an important role in which leptin influences OSA expression (Ikuyo Imayama & Bharati Prasad, 2017).Independent of obesity, leptin has been linked to a decrease in ventilatory drive and a hypercapnic response, suggesting that it may play a significant role in the development of human obesity hypoventilation syndrome (Phipps et al., 2002). Through the brain and peripheral nervous system via the carotid body chemoreflex, leptin controls ventilatory function (Bassi et al., 2015). In the carotid body, persistent intermittent hypoxia raises leptin signaling (Messenger et al., 2013).Alteration in ventilatory function and upper airway resistance as well as sleep architecture is disrupt by leptin(I. Imayama & B. Prasad, 2017).

Role of leptin in PCOS associated Inflammation

Inflammatory mediators such as C-reactive protein (CRP), interleukin 18 (IL-18), tumor necrosis factor (TNF-α), interleukin 6 (IL-6), white blood cell count (WBC), monocyte chemo attractant protein-1 (MCP-1) and macrophage inflammatory protein-1α (MIP-1α) are found to be higher in PCOS women (Rudnicka et al., 2021).Obesity and hyperinsulinemia is also responsible for increasing these inflammatory responses. In order to stimulate follicle growth, various inflammatory factors are secreted by infiltrating and inherent white cells in the PCOS ovary (Rudnicka et al., 2021). Tumor necrosis factor-α (TNFα) is a proinflammatory cytokine that has higher levels in circulation in obese people and in PCOS patients who are independent to obese. Actually, the first indication that PCOS is a proinflammatory state was the finding of elevated TNFα in PCOS patients. TNFα is a known mediator of insulin resistance in diabetic syndromes associated with obesity, as it increases the serine phosphorylation of insulin receptor substrate-1 (IRS-1) in insulin-sensitive tissues (Rui et al., 2001). As a result, the insulin-sensitive glucose transport protein GLUT 4 expresses less (Stephens & Pekala, 1992). Thus TNFα is a prime candidate to start these molecular processes in PCOS because it can cause an increase in serine phosphorylation which is involve in insulin signaling pathway also described above (González, 2012).

Adipose tissue in this case is the source of the endocrine cytokine IL-6, which stimulates the liver to produce CRP (C-reactive protein), an acute phase reactant. By encouraging lipid uptake into foamy macrophages within atherosclerotic plaques, CRP also performs a functional role (Aboeldalyl et al., 2021). Consuming glucose causes an inflammatory response in PCOS, as shown by elevated oxidative stress related to ROS and elevated NFκB activation that occurs

independently of obesity. Consuming glucose also affects the release of TNFα and IL-6 from circulating MNC (mononuclear cells) in PCOS. Proinflammatory signaling, which is implicated in the development of insulin resistance and atherogenesis, is the result of diet-induced inflammation in PCOS. Proinflammatory stimuli upregulate CYP17, the ovarian steroidogenic enzyme that produces androgens. The poor quality oocytes are formed by the presence of higher level of TNFα and IL-6 (Lee et al., 2000).The presence of androgens in the blood is strongly correlated with molecular indicators of inflammation and oxidative stress. TNFα is a proinflammatory cytokine that can promote the growth of androgen-producing theca cells in vitro and result in a disorder of the hypothalamic-pituitary-ovarian axis secretion (Tarkun et al., 2006). In women with PCOS, it's possible that MNC recruited into the polycystic ovary will trigger a local inflammatory response that will increase the production of ovarian androgen (González, 2012). Along with this leptin is stimulating the oxidative burst and chemotactic responses that goes to further mediate the inflammatory infiltrate as well as proliferation of circulating monocyte, phagocytic function and production of proinflammatory cytokines (TNF-α, IL-6, and IL-12)are also induced (Conde et al., 2010; Napoleone et al., 2007). By stimulating the synthesis of IL-2 and IFN-g CD41 T lymphocyte activation toward Th1 phenotype is modulate by leptin. Leptin could be one of the inflammatory mediators involved in other inflammatory disorders as well as autoimmune diseases (Pérez-Pérez et al., 2020). So we found that the potential involvement of leptin in immune-mediated disorders linked to obesity presents a fascinating avenue for further research (Martín-Romero et al., 2000). Fig.4.



"Fig 4". Role of leptin in PCOS causes Inflammation. Women intake high carbohydrate diet such as junk foods etc causes adiposity and more insulin secretion. This adiposity inflammation further increase the leptin level causes stress due to ROS. This initiates the inflammatory T cell responses such as IL-33, IL-88 as well as TNF-α and increase neutrophils-to-lymphocyte ratio (NLR), and mean platelet volume (MPV). Further causes hyperandrogenism imbalance LH/ FSH ratio, poor oocytes quality and decrease follicular growth associated by Insulin resistance and hyperinsulinemia in PCOS women.

Leptin causes Oxidative stress in PCOS

High production of reactive oxygen species (ROS) forms the oxidative stress which increased in PCOS women (Siddiqui et al., 2022). PCOS women have higher levels of oxidative stress markers in their serum and follicular fluid, which could be one of the causes of their low-quality (Y. Liu et al., 2021) oocytes (Dumesic et al., 2015). ROS cause Lipid peroxidation and oxidative

damage to DNA. Cancer is a result of DNA oxidative damage as well. According to a study, ovarian cancer in PCOS patients is associated with oxidative stress-induced DNA damage (Dinger et al., 2005). Impaired insulin resistance and glucose tolerance are two PCOS side effects that can lead to diabetes. In order to determine hyperandrogenism, insulin resistance and hyperinsulinemia are essential because they cause theca cells in the ovaries to secrete an excess of LH and androgen (Polak et al., 2017). The body mass index (BMI) is higher in up to 90% of PCOS women, which exacerbates insulin resistance and moves the condition closer to diabetes(Manco et al., 2014). Therefore, it suggests that obesity is a fundamental factor in impaired insulin metabolism, which hastens the development of diabetes in PCOS. Central adiposity, or excess body fat in the abdomen, hips, and thighs, is typically observed in PCOS patients (Kirchengast & Huber, 2001). Increased androgen secretion, hyperadiponectinemia, cytokine secretion, oxidative stress, and hyperinsulinemia are all facilitated by abdominal adiposity (Murri et al., 2013). Hyperglycemia can produce ROS, as multiple studies on peripheral blood leukocytes have shown (González et al., 2006). Increased oxidative stress (Sabuncu et al., 2001) results in the release of pro-inflammatory cytokines, which raise the risk of cardiovascular diseases and cause IR and hyperandrogenism (Victor et al., 2009). In addition to damaging DNA, lipids, and proteins, these ROS also cause tissue damage. DNA oxidative damage is also connected to carcinogenesis. DNA damage can be caused by reactive oxygen species such as hydrogen peroxide, hydroxyl radicals, and superoxide radicals (Ziech et al., 2011). ROS induces point mutations, DNA strand breaks, DNA–protein cross-linking, and DNA cross-linking, which results in DNA damage and genetic alterations. Therefore, in women with PCOS, damage to DNA raises the risk of ovarian and endometrial cancer. Moreover, OS induces epigenetic modifications and DNA methylation that silence tumor suppressor(Franco et al., 2008) genes (Donkena et al., 2010). Thus, elevated OS is primarily to blame for PCOS women's increased risk of gynecological cancers. In contrast, OS causes pathological conditions linked to PCOS, including obesity, IR, and hyperandrogenemia. Patients with obesity are known to have elevated levels of oxidative stress; consequently, it is expected that they will exhibit a marked increase in oxidative stress markers, which are correlated with obesity markers like waist circumference and BMI (Zuo et al., 2016). Markers such as MDA, thiobarbituric reactive substances (TBARS), oxidized low-density lipoprotein (ox. LDL), and advanced oxidation protein products (AOPP) are used to determine the extent of lipid and protein peroxidation. In obese PCOS patients, the level of these markers rises suddenly, whereas antioxidant markers like glutathione peroxidase (GSH-Px) and superoxide dismutase (Couillard et al., 2005)(SOD)

decrease (Ozata et al., 2002). In the end, this suggests that obesity is primarily responsible for the elevation of oxidative stress levels in PCOS. Because ROS are produced by hyperglycemia and elevated levels of free fatty acids, IR causes OS. In PCOS, there is a strong correlation between rising androgen levels and inflammatory markers and OS (Yilmaz et al., 2005). Leptin can cause phagocytic cells to produce (Singh et al., 2010) ROS (Caldefie-Chezet et al., 2001) as well as nonphagocytic (Gajewski et al., 2016) cells (Yamagishi et al., 2003). ROS is produced because high leptin (Yang & Barouch, 2007)activates NADPH (Morawietz & Bornstein, 2006) oxidase(Dong et al., 2006). FIG. 5

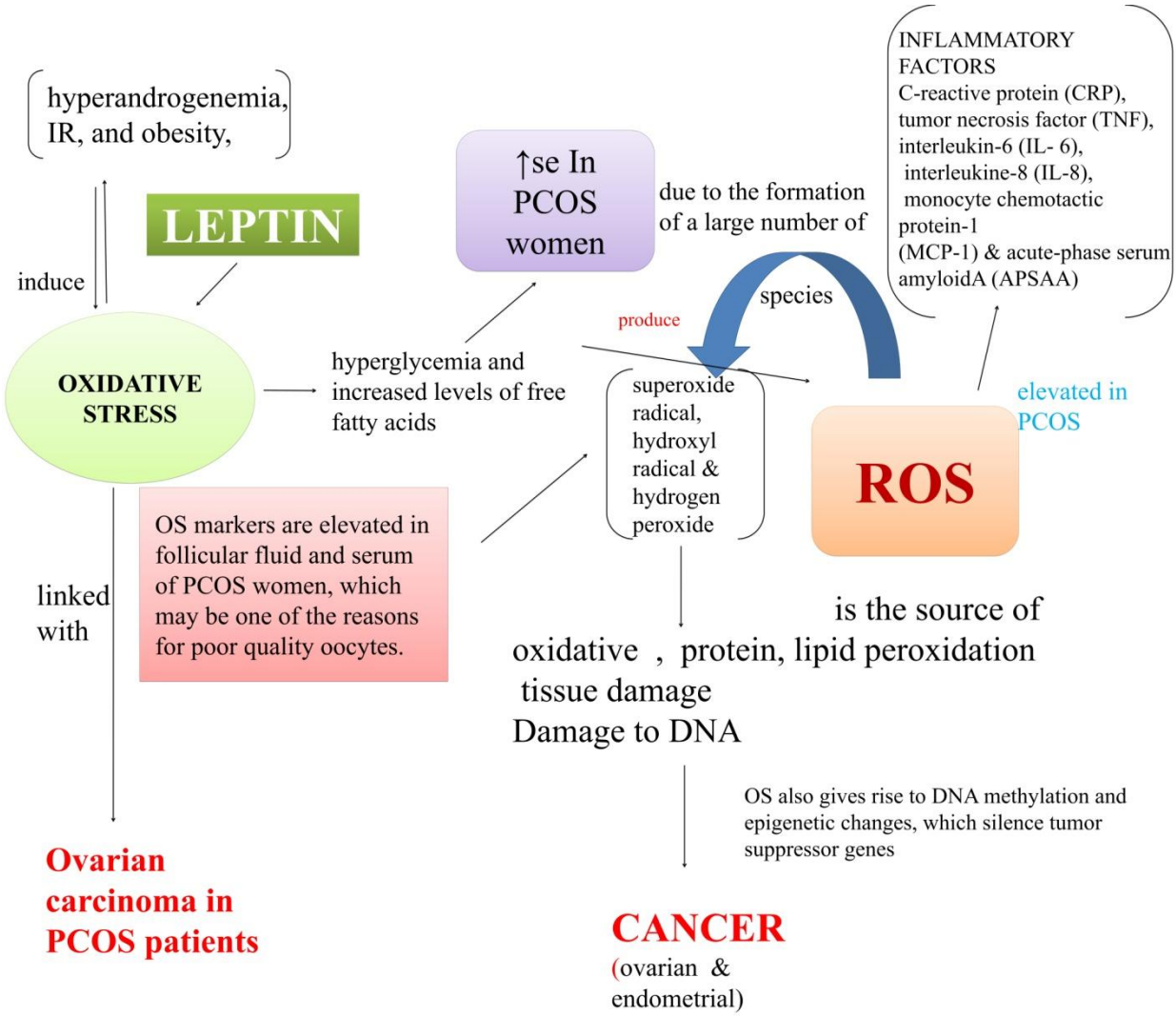


Fig.5: Role of leptin in PCOS associated Oxidative Stress. Leptin activate oxidative stress in PCOS women. This oxidative stress (OS) is associated via IR, obesity and hyperandrogenemia, increase in follicular fluid and serum which further cause's poor oocytes quality. OS also activates superoxide radical, hydroxyl radical and hydrogen peroxide produced by ROS *(*Reactive Oxygen Species). This ROS increase the inflammatory factors *such as C-reactive* protein (CRP), tumor necrosis factor (TNF), interleukin-6 (IL- 6), interleukine-8 (IL-8), monocyte chemotactic protein-1(MCP-1) & acute-phase serum amyloidA (APSAA). This ROS is the source of oxidative, protein lipid peroxidation causes tissue and DNA damage further causes Ovarian and endothelial cancer.

Leptin causes Cardiovascular Disease

In the world, cardiovascular disease (CVD) is the primary cause of morbidity and death for women (Laslett et al., 2012). Women who have caused by polycystic ovary syndrome (PCOS) tend to have a higher prevalence of risk factors for cardiovascular disease (CVD) (Daan et al., 2014). Even after accounting for age, smoking, BMI, center, and ethnic origin, CV risk factors are higher in hyper androgenic women in comparision to nonhyperandrogenic women. These variations may also be the result of variations in androgen levels across the general population among various ethnic backgrounds (Kim et al., 2012). The rise in T2DM is accompanied by an increase in the prevalence of diabetic cardiomyopathy. Cardiovascular remodeling processes leading to diabetic cardiomyopathy are a major cause of disease-related deaths in patients with type 2 diabetes (T2DM), with an incidence of 19–26% for heart failure (Jia et al., 2018). PCOS is associated with higher levels of subclinical atherosclerosis (e.g., increased coronary artery calcium, dyslipidaemia, and hypertension) and CVD risk factors (e.g., increased carotid intima-media thickness, hypertension).Women with PCOS experience hypertension due to hyperaldosteronism, which triggers the renin-angiotensin system (Cascella et al., 2006).

Obese people are more likely to develop cardiovascular diseases because of low-grade systemic inflammation, which is thought to be exacerbated by elevated leptin levels (Poetsch et al., 2020). Higher levels of leptin, regardless of BMI and possible mediators, were linked to a higher risk of heart failure in men without a history of coronary heart disease (Wannamethee et al., 2011). The relationship between leptin and congestive heart failure was eliminated when BMI was taken into account, but the relationship to a higher risk of developing cardiovascular disease (Osibogun et al., 2020)was only slightly reduced (Lieb et al., 2009).Leptin also has a major impact on

reproductive processes. Hyperleptinemia has been connected to the occurrence and severity of heart failure (HF) and coronary heart disease (CHD), and leptin is implicated in CVD events. Leptin may make myocardiocytes more susceptible to apoptosis (Vilariño-García et al., 2024). The heart may produce leptin, which has both autocrine and paracrine effects (An & Rodrigues, 2006). However, evidence from studies on rodents study suggests that animals that have lacking leptin or LepR may show heart-protective effects. The activation of endothelial precursor cells, endothelial nitric oxide synthase (eNOS), and coronary artery vasodilation(Kang et al., 2020) are all responsible for this protection. These investigations also found that in comparision to men, women have higher levels of leptin (Zhao et al., 2021).A higher cardiac output caused by obese people's increased blood volume, which in turn causes stress and structural remodeling. Further lead to cardiac hypertrophy. Localized Angiotensin II-induced ascending aortic aneurysms and cardiac remodeling can be lessened by a leptin antagonist. Moreover, improvements in cardiac function have been shown with the use of neutralizing antibodies against the leptin receptor. However, normal cardiac thickness is restored when leptin levels are met. In the elderly people, Plasma leptin levels and the prevalence of cardiovascular disease, heart failure, and overall mortality caused. Compared to mice with mutant LepR obesity, hyperleptinemia has been seen in diet-induced obese mice, offering protection via the activation of the STAT3 pathway and beyond. In the early stages after leptin administration, the pro-hypertrophic effect of leptin is mediated primarily by calcineurin, and the phosphatase can be activated via both a novel Ca2+-independent/RhoA-dependent mechanism and a Ca2+-dependent mechanism. Leptin activates calcineurin in a Ca2+-dependent manner by inhibiting NKA, which raises intracellular Na+ concentrations and then increases intracellular Ca2+ levels through reverse-mode NCX activity. On the other hand, calcineurin activation that is not dependent on Ca2+ caused by leptin-induced RhoA activation. Regardless of the mechanism of calcineurin activation, calcineurin-dependent translocation of p38 MAPK appears to play a crucial role in mediating the hypertrophic response to leptin, even though increased NFAT translocation into nuclei is a major effect of calcineurin activation (Rajapurohitam et al., 2012).Calcium ion is essential for the heart's excitation-contraction coupling process, which starts with β-adrenergic signals activating Na+/Ca2+ exchange channels via protein kinase A (PKA) signaling, which depolarizes the sarcolemma. Along with this leptin may make myocardiocytes more susceptible to apoptosis as well as leptin may protect against stressful condition of the body (Yu et al., 2014). Actually, when sufficient levels of leptin are reached, the disruption of leptin signaling resolves the lipid accumulation and increased induction of cardiomyocyte apoptosis (Martinez-Abundis et al., 2012). Moreover, this

deficiency in leptin signaling accelerated cardiac damage from myocardial infarction in mice with cardiomyocyte-specific LepR deletions by causing increased cardiac hypertrophy, apoptosis, impaired cardiac structure and function, and impaired energy, glucose, and fatty acid metabolism (Dixon et al., 2009).

Conclusion

PCOS, a common condition of fertile aged women, concerned with endocrine and metabolic dysfunctions and most commonly linked with obesity, insulin resistance, T2DM, oligomenorrhea, ammenhrhoea, infertility, hormonal imbalance, OSA, inflammation, cardiovascular diseases and oxidative stress and ROS which causes further endometrium cancer. Leptin and their isoforms, commonly known as a satiety hormone, was also found to be enhanced in PCOS women and presumably play an important role in pathogenesis and worsening of such conditions. In general, leptin is considered to control the hunger but in PCOS and obese condition over expression of leptin gene causes insulin resistance as well as T2DM. The insulin resistance (IR) and T2DM further disbalance the steroidogenesis pathway (responsible to maintain the hormonal regulation in women) and causes over production of GnRH, which increases the binding of LH in theca cell, compare to FSH on granulosa cell. Increased LH elevate androgen level in women, and at this point leptin try to balance the GnRH by negative feedback process, also help to regulate the reproduction process in PCOS women. So, leptin agonists could be one of the novel prospective therapeutic modules that could restore menstrual cycle. Leptin and their agonist may also be useful to promote follicular growth and maturation, by supporting various mechanisms such as decrease the oxidative stress (OS) markers and ROS, which are found to be elevated in PCOS. There are two ways to produce more oxidative stress - one is insulin resistance, obesity with hyperandrogenism, and another is leptin which provoke the oxidative stress. ROS (superoxide, hydroxyl radical and hydrogen peroxide) elevates various inflammatory mediators such as CRP, TNF, IL-6, IL-8, MCP-1, APSAA and damage the tissue as well DNA, such mediators of inflammatory were also reported to be enhanced by elevated leptin level, and LH/FSH imbalance was tried to be regulated by leptin, as increased LH formation is reported. The leptin’s imbalance may also be involved in the progression or development of ovarian and endometrium cancer. Therefore, a genuine attempt towards the establishment of the role of agonist and antagonist of leptin and their isomers need to

investigated, which could be as useful as adrenergic receptor antagonist/agonist or 5HT agonist/antagonist in various pathological conditions, similarly leptin agonist/antagonist could not only be useful for treatment but also as biomarker for the early diagnosis PCOS and or related pathological conditions like endometrium and ovarian cancer.

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