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REVIEW

The Role of Estrogen in Insulin Resistance

A Review of Clinical and Preclinical Data

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Insulin resistance results when peripheral tissues, including adipose, skeletal muscle, and liver, do not respond appropriately to insulin, causing the ineffective uptake of glucose. This represents a risk factor for the development of type 2 diabetes mellitus. Along with abdominal obesity, hypertension, high levels of triglycerides, and low levels of high-density lipoproteins, insulin resistance is a component of a condition known as the metabolic syndrome, which significantly increases the risk of developing cardiometabolic disorders. Accumulating evidence shows that biological sex has a major influence in the development of cardiometabolic disturbances, with females being more protected than males. This protection appears to be driven by female sex hormones (estrogens), as it tends to disappear with the onset of menopause but can be re-established with hormone replacement therapy. This review evaluates current knowledge on the protective role of estrogens in the relevant pathways associated with insulin resistance. The importance of increasing our understanding of sex as a biological variable in cardiometabolic research to promote the development of more effective preventative strategies is emphasized. *(Am J Pathol 2021, 191: 1490–1498; https://doi.org/10.1016/j.ajpath.2021.05.011)*

The aim of this review is to summarize the current knowledge on the protective effect of estrogens in maintaining insulin sensitivity, with a specific focus on current preclinical studies. It provides an overview of insulin signaling, as well as its correlation with diabetes mellitus and cardiovascular diseases. The second part of the review specifically focuses on preclinical studies looking at the various insulin-sensitive tissues and delineates relevant pathways that might be influenced by estrogens (estradiol).

Insulin: Mechanisms of Action

Postprandial elevations in blood glucose concentration are sensed by pancreatic β-cells, which release insulin into the circulation. Circulating insulin binds to insulin receptors (IRs) that are expressed by virtually all mammalian cells (Figure 1). The IR is a heterotetrameric glycoprotein composed of two αβ dimers. Insulin binds to the α-subunits, inducing autophosphorylation at sites along the β-subunits. Autophosphorylation of Y^{1158}, Y^{1160}, and Y^{1162} is of particular significance as this activates the catalytic domain of the IR tyrosine kinase.[1] Insulin receptor substrates (IRSs) 1 to 4, as well as other cellular proteins, are targeted for phosphorylation by the activated IR.[2] The IRSs are signaling adaptor proteins responsible for mediating interactions between the activated IR and components involved in the intracellular signal cascades, such as Src-homology 2 domain-containing cellular proteins.[3] Despite the underlying homology of IRS isoforms, the functions of the major IRS proteins, IRS1 and IRS2, are distinct; IRS1 is necessary for insulin-mediated glucose uptake and metabolism, whereas IRS2 plays a role in the regulation of lipid metabolism.[4]

Src-homology 2 domain-containing cellular proteins that can associate with the IRS homologs include the class I

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phosphatidylinositol 3-kinase (PI3K) enzymes. Activated class I PI3K catalyzes the phosphorylation of the inositol ring in membrane-bound phosphatidylinositol(4,5)-bisphosphate to phosphatidylinositol(3,4,5)-trisphosphate.[5] Phosphatidylinositol(3,4,5)-trisphosphate, in turn, recruits intracellular molecules with pleckstrin homology (PH) domains, including 3-phosphoinositide–dependent protein kinase 1.[6] Activated 3-phosphoinositide–dependent protein kinase 1 phosphorylates phosphatidylinositol (3,4,5)-trisphosphate–bound Akt protein kinase at Y^{308} of the catalytic activation loop.[6] Synergistic activation of Akt by mechanistic target of rapamycin (mTOR) significantly enhances its kinase activity.[7] mTOR is a serine/threonine kinase that can form complexes with regulatory-associated protein of mTOR (raptor) or rapamycin-insensitive companion of mTOR (rictor), forming mTOR complex 1 or mTOR complex 2, respectively. Notably, mTOR complex 2 is responsible for the full activation of Akt.[7] The association of class I PI3K and IRS and the subsequent series of phosphorylation events are illustrated in Figure 1.

Akt facilitates insulin action via phosphorylation of the protein Akt substrate of 160 kDa,[8] glycogen synthase kinase-3,[9] and forkhead box O (FoxO) transcription factors[10] (Figure 1). Akt-mediated phosphorylation of Akt substrate of 160 kDa inhibits its GTPase-activating protein activity, causing an increased concentration of GTP-bound Rab proteins that are involved in mediating vesicle translocation of glucose transporter protein (GLUT) 4 proteins.[8] As a result, GLUT4 translocates to the plasma membrane, and increases glucose uptake.[11]

In the absence of insulin signaling, glycogen synthase kinase-3 phosphorylates and inhibits glycogen synthase.[9] However, in the presence of insulin, Akt-mediated phosphorylation of glycogen synthase kinase-3 inhibits its kinase activity, resulting in the activation of glycogen synthase, facilitating the storage of intracellular glucose as glycogen.[9] The Akt-dependent inactivation of glycogen synthase kinase-3 also leads to the activation of sterol-regulatory element binding protein transcription factors, which promote the expression of factors involved in fatty acid and triglyceride biosynthesis.[12]

The FoxO family of transcription factors (FoxO1, FoxO3, FoxO4, and FoxO6) contains three potential Akt phosphorylation motifs (RxRxxS/T), except for FoxO6, which lacks a phosphorylation site at the COOH-terminal.[10] The FoxO family promotes the transcription of a variety of gene targets that are dependent on the specific cell type. In the liver, FoxO1 promotes the transcription of glucose-6-phosphatase, phosphoenolpyruvate carboxykinase, and pyruvate dehydrogenase kinase-4, specifically up-regulating gluconeogenesis.[13,14] Activation of IR down-regulates FoxO activity, thereby decreasing gluconeogenesis.

To summarize, insulin-dependent IR activation leads to an increased glucose transport via GLUT translocation/activation, increased glycogenesis via activation of glycogen synthase, increased lipid biosynthesis associated with activation of sterol-regulatory element binding proteins, and decreased gluconeogenesis via diminished FoxO activity and enhanced sterol-regulatory element binding protein activity.

Insulin Resistance, Mechanisms of Action, and Correlation with Diabetes and Cardiovascular Disease

Insulin resistance is characterized by the inability of circulating insulin to effectively regulate the uptake and/or

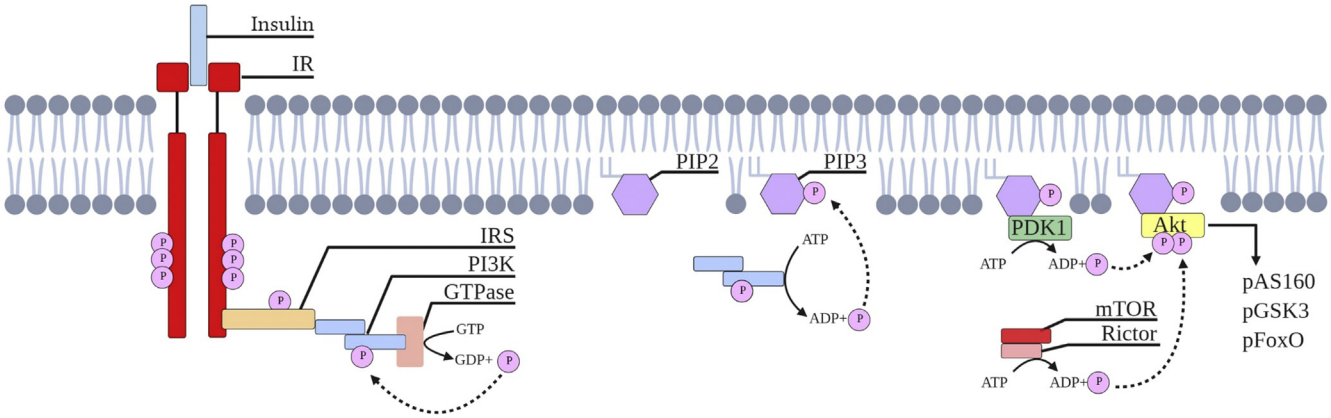

Figure 1 Insulin associates with the extracellular α-subunits of the insulin receptor (IR), facilitating autophosphorylation of β-subunits, in turn activating the catalytic domain of the IR tyrosine kinase, which phosphorylates insulin receptor substrates (IRSs). IRS isoforms associate with the Src-homology 2 domain of class I phosphatidylinositol 3-kinase (CI-PI3K). CI-PI3K associates with Ras GTPase and is activated by Ras GTPase-mediated phosphorylation. Activation of CI-PI3K permits phosphatidylinositol(4,5)-bisphosphate (PIP2) phosphorylation to generate phosphatidylinositol(3,4,5)-trisphosphate (PIP3). PIP3 associates with the pleckstrin homology (PH) domain of 3-phosphoinositide–dependent protein kinase 1 (PDK1), thereby activating it and facilitating its subsequent phosphorylation of PIP3-bound Akt protein kinase. Mechanistic target of rapamycin (mTOR) serine/threonine kinase associated with rapamycin-insensitive companion of mTOR (rictor; mTOR complex 2) synergistically phosphorylates Akt to enhance its activation. Akt further facilitates insulin action via phosphorylation of Akt substrate of 160 kDa (pAS160), glycogen synthase kinase-3 (pGSK3), and forkhead box O (pFoxO) transcription factors, which function to increase glycogenesis and lipid biosynthesis and/or decrease gluconeogenesis. Image generated with BioRender.com (Toronto, ON, Canada).

utilization of glucose by insulin-sensitive tissues and organs. In normal conditions, an increase in blood glucose levels stimulates insulin production from pancreatic β-cells, as well as the inhibition of glucose production in the liver. However, insulin-resistant individuals do not respond to this signaling process, and paradoxically show an increase in both hepatic glucose production and insulin secretion, which can induce or aggravate hyperglycemia.[15] The factors that promote the emergence of insulin resistance include altered insulin signaling, hyperinsulinemia, hyperlipidemia, and obesity. These factors are also associated with chronic low-grade inflammation characteristic of type 2 diabetes mellitus.

In addition to the regulation of blood glucose levels, insulin is also involved in the regulation of lipid metabolism, particularly in hepatic cells and adipocytes. In the liver, insulin resistance can increase lipogenesis, resulting in the development of nonalcoholic fatty liver disease.[15] Nonalcoholic fatty liver disease involves the accumulation of fat in the liver, and it is recognized as a central component of the metabolic syndrome.[16] Impaired lipid metabolism results in the deposition of surplus lipids in nonadipose tissues, which impairs insulin signaling and promotes β-cell hyperplasia.[17] As a result, insulin resistance–induced β-cell glucolipotoxicity interferes with an effective insulin secretion response, further exacerbating insulin resistance as well as glucose and lipid regulation.[17]

Obesity is another factor that is strongly associated with the development of insulin resistance, and fat distribution plays a determinant role in the pathogenesis. Specifically, the accumulation of visceral abdominal fat is considered a risk factor for metabolic syndrome and cardiovascular diseases (CVDs),[18–20] and surgical reduction of visceral abdominal fat can significantly improve insulin sensitivity.[18,21]

Compromised insulin action and/or insulin secretion contributes to the development and sustenance of hyperglycemia, hyperlipidemia, hypertension, and obesity, which are all characteristic of metabolic syndrome.[22,23] The major consequence of metabolic syndrome is a significantly elevated risk of developing type 2 diabetes mellitus and/or CVDs.[24,25]

Overview of Sex Differences in Insulin Resistance

Men are more susceptible to develop metabolic syndrome than premenopausal women; however, protection in women is significantly reduced when estrogen levels decrease.[26] Consistent with these findings, when compared with premenopausal women, women after menopause and the respective age-matched men present with increased insulin resistance, as measured by homeostatic model assessment–insulin resistance.[27] Menopause is a potential risk factor for developing insulin resistance independent of age, likely due to the reduction in circulating estrogens.[28] In support of this hypothesis, it has been shown that surgically induced menopause increases the risk of developing insulin resistance and metabolic syndrome.[29] Clinical studies show that post-menopausal women are more susceptible than premenopausal women to develop dyslipidemia, an increase in body weight (evaluated through body mass index and waist circumference), and impaired glucose tolerance (as shown by their levels of hyperinsulinemia and increased fasting glucose levels).[28–31]

Metabolic disturbances, such as insulin resistance, tend to dramatically increase with the onset of menopause, and estrogen replacement therapy significantly reduces the risk of metabolic syndrome.[28,32] However, there have been conflicting results regarding the effect of hormone replacement therapy (HRT) on glucose homeostasis and insulin sensitivity. These results can be explained by differences in the population examined, the type of hormonal regimen in HRT, differences in the way of administering HRT, as well as differences in measuring insulin sensitivity.[33] Moreover, the timing of HRT matters; treatment with HRT in early menopausal women has beneficial effects compared with HRT started in established postmenopausal women, in whom hormone treatment has no effect or even detrimental effect in terms of glucose homeostasis and insulin sensitivity.[34] Despite these controversial results, a meta-analysis of the available data has shown that exogenous estrogen confers a significant improvement in insulin sensitivity and a reduction of new onset of diabetes in women receiving estrogen replacement therapy.[35] Consistent with these findings, a recently published cross-sectional analysis on the effect of hormonal replacement therapy on metabolic syndrome in Korean women with or without diabetes showed that estrogens significantly alleviate etiological factors of the metabolic syndrome in both groups.[32]

Taken together, these studies highlight the protective role of estrogens in women's metabolic health, specifically with respect to distribution of body fat mass, mobilization of fatty acids, as well as the response to glucose by the various glucose-sensitive tissues and organs.[36,37] Furthermore, reductions in estrogens can significantly impact energy metabolism and general metabolic homeostasis.

Association between Insulin Resistance and Low-Grade Inflammatory State and the Role of Estrogens

Organs and tissues involved in glucose metabolism both express and respond to inflammatory mediators.[38] The immune system is significantly influenced by metabolic stimuli and relies on energetic support by inducing catabolism and repressing anabolic processes induced by insulin.[38] Insulin resistance is associated with a low-grade inflammatory state, which may lead to an increased risk of cardiometabolic diseases.[39] Estrogens are involved in the regulation of

metabolic processes related to energy balance, and can influence inflammatory responses.[39] Many inflammatory components, such as macrophages and monocytes, are activated by estrogen through estrogen receptors expressed in these cells.[39] Furthermore, there is an association between reduced levels of estrogen in post-menopausal women and an increased inflammatory state. Post-menopausal women have increased lymphocyte and monocyte counts, increased expression of proinflammatory cytokines, and increased senescent inflammatory cells, which is usually associated with an improper immunologic function, compared with premenopausal women.[40]

These results are in accordance with other clinical studies that confirm the association between reduced levels of estrogens and an increased proinflammatory state.[41,42]

Taken together, these findings suggest that estrogens might protect from the development of insulin resistance by both modulating the metabolic processes involved in energy balance and down-regulating and/or repressing inflammation.

Analysis of Sex Differences in Insulin Resistance Using Animal Models

Several mouse models of insulin resistance have been generated, and an extensive description of their characteristics and associated advantages and disadvantages has been published in a review by Nandi et al.[43] Ovariectomies are often performed in animal models to study the underlying mechanisms by which sexual dimorphisms affect biochemical processes.[44] This procedure results in a significant reduction in circulating estrogen levels and represents a viable option to study the impact of female sex hormones in metabolic disorders and insulin resistance in any animal model.[45] Alternatively, treatment with exogenous sex hormones can be used to study the effects of increasing estrogen concentrations. In general, the results from experiments performed in such animal models appear to approximate observations from clinical studies in humans.

Sex Differences in Insulin Resistance in the Pancreas

Hyperinsulinemia is an early indicator of the development of insulin resistance. This condition is established when there is increased insulin secretion by pancreatic β-cells in response to increased blood glucose levels. Impaired lipid metabolism, induced by insulin resistance, leads to adaptive β-cell hyperplasia in a compensatory attempt to increase insulin production.[46] Hyperglycemic and hyperlipidemic conditions, along with a chronically increased demand for insulin, can significantly compromise the function and viability of β-cells.[47]

Ovariectomized C57BL/6 mice develop impaired glucose tolerance when compared with sham-operated controls.[45] Total pancreatic β-cell insulin content, as well as glucose-stimulated insulin secretion from isolated pancreatic islets, is significantly lower in ovariectomized mice relative to their respective sham controls. Supplementation with exogenous estradiol rescues these effects.[45]

The Zucker diabetic fatty rat is a rodent model that presents with sexual dimorphism. Male obese rats become diabetic, and female counterparts remain normoglycemic.[44] Male Zucker diabetic fatty rats have a significant impairment in glucose-stimulated insulin secretion that can be remarkably improved with estradiol supplementation.[48] Male Zucker diabetic fatty rats treated with estradiol also have reduced levels of free fatty acids (FFAs) and triglycerides in the pancreatic islets, suggesting a reduction of lipotoxicity and β-cell failure.[48]

Similar to the Zucker diabetic fatty rats, male New Zealand obese (NZO) mice develop overt diabetes when compared with their female counterparts, which remain normoglycemic.[44] Estrogen deficiency (ovariectomy) in the female NZO mice promotes the development of a diabetic phenotype, with mice showing impaired oral glucose tolerance, and a significant reduction of β-cell mass, when compared with sham-operated females.[49] The observed phenotype in ovariectomized female NZO mice is similar to what is observed in male NZO mice.[49] Ovariectomized NZO mice fed a high-fat and carbohydrate-free diet show significant body weight gain when compared with sham-operated female controls. Insulin levels are also significantly higher in the ovariectomized group when compared with sham controls, indicating that the loss of estrogens plays a role in the development of insulin resistance.[49] Consistent with these findings, glucose tolerance and insulin sensitivity are impaired in ovariectomized NZO females, compared with the sham-operated controls. Estrogen supplementation improves glucose tolerance, reduces fasting levels of insulinemia, and reduces insulin resistance (homeostatic model assessment—insulin resistance) assessments in ovariectomized Wistar rats, compared with the ovariectomized nonsupplemented controls.[36]

Sex Differences in Insulin Resistance in the Liver

The liver plays an important role in glucose homeostasis as it is a central tissue for glucose production through both gluconeogenesis and glycogenolysis. Hepatic glucose production is mainly regulated by FoxO1, a transcription factor that promotes the expression of glucose-6-phosphatase.[50] Insulin signaling can attenuate hepatic glucose production by inhibiting FoxO1 via downstream Akt activation.[14] In liver-specific FoxO1 knockout mice, glucose tolerance is impaired in both males and females, suggesting that FoxO1 plays an important role in modulating gluconeogenesis.[51]

Treatment with estrogen pellets in liver-specific FoxO1 knockout males and ovariectomized females significantly improved glucose tolerance.[51] This suggests that estrogen may signal through the estrogen receptor-α present in hepatic cells. These results are consistent with other studies that show a protective effect of estrogen in terms of hepatic insulin resistance and glucose production by signaling through estrogen receptor-α.[52–54]

Male Wistar rats fed a high-fat diet develop insulin resistance, whereas females do not experience a significant induction.[16] Additionally male, but not female, Wistar rats accumulate hepatic lipid, a hallmark of nonalcoholic fatty liver disease, and have an impaired hepatic insulin response, as measured with homeostatic model assessment—insulin resistance. Lipogenesis also induces an increase in fatty acid synthesis, in turn causing an increase in triglycerides in the form of very-low-density lipoprotein.[55,56] The excess of circulating lipids can have detrimental effects on other tissues and promote CVDs. A study in C57BL/6 mice showed that ovariectomized mice fed a high-fat diet developed insulin resistance along with increased hepatic glucose and triglyceride production. Treatment with estradiol significantly improved insulin resistance and prevented triglyceride accumulation.[55] Similarly, C57BL/6 mice treated with estradiol have reduced lipid accumulation and reduced insulin resistance when compared with untreated male controls.[54]

Finally, the concentration of circulating insulin is regulated by the degradation of hepatic insulin, primarily by the insulin-degrading enzyme in the liver. Enhanced insulin degradation can promote insulin resistance. Ovariectomized C57BL/6 mice present with higher insulin-degrading enzyme levels when compared with the sham controls. Exogenous estrogen supplementation significantly decreases insulin-degrading enzyme levels.[45]

Sex Differences in Insulin Resistance in the Adipose Tissue

Estrogens can prevent the accumulation of visceral abdominal fat in premenopausal women, although this protection is lost following menopause.[19] Consistent with these findings, premenopausal obese women are less prone to develop insulin resistance and altered glucose tolerance than lean age-matched men, hinting that estrogen might exert its protective effect by influencing the pathways that control fat distribution.[56–58]

It is now understood that adipose tissue is an endocrine organ and that adipocytes can directly regulate the pathways involved in energy homeostasis.[57,58] Adipocytes of the intra-abdominal depot of C57BL/6 female mice are more insulin-sensitive than those of male mice as they have significantly enhanced activation of Akt and extracellular signal-regulated kinases compared with males, when stimulated with low doses of insulin.[59] Additionally, female adipocytes highly express genes involved in glucose and lipid metabolism compared with males.[59] Male and ovariectomized female C57BL/6J mice fed with high-fat diet present with insulin resistance, have increased adipocyte size, and are less protected from adipocyte oxidative stress compared with sham-operated females or ovariectomized females supplemented with estrogen.[60]

Sex Differences in Insulin Resistance in the Skeletal Muscle

Skeletal muscle plays a key role in insulin-stimulated glucose absorption: approximately 85% to 90% of all postprandial glucose uptake occurs at the skeletal muscle tissue.[61,62] Therefore, this tissue is a significant contributor to the development of insulin resistance. Glucose and FFA are transported into skeletal muscle tissue via GLUT4 and CD36, respectively.[63] Hyperlipidemia has been shown to suppress CD36 translocation in skeletal muscle tissue, and consequently heightens the risk of type 2 diabetes mellitus because of impaired lipid metabolism and increased FFA concentration.[64]

Sex differences in insulin action have been investigated by Hevener et al[64] using Wistar rats infused with a lipid emulsion (liposyn) to increase FFA levels. The liposyn infusion rate to attain a fourfold increase in FFA is approximately one-third higher among female rats when compared with males, indicating that female Wistar rats have a substantially greater FFA clearance compared with males.[64] After liposyn infusion, IRS1 activation is decreased by 30% and class I PI3K activity is decreased by 48% among male rats when compared with female rats, suggesting the potential role of estrogens in improving insulin sensitivity.[64]

A potential determinant of sex differences in insulin resistance in the skeletal muscle tissue is through γABA_B receptor impairment.[65] γABA_B receptor is crucial for the maintenance of glucose-stimulated insulin secretion and glucose homeostasis. Male BALB/c $\gamma ABA_{B1}R$ subunit knockout mice are more susceptible to insulin resistance in skeletal muscle tissue than female BALB/c $\gamma ABA_{B1}R$ subunit knockout mice.[65,66]

Mitochondrial dysfunction may play a role in the development of insulin resistance in the skeletal muscle. Male Wistar rats fed a high-fat diet have significantly more oxidative damage in their skeletal muscle tissues than females.[67] This is associated with higher mitochondrial biogenesis in males when compared with female controls as a way to compensate the deleterious effects of insulin resistance on oxidative metabolism.[67] Another study investigated the protective effects of estrogen in the skeletal muscle by measuring glucose uptake in the skeletal muscle tissue of male, female, and ovariectomized Sprague-Dawley rats.[68] Autoradiographic analysis of glucose transport activity in soleus muscle strips from the legs of the

Table 1 Summary of the Protective Effects of Estrogens on Specific Organs and Tissues

Organ/tissue	Effects of estrogen
Pancreas	Improves fasting insulinemia Improves GSIS
Liver	Modulates gluconeogenesis Improves hepatic insulin response Reduces hepatic insulin degradation
Adipose tissue	Improves insulin sensitivity in adipocytes Reduces adipocyte oxidative stress
Skeletal muscle	Improves insulin-stimulated glucose absorption
Cardiac tissue	Mitigates insulin resistance—induced cardiomyopathy Improves cardiac function
Vascular endothelium	Improves nitric oxide production Increases vasodilation response

GSIS, glucose-stimulated insulin secretion.

experimental rats on a high-fructose diet revealed that the female rats had significantly greater glucose uptake in the skeletal muscle tissue compared with males or ovariectomized female rats, suggesting the presence of sex differences in skeletal muscle tissue insulin sensitivity.[68] Additionally, females showed significantly greater insulin-stimulated activation of IRβ, IRS1 phosphorylation, Akt phosphorylation, and Akt substrate of 160 kDa phosphorylation when compared with males.[68] This finding is supported by another study that observed a significant overexpression of GLUT4 among female Wistar rats fed a high-fat diet, when compared with male Wistar rats.[69]

A recent study further elaborated on the mechanism of sex differences in obesity-induced insulin resistance using male and female C57BL/6 mice fed either a high-fat diet or a regular chow diet.[55] Increased insulin sensitivity in skeletal muscle and greater adiposity were associated with significantly greater glucose uptake among the female mice, thus consolidating previously discussed mechanisms of sex differences in insulin resistance in the skeletal muscle tissue.[55] Furthermore, quantification of ectopic diacylglycerol and triacylglycerol levels by liquid chromatography—tandem mass spectrometry analysis revealed significant reductions in the skeletal muscle tissue of female mice, which is likely a result of unhindered FFA metabolism.[55] Estradiol supplementation improved insulin sensitivity in the skeletal muscle tissue with associated enhanced IRS1 phosphorylation and Akt2 phosphorylation in both male and female mice.[55]

Sex Differences in Insulin Resistance in the Cardiac Tissue

Insulin resistance is a main driving factor in the development of diabetic cardiomyopathy, a complication of diabetes that can lead to heart failure independent of other cardiovascular risk factors.[70] The hallmark of diabetic cardiomyopathy is diastolic dysfunction.[71]

The effect of estrogen treatment on cardiac function in bilateral ovariectomized, insulin-resistant Wistar rats shows that cardiac ejection fraction and fractional shortening are significantly reduced among ovariectomized rats, indicating that loss of estrogens in an insulin-resistant background results in cardiac contractile dysfunction.[72] Estrogen supplementation in these mice significantly attenuates cardiac autonomic dysfunction, restores systolic blood pressure, and improves cardiac contractile performance.[72] Moreover, Western blot analysis indicates that ovariectomized rats treated with estrogen have significantly higher expression of B-cell lymphoma 2, an anti-apoptotic protein, while simultaneously decreasing expression of bcl-2—like protein 4, a pro-apoptotic protein, thereby highlighting the role of attenuating cardiomyocyte apoptosis among insulin-resistant rats.[72]

A protective role for estrogen in the attenuation of cardiac dysfunction and rescue of insulin action can be seen in studies performed in insulin-resistant H9c2 cardiomyocytes, *db/db* mice, and ovariectomized Wistar rats, whose results are consistent with the existence of female sex differences protecting against insulin resistance in cardiac tissue.[72,73] Additional clinical and preclinical research focusing primarily on sex differences in the pathology of insulin resistance in the cardiac tissue and the respective role of estrogen are needed to further our understanding of the extent of these effects.

Sex Differences in Insulin Resistance in the Endothelium

Insulin promotes endothelial nitric oxide production via signaling through the PI3K-Akt pathway. Nitric oxide induces the vasodilation of blood vessels, thereby increasing blood flow and glucose uptake by the various organs and tissues.[74,75] Nitric oxide also prevents leukocyte adhesion and platelet aggregation as well as smooth muscle cell proliferation.[74,75] Endothelial dysfunction is a feature of insulin resistance and is characterized by a reduced production of nitric oxide by endothelial cells, which can trigger the processes that lead to atherosclerosis and to the development of CVDs.[73,75] Several clinical trials have shown that post-menopausal women have significant endothelial dysfunction compared with premenopausal women, suggesting that depletion of estrogens might be detrimental to the endothelial vascular tissue.[76—78] Studies conducted in women with polycystic ovary syndrome, a condition characterized by hyperandrogenism where most patients present with insulin resistance, show significant alterations in endothelial function.[79,80] These findings suggest that a reduction in estrogens along with insulin resistance could be detrimental and significantly increase the risk of developing CVDs.[79,80] Research conducted on male

insulin-resistant Zucker rats treated with estradiol showed a significant improvement in endothelial function by reducing vasoconstriction and increasing vasodilation responses as well as inducing nitric oxide synthase expression.[81] Consistent with these results, ovariectomized Wistar rats treated with estradiol showed a reduced vasoconstrictor response of mesenteric arteries compared with the ovariectomized Wistar rats without estrogen treatment.[82]

Conclusion

Insulin resistance is one of the main components of the metabolic syndrome, a condition that increases the risk of developing type 2 diabetes mellitus and cardiovascular diseases. Accumulating clinical and preclinical data suggest that sex differences in cardiometabolic risk do exist; however, this is still an underrepresented and understudied area of research. Estrogen seems to play a role on various insulin-sensitive tissues and organs by improving and/or modulating glucose homeostasis (Table 1).[51]

However, the specific action in these organs and tissues as well as the underlying mechanisms and pathways are yet to be identified. This review summarizes current knowledge on the potential protective role of estrogen in the development of insulin resistance and its consequences. We acknowledge that there is a considerable gap in understanding the molecular mechanisms underlying how these protective effects are exerted, and more studies are needed to delineate these pathways. This highlights the importance of including sex as a biological variable in preclinical studies, as stated by the NIH's mandate,[83] which will lead to a more detailed comprehension of the pathogenesis of cardiometabolic diseases and subsequently improve contemporary management and prevention of these conditions.

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