Energy metabolism and fertility—a balance preserved for female health

Sara Della Torre, Valeria Benedusi, Roberta Fontana and Adriana Maggi

Abstract | In female animals, energy metabolism and fertility are tightly connected, and reciprocally regulated. However, the relative contributions of metabolic and reproductive pathways have changed over the course of evolution. In oviparous animals, metabolic factors take precedence over fertility, enabling egg production to be inhibited in a nutritionally poor environment. By contrast, in placental mammals, the opposite occurs: the need to feed a developing embryo and neonate forces metabolic pathways to adapt to these reproductive needs. This physiological necessity explains why in female mammals alterations of gonadal activity, including age-dependent cessation of ovarian functions, are associated with a disruption of metabolic homeostasis and consequent inflammatory reactions that trigger the onset of metabolic, cardiovascular, skeletal and neural pathologies. This Review discusses how metabolic homeostasis and reproductive functions interact to optimize female fertility and explains the pathogenic mechanisms underlying the disordered energy metabolism associated with human ovarian dysfunction owing to menopause, polycystic ovary syndrome and Turner syndrome. Finally, this article highlights how hormone replacement therapy might aid the restoration of metabolic homeostasis in women with ovarian dysfunction.

Della Torre, S. *et al. Nat. Rev. Endocrinol.* **10**, 13–23 (2014); published online 22 October 2013; doi:10.1038/nrendo.2013.203

Introduction

Although women have a greater life expectancy than men, the gap is narrowing.[1] From 1990 to 2011 the difference in life expectancy between men and women dropped from an average of 6.61 to 4.67 years in European countries and from 7 to 5 years in the USA.[2] The fact that women develop an increased susceptibility to weight gain as they age[3] could potentially contribute to this phenomenon, because obesity is a well-known risk factor for a large number of metabolic, cardiovascular and skeletal disorders.[4] Understanding the biological basis of weight gain in ageing women is, therefore, relevant to define appropriate strategies to combat obesity in this population.

One interesting hypothesis suggests that the female-specific propensity to weight gain (and, thereby, to metabolic dysfunction) might be the consequence of adaptations that enable reproduction and nurturing of offspring in food-scarce environments.[3] Uncontrolled reproduction in a nutritionally poor environment would lead to competition for food between mothers and their offspring, which ultimately might result in extinction of the species. Female fertility and energy metabolism are, therefore, tightly interconnected; during the reproductive period of life, the physiological activity of the gonads, with their cyclic production of sex hormones, ensures a continuous regulation of food intake and energy expenditure. However, with the cessation of ovarian function, gonadal control over energy metabolism decreases, with negative consequences. In women, weight gain and obesity are most prevalent around and after menopause.[5] Moreover, pathologies that involve ovarian dysfunction, such as polycystic ovary syndrome (PCOS) and Turner syndrome, are generally associated with metabolic disorders.[6,7] Thus, an obesogenic milieu might be particularly metabolically harmful in species adapted to survive in nutrient-poor environments.

The molecular mechanisms involved in the interactions between fertility and metabolism have been little investigated in mammals. However, the remarkable similarities in these mechanisms among oviparous species demonstrate that strong selection pressure has favoured the preservation of pathways that coordinate reproductive functions with energy availability. Indeed, mechanisms that limit female gonadal activity during times of calorie restriction should have been positively selected for in all species during evolution.[8] An appreciation of the role of evolution in shaping these reproductive and metabolic pathways might, therefore, improve our understanding of human female physiology.

In this Review, we discuss how the reciprocal interactions between pathways that control fertility and energy metabolism are organized and underline the key roles of molecules such as estrogens and insulin-like growth factors (IGFs) in these pathways. We also discuss how this novel perspective could challenge current therapeutic strategies—amelioration of metabolic disorders, for example, might become an important goal of hormone replacement therapy (HRT), particularly in ageing women.

Department of Pharmacological and Biomolecular Sciences (**S. Della Torre, V. Benedusi**), Centre of Excellence on Neurodegenerative Diseases (**A. Maggi**), University of Milan, Via Balzaretti 9, 20133 Milan, Italy. Department of Drug Discovery and Development, Italian Institute of Technology, Via Morego 30, 16163 Genoa, Italy (**R. Fontana**).

Correspondence to: A. Maggi
adriana.maggi@unimi.it

Competing interests
A. Maggi declares an association with the following company: Pfizer. See the article online for full details of the relationship. The other authors declare no competing interests.

Key points

- Metabolic and reproductive pathways are tightly associated, and this relationship has been conserved throughout evolution
- Reproductive disorders can lead to changes in metabolic function
- Similarly, metabolic disorders can underlie changes in reproductive function
- Hormone replacement therapy for reproductive disorders might also have beneficial effects on energy metabolism

Nutritional status—links to fertility

Mechanisms in oviparous species

In oviparous species, the most important yolk proteins are vitellogenins, a family of large glycoproteins that provide nutrients (such as amino acids, carbohydrates, phosphates and sulphates) to the embryo, as well as lipids, hormones, vitamins and metals.[9] Synthesis of vitellogenins takes place in metabolic organs that are functionally comparable to liver.[10] Vitellogenins also have a key role in fat storage and mobilization; thus, egg maturation ceases when energy availability is restricted. Moreover, vitellogenins and the molecular pathways directing their synthesis are well-conserved across all oviparous species, from invertebrates to vertebrates.[11] In liver-like tissues, stimuli from local sources and the nervous system (such as insulin-like peptides,[12] amino acids and nutritional signalling factors, such as target of rapamycin[13]),[12,14] control vitellogenin production in concert with gonadal hormones (ecdysone and estrogens) to signal the state of egg development and maturation (Figure 1).

Mechanisms in placental mammals

In mammals, including primates, severe malnutrition and allostatic overload reduce fertility.[15] Reproduction is still arrested in nutritionally unfavourable settings, but the mechanisms involved have increased complexity compared with those in oviparous species.

In the mammalian liver, estrogen-regulated synthesis of apolipoproteins[16] seems to have a role in maintaining reproductive capacity, as defective hepatic production of VLDL leads to female sterility.[17] Interestingly,

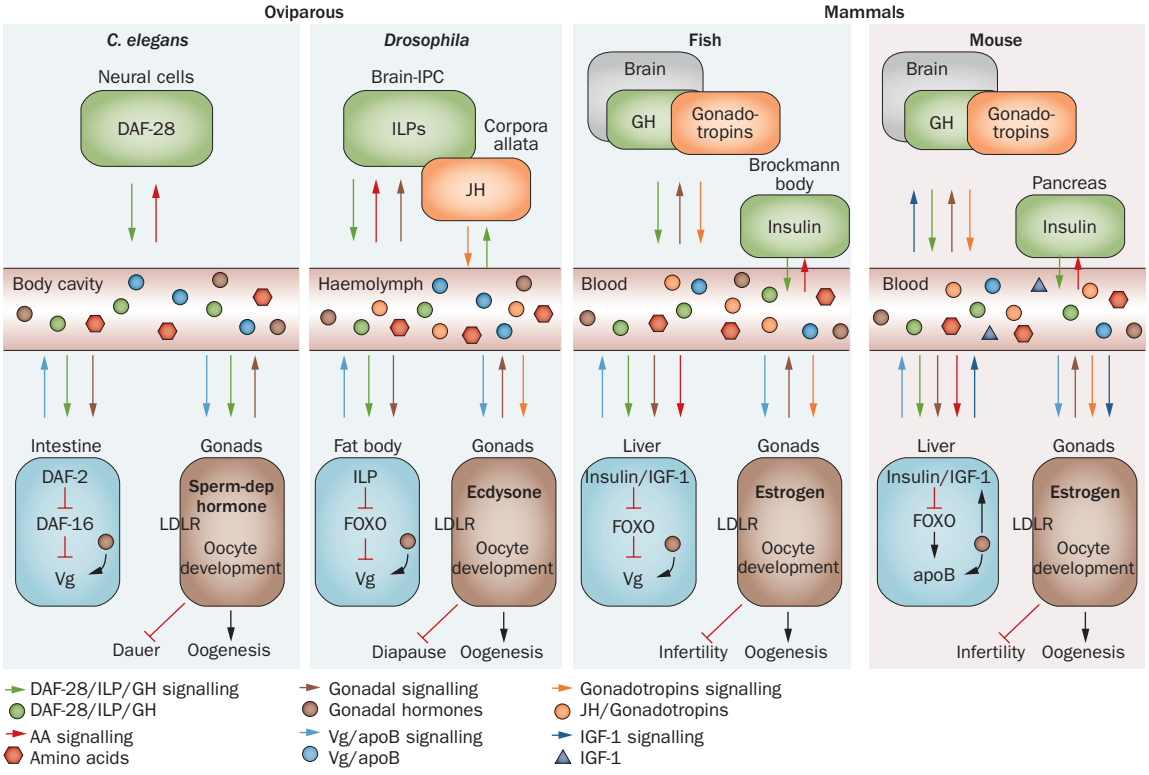

Figure 1 | The mutual control of nutritional status and reproduction throughout evolution. In oviparous animals, vitellogenin synthesis takes place in the organ functionally comparable to the mammalian liver,[9] and is regulated by nutritional cues together with gonadal hormones (such as ecdysone or estrogens).[13] Once synthesized, these lipid transport proteins are secreted into the circulatory system to reach the gonads where they regulate the maturation of the egg. Vitellogenins and the proteins they transport provide the embryo with energy necessary for its development. In all oviparous animals, therefore, nutritional cues have a direct control over reproduction regulating oogenesis, or inducing infertility or a dormant fertility state (dauer and diapause). In placental mammals, the liver regulates gonadal function through the synthesis of the lipid transport protein apoB (a member of the vitellogenin family) and the synthesis of IGF-1.[144–146] Estrogens, together with dietary intake, regulate liver production of both apoB and IGF-1. In addition, estrogens have a strong influence on the activity of all organs relevant for energy metabolism and provide the embryo with the energy molecules necessary for its growth. In mammals the control of energy metabolism on reproduction, typical of oviparous animals, is maintained, but reproductive functions become the key driver of metabolic functions. Abbreviations: AA, amino acids; apoB, apolipoprotein B-100; E2, estradiol; GH, growth hormone; IGF, insulin-like growth factor; ILPs, insulin-like peptides; IPC, insulin-producing cells; JH, juvenile hormones; LDLR, LDL receptor; Sperm-dep, sperm dependent; Vg, vitellogenin.

apolipoprotein B-100 (apoB100) contains a vitellogenin domain,[18] and placenta formation is impaired in *apoB*-knockout mice.[19] Moreover, dietary intakes of amino acids can also regulate fertility.[20] Hepatic synthesis of IGF-1, which is essential for the reproductive cycle, is regulated by amino-acid-dependent activation of estrogen receptor (ER) α in the liver.[21] These mechanisms resemble those in oviparous species. However, in placental animals, the central regulation of energy expenditure and reproduction takes precedence over these ancestral, nutrition-based, mechanisms of fertility control. Peripheral messages converge in the brain nuclei responsible for their integration and for production of the efferent signals that ultimately control allostatic regulation in the organism. Several tissues and organs communicate nutritional status to the central nervous system. White adipose tissue secretes the anorexigenic hormone leptin in amounts proportional to the amount of energy stored as fat.[22] The stomach produces the orexigenic hormone ghrelin.[23] The intestine secretes peptide YY in response to food intake, which induces satiety.[24] Finally, the pancreas releases insulin, which has a similar action to leptin in the hypothalamus.[25] All these signals converge and are integrated in the brainstem and arcuate nucleus which, together with other hypothalamic nuclei, regulate both ovulation and energy homeostasis. For example, sensors of energy status in the arcuate nucleus—such as cocaine and amphetamine-regulated transcript (CART)–pro-opiomelanocortin (POMC) neurons and agouti-related peptide (AgRP)–neuropeptide Y (NPY) neurons—direct the synthesis of gonadotropins in the pituitary by regulating the activity of gonadotropin-releasing hormone (GnRH) neurons located in the preoptic area (Figure 2).[26]

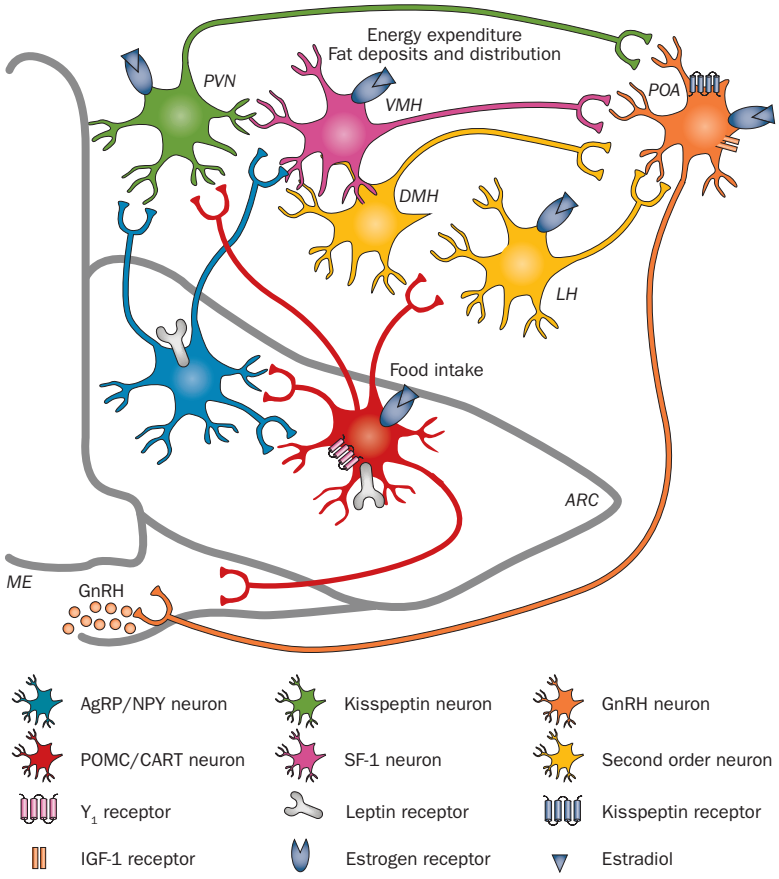

Figure 2 | ERα in hypothalamic circuits regulates energy metabolism and reproduction. Estrogen inhibits food intake via ERα signalling on POMC/CART neurons (red) that signal to second-order neurons (yellow) in the lateral hypothalamus, to kisspeptin neurons (green) in the paraventricular nucleus and to orexigenic AgRP/NPY neurons (blue) in the arcuate nucleus. Conversely, AgRP/NPY neurons, when activated, inhibit the POMC-CART neuron possibly through the Y_1 receptor. Estrogens act on SF-1 neurons (purple) in the ventromedial hypothalamus to regulate energy expenditure and fat distribution. ERα in the arcuate nucleus is a critical regulator of food intake, whereas ERα in the ventromedial hypothalamus regulates energy expenditure.[39] Finally, POMC/CART (through kisspeptin and second-order neurons) and SF-1 neurons signal energetic status to GnRH neurons (orange) that are responsible for the pituitary release of gonadotropins, thereby regulating plasma estrogen levels. AgRP/NPY and POMC/CART neurons express receptors for leptin that allow them to be sensitive to the amount of energy stored in fat. Abbreviations: AgRP, agouti-related protein; CART, cocaine and amphetamine-regulated transcript; DMH, dorsomedial hypothalamus; ERα, estrogen receptor α; GnRH, gonadotrophin releasing hormone; LH, lateral hypothalamus; ME, median eminence; NPY, neuropeptide Y; POA, preoptic area; POMC, pro-opiomelanocortin; PVN, paraventricular nucleus; SF-1, steroidogenic factor-1; VMH, ventromedial hypothalamus.

In placental mammals, the nutritional burden associated with development of the embryo and growth of the offspring has been transferred to the mother. Consequently, the regulatory mechanisms linking energy availability and reproductive function had to be adapted to take into account the variable energy demands of each stage of the reproductive cycle (periodic ovulation, pregnancy and lactation).[26] Thus, the need to have highly reciprocal control of both energy sensing and reproduction might have favoured the selection of ERα and ERβ as the nexus through which these two functions are linked. Indeed, these receptors can be activated by nutritional signalling molecules (such as amino acids and IGF-1), as well as gonadal hormones,[21] and can regulate expression of a diverse range of genes.[26] ERα and ERβ are exceptionally versatile sensory and regulatory effectors that are widely expressed in mammalian tissues.[27]

The essential role of estrogens and their receptors in reproduction has long been known. However, emerging evidence suggests that estrogen signalling also has a central role in the control of energy metabolism. The concentration of various circulating estrogen metabolites such as estradiol, estrone, estriol and their ratio depends on fertility status.[28] The exact role of each of these metabolites is unclear; therefore, in this Review, the term estrogens refers to all estrogenic compounds. The major naturally occurring estrogens in women are estrone, estradiol (which has the most potent effect on ERα and ERβ), and estriol (which has the least potency). In addition, pregnant women produce estetrol. Estradiol is the predominant estrogen produced during the reproductive years (mean levels of which rise from a nadir of 106.5 ± 25.7 pmol/l of plasma during menstruation, to a peak of 1167.4 ± 99.1 pmol/l of plasma around mid-cycle). Changes in estrone concentrations follow a similar, although less marked, pattern (from a nadir of 148.0 ± 14.8 pmol/l during menstruation, to a peak

of 628.8 ± 48.1 pmol/l at mid-cycle).[29] With menopause, estradiol levels decrease markedly, although estrone levels remain comparable to those observed in fertile women. The transcriptional programme regulated by ERα and ERβ might be modulated in response to changing levels of circulating hormones, which characterize the different stages of female fertility.[26,30,31] Accordingly, the set of genes controlled by ERα and ERβ might substantially change in response to different concentrations and ratios of hormones. Hence, in the same target tissue, the intracellular activity of these receptors might vary at each reproductive stage or during the estrous cycle, leading to an alternate activation of pathways that control energy metabolism.[26]

After loss of estrogen production due to ovariectomy[32] or menopause,[33] adipose tissue mass rapidly increases, and its distribution changes. These effects can be reversed by the administration of HRT.[32] The influence of estrogens on energy metabolism is believed to be largely mediated by ERα. Mice lacking ERα have increased body weight and food intake compared with wild-type littermates,[34] and administration of ERα-selective agonists (but not ERβ-selective agonists) has anorexigenic effects in these mice.[35,36] Interestingly, mice lacking ERβ have variable degrees of reduced fertility, show abnormal follicular maturation and very few *corpora lutea*,[37] whereas mice lacking ERα are infertile and have markedly hypoplastic reproductive organs and tissues,[27] again highlighting the close association between regulation of energy balance and fertility.

Estrogen-mediated metabolic effects occur at multiple levels. In the brainstem, estrogens potentiate or attenuate the effect of various peptides that signal satiety or hunger, respectively (such as cholecystokinin, which is released from the small intestine in response to food ingestion,[38] or ghrelin,[39] production of which is stimulated by fasting). In the arcuate and ventromedial nuclei, estrogens stimulate POMC and steroidogenic factor 1 (SF-1) neurons,[40] thereby repressing the synthesis of orexigenic neuropeptides (such as AgRP and NPY).[41] POMC expression is regulated by ERα; in mice lacking this receptor, leptin and insulin no longer increase POMC expression.[42] Moreover, mice lacking ERα specifically in POMC neurons are hyperphagic and have reduced sensitivity to leptin, despite POMC expression being maintained in the hypothalamus.[40] Thus, the anorexigenic effect of estrogens on POMC neurons is clearly mediated by ERα, although whether the underlying mechanism involves direct regulation of POMC expression or potentiated effects of other anorexigenic hormones, such as leptin, or both remains unclear. POMC neurons also express CART, and, to our knowledge, no data describe whether ERα or ERβ (or both) are involved in CART regulation, even if CART expression is modulated by estradiol.[43] AgRP and NPY are essential targets for the anorexigenic effect of estrogens. AgRP–NPY neurons do not express ERα *in vivo*.[41] However, given that ERα is abundantly expressed in the arcuate nucleus of the hypothalamus,[44] whereas expression of ERβ is barely detectable at this location, estrogens might conceivably regulate AgRP–NPY neurons through ERα on other neuronal subtypes (such as kisspeptin neurons in the ventromedial hypothalamus that innervate AgRP–NPY neurons). The mechanisms through which estrogens regulate GnRH neurons to control fertility are unclear. Estrogen decreases GnRH expression in GT1-7 cells (a hypothalamic cell line that expresses ERα and ERβ), an effect that might be mediated by ERα.[45] Although GnRH neurons seem to express only ERβ *in vivo*,[46] a role for both ERα and ERβ receptors in regulating GnRH neuronal activity as well as GnRH secretion remains probable. Different mechanisms are potentially required, including direct actions of estrogen through ERβ-expressing GnRH neurons, and indirect actions of estrogen through ERα-expressing afferents of GnRH neurons.

Moreover, estrogen signalling potentiates leptin sensitivity, possibly by increasing expression of the leptin receptor in the hypothalamus.[47] The overall effects of increased estrogen signalling are induction of an anorexigenic response and fat redistribution to subcutaneous rather than visceral depots. In the arcuate nucleus, anteroventricular periventricular nucleus and preoptic area, estrogen signalling integrates with afferent signals from peripheral organs and tissues, such as the stomach, pancreas and adipose tissue and, through kisspeptin neurons, controls the release of GnRH[48] in response to an individual's metabolic status. These effects are mediated mainly by ERα, which is abundantly expressed in these hypothalamic nuclei (Figure 2).[49]

ERα and ERβ are expressed and active in all metabolic organs. In adipose tissue, estrogens increase subcutaneous fat deposition in lower body areas and decrease lipolytic activity (which maintains fat stores in case periods of food scarcity occur during pregnancy or lactation). When estrogen signalling decreases, the subcutaneous fat redistributes to visceral areas;[31,34,50] this phenomenon has been observed in women after natural or oophorectomy-induced menopause;[32,33] in animal models of selective ablation of ERα;[27,34] in humans with loss-of-function ERα polymorphisms;[51] and in ovariectomized mice, the phenotype of which can be partially rescued by treatment with 17β-estradiol.[32] Studies in cultured adipocytes show that estrogens have a direct antilipogenic and prolipolytic activity *in vitro*, associated with inducing the expression of hormone-sensitive lipases and decreasing the activity of lipoprotein lipase.[52,53] These same effects of estrogens in fatty acid metabolism are observed in animal models.[52–54] However, the exact contributions of ERα and ERβ to these activities remain to be defined. Although the results of studies in mice genetically engineered to lack ERα suggest that ERα is the main receptor involved in the control of adipose tissue distribution,[34] other studies, mainly in cultured adipocytes, suggest that estradiol also has antilipogenic and antiadipogenic effects mediated by ERβ.[55]

ERα involvement in the control of lipid metabolism is certainly relevant in the liver,[26] where this receptor isoform is predominant.[27] In addition to controlling the synthesis of lipid transport proteins, such as VLDL, hepatic ERα signalling also regulates the expression of numerous genes, the products of which are involved

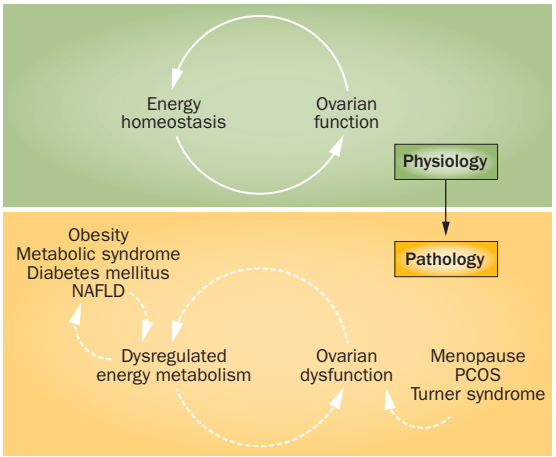

Figure 3 | Reciprocal regulation of energy metabolism and reproduction. Reproduction and energy metabolism are controlled to guarantee a metabolic status that is finely tuned to reproductive needs. The alteration of ovarian functions that characterizes menopause and other endocrine disorders changes these metabolic pathways, which might lead to obesity, metabolic syndrome, diabetes mellitus or NAFLD. Changes in energy metabolism might further impair reproductive activity. Abbreviations: NAFLD, nonalcoholic fatty liver disease; PCOS, polycystic ovary syndrome.

in pathways of cholesterol and fatty acids synthesis, according to the phase of the menstrual cycle and fertility status.[26] The oscillation of lipid biosynthesis induced during the menstrual cycle by fluctuating estrogen levels is necessary to maintain healthy fat metabolism. Menopause, ovariectomy and suppression of ERα activity in the liver are all associated with increased hepatic fat deposition.[26] These observations suggest that the changes in estrogen metabolism that accompany each stage of reproductive activity in women might regulate ERα transcriptional activity in the liver, thereby promoting the release or the storage of energy in the form of fat, as necessary for reproductive function.[26]

ERα and ERβ are expressed in all tissues relevant to glucose metabolism, including skeletal muscle and liver.[27] However, the most studied extragonadal site of estrogen action is the pancreas, where its important protective effect has been known for decades.[56] In pancreatic β cells, estrogens have an antiapoptotic action,[57] repress both lipid biosynthesis and the accumulation of fat, preventing lipotoxicity,[58] and directly stimulate insulin biosynthesis.[59] This last effect might be important in late pregnancy, when high estrogen levels could synergize with high prolactin levels to promote β-cell insulin production, to meet the increased metabolic demand.[60] Of particular relevance with regard to the potential therapeutic use of hormonal interventions is that the beneficial effects of estrogens on pancreatic β cells are observed with physiological plasma estrogen levels, as estrogen concentrations above or below the physiological range are associated with metabolic dysfunction.[61] Animal studies have shown that after ovariectomy, sensitivity to insulin is progressively impaired. The administration of estradiol to reach early gestational concentrations of the hormone (734–918 pmol/l) improves insulin sensitivity whereas a 100-fold higher physiological dose than normal cycle concentration decreases insulin sensitivity.[62] Randomized controlled clinical trials should be carried out to better evaluate the long-term effects of oral contraceptives or HRT in women. The use of contraceptives can deteriorate glucose tolerance;[63] however, low estrogen doses (for example 0.625 mg/day), when administered orally can improve tolerance.[64] Moreover, the Heart Estrogen Progestin replacement study[65] and the Women's Health Initiative found a lower incidence of diabetes mellitus in women taking HRT.[66] Finally, high estrogen levels during late pregnancy (1,278–7,192 pg/ml)[67] decrease insulin sensitivity, which might lead to gestational diabetes mellitus. However, the mechanism mediating these effects requires clarification. Normalization of glycaemia must also take into consideration hormonal status, because sex hormones counteract the effects of insulin.[68]

Metabolic effect of reproductive status

Menopause

Given that mammalian reproductive functions also regulate energy homeostasis, the fact that cessation of ovarian function leads to the manifestation of metabolic disorders is not surprising. Indeed, postmenopausal women have increased vulnerability to a large number of pathologies, including disorders of the cardiovascular, skeletal, immune and nervous systems.[69] How menopause triggers the onset of such diverse pathologies is unclear; perhaps declining levels of circulating estrogens weaken ERα and ERβ activity and thereby cause subtle alterations of energy metabolism in multiple tissues. Over time, such changes could lead to overt pathology owing to the diversity of organs involved. However, in our opinion, for each individual woman, the risk of developing a specific disease is influenced by genetic and environmental factors, as well as her overall health status (Figure 3).

Altered lipid metabolism

Menopause is associated with increased body weight, decreased lean mass[70] and abdominal fat accumulation.[33,71] Several enzymes involved in fat turnover are reduced in adipose tissue from postmenopausal women (such as acetyl-coenzyme A carboxylase 1, long-chain-acyl-coenzyme A dehydrogenase and hormone-sensitive lipase).[72] Increased insulin resistance is also evident, possibly prompted by nonphysiological fatty acid deposits,[73] high levels of circulating free fatty acids, and increased production of reactive oxygen species from mitochondrial β-oxidation of fatty acids.[74] Macrophages are recruited by the increased fat mass[75] and, in combination with adipocytes, secrete proinflammatory cytokines and chemokines.[76–78] Fatty acids also accumulate in the liver, where they facilitate the development of diffuse hepatic steatosis, characteristic of postmenopausal women.[79] This steatosis further contributes to the proinflammatory state, which facilitates the onset of the pathologies associated with climaterium such as atherosclerosis, osteoporosis and metabolic dysfunctions. Furthermore,

in the liver, cessation of estrogenic control of cholesterol, fatty acid[26] and lipoprotein metabolism leads to a decrease in production of HDL2 (large, antiatherogenic lipid particles)[80] and an increase of HDL3 (small, proatherogenic particles).[79,80] Total cholesterol, LDL, triglycerides,[81,82] apoB100 and apoB100-containing lipoproteins and lipoprotein(a),[83] a complex of an LDL-like particle and apoA,[81] are also increased, which contribute to the development of an atherogenic lipid profile and an increased risk of cardiovascular disease. The loss of estrogen's anti-inflammatory action in circulating monocytes and microglia[84,85] is another important pathophysiological element that, together with the altered lipid transport associated with reduced levels of apoE, might lead to brain neurodegenerative pathologies. For example, the incidence of disorders such as Alzheimer disease is similarly low in both female and male individuals ≤50 years old, but increases after this age and thereafter is considerably higher in women.[86]

Impaired muscle and bone physiology

After menopause, skeletal muscle function is reduced, as is muscle strength and mass, an effect that is reversed by HRT.[87] Additionally, the decline in expression of the glucose transporter GLUT-4,[88] which is associated with impaired muscle ERα activity, together with a generalized proinflammatory milieu might participate in the increased risk of insulin resistance typical in postmenopausal women. The proinflammatory state and increased levels of circulating cytokines (IL-1, IL-6 and TNF) also have repercussions in other organs, such as bone, where they contribute to a decrease in BMD and increase in osteoclast number.[89]

Nonalcoholic fatty liver disease

Nonalcoholic fatty liver disease (NAFLD) is a metabolic condition that leads to liver damage. The spectrum of NAFLD ranges from simple steatosis, through nonalcoholic steatohepatitis and cirrhosis, to liver failure and hepatocellular carcinoma. NAFLD is characterized by hepatic lipid accumulation (fat comprises 5–10% of the total organ weight), which is associated with insulin resistance in the liver,[90] increased lipogenesis[90] and reduced secretion of triglycerides.[91] Insufficient suppression of lipolysis in adipocytes[72] further contributes to the formation of lipid deposits in the liver. These alterations result in impaired mitochondrial fatty acid oxidation[92] and upregulation of both peroxisomal β-oxidation and microsomal ω-oxidation. Together with production of lipotoxic lipid intermediates, increased production of ROS, and induction of a proinflammatory response, these changes further contribute to progression of NAFLD and insulin resistance.[93]

NAFLD can occur in individuals of all ages and ethnic groups, and has a prevalence of about 30%;[94] this disorder is more common in men, in whom it has a 2.0–3.5-fold higher prevalence than in women.[95,96] After menopause, the incidence of NAFLD increases significantly to reach the levels seen in men.[97] HRT reduces the risk of hepatic steatosis[98] and the prevalence of NAFLD[79] in this population. These and other data, including the association of NAFLD with altered ovarian function,[99] suggest that estrogens protect against the development of NAFLD.

The exact aetiology of NAFLD in postmenopausal women is unclear. However, estrogen deficiency certainly contributes to the loss of inhibition of *de novo* fatty acid synthesis,[26,100] decreased VLDL-mediated export of lipids,[97,101] and reduced fatty acid oxidation[97,100] in the liver. This view is supported by studies in ovariectomized rodents, in which administration of estrogens prevented hepatic fat accumulation by inhibiting expression of genes that encode proteins involved in lipogenesis, including sterol regulatory element-binding protein 1c, peroxisome proliferator-activated receptor (PPAR) γ, stearoyl-coenzyme A desaturase 1, acetyl-coenzyme A carboxylase and fatty acid synthase.[26,100] Estrogens also facilitate VLDL-mediated export of lipids from the liver, by increasing hepatic VLDL-triglyceride production and expression of microsomal triglyceride transfer protein.[97,101] Finally, estrogens sustain the β-oxidation of fatty acids by inducing expression of PPAR-α.[100]

Polycystic ovary syndrome

NAFLD is often associated with PCOS,[102] which affects up to 10% of women of reproductive age.[103] PCOS is a major metabolic and reproductive disorder characterized by hyperandrogenism, chronic anovulation and polycystic ovaries,[104] as well as metabolic disturbances—50% of women with PCOS have overweight or obesity,[105] and dyslipidaemia (increased levels of LDL and decreased levels of HDL) is also commonly observed.[106] In the USA, 39% of women with PCOS have hepatic steatosis,[102] and 50% have insulin resistance and metabolic syndrome.[6] Indeed, insulin resistance, independent of obesity, is the metabolic disorder that is most strongly correlated with PCOS.[107] This decreased insulin signalling might be due to serine hyperphosphorylation of the insulin receptor and its substrate IRS-1.[108]

The cause of anovulation in women with PCOS has not been clearly identified, but might involve an increase in GnRH pulse frequency, possibly caused by low progesterone levels in these patients, which augment both luteinizing hormone release from the pituitary and ovarian androgen production.[109] Insulin might increase luteinizing hormone production and synergize with it to stimulate androgen synthesis by ovarian theca cells, thereby further impairing the development of ovarian follicles.[110,107] This observation further emphasizes the tight interconnection between metabolic and reproductive disturbances in patients with PCOS. The presence of metabolic disorders worsens the clinical and biochemical manifestations of PCOS. Being overweight contributes to hyperandrogenism, insulin resistance and dyslipidaemia;[105,110] conversely, a reduction in excess body weight ameliorates these abnormal metabolic and reproductive features of PCOS.[105]

The oral contraceptive pill has been the mainstay of therapy for women with PCOS for several years—combinations of estrogens and progestins ameliorate hirsutism, acne and oligomenorrhoea in these patients.[110,111]

Additionally, HRT, possibly by reducing abdominal fat deposits, is thought to counteract the worsening of hyperinsulinaemia that occurs with ageing in women with PCOS.[112] Concerns about the use of oral contraceptive therapy in patients with PCOS have been raised by the observation of increased triglyceride and cholesterol levels,[113] a phenomenon that is not in line with either observations from experimental studies of HRT or with the notion that endogenous estrogens protect against dyslipidaemia.[81] Further studies should be carried out to clarify the extent to which these alterations in patients' lipid profiles were related to the specific compound used (ethinyl estradiol), its dosage and route of administration.

Metformin, an antidiabetic agent, can effectively restore ovarian function in women with metabolic disorders—indeed, metformin not only reduces insulin levels but also directly stimulates ovarian steroidogenesis.[114] However, the effects of this therapy on reproductive outcomes seem to be limited, as live birth rates do not improve in metformin-treated women with PCOS (despite improved clinical pregnancy rates).[115] Metformin in combination with oral contraceptives can also mitigate the reproductive and metabolic symptoms of PCOS.[111] This observation, which is in line with the theory that gonadal hormones are essential for a well-balanced metabolism, also requires confirmation in additional studies including a statistically relevant number of patients with PCOS.[111]

Turner syndrome

Turner syndrome is a common genetic disorder affecting 1 in 2,500 live-born girls[116] that is caused by the total or partial absence of one X chromosome (genotype 45,XO). The most common features of Turner syndrome are infertility due to gonadal dysgenesis, short stature,[117] metabolic disorders, webbed neck and other physical abnormalities.[118] Reduced dosage of genes on the X chromosome, which in 46,XX female individuals escape X-inactivation (and are, therefore, functionally diploid), is thought to cause most Turner syndrome features.[119] However, candidate X-chromosome genes responsible for specific Turner syndrome features are yet to be identified.[120]

The clinical features of patients with Turner syndrome include obesity,[121] low lean body mass and increased BMI, waist circumference[122] and visceral adipose tissue;[99] moreover, triglycerides and LDL levels are elevated, but HDL levels are decreased.[123] Patients with Turner syndrome also have smaller lipid particle sizes than do 46,XX individuals.[123] Interestingly, the extent of the differences in lipid levels and particle sizes between 46,XX and 45,XO individuals is very similar to that between men and women.[81] Thus, haploinsufficiency of X-chromosome genes whose products are involved in lipid metabolism is probably the cause of dyspilidaemia in patients with Turner syndrome, in agreement with the fact that men generally have a more atherogenic lipid profile than do women.[124] As a result, patients with Turner syndrome have a sevenfold increased risk of mortality from ischaemic heart disease.[121]

Moreover, 80% of patients with Turner syndrome have abnormal liver function,[125] which is associated with intracellular hepatic lipid accumulation,[99,125] elevated levels of liver enzymes,[126] and increased incidence of NAFLD,[99] cirrhosis,[122] liver hyperplasia and inflammation.[127] The hepatic abnormalities seem to be caused by the lack of estradiol production, as estradiol treatment ameliorates these abnormalities.[128]

Although many clinical features in patients with Turner syndrome recapitulate the metabolic syndrome, paradoxically, such patients have decreased levels of fasting glucose, insulin and leptin (despite their high visceral adiposity), even compared with women who have premature ovarian failure.[129] However, the low insulin levels are thought to result from defective β-cell secretory function[130] or a glucose-storage defect,[131] and the decreased leptin levels are probably attributable to low fasting insulin levels.[132] Impaired glucose homeostasis is also frequent[121,122] and results in an increased risk of diabetes mellitus,[122,126] which is the cause of death in 25% of patients with Turner syndrome.[133]

Current guidelines for the treatment of Turner syndrome recommend growth hormone therapy and suggest the initiation of estrogen-based HRT in patients aged 12–14 years.[134] Patients with Turner syndrome require high doses of growth hormone to achieve optimal development because they are resistant to its metabolic effects.[7,135] Growth hormone therapy aids the decrease of adiposity and abdominal fat, and increases lean mass and circulating IGF-1 levels,[7] whereas HRT is necessary for female sexual development, normalization of BMD and improvement of neurocognitive functions.[118] Furthermore, estrogen therapy has important beneficial effects on the metabolic derangements associated with Turner syndrome, resulting from decreasing visceral adipose tissue,[7] increasing HDL levels[121] and maintaining normal liver metabolism.[128] Indeed, estradiol therapy improves liver function[128] and regulates the release of growth factors and antiapoptotic factors, which maintain the integrity of hepatocytes and promote their proliferation.[136]

Current and future aims of HRT

Estrogens regulate both reproductive and metabolic functions, which have adapted to the reproductive cycle with fluctuating production of the metabolites relevant for energy storage or utilization.[26] Estradiol is the molecule most likely to be responsible for the beneficial effects of estrogens on metabolism, because it is the most potent and predominant form of estrogen during the fertile age, and its levels substantially decrease after menopause. However, the literature on levels of individual estrogen metabolites and their physiological effects is limited and often conflicting. Furthermore, we still do not understand the exact consequences of either the termination of ovarian activity or the oscillation of metabolic functions. Experimental and clinical observations show that ovariectomy disrupts the rhythm of ERα activation in the liver[26] and induces rapid disorganization of lipid metabolism characterized by accumulation of hepatic fat deposits.[26,79] Decreased circulating estrogens and the lack of estrogen receptor oscillatory activity that occurs after menopause might trigger a derangement of energy metabolism in organs that are targets of estrogen action, such as adipose

tissue, muscle and hypothalamus. Functional deterioration leading to the onset of specific pathologies (cancer, immune, neuropsychiatric, cardiovascular and cerebrovascular diseases) are also observed with desynchrony of the circadian rhythm, which is generated by clock genes and proteins that regulate sleep and wakefulness, body temperature, blood pressure, digestive secretion, immune responses and metabolism.[137–141] In analogy with such findings, the observed lack of efficacy of HRT with regard to menopause-associated dysmetabolism might plausibly be caused by their inability to reinstate the oscillatory activity of ERα and ERβ in sexually mature individuals[21] and the consequent reciprocal control of genes regulating both fertility and energy metabolism.

The aim of all forms of HRT used to date has been to keep hormone levels constant. However, in some organs, cyclical modulation of ERα and ERβ signalling might be necessary to activate the expression of large numbers of genes whose products maintain energy homeostasis. For example, hepatic ERα becomes associated with distinct classes of promoters during different phases of the estrous cycle,[26] which might be necessary to poise ERα signalling for rapid selection of the transcriptome most appropriate for the specific energy requirements of each stage of the reproductive cycle. Previous studies in our laboratory suggest that selective estrogen receptor modulators, either alone or in combination with natural hormones, might have a substantial effect on the relative phasing and intensity of ERα and ERβ activity in target organs.[26,142] These findings raise the possibility that the complex actions of ERα and ERβ in the whole organism might eventually be reproduced pharmacologically. However, currently a clear understanding of the pattern of ERα and ERβ activity that would have the most favourable effect on women's health during ageing is lacking. In the absence of such knowledge, we believe that the analysis of the effects of existing forms of HRT on a single parameter (such as the period or amplitude of ERα or ERβ activity in different organs) is not sufficient to establish the superiority of one treatment over another. Specific methodologies and algorithms for the comparative analysis of multivariate parameters of synthetic ERα and ERβ ligand activity should also be developed.[143] Initial animal studies have shown that screening, based on the whole-body analysis of ERα and ERβ, by molecular imaging is a viable method.[143] This innovative technique might aid the development of novel HRT modalities better able to mimic estrogens' effects during the reproductive cycle.

Conclusions

This Review underlines the close link between energy metabolism and reproduction, to provide a comprehensive view of the role of estrogens in mammalian physiology. Given the association between energy production and reproductive activity, a main aim of HRT should be restoration of the metabolic functions characteristic of women still undergoing reproductive cycling. However, in postmenopausal women, current forms of HRT cannot reinstate the low morbidity from skeletal, cardiovascular and metabolic diseases typical in women of fertile age. Improved understanding of the exact physiological role of estrogens is, therefore, required to develop new types of HRT that can address metabolic as well as hormonal derangements.

Review criteria

The articles selected for this Review were English-language, full-text articles and abstracts identified by searching PubMed and personal databases. The keywords used included "estrogen", "estrogen receptor", "estradiol", "fertility", "reproduction", "energy", "metabolism", "menopause", "ovarian activity", "ovariectomy", "hormone replacement therapy", "SERMs", "obesity", "steatosis", "lipids", "insulin resistance", "apolipoproteins", "POMC", "NPY", "AgRP", "GnRH", "CART", "SF-1" "neurons", "PYY", "ghrelin", "leptin", "fat", "amino acids", "IGF-1", "GH", "hypothalamus", "liver", "adipose tissue", "pancreas", "metabolic syndrome", "NAFLD", "PCOS", "Turner syndrome", "fatty acids", "β-oxidation", "lipogenesis", "LH", "androgens", "oral contraceptive", "metformin", "circadian rhythm", "vitellogenin", "fat body", "juvenile hormones", "ecdysone", "Drosophila", "mouse", "insulin-like-peptides", "mTOR", "gonadotropins", and "FOXO".

1. Trovato, F. & Heyen, N. B. A varied pattern of change of the sex differential in survival in the G7 countries. *J. Biosoc. Sci.* **38**, 391–401 (2006).
2. WHO. Life *expectancy*: Life *expectancy by WHO region* [online], http://apps.who.int/gho/data/view.main.690?lang=en (2013).
3. Shapira, N. Women's higher health risks in the obesogenic environment: a gender nutrition approach to metabolic dimorphism with predictive, preventive, and personalised medicine. *EPMA J.* **4**, 1 (2013).
4. Kopelman, P. G. Obesity as a medical problem. *Nature* **404**, 635–643 (2000).
5. Wing, R. R., Matthews, K. A., Kuller, L. H., Meilahn, E. N. & Plantinga, P. L. Weight gain at the time of menopause. *Arch. Intern. Med.* **151**, 97–102 (1991).
6. Essah, P. A. & Nestler, J. E. The metabolic syndrome in polycystic ovary syndrome. *J. Endocrinol. Invest.* **29**, 270–280 (2006).
7. Gravholt, C. H. Epidemiological, endocrine and metabolic features in Turner syndrome. *Eur. J. Endocrinol.* **151**, 657–687 (2004).
8. Hansen, M., Flatt, T. & Aguilaniu, H. Reproduction, fat metabolism, and life span: what is the connection? *Cell Metab.* **17**, 10–19 (2013).
9. Romano, M., Rosanova, P., Anteo, C. & Limatola, E. Vertebrate yolk proteins: a review. *Mol. Reprod. Dev.* **69**, 109–116 (2004).
10. Hagedorn, H. H., Fallon, A. M. & Laufer, H. Vitellogenin synthesis by the fat body of the mosquito Aedes aegypti: evidence of transcriptional control. *Dev. Biol.* **31**, 285–294 (1973).
11. Chen, J. S., Sappington, T. W. & Raikhel, A. S. Extensive sequence conservation among insect, nematode, and vertebrate vitellogenins reveals ancient common ancestry. *J. Mol. Evol.* **44**, 440–451 (1997).
12. DePina, A. S. *et al.* Regulation of Caenorhabditis elegans vitellogenesis by DAF-2/IIS through separable transcriptional and posttranscriptional mechanisms. *BMC Physiol.* **11**, 11 (2011).
13. Roy, S. G., Hansen, I. A. & Raikhel, A. S. Effect of insulin and 20-hydroxyecdysone in the fat body of the yellow fever mosquito, *Aedes aegypti*. *Insect Biochem. Mol. Biol.* **37**, 1317–1326 (2007).
14. Hagedorn, H. H. & Fallon, A. M. Ovarian control of vitellogenin synthesis by the fat body in *Aedes aegypti*. *Nature* **244**, 103–105 (1973).
15. Mircea, C. N., Lujan, M. E. & Pierson, R. A. Metabolic fuel and clinical implications for female reproduction. *J. Obstet. Gynaecol. Can.* **29**, 887–902 (2007).
16. Tam, S. P., Archer, T. K. & Deeley, R. G. Biphasic effects of estrogen on apolipoprotein synthesis in human hepatoma cells: mechanism of antagonism by testosterone. *Proc. Natl Acad. Sci. USA* **83**, 3111–3115 (1986).

17. Frykman, P. K., Brown, M. S., Yamamoto, T., Goldstein, J. L. & Herz, J. Normal plasma lipoproteins and fertility in gene-targeted mice homozygous for a disruption in the gene encoding very low density lipoprotein receptor. *Proc. Natl Acad. Sci. USA* **92**, 8453–8457 (1995).
18. Babin, P. J., Bogerd, J., Kooiman, F. P., Van Marrewijk, W. J. & Van der Horst, D. J. Apolipophorin II/I, apolipoprotein B, vitellogenin, and microsomal triglyceride transfer protein genes are derived from a common ancestor. *J. Mol. Evol.* **49**, 150–160 (1999).
19. Farese, R. V. Jr, Ruland, S. L., Flynn, L. M., Stokowski, R. P. & Young, S. G. Knockout of the mouse apolipoprotein B gene results in embryonic lethality in homozygotes and protection against diet-induced hypercholesterolemia in heterozygotes. *Proc. Natl Acad. Sci. USA* **92**, 1774–1778 (1995).
20. Macomber, D. Studies of reproduction in the rat. 1. The effect of changes in the protein upon fertility, pregnancy and lactation. *N. Engl. J. Med.* **209**, 1105–1109 (1933).
21. Della Torre, S. *et al.* Amino acid-dependent activation of liver estrogen receptor α integrates metabolic and reproductive functions via IGF-1. *Cell Metab.* **13**, 205–214 (2011).
22. Quennell, J. H. *et al.* Leptin indirectly regulates gonadotropin-releasing hormone neuronal function. *Endocrinology* **150**, 2805–2812 (2009).
23. Wren, A. M. *et al.* Ghrelin enhances appetite and increases food intake in humans. *J. Clin. Endocrinol. Metab.* **86**, 5992 (2001).
24. Vincent, R. P. & le Roux., C. W. The satiety hormone peptide YY as a regulator of appetite. *J. Clin. Pathol.* **61**, 548–552 (2008).
25. Baskin, D. G. *et al.* Insulin and leptin: dual adiposity signals to the brain for the regulation of food intake and body weight. *Brain Res.* **848**, 114–123 (1999).
26. Villa, A. *et al.* Tetradian oscillation of estrogen receptor α is necessary to prevent liver lipid deposition. *Proc. Natl Acad. Sci. USA* **109**, 11806–11811 (2012).
27. Couse, J. F. & Korach, K. S. Estrogen receptor null mice: what have we learned and where will they lead us? *Endocr. Rev.* **20**, 358–417 (1999).
28. Chervenak, J. Bioidentical hormones for maturing women. *Maturitas* **64**, 86–89 (2009).
29. Baird, D. T. & Guevara, A. Concentration of unconjugated estrone and estradiol in peripheral plasma in nonpregnant women throughout the menstrual cycle, castrate and postmenopausal women and in men. *J. Clin. Endocrinol. Metab.* **29**, 149–156 (1969).
30. Barkley, M. S., Geschwind, I. I. & Bradford, G. E. The gestational pattern of estradiol, testosterone and progesterone secretion in selected strains of mice. *Biol. Reprod.* **20**, 733–738 (1979).
31. Lu, K. H., Hopper, B. R., Vargo, T. M. & Yen, S. S. Chronological changes in sex steroid, gonadotropin and prolactin secretions in aging female rats displaying different reproductive states. *Biol. Reprod.* **21**, 193–203 (1979).
32. Stubbins, R. E., Holcomb, V. B., Hong, J. & Nunez, N. P. Estrogen modulates abdominal adiposity and protects female mice from obesity and impaired glucose tolerance. *Eur. J. Nutr.* **51**, 861–870 (2012).
33. Tchernof, A. *et al.* Ovarian hormone status and abdominal visceral adipose tissue metabolism. *J. Clin. Endocrinol. Metab.* **89**, 3425–3430 (2004).
34. Heine, P. A., Taylor, J. A., Iwamoto, G. A., Lubahn, D. B. & Cooke, P. S. Increased adipose tissue in male and female estrogen receptor-α knockout mice. *Proc. Natl Acad. Sci. USA* **97**, 12729–12734 (2000).
35. Roesch, D. M. Effects of selective estrogen receptor agonists on food intake and body weight gain in rats. *Physiol. Behav.* **87**, 39–44 (2006).
36. Santollo, J., Katzenellenbogen, B. S., Katzenellenbogen, J. A. & Eckel, L. A. Activation of ERα is necessary for estradiol's anorexigenic effect in female rats. *Horm. Behav.* **58**, 872–877 (2010).
37. Krege, J. H. *et al.* Generation and reproductive phenotypes of mice lacking estrogen receptor β. *Proc. Natl Acad. Sci. USA* **95**, 15677–15682 (1998).
38. Asarian, L. & Geary, N. Estradiol enhances cholecystokinin-dependent lipid-induced satiation and activates estrogen receptor-α-expressing cells in the nucleus tractus solitarius of ovariectomized rats. *Endocrinology* **148**, 5656–5666 (2007).
39. Sakurazawa, N., Mano-Otagiri, A., Nemoto, T. & Shibasaki, T. Effects of intracerebroventricular ghrelin on food intake and Fos expression in the arcuate nucleus of the hypothalamus in female rats vary with estrous cycle phase. *Neurosci. Lett.* **541**, 204–208 (2013).
40. Xu, Y. *et al.* Distinct hypothalamic neurons mediate estrogenic effects on energy homeostasis and reproduction. *Cell Metab.* **14**, 453–465 (2011).
41. Olofsson, L. E., Pierce, A. A. & Xu, A. W. Functional requirement of AgRP and NPY neurons in ovarian cycle-dependent regulation of food intake. *Proc. Natl Acad. Sci. USA* **106**, 15932–15937 (2009).
42. Hirosawa, M. *et al.* Ablation of estrogen receptor α (ERα) prevents upregulation of POMC by leptin and insulin. *Biochem. Biophys. Res. Commun.* **371**, 320–323 (2008).
43. Xu, Y. *et al.* Role of cocaine- and amphetamine-regulated transcript in estradiol-mediated neuroprotection. *Proc. Natl Acad. Sci. USA* **103**, 14489–14494 (2006).
44. Simerly, R. B., Chang, C., Muramatsu, M. & Swanson, L. W. Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an *in situ* hybridization study. *J. Comp. Neurol.* **294**, 76–95 (1990).
45. Roy, D., Angelini, N. L. & Belsham, D. D. Estrogen directly represses gonadotropin-releasing hormone (GnRH) gene expression in estrogen receptor-α (ERα)- and ERβ-expressing GT1–7 GnRH neurons. *Endocrinology* **140**, 5045–5053 (1999).
46. Temple, J. L., Laing, E., Sunder, A. & Wray, S. Direct action of estradiol on gonadotropin-releasing hormone-1 neuronal activity via a transcription-dependent mechanism. *J. Neurosci.* **24**, 6326–6333 (2004).
47. Clegg, D. J., Brown, L. M., Woods, S. C. & Benoit, S. C. Gonadal hormones determine sensitivity to central leptin and insulin. *Diabetes* **55**, 978–987 (2006).
48. Oakley, A. E., Clifton, D. K. & Steiner, R. A. Kisspeptin signaling in the brain. *Endocr. Rev.* **30**, 713–743 (2009).
49. Scott, C. J. *et al.* The distribution of cells containing estrogen receptor-α (ERα) and ERβ messenger ribonucleic acid in the preoptic area and hypothalamus of the sheep: comparison of males and females. *Endocrinology* **141**, 2951–2962 (2000).
50. Lovejoy, J. C., Champagne, C. M., de Jonge, L., Xie, H. & Smith, S. R. Increased visceral fat and decreased energy expenditure during the menopausal transition. *Int. J. Obes. (Lond.)* **32**, 949–958 (2008).
51. Okura, T. *et al.* Association of polymorphisms in the estrogen receptor α gene with body fat distribution. *Int. J. Obes. Relat. Metab. Disord.* **27**, 1020–1027 (2003).
52. D'Eon, T. M. *et al.* Estrogen regulation of adiposity and fuel partitioning. Evidence of genomic and non-genomic regulation of lipogenic and oxidative pathways. *J. Biol. Chem.* **280**, 35983–35991 (2005).
53. Lundholm, L. *et al.* Key lipogenic gene expression can be decreased by estrogen in human adipose tissue. *Fertil. Steril.* **90**, 44–48 (2008).
54. Gao, H. *et al.* Long-term administration of estradiol decreases expression of hepatic lipogenic genes and improves insulin sensitivity in ob/ob mice: a possible mechanism is through direct regulation of signal transducer and activator of transcription 3. *Mol. Endocrinol.* **20**, 1287–1299 (2006).
55. Foryst-Ludwig, A. & Kintscher, U. Metabolic impact of estrogen signalling through ERα and ERβ. *J. Steroid Biochem. Mol. Biol.* **122**, 74–81 (2010).
56. Nelson, W. O. & Overholser, M. The effect of estrogenic hormones on experimental pancreatic diabetes in the monkey. *Endocrinology* **20**, 473–480 (1936).
57. Le May, C. *et al.* Estrogens protect pancreatic β-cells from apoptosis and prevent insulin-deficient diabetes mellitus in mice. *Proc. Natl Acad. Sci. USA* **103**, 9232–9237 (2006).
58. Tiano, J. P. *et al.* Estrogen receptor activation reduces lipid synthesis in pancreatic islets and prevents β cell failure in rodent models of type 2 diabetes. *J. Clin. Invest.* **121**, 3331–3342 (2011).
59. Alonso-Magdalena, P. *et al.* Pancreatic insulin content regulation by the estrogen receptor ER α. *PLoS ONE* **3**, e2069 (2008).
60. Wong, W. P. *et al.* Extranuclear estrogen receptor-α stimulates NeuroD1 binding to the insulin promoter and favors insulin synthesis. *Proc. Natl Acad. Sci. USA* **107**, 13057–13062 (2010).
61. Nadal, A., Alonso-Magdalena, P., Soriano, S., Quesada, I. & Ropero, A. B. The pancreatic β-cell as a target of estrogens and xenoestrogens: Implications for blood glucose homeostasis and diabetes. *Mol. Cell Endocrinol.* **304**, 63–68 (2009).
62. Gonzalez, C., Alonso, A., Diaz, F. & Patterson, A. M. Dose- and time-dependent effects of 17β-oestradiol on insulin sensitivity in insulin-dependent tissues of rat: implications of IRS-1. *J. Endocrinol.* **176**, 367–379 (2003).
63. Wynn, V. & Doar, J. W. Some effects of oral contraceptives on carbohydrate metabolism. *Lancet* **2**, 715–719 (1966).
64. Lindheim, S. R. *et al.* A possible bimodal effect of estrogen on insulin sensitivity in postmenopausal women and the attenuating effect of added progestin. *Fertil. Steril.* **60**, 664–667 (1993).
65. Kanaya, A. M. *et al.* Glycemic effects of postmenopausal hormone therapy: the Heart and Estrogen/progestin Replacement Study. A randomized, double-blind, placebo-controlled trial. *Ann. Intern. Med.* **138**, 1–9 (2003).
66. Margolis, K. L. *et al.* Effect of oestrogen plus progestin on the incidence of diabetes in postmenopausal women: results from the Women's Health Initiative Hormone Trial. *Diabetologia* **47**, 1175–1187 (2004).
67. Abbassi-Ghanavati, M., Greer, L. G. & Cunningham, F. G. Pregnancy and laboratory studies: a reference table for clinicians. *Obstet. Gynecol.* **114**, 1326–1331 (2009).

68. Jovanovic, L. Advances in diabetes for the millennium: diabetes in women. *MedGenMed* **6**, 3 (2004).
69. Komm, B. S. A new approach to menopausal therapy: the tissue selective estrogen complex. *Reprod. Sci.* **15**, 984–992 (2008).
70. Douchi, T. *et al.* Relative contribution of aging and menopause to changes in lean and fat mass in segmental regions. *Maturitas* **42**, 301–306 (2002).
71. Ley, C. J., Lees, B. & Stevenson, J. C. Sex- and menopause-associated changes in body-fat distribution. *Am. J. Clin. Nutr.* **55**, 950–954 (1992).
72. Misso, M. L. *et al.* Differential expression of factors involved in fat metabolism with age and the menopause transition. *Maturitas* **51**, 299–306 (2005).
73. Song, M. J., Kim, K. H., Yoon, J. M. & Kim, J. B. Activation of Toll-like receptor 4 is associated with insulin resistance in adipocytes. *Biochem. Biophys. Res. Commun.* **346**, 739–745 (2006).
74. Lin, Y. *et al.* The hyperglycemia-induced inflammatory response in adipocytes: the role of reactive oxygen species. *J. Biol. Chem.* **280**, 4617–4626 (2005).
75. Weisberg, S. P. *et al.* Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Invest.* **112**, 1796–1808 (2003).
76. Suganami, T. *et al.* Role of the Toll-like receptor 4/NF-kappaB pathway in saturated fatty acid-induced inflammatory changes in the interaction between adipocytes and macrophages. *Arterioscler. Thromb. Vasc. Biol.* **27**, 84–91 (2007).
77. Xu, H. *et al.* Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J. Clin. Invest.* **112**, 1821–1830 (2003).
78. Hotamisligil, G. S. Inflammation and metabolic disorders. *Nature* **444**, 860–867 (2006).
79. Clark, J. M., Brancati, F. L. & Diehl, A. M. Nonalcoholic fatty liver disease. *Gastroenterology* **122**, 1649–1657 (2002).
80. Stevenson, J. C., Crook, D. & Godsland, I. F. Influence of age and menopause on serum lipids and lipoproteins in healthy women. *Atherosclerosis* **98**, 83–90 (1993).
81. Li, Z. *et al.* Effects of gender and menopausal status on plasma lipoprotein subspecies and particle sizes. *J. Lipid Res.* **37**, 1886–1896 (1996).
82. Campos, H., McNamara, J. R., Wilson, P. W., Ordovas, J. M. & Schaefer, E. J. Differences in low density lipoprotein subfractions and apolipoproteins in premenopausal and postmenopausal women. *J. Clin. Endocrinol. Metab.* **67**, 30–35 (1988).
83. Jenner, J. L. *et al.* Effects of age, sex, and menopausal status on plasma lipoprotein(a) levels. The Framingham Offspring Study. *Circulation* **87**, 1135–1141 (1993).
84. Vegeto, E. *et al.* Estrogen prevents the lipopolysaccharide-induced inflammatory response in microglia. *J. Neurosci.* **21**, 1809–1818 (2001).
85. Pozzi, S., Benedusi, V., Maggi, A. & Vegeto, E. Estrogen action in neuroprotection and brain inflammation. *Ann. NY Acad. Sci.* **1089**, 302–323 (2006).
86. Henderson, V. W. Estrogen-containing hormone therapy and Alzheimer's disease risk: understanding discrepant inferences from observational and experimental research. *Neuroscience* **138**, 1031–1039 (2006).
87. Dionne, I. J., Kinaman, K. A. & Poehlman, E. T. Sarcopenia and muscle function during menopause and hormone-replacement therapy. *J. Nutr. Health Aging* **4**, 156–161 (2000).
88. Moreno, M. *et al.* Chronic 17β-estradiol treatment improves skeletal muscle insulin signaling pathway components in insulin resistance associated with aging. *Age (Dordr.)* **32**, 1–13 (2010).
89. Kimble, R. B. *et al.* Simultaneous block of interleukin-1 and tumor necrosis factor is required to completely prevent bone loss in the early postovariectomy period. *Endocrinology* **136**, 3054–3061 (1995).
90. Bugianesi, E., McCullough, A. J. & Marchesini, G. Insulin resistance: a metabolic pathway to chronic liver disease. *Hepatology* **42**, 987–1000 (2005).
91. Charlton, M., Sreekumar, R., Rasmussen, D., Lindor, K. & Nair, K. S. Apolipoprotein synthesis in nonalcoholic steatohepatitis. *Hepatology* **35**, 898–904 (2002).
92. Kohjima, M. *et al.* Re-evaluation of fatty acid metabolism-related gene expression in nonalcoholic fatty liver disease. *Int. J. Mol. Med.* **20**, 351–358 (2007).
93. Malaguarnera, M., Di Rosa, M., Nicoletti, F. & Malaguarnera, L. Molecular mechanisms involved in NAFLD progression. *J. Mol. Med. (Berl.)* **87**, 679–695 (2009).
94. Szczepaniak, L. S. *et al.* Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am. J. Physiol. Endocrinol. Metab.* **288**, E462–E468 (2005).
95. Omagari, K. *et al.* Fatty liver in non-alcoholic non-overweight Japanese adults: incidence and clinical characteristics. *J. Gastroenterol. Hepatol.* **17**, 1098–1105 (2002).
96. Weston, S. R. *et al.* Racial and ethnic distribution of nonalcoholic fatty liver in persons with newly diagnosed chronic liver disease. *Hepatology* **41**, 372–379 (2005).
97. Wilfred de Alwis, N. M. & Day, C. P. Genes and nonalcoholic fatty liver disease. *Curr. Diab. Rep.* **8**, 156–163 (2008).
98. McKenzie, J. *et al.* Effects of HRT on liver enzyme levels in women with type 2 diabetes: a randomized placebo-controlled trial. *Clin. Endocrinol. (Oxf.)* **65**, 40–44 (2006).
99. Ostberg, J. E. *et al.* Excess visceral and hepatic adipose tissue in Turner syndrome determined by magnetic resonance imaging: estrogen deficiency associated with hepatic adipose content. *J. Clin. Endocrinol. Metab.* **90**, 2631–2635 (2005).
100. Paquette, A., Wang, D., Jankowski, M., Gutkowska, J. & Lavoie, J. M. Effects of ovariectomy on PPAR α, SREBP-1c, and SCD-1 gene expression in the rat liver. *Menopause* **15**, 1169–1175 (2008).
101. Barsalani, R., Chapados, N. A. & Lavoie, J. M. Hepatic VLDL-TG production and MTP gene expression are decreased in ovariectomized rats: effects of exercise training. *Horm. Metab. Res.* **42**, 860–867 (2010).
102. Gambarin-Gelwan, M. *et al.* Prevalence of nonalcoholic fatty liver disease in women with polycystic ovary syndrome. *Clin. Gastroenterol. Hepatol.* **5**, 496–501 (2007).
103. Ehrmann, D. A. Polycystic ovary syndrome. *N. Engl. J. Med.* **352**, 1223–1236 (2005).
104. Broekmans, F. J. & Fauser, B. C. Diagnostic criteria for polycystic ovarian syndrome. *Endocrine* **30**, 3–11 (2006).
105. Lim, S. S., Norman, R. J., Davies, M. J. & Moran, L. J. The effect of obesity on polycystic ovary syndrome: a systematic review and meta-analysis. *Obes. Rev.* **14**, 95–109 (2013).
106. Wild, R. A., Rizzo, M., Clifton, S. & Carmina, E. Lipid levels in polycystic ovary syndrome: systematic review and meta-analysis. *Fertil. Steril.* **95**, 1073–1079 e1–11 (2011).
107. Diamanti-Kandarakis, E. & Dunaif, A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. *Endocr. Rev.* **33**, 981–1030 (2012).
108. Corbould, A., Zhao, H., Mirzoeva, S., Aird, F. & Dunaif, A. Enhanced mitogenic signaling in skeletal muscle of women with polycystic ovary syndrome. *Diabetes* **55**, 751–759 (2006).
109. Panidis, D. *et al.* Serum luteinizing hormone levels are markedly increased and significantly correlated with delta 4-androstenedione levels in lean women with polycystic ovary syndrome. *Fertil. Steril.* **84**, 538–540 (2005).
110. Teede, H., Deeks, A. & Moran, L. Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. *BMC Med.* **8**, 41 (2010).
111. Hoeger, K. *et al.* The impact of metformin, oral contraceptives, and lifestyle modification on polycystic ovary syndrome in obese adolescent women in two randomized, placebo-controlled clinical trials. *J. Clin. Endocrinol. Metab.* **93**, 4299–4306 (2008).
112. Pasquali, R. *et al.* The natural history of the metabolic syndrome in young women with the polycystic ovary syndrome and the effect of long-term oestrogen-progestagen treatment. *Clin. Endocrinol. (Oxf.)* **50**, 517–527 (1999).
113. Gode, F. *et al.* Alteration of cardiovascular risk parameters in women with polycystic ovary syndrome who were prescribed to ethinyl estradiol-cyproterone acetate. *Arch. Gynecol. Obstet.* **284**, 923–929 (2011).
114. Mansfield, R., Galea, R., Brincat, M., Hole, D. & Mason, H. Metformin has direct effects on human ovarian steroidogenesis. *Fertil. Steril.* **79**, 956–962 (2003).
115. Tang, T., Lord, J. M., Norman, R. J., Yasmin, E. & Balen, A. H. Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. *Cochrane Database of Systematic Reviews*, Issue 1. Art. No.: CD003053. http://dx.doi.org/10.1002/14651858.CD003053.pub3
116. Stochholm, K., Juul, S., Juel, K., Naeraa, R. W. & Gravholt, C. H. Prevalence, incidence, diagnostic delay, and mortality in Turner syndrome. *J. Clin. Endocrinol. Metab.* **91**, 3897–3902 (2006).
117. Rao, E. *et al.* Pseudoautosomal deletions encompassing a novel homeobox gene cause growth failure in idiopathic short stature and Turner syndrome. *Nat. Genet.* **16**, 54–63 (1997).
118. Bondy, C. A. Care of girls and women with Turner syndrome: a guideline of the Turner Syndrome Study Group. *J. Clin. Endocrinol. Metab.* **92**, 10–25 (2007).
119. Zinn, A. R., Page, D. C. & Fisher, E. M. Turner syndrome: the case of the missing sex chromosome. *Trends Genet.* **9**, 90–93 (1993).
120. Mortensen, K. H., Andersen, N. H. & Gravholt, C. H. Cardiovascular phenotype in Turner syndrome—integrating cardiology, genetics, and endocrinology. *Endocr. Rev.* **33**, 677–714 (2012).
121. Gravholt, C. H. *et al.* Glucose metabolism, lipid metabolism, and cardiovascular risk factors in adult Turner's syndrome. The impact of sex hormone replacement. *Diabetes Care* **21**, 1062–1070 (1998).
122. Gravholt, C. H., Juul, S., Naeraa, R. W. & Hansen, J. Morbidity in Turner syndrome. *J. Clin. Epidemiol.* **51**, 147–158 (1998).

123. Van, P. L., Bakalov, V. K. & Bondy, C. A. Monosomy for the X-chromosome is associated with an atherogenic lipid profile. *J. Clin. Endocrinol. Metab.* **91**, 2867–2870 (2006).
124. Williams, C. M. Cardiovascular risk factors in women. *Proc. Nutr. Soc.* **56**, 383–391 (1997).
125. Koulouri, O., Ostberg, J. & Conway, G. S. Liver dysfunction in Turner's syndrome: prevalence, natural history and effect of exogenous oestrogen. *Clin. Endocrinol. (Oxf.)* **69**, 306–310 (2008).
126. El-Mansoury, M. *et al.* Elevated liver enzymes in Turner syndrome during a 5-year follow-up study. *Clin. Endocrinol. (Oxf.)* **68**, 485–490 (2008).
127. Roulot, D. *et al.* Vascular involvement of the liver in Turner's syndrome. *Hepatology* **39**, 239–247 (2004).
128. Elsheikh, M., Hodgson, H. J., Wass, J. A. & Conway, G. S. Hormone replacement therapy may improve hepatic function in women with Turner's syndrome. *Clin. Endocrinol. (Oxf.)* **55**, 227–231 (2001).
129. Ostberg, J. E., Attar, M. J., Mohamed-Ali, V. & Conway, G. S. Adipokine dysregulation in turner syndrome: comparison of circulating interleukin-6 and leptin concentrations with measures of adiposity and C-reactive protein. *J. Clin. Endocrinol. Metab.* **90**, 2948–2953 (2005).
130. Bakalov, V. K. *et al.* Impaired insulin secretion in the Turner metabolic syndrome. *J. Clin. Endocrinol. Metab.* **89**, 3516–3520 (2004).
131. Caprio, S. *et al.* Insulin resistance: an early metabolic defect of Turner's syndrome. *J. Clin. Endocrinol. Metab.* **72**, 832–836 (1991).
132. Kolaczynski, J. W. *et al.* Acute and chronic effects of insulin on leptin production in humans: Studies *in vivo* and *in vitro*. *Diabetes* **45**, 699–701 (1996).
133. Naeraa, R. W., Gravholt, C. H., Hansen, J., Nielsen, J. & Juul, S. in *Turner Syndrome in a Life-Span Perspective: Research and Clinical Aspects* (eds Albertsson-Wikiland, K. & Ranke, M.) 323–325 (Elsevier, 1995).
134. Saenger, P. *et al.* Recommendations for the diagnosis and management of Turner syndrome. *J. Clin. Endocrinol. Metab.* **86**, 3061–3069 (2001).
135. Westwood, M., Tajbakhsh, S. H., Siddals, K. W., Whatmore, A. J. & Clayton, P. E. Reduced pericellular sensitivity to IGF-I in fibroblasts from girls with Turner syndrome: a mechanism to impair clinical responses to GH. *Pediatr. Res.* **70**, 25–30 (2011).
136. Gravholt, C. H., Poulsen, H. E., Ott, P., Christiansen, J. S. & Vilstrup, H. Quantitative liver functions in Turner syndrome with and without hormone replacement therapy. *Eur. J. Endocrinol.* **156**, 679–686 (2007).
137. Shaw, D. B., Knapp, M. S. & Davies, D. H. Variations of bloodpressure in hypertensives during sleep. *Lancet* **1**, 797–799 (1963).
138. Oliver, P. L. *et al.* Disrupted circadian rhythms in a mouse model of schizophrenia. *Curr. Biol.* **22**, 314–319 (2012).
139. Vanin, S. *et al.* Unexpected features of Drosophila circadian behavioural rhythms under natural conditions. *Nature* **484**, 371–375 (2012).
140. Harrington, M. Location, location, location: important for jet-lagged circadian loops. *J. Clin. Invest.* **120**, 2265–2267 (2010).
141. Jeyaraj, D. *et al.* Circadian rhythms govern cardiac repolarization and arrhythmogenesis. *Nature* **483**, 96–99 (2012).
142. Della Torre, S. *et al.* The conundrum of estrogen receptor oscillatory activity in the search for an appropriate hormone replacement therapy. *Endocrinology* **152**, 2256–2265 (2011).
143. Rando, G. *et al.* An innovative method to classify SERMs based on the dynamics of estrogen receptor transcriptional activity in living animals. *Mol. Endocrinol.* **24**, 735–744 (2010).
144. Wangh, L. J. & Knowland, J. Synthesis of vitellogenin in cultures of male and female frog liver regulated by estradiol treatment *in vitro*. *Proc. Natl Acad. Sci. USA* **72**, 3172–3175 (1975).
145. Davis, L. K. *et al.* Induction of three vitellogenins by 17β-estradiol with concurrent inhibition of the growth hormone-insulin-like growth factor 1 axis in a euryhaline teleost, the tilapia (*Oreochromis mossambicus*). *Biol. Reprod.* **77**, 614–625 (2007).
146. Liou, M. L. *et al.* Association of serum protein levels with egg productivity in Taiwan red-feathered country chickens. *Anim. Reprod. Sci.* **100**, 158–171 (2007).
147. Carnevali, O., Mosconi, G., Ridolfi, S. & Polzonetti-Magni, A. M. Growth hormone and insulin-like growth factor-I in inducing vitellogenin synthesis by frog hepatocytes. *Ann. NY Acad. Sci.* **839**, 556–557 (1998).
148. Mommsen, T. P., Moon, T. W. & Plisetskaya, E. M. Effects of arginine on pancreatic hormones and hepatic metabolism in rainbow trout. *Physiol. Biochem. Zool.* **74**, 668–678 (2001).
149. Mehta, K. D., Chen, W. J., Goldstein, J. L. & Brown, M. S. The low density lipoprotein receptor in Xenopus laevis. I. Five domains that resemble the human receptor. *J. Biol. Chem.* **266**, 10406–10414 (1991).

Acknowledgements
The authors are sincerely grateful to Enzo Nisoli for his important, thoughtful suggestions and critical assessment of the manuscript, and to the European Research Council (ERC-Advanced Grant 322977 and INMiND Collaborative Project FP7-HEALTH-011.2.2.1-2 to A. Maggi) and Pfizer (grant IIR WS897258 to A. Maggi) for their research support.

Author contributions
All authors researched the data for the article and contributed substantially to discussions of its content. A. Maggi wrote the manuscript, and S. Della Torre, V. Benedusi and R. Fontana reviewed and/or edited the manuscript before submission.