Energy metabolism and fertility - a balance preserved for female health.

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Title: ENERGY METABOLISM AND REPRODUCTION, AN ANCESTRAL BALANCE TO BE PRESERVED FOR WOMEN HEALTH

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Running title: Energy metabolism and fertility

Key points:

- Metabolism and reproduction are tightly associated in phylogenesis
- Reproductive disorders cause metabolic alterations
- Hormone replacement therapies should aim to energy metabolism maintenance

Summary

In all female animals energy metabolism and fertility are tightly connected and reciprocally regulated. However, gradual changes occur in their relative influence during evolution: in oviparous, the metabolism predominates to limit the production of eggs in a nutritionally poor environment; in eutherians the burden of sustenance of the embryo and newborn prevails forcing mammalian metabolic pathways to adapt to the reproductive needs. We here review how the mechanisms of interaction between these two fundamental biological functions stratified during phylogenesis in the attempt to explain the etiology of the pathologies of the energy metabolism associated with human ovarian dysfunction and to provide a novel perspective for the design of efficacious hormone replacement therapies.

Introduction

In Western Countries life expectancy of women is not augmenting at the same pace as men and their prospect of unhealthy life is growing[1]. The observation of women's increasing susceptibility to weight gain[2] may partly explain this phenomenon as obesity is a well known risk factor for a large number of pathologies of the metabolic, cardiovascular and skeletal systems[3]; therefore, the understanding of the biological basis for female higher propensity to gain weight is relevant to the definition of appropriate preventive strategies. A recent, interesting hypothesis for women vulnerability to metabolic dysfunctions is that current "obesogenic" milieu may be particularly harmful for organisms whose energy homeostasis has been perfected during evolution to reproduce and nurture the offspring in an environment characterized by food scarcity[2].

In all animal species female fertility and energy metabolism are tightly connected; in women this is substantiated by large epidemiological evidence showing that the cessation of ovarian function is associated with a positive energy balance[4] and the highest prevalence of obesity and overweight is at the time of menopause[4]. A further element indicating a relationship between women reproductive functions and energy metabolism is provided by the observation that pathologies involving dysfunctional ovaries, like polycystic ovary syndrome or Turner syndrome (TS), are generally associated with metabolic disorders[5, 6].

The mechanisms invoIved in the reciprocal interaction between fertility and metabolism are little investigated in mammals, but not in oviparous where remarkable similarities among species demonstrate that, throughout evolution, a strong selective pressure favored the preservation of the molecular machinery coordinating reproductive functions on energy availability (BOX 1). Thus a strategic approach to improve our current understanding of women's physiology could be the study of the connections between reproduction and energy metabolism in the context of evolution. To that end, we here review how, during phylogenesis, the reciprocal interactions among organs for the control of fertility and energy metabolism were organized and we underline the key role played by

molecules like estrogens and insulin-like growth factors (IGFs) in this functional cross-coupling. We also discuss how this novel perspective may challenge current therapeutic strategies and suggest indications for future intervention.

Reproductive functions and energy metabolism: an indissoluble bond in the phylogenesis.

Reproduction in a nutritionally poor environment leads to a competition for food between mother and offspring that may lead to extinction. Hence, mechanisms limiting the activity of female gonads during calorie restriction should have been positively selected during evolution[7]. This is indeed the case as in all oviparous species the synthesis of the egg yolk proteins takes place mainly in metabolic organs[8]: this is a very simple and efficient way to guarantee that egg maturation, and thus reproduction, does not occur in case of energy restrictions.

The most important yolk proteins are vitellogenins (Vg), glycoproteins that provide the embryo with all nutritional reserves indispensable for its development: amino acids (AA), carbohydrates, phosphates, sulphates and molecules such as lipids, hormones, vitamins and metals transported by Vg[9].

Vg are members of a family of large lipid transfer proteins very well conserved from invertebrates to oviparous vertebrates[10] and even present in the mammalian genome (Apolipoprotein B, ApoB)[11]. Vg are synthesized in tissues functionally comparable to liver, thus with a key role in fat storage and mobilization (BOX 1). Given the central function of Vg, the molecular pathways directing their synthesis are extremely well conserved allthrough *phyla* (BOX 1): in the liver-like tissues, stimuli (insulin-like peptides[12], AA and factors related to nutritional signaling such as target of rapamycin, TOR[13]) which originate from the nervous system and locally[12, 14] control Vg production in concert with gonadal hormones (ecdysone and estrogens) signaling the status of egg development and maturation (Fig.1).

Reciprocal regulation of reproductive and metabolic functions in placental organisms.

Energy homeostasis and reproductive functions in mammals.

In mammals and primates severe malnutrition (e.g. in anorexic patients) or allostatic overload (e.g. athletes under strenuous exercise) lead to fertility deficits[15]; thus also in eutherians reproduction is arrested in a nutritionally unfavorable setting, but the mechanisms involved appear to have grown in complexity with respect to oviparous.

In the liver, the estrogen-regulated synthesis of apolipoproteins[16] appears to maintain a role in reproduction as suggested by the observation that a defective hepatic production of very low density lipoproteins (VLDL) causes female sterility[17] and that in ApoB KO mice the formation of the placenta is impaired[18]. Moreover, dietary AA regulate fertility as indicated by experimental studies[19] where it was demonstrated that the synthesis of hepatic IGF-1, essential for the completion of the reproductive cycle, is regulated by AA-dependent activation of liver ERα[20]. In placental animals, on the other hand, a central regulation for energy expenditure and reproduction stratified over the more ancestral mechanisms with peripheral messages converging in brain nuclei responsible for their integration and ultimate allostatic regulation. Several organs in the periphery communicate the nutritional status to the Central Nervous System (CNS): the white adipose tissue with the anorexigenic leptin, secreted in proportion to the amount of body energy (fat) stores[21], the stomach with the production of ghrelin that promotes food intake[22], the intestine with the post-prandial secretion of the polypeptide YY that induces satiety[23], the pancreas with insulin which anorexigenic action overlaps with leptin at the hypothalamic level[24]. All these signals converge into the brain stem and the arcuate nucleus that, with other hypothalamic nuclei, are responsible for their integration for the coordinated regulation of ovulation and energy homeostasis. In particular, we now know that in the arcuate nucleus sensors of the energetic status like cocaine- and amphetamine-regulated transcript (CART)/proopiomelanocortin (POMC) and agouti-related protein (AgRP)/neuropeptide Y (NPY) neurons organize the synthesis of gonadotropins in the pituitary by

controlling the activity of gonadotropin-releasing hormone (GnRH) neurons located in the preoptic area (POA)[25] (fig.2).

Reproductive functions regulating mammalian metabolism.

With placentation and the translation of the burden of nutrition of the embryo and the newborn to the maternal organism, the nature of the connections between the energetic and reproductive *apparati* had to grow to include means to adapt the metabolic activities to the variable energetic requirements of each stage of reproduction (periodic ovulation, pregnancy or lactation)[26]. The necessity to perfect a system where the control of energy metabolism and reproduction is highly reciprocal, may have favored the selection of estrogen receptors (ERs) as the molecular hinge able to join these two functions indissolubly. Indeed, because of their susceptibility to be activated by nutritional signaling molecules (AA, IGF-1) and gonadal hormones (steroids)[20], their ability to regulate the expression of diverse gene programs[26] and their pervasive presence in animal tissues[27], ERs are exceptionally versatile sensors and regulators and thus unique molecules to regulate reproduction in harmony with the environmental resources.

The essential role of estrogens and ERs for reproduction has been long known, but, more recently, experimental evidence pointed to their privileged role in the control of energy metabolism. It is now well accepted that, after systemic loss of estrogens due to surgical[28] and natural menopause[29], the fat mass rapidly increases and changes its distribution; these effects are reversed by the administration of exogenous hormones[28].

The influence of estrogens on energy metabolism occurs at multiple levels. In the CNS, estrogens potentiate or attenuate, respectively, the effect of peptides signaling satiety or hunger (such as cholecystokinin released from the small intestine in response to food ingestion[30] or the gastric hormone ghrelin[31] stimulated by fasting) by acting in the brain stem; in the arcuate and ventromedial nuclei these hormones affect the inputs to POMC and steroidogenic factor 1 (SF-1) neurons[32] repressing the synthesis of orexigenic neuropeptides (such as AgRP and NPY)[33],

moreover they potentiate leptin sensitivity (possibly by increasing the expression of the hypothalamic leptin receptor gene)[34]. The overall effect is to produce an anorexigenic response and to regulate fat distribution (Fig. 2). In the arcuate and anteroventricular periventricular nuclei and in POA, estrogen signaling integrates with inputs from the periphery and, through the kisspeptin neurons, controls the release of GnRH[35] in response to the metabolic status. These effects are mediated mainly by the ERα that is abundantly expressed in the different nuclei of the hypothalamus[36].

In the periphery, ERs are expressed and active in most metabolic organs. In the adipose tissue, the presence of estrogens favors subcutaneous fat deposition in the lower body areas with low lipolytic activity (to provide resistance to long periods of food scarcity needed in case of child bearing or lactation). When estrogen signaling decreases fat redistributes to the visceral areas[29, 37, 38]; this was demonstrated by the study of: *i.*) the consequences of natural or surgical menopause[28, 29], *ii.*) the selective ablation of ERα (ERKO)[27, 37], *iii.*) the analysis of human ERα polymorphisms[39] and *iv.*) in rescue experiments with 17β-estradiol (E2) administration[28]. Studies in adipocytes in culture suggest that estrogens have a direct anti-lipogenic and pro-lipolytic activity by inducing a hormone-sensitive lipase and decreasing the activity of lipoprotein lipase[40, 41], an effect confirmed by the study of fatty acid metabolism in experimental animals[40-42]. The exact contribution of the two ERs in these activities remains to be defined because, while the studies in KO mice would point to ERα as the main actor in fat distribution[37], further studies, mainly in adipocytes, suggest a role for ERβ in E2 anti-lipogenic and anti-adipogenic effects[43].

ERα involvement in the control of lipid metabolism is certainly relevant in liver[26] where this receptor isoform is predominant[27]. Besides controlling the synthesis of proteins for lipid transport, we now know that numerous genes of the cholesterol and fatty acids synthetic pathways are regulated by the hepatic ERα in tight connection with the phase of the estrous cycle or the state of fertility[26]. The oscillation of lipid biosynthesis, induced during the fertile cycle by the fluctuation of

estrogens levels, was demonstrated to be necessary for the maintenance of a healthy fat metabolism: the cessation of the reproductive cycle due to aging, ovariectomy or ER liver inactivity, was shown to be associated with the formation of hepatic fat deposits[26]. These observations led to the suggestion that the changes of estrogen metabolism, that accompany and mark each stage of woman reproductive activity, might be instrumental to stimulate liver ERα to produce the energy molecules necessary to satisfy the variable needs of reproductive functions[26].

ERs are expressed in all tissues relevant for glucose metabolism, including muscle and liver[27]; but to date, the most studied was estrogen action in pancreas where its important protective effect has been known for decades[44] and include: *i.*) an antiapoptotic action of estrogens in the β-cells[45], *ii.*) the repression of lipid biosynthesis and accumulation in β-cells preventing their lipotoxic failure[46], *iii.*) a direct stimulation of insulin biosynthesis[47]. This last effect may be important in late pregnancy when estrogen high levels may synergize with prolactin to promote β-cells insulin production to meet the increased metabolic demand[48].

Reproductive dysfunctions are associated with metabolic alterations.

Menopause: a jam in a metabolism geared to reproduction.

Considering the prominent role of mammalian reproductive functions on energy homeostasis it is not surprizing that the cessation of ovarian functions entails the manifestation of metabolic disorders. Indeed, the post-menopause is characterized by women increased vulnerability to a large number of pathologies including disorders of the cardiovascular, skeletal, immune and nervous systems[49]. How climacterium may trigger the onset of such a diversity of pathologies remains to be clarified; it could be hypothesized that the declining levels of circulating estrogens, by weakening ER activities, cause subtle alterations of energy metabolism in multiple tissues which, in time, with a *domino* effect, result in overt pathology: because of the variety of organs interested in the phenomenon, for each woman the risk to develop a specific disease would be relative to her individual predisposition and health status.

Menopause is associated with increased body weight, lower lean mass[50] and abdominal accumulation of fat[29, 51]. The study of adipose tissue demonstrated a reduced expression of enzymes for fat turnover (e.g. acetyl-CoA carboxylase 1, long-chain-acyl-CoA dehydrogenase, hormone sensitive lipase)[52] and increased insulin resistance (IR), possibly prompted by the non-physiological fatty acids deposits[53], high circulating free fatty acids (FA) and the reactive oxygen species (ROS) produced by mitochondrial β-oxidation of FA[54]. Macrophages are recruited by the fat mass[55] and, in concert with adipocytes, secrete pro-inflammatory factors[56-58]. FA accumulate also in the liver facilitating hepatic steatosis[59], a feature of metabolic syndrome quite diffused in post-menopausal women, which further contributes to a pro-inflammatory response. Furthermore, in liver the cessation of estrogenic control on the metabolism of cholesterol, fatty acids[26] and lipoproteins causes a decreased production of HDL2, large, antiatherogenic lipid particles[60] and an increase of small atherogenic HDL3[60, 61], total cholesterol, LDL, triglycerides (TG)[61, 62], ApoB and ApoB containing lipoproteins and Lp(a)[63] (a complex of a LDL-like particle and ApoA)[61] contributing to the establishment of an atherogenic lipid profile and to the increased risk of cardiovascular disease. After menopause, the function of skeletal muscle is impaired as well with a loss in muscle strength and mass, an effect reversed by HRT[64]; additionally, the decreased expression of the glucose transporter GLUT-4[65] associated with impaired muscle ERα activity and the generalized pro-inflammatory status, may participate to increase the risk of IR typical of the post-menopause.

Another important element for the understanding of the pathologies associated with climacterium is the loss of estrogens anti-inflammatory action in circulating monocytes and in microglia[66, 67]. This element, together with the altered lipid transport due to reduced production of ApoE, may participate to the onset of brain neurodegenerative pathologies such as Alzheimer's disease which incidence is low at age of 50, but increases with age with a prevalence significantly higher in women[68].

The pro-inflammatory state and increased levels of circulating cytokines (interleukin-1 and 6 and tumor necrosis factor-α) have a significant repercussion in other organs like in bone where it participates to the decreased mineral density and increased osteoclast number[69].

Menopause and NAFLD: the protective role of estrogens on the hepatic manifestation of metabolic syndrome.

Non-alcoholic fatty liver disease (NAFLD) is a metabolic condition that refers to a wide spectrum of liver damage, ranging from simple steatosis through non-alcoholic steatohepatitis (NASH) to cirrhosis, liver failure and hepatocellular carcinoma. NAFLD is characterized by hepatic lipid accumulation (5 to 10% per weight) due to IR in liver[70], increased lipogenesis[70] and reduced secretion of triglycerides[71]. A deficient suppression of lipolysis in the adipocytes[52] further contributes to the formation of lipid deposits in liver. The consequences of the altered FA metabolism are impairment of mitochondrial FA oxidation[72], up-regulation of the peroximal β-oxidation and microsomal ω-oxidation, with production of lipotoxic lipid intermediates, over-production of ROS and induction of a pro-inflammatory response that further contributes to progression of NAFLD and IR[73].

NAFLD occurs at all ages and ethnic groups with a prevalence of about 30%[74]; NAFLD is more common in men (2-3.5 fold higher than in women)[75, 76], however, following menopause its incidence increases significantly becoming prominent in women[77]. HRT decreases the risk of steatosis[78] and the prevalence of NAFLD in the post-menopause [59]. These and other data, including the association of NAFLD with altered ovarian function like in Turner syndrome[79], suggest that estrogens protect from the development of NAFLD. The exact aetiology of the pathology is not well known, certainly the lack of estrogens plays a role causing: *i.*) loss of inhibition of liver *de novo* fatty acids synthesis[26, 80], *ii.*) decreased export of lipids from the liver in very-low-density lipoproteins[77, 81], *iii.*) reduced fatty acid oxidation[77, 80]. Supporting this view are studies in rodents with surgical menopause where estrogens prevented hepatic fat accumulation by: *i.*) inhibiting the

expression of genes involved in lipogenesis as sterol regulatory element-binding proteins 1c (SREBP-1c), peroxisome proliferator-activated receptor γ (PPARγ), stearoyl-CoA desaturase 1 (SCD-1), acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS)[26, 80], *ii.*) facilitating the export of lipids from the liver in VLDL, by increasing the hepatic VLDL-TG production and microsomal triglyceride transfer protein (MTTP) expression[77, 81], *iii.*) sustaining the β-oxidation of fatty acids by inducing PPARα[80].

Polycystic Ovarian Syndrome: the ovarian manifestation of metabolic syndrome.

NAFLD is often associated with Polycystic Ovary Syndrome (PCOS)[82], the most common endocrine disease affecting up to 10% of women of reproductive age[83]. PCOS is characterized by hyperandrogenism, chronic anovulation and polycystic ovaries[84]; additionally metabolic disturbances are often observed: 50% of PCOS women is overweight or obese[85] and dyslipidemia is commonly observed (increased levels of LDL and decreased levels of HDL)[86]; in USA 39% of women with PCOS have hepatic steatosis[82] and 50% of have IR and metabolic syndrome[5]. Indeed, IR is the metabolic disorder that most correlates with PCOS[87] and the increased insulin levels may explain the pathogenesis of the syndrome.

The cause of anovulation in PCOS patients has not been clearly identified, possibly involves the increase in GnRH pulse frequency, originated by an intrinsic abnormality of the GnRH pulse or by the relative low levels of progesterone augmenting LH release from the pituitary and ovarian androgen production[88]. Insulin sinergizes with LH to stimulate androgen synthesis by the ovarian theca cells[89]; this further emphasizes the tight interconnection between metabolic and reproductive disturbances in this syndrome. Additionally, metabolic disorders such as obesity worsen PCOS clinical and biochemical manifestation: overweight contributes to hyperandrogenism, insulin resistance and dyslipidemia[85, 89] and a reduction in body weight, recommended in overweight patients, ameliorates PCOS metabolic and reproductive features significantly[85].

The oral contraceptive (OC) pill represented the core PCOS therapy for several years: the combination of estrogen and progestins ameliorates hirsutism, acne and oligoamenorrhea[89, 90]. Additionally, estrogens, possibly through the reduction of abdominal fat deposits, were proposed to counteract hyperinsulinemia worsening with aging[91]. Concerns on the use of OC in PCOS patients were raised by the observation of increased TG and cholesterol levels[92]: a phenomenon not in line with HRT experimental studies and with the notion that endogenous E2 protects against dyslipidemia[61], thus suggesting that more studies should be carried out to clarify the extent to which the negative effects observed were due to the nature of the estrogenic compound used (ethynil estradiol) or to its dosage and route of administration.

The use of metformin, an antidiabetic agent, was demonstrated to be effective in the restoration of ovarian function in metabolic compromised women, indeed metformin is not only able to reduce insulin levels but can directly stimulate ovarian steroidogenesis[93]; however, the reproductive outcomes of this therapy seem to be limited[94]. Recently, Hoeger et al. demonstrate that the use of metformin and OCs in combination are beneficial for both the reproductive and metabolic sympthoms of the syndrome[90]. This observation, in line with the theory that gonadal hormones are essential for a well balanced metabolism, requires additional studies to confirm its efficacy in a statistically significant number of patients[90].

Turner Syndrome: a genetic hypoestrogenic condition and a unique metabolic defect.

TS is a common genetic disorder affecting 1 in 2500 live born females[95] caused by total or partial absence of chromosome X. The most common features of TS are infertility due to gonadal dysgenesis, short stature[96], dysmetabolism, webbed neck and other physical abnormalities[97]. The reduced dosage of those X-linked genes which in normal females escape X-chromosome inactivation and are functionally diploid is proposed to be the cause of most of the TS features[98], but candidate genes are still being identified[99].

An altered energy metabolism characterizes TS. The common clinical features of TS patients are obesity[100], low lean body mass and increased body mass index, waist circumference[101] and adipose visceral tissue[79]; triglycerides and LDL are elevated, HDL decreased[102]. TS patients have also smaller lipid particle size[102] and the degree of difference in lipid levels and lipid particle size between 46XX and 45X women is very similar to the well documented differences of these parameters in men and women[61]. Haploinsufficiency of X chromosome genes involved in lipid metabolism is probably the cause, in agreement with the fact that men show a more atherogenic profile than women. 80% of TS patients show abnormal liver function[103], accordingly TS is associated to increased intracellular hepatic lipids[79, 103] and liver enzymes[104], elevated incidence of NAFLD[79] and cirrhosis[101], hyperplasia[105] and inflammation[105]. Hepatic abnormalities seem to be generated by the lack of E2 production as E2 treatment has a protective role[106]. In addition, impaired glucose homeostasis is common[100, 101] and the increased risk of diabetes[101, 104] is cause of mortality in 25% of TS patients[107]. Considering that the lipid profile is overall more atherogenic compared with age-matched healthy controls or even with karyotypically normal women with premature ovarian failure (POF)[102, 108] it is not surprizing that TS women have a 7-fold augmented risk of mortality due to ischemic heart disease[100].

Although TS patients recapitulate many features of metabolic syndrome, paradoxically, they have lower fasting glucose and insulin and decreased leptin levels, despite of the high visceral fat, even when compared with women with POF[109]. It has been hypothesized that the low levels of insulin are probably due to defective β cell secretory function[110] or to a glucose storage defect[111] and that the decreased levels of leptin are probably due to low insulin fasting levels[112].

The current guidelines for TS treatment indicate growth hormone (GH) treatment and introduction of estrogenic therapy at 12-14 yrs[113]. TS patients need high doses of GH for correct development as they are resistant to the metabolic effects of GH[6, 114]. GH treatment contributes to decrease adiposity and abdominal fat and to increase lean mass and circulating IGF-1[6]. The HRT is necessary for

feminization, normalization of bone mineral density and improvement of neurocognitive functions[97], but estrogens have important effects also on TS metabolic derangements by decreasing visceral adipose tissue[6] and increasing HDL levels[100], but their prominent beneficial role is maintaining normal liver metabolism[106]. Indeed, E2 is able to improve liver function[106] and to regulate the release of growth and survival factors which protect hepatocytes and promote their proliferation[115].

Is there any therapy for the appropriate regulation of energy metabolism in case of ovarian failure?

Estrogens are key players in the mutual regulation of reproductive and metabolic functions which appear to be adapted to the harmonics of the reproductive cycle with a fluctuating production of the metabolites relevant for energy deposit or utilization[26]. We still lack the comprehension of the exact consequences of the termination of ovarian activity and the oscillation of metabolic functions. Experimental and clinical observations show that ovariectomy disrupts liver tetradian activation of ER[26] and induces a rapid disorganization of lipid metabolism with accumulation of hepatic fat deposits[26, 59]; likely other organs undergo similar functional changes upon decreased estrogen signaling. It is well known that functional deteriorations leading to the onset of specific pathologies (cancer, immune, neuropsychiatric, cardiovascular and cerebrovascular diseases) are observed with the desynchrony of the circadian rhythm generated by the clock genes and proteins regulating sleep and wakefulness, body temperature, blood pressure, digestive secretion, immune responses and metabolism[116-120]. In analogy with these findings, it is highly plausible that the lack of efficacy of HRTs applied so far is caused by their inability to reinstate the oscillatory activity of the ER in sexually mature animals[20] and the consequent reciprocal control of the genes regulating fertility and energy metabolism.

HRT to date has aimed at keeping hormone levels constant: however, in the different organs, a rhythmic and harmonic modulation of ER may be instrumental for the activation of large

transcriptional programs necessary to maintain energy homeostasis. This is also suggested by experiments where it was demonstrated that the hepatic ER associates with very distinct classes of promoters during the different phases of the estrous cycle[26]; this may be necessary to poise the receptor for the rapid selection of a transcriptome most suited for the production of the energy required in each stage of the reproductive physiology.

It remains to be established which are the harmonics of ER activation to be re-established for an efficacious HRT. Previous studies from our laboratory indicated that the use of SERMs or combination of natural hormones and SERMs, may have a significant effect on the relative phasing and intensity of ER activity in the target organs; this prospects the possibility to reproduce pharmacologically the desired complexity of ER action in the whole organism. What is lacking at present time is a clear view on the pattern of ER activity which would have the most favorable effects for women health during aging. In the absence of such knowledge, we believe that the mere analysis of the effects of HRTs on a single parameter (e.g. effect on the period or amplitude of ER activity in different organs) is not sufficient to establish the superiority of a treatment over others. Specific methodologies and algorithms for the comparative analysis of multivariate parameters of the activity of synthetic ER ligands should be developped as also suggested by the work of Rando et al[121].

Conclusions.

In face of the difficulties of current HRT to reinstate the lower morbidity of skeletal, cardiovascular and metabolic diseases typical of women in their fertile age, a better understanding of the exact physiological role of estrogens is mandatory. The aim of this review was to underline the indissoluble link between energy metabolism and reproduction in the effort to provide a more comprehensive view of the role of estrogens in mammalian physiology facilitating the identification of novel therapeutic targets. The view of a strict association between energy production and

reproductive activity suggests that the main goal of HRT should be the restoration of the metabolic functions characteristic of cycling women.

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BOX 1. Vitellogenin synthesis, a highly conserved mechanism in the phylogenesis.

In C. *elegans*, Vg are synthesized in the intestine, the organ responsible for food digestion and nutrients uptake[122] and are secreted in the body cavity to reach the gonads [123] where a sperm-dependent hormone stimulates its uptake. The main stimulus for Vg synthesis is DAF-28 (the hortologue of mammalian Ins/IGF-1) produced by neural cells in response to AA ingestion and gonadal hormones[12]. In insects, Vg synthesis takes place mainly in the fat body[8], a metabolic organ comparable to vertebrate liver[124] and is regulated by the juvenile hormones (JH, the insect gonadotropins) produced in the *corpora allata* and by ecdysone, a cholesterol-derived hormone, synthesized upon JH stimulation[14]. As for nematodes, the process is initiated by regulating the synthesis of insulin-like-peptides by nutritional AA and, consequently, JH production[125]. Once synthesized, Vg are secreted in the hemolymph and reach the gonads where the cell uptake occurs through the Vg receptor. The AA present in the hemolymph, together with ILPs and ecdysone, control vitellogenesis[13]. The study of anautogenous species in which reproduction is triggered by feeding clearly pointed to the involvement of TOR in JH and Vg biosynthesis[126]. In crustaceans the hepatopancreas is the site of Vg synthesis[127] that is triggered by neural and endocrine factors including ecdysone, estrogen and progesterone[127]; little is known about the control of nutrition in these species. In vertebrates such as amphibians, fishes and birds, Vg are principally synthesized in the liver where vitellogenesis is controlled by gonadal hormones (estrogen) both directly and indirectly through brain gonadotropins[128-130]. Nutritional AA have a direct influence on Vg synthesis through the IGF-1/insulin/Foxo signaling pathway[131, 132]. Once secreted by the liver in the blood stream, the Vg reach the gonads where a member of the Vg/LDL receptor family facilitates their uptake[133].

Legends to figures.

FIGURE 1

Nutritional AA as regulators of reproductive functions throughout evolution.

In invertebrates such as nematodes and insects, AA act in neural cells to regulate the synthesis of insulin-like-peptides (DAF-28 and ILPs) which, through the body cavity or the circulation, reach the liver where they modulate the production of Vg, by activating the ILP receptors, and therefore egg maturation. Effect of AA in vertebrate brains are not well investigated. Gonadal hormones (ecdysone in insects) participate in the regulation of liver production of Vg. Gonadotropins (juvenile hormones in insects) produced by neural cells intervene in the regulation of gonadal activities. In mammals the molecular mechanisms identified in lower *phyla* and based on the interaction of ILP (IGF-1), gonadotropins and gonadal estrogens are maintained in spite of an accrued complexity.

FIGURE 2

Role of ER in hypothalamic circuits regulating energy metabolism and reproduction.

Estrogens inhibit food intake by acting mainly on ERα expressed on CART/POMC neurons that signal to second order neurons in the LH and PVN and to orexigenic AgRP/NPY neurons in the arcuate (Arc). Estrogen action on SF1 neurons in the ventromedial hypothalamus (VMH) is needed for the regulation of energy expenditure and fat distribution. Thus ERα in the Arc is a critical regulator of food intake while ERα in the VMH plays a pivotal role in the regulation of energy expenditure. Completing the loop, CART/POMC and SF1 neurons signal the energetic status to GnRH neurons that are responsible for the pituitary release of gonadotropins, thus controlling reproductive functions by regulating plasma estrogen levels. Median Eminence (ME).[32]

FIGURE 3

The reciprocal regulation of energy metabolism and reproduction.

Reproduction and energy metabolism are under a tight reciprocal control in physiological conditions to guarantee a metabolic status finely tuned on reproductive needs. The alteration of ovarian functions that characterize menopause as well as other endocrine disorders determines a dysregulation of metabolic pathways which may lead to the development of obesity, MetS, diabetes or NAFLD. Conversely, the alteration of energy metabolism may further impair ovarian activity leading to a feed forward loop.

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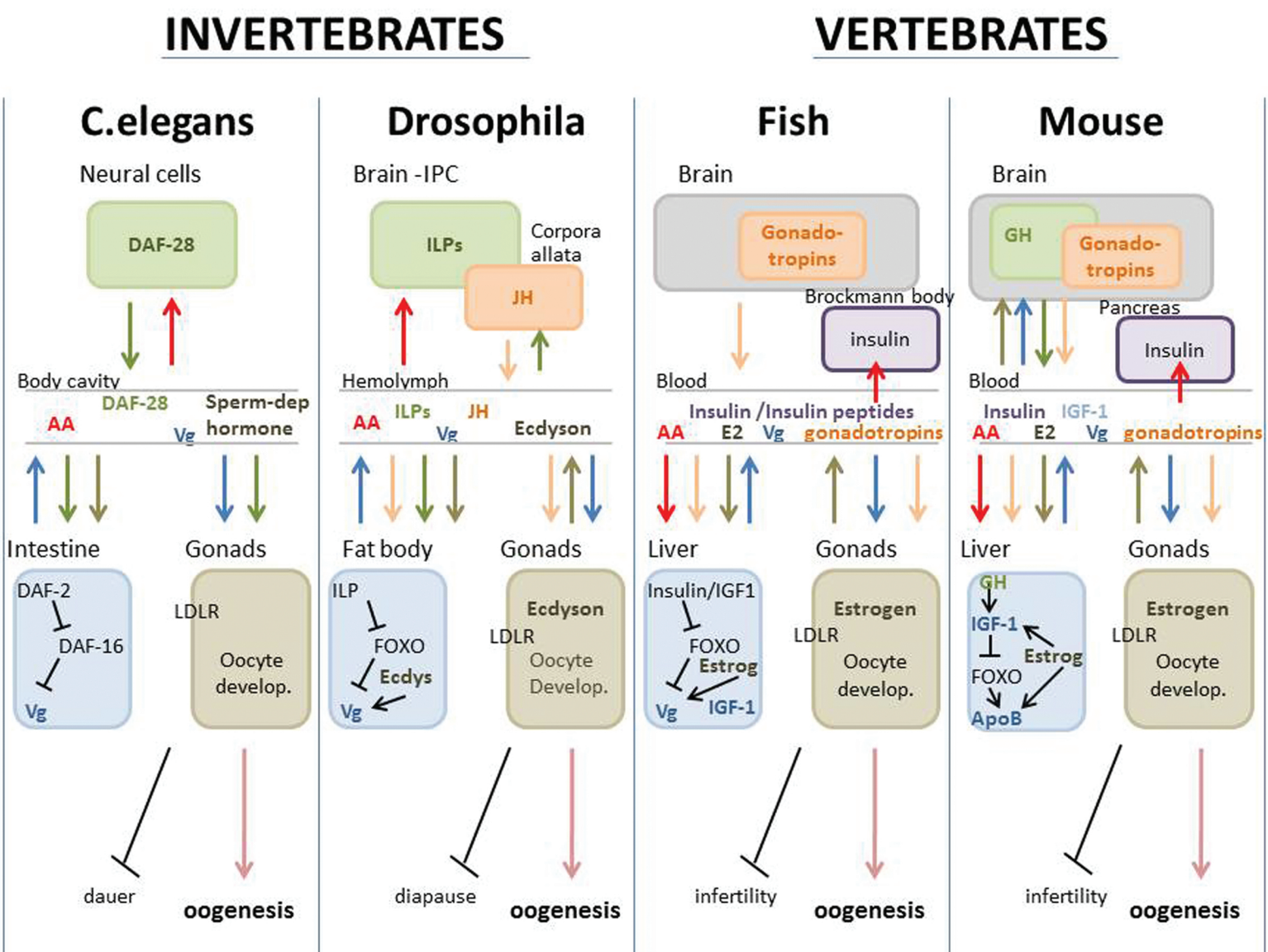
INVERTEBRATES
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Vg
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Oocyte develop.
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JH
Vg
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Gonads
ILP
FOXO
Ecdys
Vg
Ecdyson
LDLR
Oocyte Develop.
diapause
oogenesis
Fish
Brain
Gonado-tropins
Brockmann body
insulin
Blood
Insulin /Insulin peptides
AA
E2
Vg
gonadotropins
Liver
Gonads
Insulin/IGF1
FOXO
Estrog
Vg
IGF-1
Estrogen
LDLR
Oocyte develop.
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Mouse
Brain
GH
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Pancreas
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Blood
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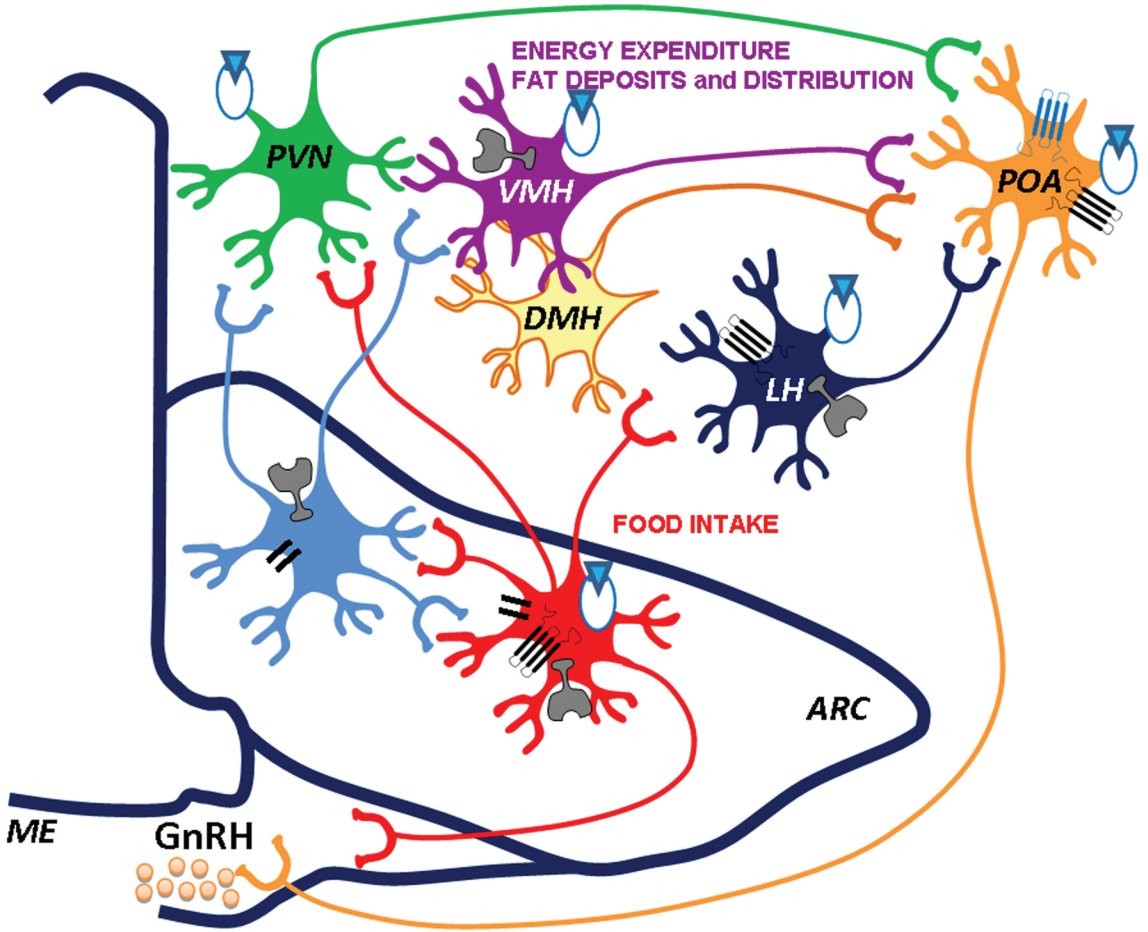


AgRP/NPY neuron

Kisspeptin neuron

GnRH neuron

POMC/CART neuron

SF1 neuron

Second order neuron

Y1 receptor

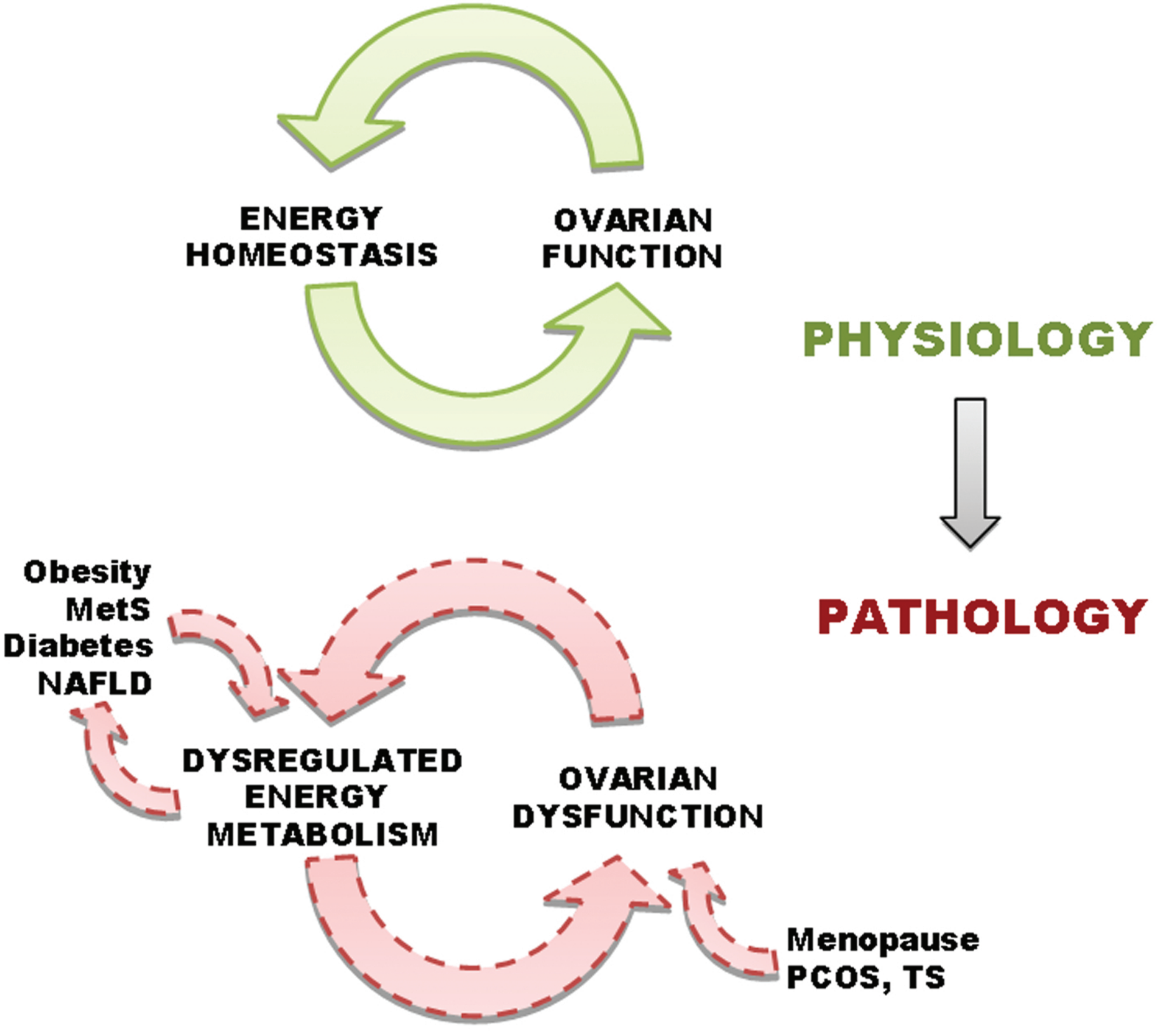
Leptin receptor

Kisspeptin receptor

Insulin receptor

Estrogen receptor

Estrogen


ENERGY HOMEOSTASIS
OVARIAN FUNCTION
PHYSIOLOGY
PATHOLOGY
Obesity
MetS
Diabetes
NAFLD
DYSREGULATED ENERGY METABOLISM
OVARIAN DYSFUNCTION
Menopause
PCOS, TS