Trends in Endocrinology & Metabolism

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REVIEWS

Review

Implications and Future Perspectives of AGEs in PCOS Pathophysiology

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Human, animal, and *in vitro* studies provide evidence that advanced glycation end-products (AGEs) may contribute to the pathogenesis of polycystic ovary syndrome (PCOS) and its metabolic and reproductive consequences. AGEs are able to induce, via activation of key intracellular signaling pathways, the generation of oxidative stress and proinflammatory cytokines, thus contributing to the adverse health impact of PCOS. This review presents the implications of AGEs in several disease pathophysiologies, including PCOS, as well as the cellular and systemic effects of AGEs on insulin resistance (IR), hyperandrogenemia, endoplasmic reticulum (ER) stress, hypoxia, and ovarian function. The gaps in our knowledge will serve as launching pad for future developments ranging from dietary and lifestyle changes to pharmaceutical interventions aiming at potential applications in women with PCOS.

Highlights

AGEs are elevated in the serum and the ovarian tissue of women with PCOS, and may contribute to the metabolic and reproductive consequences of PCOS.

Studies *in vitro* and in animal models suggest that AGEs may be associated with abnormal steroidogenesis and folliculogenesis.

Women with PCOS have low levels of the anti-inflammatory sRAGE receptors in both the serum and the ovarian follicular fluid.

AGEs could alter insulin intracellular signaling and the cellular translocation of glucose transporters, thus leading to tissue IR.

PCOS is highly prevalent and arguably constitutes the most frequently encountered endocrinopathy in reproductive-aged women [1,2]. PCOS is associated with reproductive and metabolic alterations, and constitutes the most common cause of anovulation in women, with ~70% experiencing ovulatory problems [2]. In addition, the majority of women with PCOS exhibit one or a combination of metabolic and cardiovascular risk factors [3]. Thus, there is a crucial need to establish therapeutic strategies for these metabolic aberrations observed in women with PCOS. However, the absence of any multipotential therapeutic tool targeting the primary defect of PCOS reflects the gaps in our knowledge of the underlying pathophysiological mechanisms linked with this enigmatic syndrome.

Elevated levels of AGEs, that are produced endogenously or absorbed exogenously from modern heat-processed diets, is observed in women with PCOS, and data from *in vitro* experiments, animal model, and human studies provide evidence that AGEs may contribute to the pathogenesis of PCOS and its metabolic and reproductive consequences [4–8]. This review will define AGEs and their receptors and present data relevant to the implications of AGEs in pathophysiologies related to PCOS. It will critically present data, including our own, on the role of AGEs as mediators of metabolic and reproductive alterations in PCOS [4–8] related to the cellular and systemic effects of AGEs on IR, hyperglycemia, hyperandrogenemia, ER stress, hypoxia, steroid production, and ovarian function.

AGEs and Their Receptors: Formation, Action, and Elimination

Formation of AGEs

AGEs are a group of over 20 heterogeneous compounds of which pentosidine and *N*-carboxymethyl-lysine (CML) are the most commonly studied (Figure 1) [9]. In contrast to exogenous AGEs (Box 1), *in vivo* production of AGEs occurs at a much slower pace. Endogenous AGEs are usually formed slowly under physiological conditions. Aging, hyperglycemia, obesity, oxidative stress, and hypoxia accelerate the generation of AGE precursors [10].

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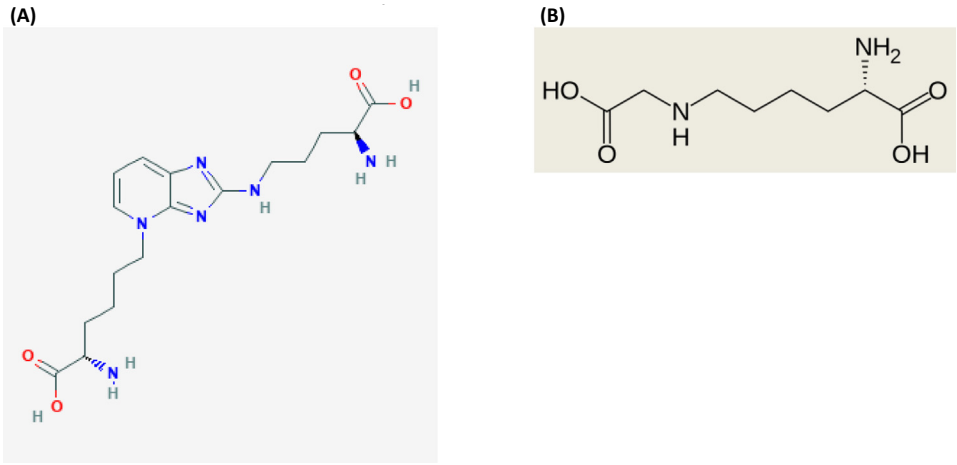

Figure 1. Structure of the Two Commonly Studied AGEs. (A) Pentosidine is a carbohydrate-derived AGE that is formed in a reaction between amino acids and the Maillard reaction products of ribose. Its molecular formula is $C_{17}H_{26}N_6O_4$. (B) *N*-Carboxymethyl-lysine (CML) is the most commonly used marker for the AGE content of different diets. Its molecular formula is $C_8H_{16}N_2O_4$.

The accumulation of AGEs has been shown to be associated with variable pathologies such as type 2 diabetes mellitus (T2DM), metabolic syndrome, cardiovascular disease (CVD), hypertension, inflammation, cancer, neurodegenerative disorders (such as Alzheimer and Parkinson disease), and PCOS [6,10,11].

Actions of AGEs

Although the exact cellular and molecular mechanisms of the role of AGEs in disease pathogenesis are undetermined, the actions of AGEs are classified as receptor-independent (such as crosslinks with the extracellular matrix) or receptor-dependent (Figure 2) [12].

Box 2 summarizes the action of AGEs on several proteins and their clearance. To better understand the biological processes underlying AGEs, endogenous or exogenous, it is crucial

Box 1. Advanced Glycation End-Products Formation

Endogenous AGEs

The formation of endogenous AGEs starts with the condensation of the carbonyl group of a reducing sugar or aldehyde with a free amino group of a protein, forming a reversible Schiff base (requires basic pH) which is ultimately transformed into a covalently bound Amadori product (requires acidic pH), which is also reversible [14]. A well-known example of an Amadori product is the glycated hemoglobin A1c, a marker for monitoring blood glucose levels [14]. The combination of increased blood glucose with oxidative and non-oxidative processes results in rearrangements of the Amadori products into the irreversible AGEs [88]. The formation of carbonyl compounds after autoxidation of monosaccharides such as fructose can also lead to the production of AGEs. Finally, lipid peroxidation forms a main source of carbonyl compounds called α-oxoaldehydes [14]. During the production process of AGEs, many intermediates are unstable, and these spontaneously degrade or undergo redox cyclization reactions, releasing ROS that can modify proteins, lipids, or nucleic acids in the extracellular and intracellular compartments [89].

Exogenous AGEs

The formation of exogenous dietary AGEs during food preparation is a very fast process, and increases with elevated temperature and sugar availability [14,90]. Dietary AGEs are orally absorbed [91], and ~50% of AGEs remain measurable in the urine until a few days following their consumption [92]. Reactive glycation products are also present in aqueous extracts of tobacco and in tobacco smoke in a form that can rapidly react with proteins to form AGEs [93].

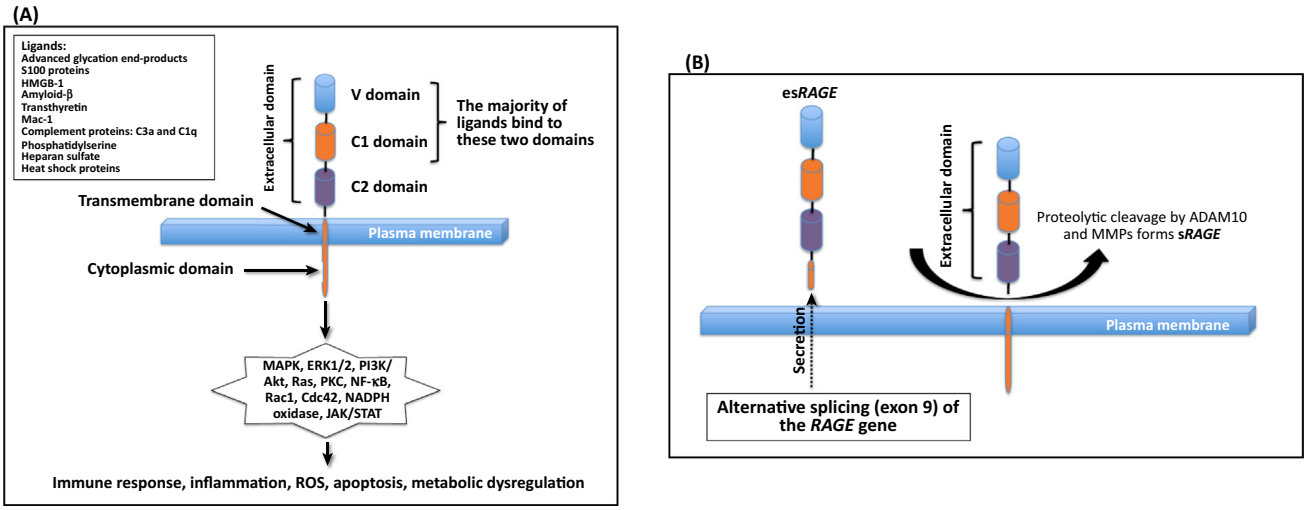

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Figure 2. Schematic Representation of RAGE and Soluble RAGE (sRAGE). (A) RAGE is an immunoglobulin superfamily receptor that binds multiple families of ligands. It consists of three domains: extracellular, transmembrane, and cytoplasmic. The extracellular domain is composed of three distinct immunoglobulin-like V, C1, and C2 domains. The V and C1 domains form a structural and functional unit where most ligands bind. Following activation of RAGE by multiple ligands, several signaling pathways are activated that lead to transcription of genes involved in inflammation and apoptosis. (B) There are two forms of sRAGE. sRAGE is formed by proteolytic cleavage actions of ADAM10 and MMPs. The second form is an alternatively spliced form called endogenous secretory (esRAGE; also known as RAGEv1). A unique 16 amino acid span in the C2 domain distinguishes esRAGE from sRAGE. Multiple factors are likely to regulate sRAGE and esRAGE production and clearance, such as gender, hormones, oxidative stress, and glycemic stress. Abbreviations: ADAM10, a distintegrin and metalloprotease 10; Cdc, cell division cycle; ERK, extracellular signal-regulated kinase; HMGB1, high mobility group box 1; JAK/STAT, Janus kinase/signal transducers and activators of transcription; Mac-1, macrophage receptor-1; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor κB; MMP, matrix metalloproteinase; PI3K, phosphatidylinositol-3-kinase; PKC, protein kinase C; Rac-1, Ras-related C3 botulinum toxin substrate 1; RAGE, receptor for advanced glycation end-products; ROS, reactive oxygen species.

to develop future *in vivo* pharmacokinetic studies pertaining to their biodistribution, trafficking, elimination, and clearance.

RAGE and Intracellular Signaling Pathways

The AGE receptor, RAGE, is an immunoglobulin superfamily receptor that binds multiple families of ligands such as amyloid β peptide, S100/calgranulin protein, HMGB1, and others [13]. RAGE is a single transmembrane domain receptor that consists of three domains:

Box 2. Action and Clearance of AGEs

Action of AGEs on Different Proteins

AGEs result in an alteration of protein structure, making them more resistant to degradation, and resulting in the accumulation of crosslinked products in cells and body tissues [94]. AGEs affect the functional properties of long-lived proteins in several important matrix components [95]. The formation of AGEs on the extracellular matrix interferes with both matrix–matrix and matrix–cell interactions [96]. Common targets of AGEs include type IV collagen [64] (a structural component of the basement membrane) and other long-lived proteins such myelin, tubulin, plasminogen activator 1, and fibrinogen [96].

Clearance of AGEs

Clearance of systemic AGEs takes place via the kidneys. Degradation of AGEs principally takes place intracellularly, thus AGEs need to be taken up into the cell before they can be degraded. Not all AGE receptors are able to bind AGEs for subsequent intracellular processing. AGE receptors involved in detoxification include AGE-R1/OST-48, AGE-R3/galectin-3, and some scavenger receptors (MSR-AII, MSR-BI, CD36) [97]. Specifically, these inhibit AGE-mediated signaling and oxidative stress by competing with RAGE for binding to AGEs, and AGE uptake takes place through receptor-mediated endocytosis promoted by phosphorylation or ubiquitinylation of the cytoplasmic side of the receptor [98].

extracellular, transmembrane, and cytoplasmic [13]. The extracellular domain is large and is composed of three distinct domains: an immunoglobulin-like V (variable-type) domain and two constant domains named C1 and C2. The V and C1 domains form a structural and functional unit where most ligands bind (Figure 2). Following activation of RAGE by ligands, several signaling pathways are activated that lead to the transcription of genes involved in inflammation and apoptosis [13].

RAGE displays a normal physiological role that has not yet been completely unraveled and, in pathological conditions, is an important player in the propagation of immune/inflammatory responses, as shown by the upregulation of RAGE in monocytes of women with PCOS as well as in inflammatory lesions of various diseases (including rheumatoid arthritis, inflammatory kidney disease, atherosclerosis, and inflammatory bowel disease) and the capacity of RAGE to bind many proinflammatory ligands [14].

RAGE signaling is complex and results in the activation of multiple pathways that are tissue-specific [15]. AGE–RAGE binding leads to the activation of key elements of the PI3K/AKT pathways or the mitogen-activated protein kinase (MAPK) signaling pathways, which include ERK1/2, p38, and JNK. In addition, p21-Ras, Rac-1, and Cdc42 can also be activated as a result of the AGE–RAGE binding [16]. AGE binding to RAGE leads to the activation of inflammatory processes (production of cytokines such as IL-1, IL-6, and IL-8, as well as many chemokines) via NADPH oxidase, as well as activation of the transcription factor NF-κB, ultimately leading to the production of reactive oxygen species (ROS) [17]. The AGE–RAGE interaction also promotes JAK-2/STAT-1 activation, another signaling pathway that promotes inflammation and cytokine production, and affects the composition and activity of proteasomal subunits [18].

Formation and Role of Soluble Receptors for AGEs (sRAGEs)

At least two forms of soluble RAGE have been detected in the circulation and in body fluids: sRAGE and esRAGE (endogenous secretory RAGE) (Figure 2) [19]. The sRAGE is generated by cell-surface proteolytic cleavage mechanisms, such as that induced by matrix metalloproteinases (MMPs) and ADAM 10 (a distintegrin and metalloprotease 10) [20,21]. This form of receptor is derived from the full-length form of RAGE, and comprises the V–C1–C2 domains [20]. sRAGE circulates and act as a decoy by binding the circulating AGEs, and therefore competitively inhibits the AGE–RAGE interaction and downstream proinflammatory signaling, thus having cytoprotective effects [19]. The second form of soluble RAGE, esRAGE, is generated by pre-mRNA alternative splicing [22]. The esRAGE, also called variant RAGE-v1, usually comprises 20% of the total soluble RAGE and contains an additional 16 amino acids in the C-terminal region of the molecule [22].

Because of its easy quantification by ELISA, sRAGE has been extensively studied. As of today, over 700 publications on PubMed pertaining to many body organs and several organ systems have tied sRAGE to (patho)physiological processes. This attention on sRAGE has the potential to make it an attractive marker in several diseases. However, normal versus abnormal circulating sRAGE levels have yet to be determined and a cut-off beyond which a 'normal physiology' becomes 'pathology' is needed.

AGEs and Metabolic Implications for PCOS

Oxidative Stress and Inflammation: Potential Links of AGEs with IR, Adiposity, and Cardiovascular Disease in PCOS

It has been estimated that 50–70% of women with PCOS have IR and hyperinsulinemia [23] which are closely associated with oxidative stress and inflammation. The AGE–RAGE system,

functioning in a bidirectional axis with generation of excess oxidative stress, is one of the major inducers of systemic oxidative stress and inflammation which are well known players in the pathogenesis of IR [24] and subsequently in the development of T2DM and cardiovascular disease.

PCOS has been considered to be a state of oxidative stress in which the antioxidant mechanisms of the body cannot deal with the excessive production of ROS. Genetic aberrations, epigenetic alterations during the developmental course of the syndrome, and the cardinal contribution of environmental factors all lead to an adverse redox status, which further exacerbates the classical characteristics of PCOS [25]. Increased ROS production by monocytes in response to hyperglycemia was observed in women with PCOS, independently of adiposity, and was related to the activation of NADPH oxidase. Furthermore, women with PCOS displayed high levels of ROS in their granulosa cells, which was accompanied by poorer reproductive outcome (including fertilization rate, good-quality embryo formation rate, and pregnancy outcome) following *in vitro* fertilization (IVF) [26]. Another system involved in oxidative stress is the glyoxalase detoxification system, which is composed of glyoxalases GLO-I and GLO-II that are ubiquitously expressed and implicated in protection against cellular damage caused by cytotoxic metabolites such as AGEs [27]. A study in PCOS animal model (androgenized model) showed that the activity of ovarian GLO-I was significantly reduced in animals fed a high-AGE diet compared to animals fed a low-AGE diet [28]. In addition, there was a negative correlation between ovarian GLO-I activity and AGE expression in the ovarian granulosa cell layer, demonstrating that ovarian GLO-I activity may be regulated by AGEs. These results suggested that modification of ovarian GLO-I activity might be a contributing factor to the reproductive dysfunction characterizing PCOS.

The binding of AGEs to RAGE rapidly activates NF-κB, PKC, and MAPK pathways, which in turn leads to increased ROS production, activation of NADPH oxidase, and increased expression of inflammatory markers [such as tumor necrosis factor α (TNF-α), IL-6, and IL-1α] [29,30]. This excessive ROS production causes a positive feedback loop by inducing RAGE expression, thus causing a vicious cycle that further exacerbates oxidative stress and inflammation [29,30]. Original data suggest that inflammatory markers are elevated in women with PCOS, possibly playing an essential role in the pathogenesis of IR because the beneficial effects of metformin were linked to a reduction in the serum levels of inflammatory markers [31]. Nevertheless, a recent meta-analysis emphasized the need for further well-designed studies [32]. Intake of a high-AGE diet could also contribute to an elevated level of serum AGEs which has been shown to correlate with inflammatory markers such as high-sensitivity C-reactive protein (CRP), fibrinogen, 8-isoprostanes (a marker of lipid peroxidation), TNF-α, and vascular adhesion molecule-1 (VAM-1) [33].

Although still contentious [34], women with PCOS have a higher prevalence of subclinical atherosclerosis due to endothelial dysfunction [35–38], which was observed even in young, non-obese women with PCOS [39,40] and that was independent of metabolic disturbances [41]. Endothelial dysfunction is promoted by high levels of IL-6 and TNF-α and by low expression/activity of endothelial nitric oxide synthase (eNOS) which leads to decreased nitric oxide (NO) synthesis and decreased vasodilation [42]. In addition, women with PCOS have elevated levels of adhesion molecules that are known to be markers of endothelial dysfunction, such as endothelin 1 [43,44]. Interestingly, endothelin 1 levels were found to be positively and strongly associated with AGE levels in women with PCOS, suggesting that the detrimental effect of AGEs on endothelial cells may involve increased endothelin 1 production [45]. In addition, studies *in vitro* have shown that AGEs and TNF-α can upregulate RAGE mRNA and

protein levels in human vascular endothelial cells via two distinct nuclear complexes, p65/p50 NF-κB and Sp-1/ERα [46].

Because proatherogenic AGEs were found to be elevated in the circulation of women with PCOS, our group investigated whether metformin treatment of women with PCOS could alter plasma AGE levels [47]. That study involved 22 healthy women without PCOS and 22 patients with PCOS who received 1700 mg of metformin daily for 6 months. The results showed that AGE levels as well as testosterone levels and free androgen index were significantly reduced after metformin administration, although body mass index (BMI) remained unchanged [47].

Together, the data to date suggest that targeting the AGE–RAGE system has future clinical potential in women with PCOS, a high-risk group for T2DM and cardiovascular disease.

AGEs Alter Insulin Cell Signaling and Glucose Transporters

The molecular pathophysiology of IR in PCOS is related to a variety of defects, including a post-binding receptor defect [48], low levels of IRS-1 expression [49], impaired IRS-1 phosphorylation, reduced activity of the serine/threonine kinase AKT2, and altered glucose transporter GLUT-4 translocation to the plasma membrane [50–52]. Women with PCOS demonstrate changes in the functionality of the downstream components of the insulin signaling cascade such as GLUT-4, which is necessary for glucose uptake into the cell [50–54]. AGEs and RAGE affect insulin signaling and glucose metabolism in insulin-sensitive cells and tissues, including ovarian cells. Morphological changes of adipocytes induced by RAGE overexpression were associated with a decrease in *Glut4* gene expression and attenuation of insulin signaling [55]. On the other hand, $Rage^{-/-}$ mice had significantly higher *Glut4* mRNA expression in epididymal fat compared to wild-type mice [55]. The effect of AGEs on glucose transport was investigated in the human granulosa KGN cell line where cells were treated with human glycated albumin (HGA), a representative of AGEs for *in vitro* studies, in the presence of insulin [56]. Insulin-mediated AKT phosphorylation was inhibited in the presence of HGA. In addition, HGA treatment suppressed insulin-induced GLUT-4 translocation from the cytoplasm to the membrane compartments of KGN cells [57].

Several studies evaluated the AGE-induced impairment of insulin signaling in muscle cells and pancreatic β cells as well as the mechanisms behind this impairment. These studies were very helpful for our understanding for the AGE-induced IR. In skeletal muscle cells, chronic exposure to HGA selectively inhibits the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB) pathway in the insulin signaling cascade while leaving the Ras–ERK activation and mitogenic action of the hormone unaltered [57]. In addition, HGA induced an alteration of insulin metabolic signals, which was correlated with an increase in serine/threonine phosphorylation of IRS specifically mediated by the activation of PKCα [57]. Of note, the PKC family of serine/threonine kinases is implicated in the development of IR [58]. IRS serine/threonine phosphorylation by HGA-activated PKC inhibited insulin-stimulated glucose metabolism without changes in growth-related pathways regulated by insulin. In tibialis muscle of mice on a high-AGE diet, insulin-induced glucose uptake and PKB phosphorylation were reduced, which was paralleled by a 2.5-fold increase in PKCα activity. AGEs were also able to induce IR in these mice *in vivo* because insulin tolerance tests revealed a significant impairment of insulin sensitivity [59]. Methylglyoxal (MGO) is the most reactive AGE precursor and its intracellular formation is an essential source of intracellular AGEs. In L6 muscle cells, short exposure to MGO induced an inhibition of insulin-stimulated phosphorylation of PKB and ERK1/2 without affecting insulin receptor tyrosine phosphorylation. This indicates that AGEs lead to inhibition of insulin-induced signaling [59]. These findings suggest that AGEs impair insulin signaling and glucose

metabolism in insulin-sensitive tissues and organs via several mechanisms, one of which is the formation of multimolecular complex including RAGE/IRS-1/Src and PKCα. In addition, the deleterious actions of these molecules generated by metabolic dysregulation will worsen the IR process by causing β cell malfunction, at least in animals.

These data suggest that AGEs may alter insulin cell signaling and glucose transporter translocation in several pathological conditions including PCOS.

AGEs and Reproductive Implications for PCOS

Alterations in the AGE system Are Related to Reproductive Impairment in Women with PCOS

In 2005 it was demonstrated for the first time that, independently of hyperglycemia, overweight women with PCOS have increased serum AGE levels and elevated RAGE expression in their monocytes, as assessed by flow cytometry, compared to women without PCOS [5]. Then in 2008 the same group of investigators from Greece showed that lean non-insulin-resistant women with PCOS also have elevated serum AGE levels compared to women with isolated PCOS components such as hyperandrogenemia with or without PCO morphology, and with or without anovulation [4].

When assessed at the ovarian tissue level by immunohistochemistry, RAGE and AGE-modified proteins were differently expressed in women with or without PCOS [6]. In that study, ovaries of women without PCOS exhibited no staining differences between granulosa cells and theca interna cells; however, in the ovaries of women with PCOS, granulosa cells displayed stronger RAGE expression compared to theca interna cells [6]. In addition, NF-κB p65 subunit was only observed in granulosa cell nuclei in the ovaries of women with PCOS [6]. Interestingly, the relationship between −429T > C and −374T > A single-nucleotide polymorphisms (SNPs) of the *RAGE* gene and susceptibility to PCOS was analyzed by PCR–restriction fragment length polymorphism assay, and showed no significant differences between women with or without PCOS [60]. These findings suggest that these harmful molecules and the proinflammatory multiligand receptor RAGE have a pathological significance in the reproductive abnormalities, in particular ovarian dysfunction, in women with PCOS.

In addition, several studies assessed the relationship between sRAGE and PCOS. Compared to women without PCOS, those with PCOS had significantly lower follicular fluid sRAGE levels [61,62]. However, these studies were performed in women who underwent IVF, and the results cannot be easily extrapolated to the normal physiologic state where women are not exposed to high doses of gonadotropins and other hormonal medications. The implications of these findings suggest that there may be alterations in anti-inflammatory receptors for sRAGEs in PCOS, and the role of sRAGE in the PCOS-related reproductive alterations needs further evaluation.

AGEs and Folliculogenesis/Ovulation

In the ovaries of women with PCOS there is abnormal collagen synthesis and an increased volume and density of ovarian stroma. It has been shown that the ovaries of women with PCOS have alterations in enzymes responsible for collagen synthesis, for example an increase in lysyl oxidase (LOX) expression [63].

AGE signaling could be involved in the regulation of ovarian follicular extracellular matrix organization in PCOS. The interaction between AGEs and LOX could explain some of the ovarian tissue changes observed in PCOS. For instance, the deposition of excess collagen in

PCOS tissue may be partly due to AGE-mediated stimulation of LOX activity [64]. In addition, dietary AGEs can reduce the activity of glyoxalase I in the ovary of a PCOS rat model, an effect that could be reversed by a low-AGE diet [65]. These finding suggest that AGEs probably play a significant role in the intraovarian pathophysiology in PCOS, partly via LOX and glyoxalase I [64].

Activation of MAPK/ERK has been implicated in the regulation of oocyte maturation, and thus this effect is crucial in normal follicle development and proper ovulation [66]. In light of this information, Kandaraki *et al.* [67] studied the *in vitro* interference of AGEs with luteinizing hormone (LH)-induced MAPK/ERK signaling pathway in KGN granulosa cells. A direct and specific effect of AGEs was observed in this pathway, leading to reduced activation of ERK1/2. In addition, follicle-stimulating hormone (FSH) has been shown to act through the ERK pathway to increase cyclin D2 expression and granulosa cell proliferation [68]. FSH-induced phosphorylation of MEK1/2 and ERK1/2 was also affected by the presence of AGEs, leading to reduced activation of these molecules in KGN granulosa cells [67]. Because ERK1/2 activation is crucial for FSH-mediated granulosa cell mitogenesis, the inhibitory effect of AGEs could potentially disturb follicular development.

Anti-Mullerian hormone (AMH) signals intracellularly via phosphorylating SMADs 1/5/8 [69,70] and plays an important role in folliculogenesis by suppressing the differentiation of granulosa cells, thus protecting the follicles from growing and ultimately becoming atretic [71]. This inhibitory role of elevated AMH levels, which are associated positively with elevated serum AGEs in anovulatory hyperandrogenemic PCOS women [72], may contribute to anovulation. Further support for a possible link between these molecules and anovulation in PCOS comes from the observation of increased expression and production of AMH and its receptor by granulosa cell in PCOS [73,74], given the strong positive correlation between AGEs per follicle and the AMH/follicle ratio according to ovulation status [72]. The effect of AGEs on pathways (such as SMADs 1/5/8) and genes (LH receptor, *LHR*; *AMH*; and the AMH receptor, *AMHR2*) involved in ovarian follicular development was recently studied in human luteinized granulosa cells [75]. Granulosa cells treated with AGEs *in vitro* displayed a significant increase in *LHR* mRNA levels and an increase in *AMHR2* mRNA levels, but no change in *AMH* mRNA levels. In addition, KGN granulosa cells treated with recombinant AMH (rAMH) showed a marked increase in the phosphorylation of SMADs 1/5/8 in the presence of AGEs *in vitro* (Figure 3). Women with PCOS have elevated AMH production [76,77] and AMH-induced SMAD 1/5/8 signaling in their granulosa cells [73], which is partly responsible for the abnormal follicular development. Interestingly, AGEs, which are elevated in PCOS [6], induced excess AMH-induced SMAD 1/5/8 phosphorylation. Thus, it is plausible that AGEs, which are usually elevated in PCOS [4–6], could be partly responsible for the elevation of AMH-induced SMAD 1/5/8 signaling, ultimately leading to ovulatory dysfunction in PCOS.

AGEs and Steroidogenesis

Whether the elevated AGEs in PCOS directly or indirectly alter steroidogenesis is still under research, although some descriptive studies have shown an association. AGE levels in serum and follicular fluid negatively correlated with serum estradiol (E2) levels in women with PCOS undergoing IVF [78]. Luteinized granulosa cells treated with AGEs *in vitro* had significant increases in *CYP11A1*, *HSD3B*, *STAR*, and *CYP17A1* mRNA expression levels, as well as significant oversecretion of E2, but there was no effect on *CYP19A1* mRNA levels [79] (Figure 4). These findings clearly imply a relationship between AGEs and circulating steroid levels as well as with steroid gene expression. There is a big gap in our knowledge on this topic, and further studies will be necessary to determine whether the elevated AGEs in PCOS are in part responsible for the abnormal steroidogenesis in theca cells and non-luteinized granulosa cells.

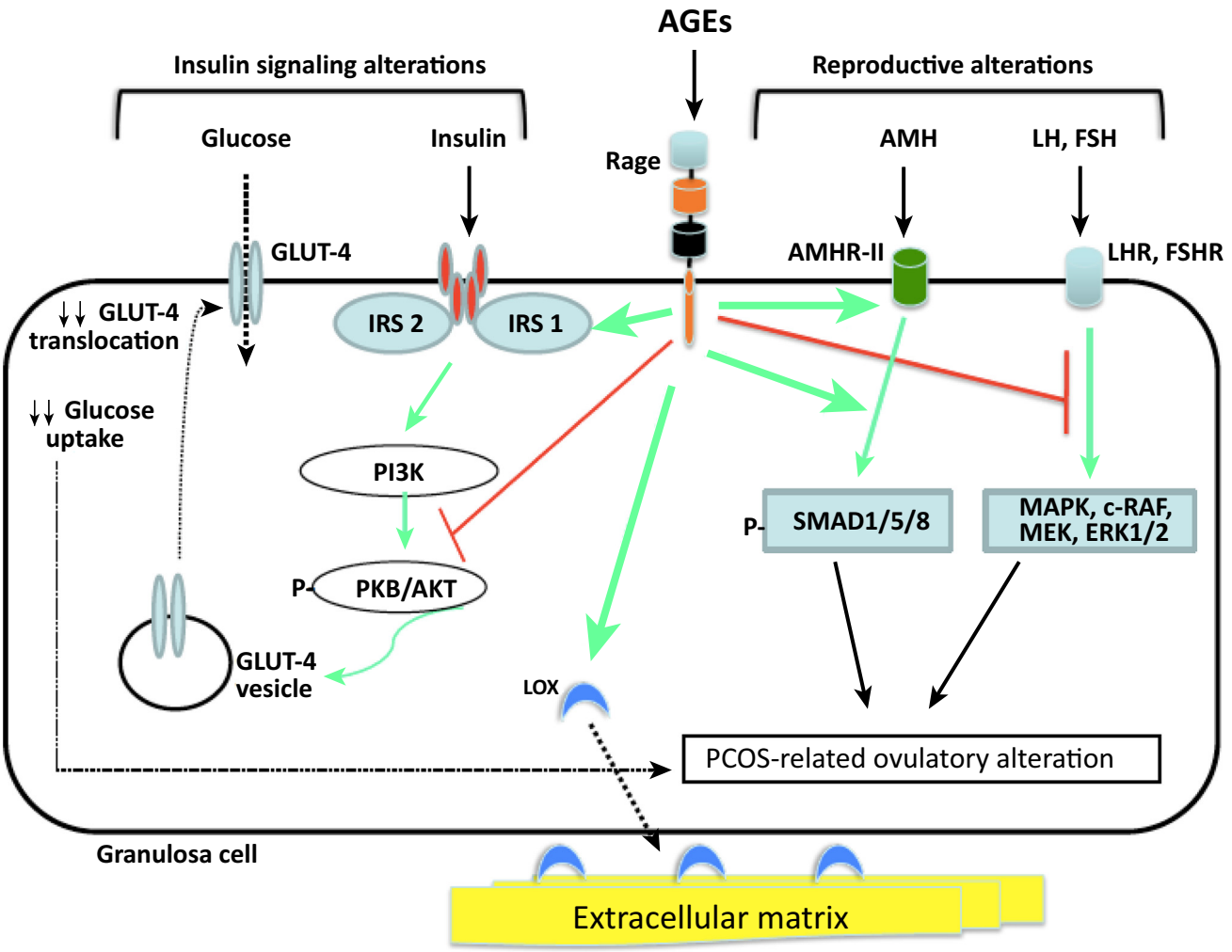

Figure 3. Schematic Diagram Illustrating the Potential Metabolic and Reproductive Alterations Caused by Advanced Glycation End-Products (AGEs) in Granulosa Cells. AGEs are related to ovulatory dysfunction by impairing the actions of LH and FSH via attenuating their intracellular signaling MAPK, c-Raf, MEK, and ERK1/2. AGEs may also contribute to polycystic ovary syndrome (PCOS)-related ovarian dysfunction by upregulating AMHR-II and its intracellular signaling via SMAD1/5/8 phosphorylation (P). AGE–RAGE activation induces LOX expression/release and its binding to the extracellular matrix. The insulin signaling alterations induced by AGEs could occur at the level of IRS and PI3K activation of AKT, all of which lead to impaired GLUT-4 translocation to the cell membrane, ultimately contributing to insulin resistance. Green arrows indicate induction by AGEs.

AGEs and Hyperandrogenism

It is well established that the abnormal steroidogenesis in PCOS can lead to elevated androgen synthesis and hyperandrogenism [80]. Interestingly, AGEs are associated with hyperandrogenemia in PCOS, as shown in a positive correlation between serum AGEs and testosterone (T) levels in women with PCOS ($r = 0.73$, $P < 0.0001$), even after controlling for BMI [5]. Women with PCOS given either high-AGE or low-AGE isocaloric diets for 2 months had different androgen levels [81]. Women with PCOS on a high-AGE diet had significantly increased serum AGEs and higher T, free androgen index (FAI), and androstendione levels compared to women with PCOS on a low-AGE diet. In Chinese women with PCOS, an inverse association between sRAGE and hyperandrogenism has been reported [82]. Studies in animals showed similar

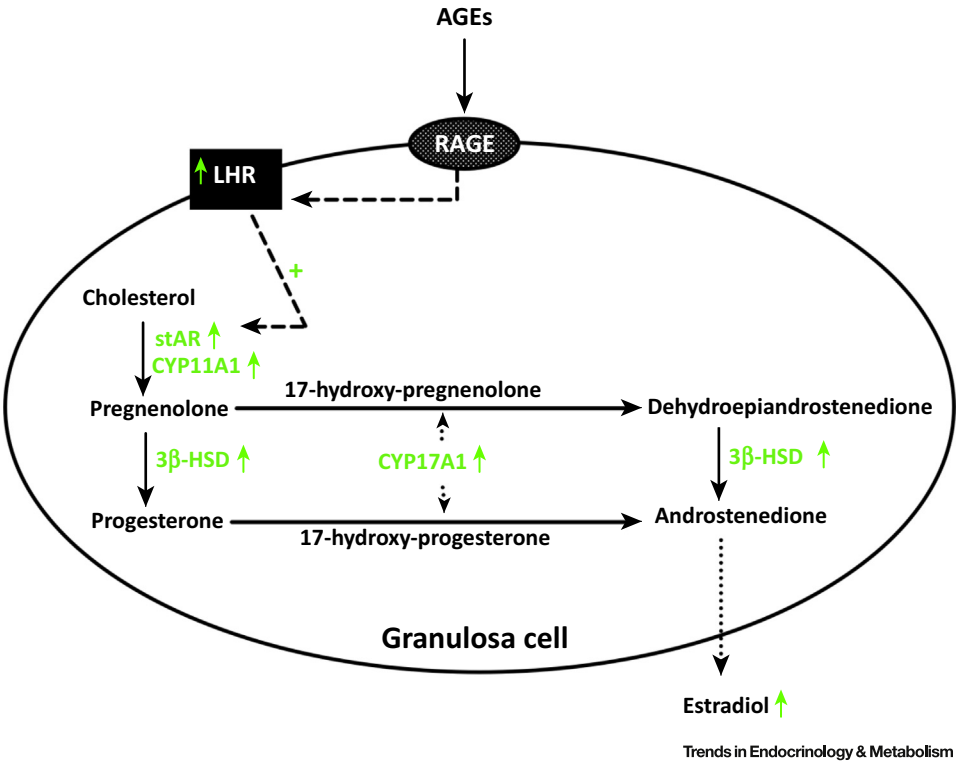

Figure 4. Schematic Diagram Illustrating the Potential Effect of Advanced Glycation End-Products (AGEs) on Steroid Gene Expression in Human Granulosa Cells. AGEs may affect steroidogenesis by upregulating luteinizing hormone receptor (LHR), which is known to induce steroidogenic acute regulatory protein (StAR) and P450 side-chain cleavage enzyme (CYP11A1). AGEs also upregulate 17α-hydroxylase/17,20 lyase (CYP17A1) and 3β-hydroxysteroid dehydrogenase (3β-HSD), ultimately leading to more production and release of estradiol. Upward green arrows indicate upregulation by AGEs.

Outstanding Questions

Are dietary AGEs directly harmful to the ovaries?

Do changes in lifestyle and dietary composition protect women with PCOS from the harmful effects of AGEs?

Are insulin-sensitizers protective against the proatherogenic effect of AGEs in PCOS?

results. For example, Wistar rats fed high-AGE or low-AGE diets for 6 months showed different androgen levels. In the high-AGE diet group there was elevated AGE deposition in ovarian theca interna cells, increased RAGE staining in granulosa cells, and higher plasma T levels compared to the low-AGE diet group [83]. In addition, female Wistar rats on high-AGE for 3 months showed a significant elevation of plasma T but reduced levels of E2 and P4 compared to rats on low-AGE diet for the same period of time [84]. Thus, this strong association between endogenous/exogenous AGEs and hyperandrogenemia is compelling, and leads to the hypothesis that lowering systemic AGEs, whether by a low-AGE diet or by AGE inhibitors, in women with PCOS could alleviate the symptoms caused by hyperandrogenemia such as hirsutism.

Concluding Remarks

Clinical studies to date have mostly been based on small cohorts of women with PCOS in addition to animal and *in vitro* studies. Our understanding of the action of AGEs in the ovaries of women with or without PCOS is still in its infancy. There is thus a crucial need to investigate the effect of AGEs on folliculogenesis and steroidogenesis. There is also a need for studies pertaining to targeting AGEs and their receptors as potential therapies for metabolic and reproductive alterations in women with PCOS because targeting the AGE–RAGE system could

represent an alternative mechanistic pathway for treating anovulation, and possibly IR and hyperandrogenemia, in women with PCOS.

As of today, with respect to women with PCOS seeking pregnancy, the use of fertility treatments including assisted reproductive technology are costly and are frequently associated with poorer success in women with PCOS compared to women without PCOS because they produce poorer-quality oocytes [85], have a lower fertilization rate [86], and generate poorer-quality embryos [87]. Because observational studies have shown a correlation between serum/follicular fluid AGE levels and adverse IVF outcomes, this raises an important question: could the elevated AGEs in PCOS be responsible for the suboptimal outcome in assisted reproductive technology? Well-designed randomized trials will be necessary to answer this question. These largely underappreciated inflammatory molecules are expected to provide a crucial missing piece to our understanding of PCOS-related reproductive and metabolic dysfunction.

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