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INVITED REVIEW

Erythropoietin regulates metabolic response in mice via receptor expression in adipose tissue, brain, and bone

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Erythropoietin (EPO) acts by binding to erythroid progenitor cells to regulate red blood cell production. While EPO receptor (Epor) expression is highest on erythroid tissue, animal models exhibit EPO activity in nonhematopoietic tissues, mediated, in part, by tissue-specific Epor expression. This review describes the metabolic response in mice to endogenous EPO and EPO treatment associated with glucose metabolism, fat mass accumulation, and inflammation in white adipose tissue and brain during diet-induced obesity and with bone marrow fat and bone remodeling. During high-fat diet-induced obesity, EPO treatment improves glucose tolerance, decreases fat mass accumulation, and shifts white adipose tissue from a pro-inflammatory to an anti-inflammatory state. Fat mass regulation by EPO is sex dimorphic, apparent in males and abrogated by estrogen in females. Cerebral EPO also regulates fat mass and hypothalamus inflammation associated with diet-induced obesity in males and ovariectomized female mice. In bone, EPO contributes to the balance between adipogenesis and osteogenesis in both male and female mice. EPO treatment promotes bone loss mediated via Epor in osteoblasts and reduces bone marrow adipocytes before and independent of change in white adipose tissue fat mass. EPO regulation of bone loss and fat mass is independent of EPO-stimulated erythropoiesis. EPO nonhematopoietic tissue response may relate to the long-term consequences of EPO treatment of anemia in chronic kidney disease and to the alternative treatment of oral hypoxia-inducible factor prolyl hydroxylase inhibitors that increase endogenous EPO production. Published by Elsevier Inc. on behalf of ISEH – Society for Hematology and Stem Cells.

Erythropoietin (EPO), produced in the kidney, is the primary regulator of erythropoiesis [1,2]. EPO is regulated by hypoxia [3]. Hypoxia-inducible factor (HIF) heterodimer (ARNT/HIF-α; primarily HIF-2α for EPO) induces EPO by binding to the EPO gene hypoxia-responsive element [4–6]. HIF-α is stable and active under hypoxia and is targeted at normoxia by oxygen-dependent prolyl hydroxylase-domain (PHD) enzymes and factor-inhibiting HIF-1 [7,8]. Proline hydroxylation by PHD2 targets HIF-α for ubiquitination by Von Hippel–Lindau protein and proteasome degradation [7,9,10]. Mutations in genes for PHD2, VHL, and HIF2A, as well as EPO and EPO receptor (Epor), contribute to congenital erythrocytosis [11] and suggest alternate modalities to stimulate erythropoiesis. Recently, HIF-prolyl hydroxylase inhibitors, small molecule oral agents that stimulate production of endogenous erythropoietin, have been approved in China and Japan for treatment of anemia associated with chronic kidney disease [12,13], although adverse events with long-term administration remain unknown [14].

Animal models suggest that EPO can promote a nonhematopoietic response mediated via Epor expression beyond erythroid tissue and include protection against ischemic stress and injury in brain, vascular

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endothelium, heart, and skeletal muscle [15,16]. The nonhematopoietic EPO responses may also relate to EPO production by HIF-prolyl hydroxylase inhibitors. Reviewed here is the metabolic response to endogenous and exogenous EPO such as glucose tolerance, anti-inflammatory response in white adipose tissue (WAT) and brain, gender-specific fat mass regulation particularly during diet-induced obesity in mice, and adipogenic/osteogenic balance in bone maintenance [17–21].

EPO receptor and EPO-stimulated signaling beyond erythropoiesis

EPO stimulates erythroid progenitor cell survival, proliferation, and differentiation regulating the production of two million erythrocytes per second in the human body. Mice that lack Epor die in utero of severe anemia [22,23]. EPO binding to the cell surface Epor homodimer on erythroid progenitor cells activates Epor cytoplasmic domain associated JAK2 and phosphorylation of Epor, STAT, AKT, ERK, and other downstream signaling pathways [24,25]. EPO binding to Epor induces erythroid transcription factors GATA1 and TAL1, which also transactivate Epor via a GATA-binding site and three TAL1-binding (E-boxes) motifs in the proximal promoter. Epor expression and EPO sensitivity is greatest on erythroid progenitor cells [26–29].

The GATA motif and E-boxes also provide for Epor expression in select nonhematopoietic tissues including the endothelial/cardiovascular system, brain, skeletal muscle, fat depots, and bone [15,16], and can be transactivated in part by other GATA proteins including GATA2, GATA3, and GATA4 [30–33]. Endothelial cells expressing Epor induced by reduced oxygen and/or nitric oxide [34–36] exhibit EPO proliferative and chemotactic response [37,38]. In mice, Epor is required for vessel network development and for EPO-stimulated, endothelial nitric oxide synthase (eNOS)-mediated cardioprotection [39–42]. In neural cells, Epor is transactivated by GATA3, which is critical for morphological development of the nervous system [31,43]. In rodents, endogenous EPO contributes to maintenance and proliferation of neural progenitor cells and neuroprotection [31,44–46], and exogenous EPO is neuroprotective for brain ischemia and injury [31,47,48]. In skeletal muscle myoblasts, Epor induced by GATA3, GATA4, and TAL1 and E-box-binding muscle regulator transcription factors MyoD and Myf5 [30,32] promotes transplanted myoblast survival and restored dystrophin expression in mdx mice [49,50]. Endogenous EPO and exogenous EPO contribute to skeletal muscle repair in mice [49,51].

EPO activity in nonhematopoietic tissue and regulation of fat mass

Epor knockout mice can be rescued from death in utero by an erythroid-specific Epor transgene driven by GATA1 erythroid transcription regulatory regions, resulting in mice with erythroid-restricted Epor ($\Delta EPOR_E$) [52]. $\Delta EPOR_E$ mice have no gross morphological defects, indicating that nonhematopoietic Epor expression is not required for life [52]. Although food intake is comparable between $\Delta EPOR_E$ and wild-type mice on a C57BL/6 background, $\Delta EPOR_E$ mice are glucose intolerant and become obese and insulin resistant with decreased metabolic rate and locomotor activity (Figure 1) [17]. By age 8 months, female $\Delta EPOR_E$ mice exhibit a 150% increase in fat mass and a 55% increase in body weight, and male $\Delta EPOR_E$ mice exhibit a 40% increase in fat mass and a 20% increase in body weight compared with wild-type mice [17]. Despite increases in fat mass, adipocyte size distribution in $\Delta EPOR_E$ gonadal fat pads shifted to smaller cell size [17], indicating a disproportionate increase in adipocyte number with loss of nonhematopoietic Epor.

In wild-type mice, Epor expression in white adipose tissue is 60% the level of erythroid tissue (spleen) and,

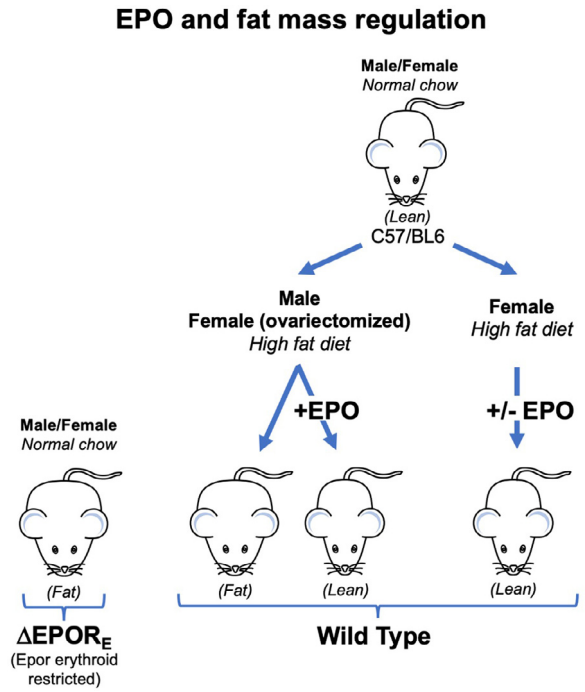

Figure 1. Erythropoietin contributes to fat mass regulation. $\Delta EPOR_E$ mice with Epor restricted to erythroid tissue exhibit accelerated body weight gain because of increased fat mass and become obese. Conversely, C57BL/6 male mice fed a high-fat diet become obese, while EPO treatment concomitant with a high-fat diet increases hematocrit and protects against diet-induced obesity. In female mice, estrogen provides protection against diet-induced obesity, and EPO treatment increases hematocrit without change in fat mass. Ovariectomy in female mice abrogates the estrogen anti-obesity activity, and ovariectomized mice fed a high-fat diet become obese. Ovariectomized mice on a high-fat diet concomitant with EPO treatment exhibit an increased hematocrit and protection against diet-induced obesity.

in brown adipose tissue, is an order of magnitude lower [17]. Mice (C57BL/6 background) with adipocyte deletion of Epor also exhibited increase fat mass accumulation, insulin resistance, and reduced oxygen consumption and activity [53]. These mice exhibit an increase of 20% in body weight because of increased fat mass by 30 weeks, compared with littermate control mice, and increased susceptibility to high-fat diet-induced obesity, glucose intolerance, and insulin resistance. Insulin activation of the serine/threonine kinase AKT (also known as protein kinase B) in adipocytes is required to stimulate glucose transporter 4 translocation to the membrane to increase glucose uptake [54]. In erythroid cells EPO stimulates AKT signaling to promote survival, proliferation, and differentiation downstream of EPOR activation [55]. EPO treatment also activates AKT in adipocytes, but not in mice that lack Epor in adipocytes and also manifest reduced AKT phosphorylation compared with control mice [53]. These mouse models illustrate that both endogenous and exogenous EPO activity contributes to regulation of fat mass and glucose homeostasis, in part via direct adipocyte EPO response to affect insulin signaling that may also be influenced by mouse background strain [53,56].

Exogenous EPO modulates body weight and fat mass accumulation

Male mice treated with EPO exhibit increased hematocrit and decreased body weight when fed normal chow or reduced weight gain and fat mass accumulation when fed a high-fat diet (Figure 1) [17,57]. Further evidence that elevated serum EPO increased hematocrit and decreased blood glucose and body weight is provided by mice treated with EPO and transgenic mice with constitutive high human EPO [57]. Gene electrotransfer in skeletal muscle to increase EPO expression in obese mice also revealed increased erythropoiesis and reduced body weight and fat mass, improved glucose tolerance, and increased fat metabolism [58]. In contrast, $\Delta EPOR_E$ mice with EPO receptor restricted to erythroid tissue and mice with targeted deletion of Epor in adipocytes exhibited no significant changes in fat mass/body weight with EPO-stimulated erythropoiesis [17,53]. This indicates that exogenous EPO regulation of body weight/fat mass is independent of EPO-stimulated erythropoiesis and is mediated by EPO activity in nonhematopoietic tissue, especially in adipose tissue.

EPO treatment during a high-fat-diet in mice increased metabolic activity and white adipose tissue cellular respiration capacity, fatty acid utilization, mitochondrial biogenesis and fatty acid oxidation-associated gene expression, metabolic regulator Pgc-1α, and cytochrome C protein compared with vehicle-treated and pair-fed diet-induced obese mice [53]. Analogous changes were observed in EPO-treated mouse and human adipocyte cultures. In contrast, these activities and gene expression were reduced in white adipose tissue of mice with adipocyte deletion of Epor [53]. EPO-associated response in cellular mitochondrial respiration and oxidative metabolism extend the role of EPO/Epor beyond regulation of erythropoiesis and oxygen transport capacity. Nonerythroid EPO activity contributes to increased energy expenditure in white adipose tissue and enhances the ability of adipocytes to metabolize fatty acid and to potentially protect against obesity.

Brown adipose tissue with high mitochondria content maintains body temperature by release of chemical energy as heat via nonshivering thermogenesis [59]. The browning of white adipose tissue is characterized by increased levels of uncoupling protein UCP1, which uncouples electron transport from oxidative phosphorylation to generate heat [59,60]. Increasing beige adipocytes in white adipose tissue is of particular interest and has the potential to utilize energy-dissipating thermogenesis to reduce fat storage and promote a lean phenotype. EPO treatment in mice increased expression and protein of brown fat-associated genes including UCP1 in adipocytes from subcutaneous fat independent of change in body weight [53]. Corresponding expression was decreased in mice with targeted deletion of Epor in adipocytes that was unchanged with EPO treatment. Primary adipocyte cultures also exhibit an analogous EPO-stimulated increase in brown fat-associated genes. Citrate synthase, the first enzyme in the tricarboxylic acid cycle, is an indicator of mitochondrial function. EPO treatment in mice increased citrate synthase activity in adipocytes from white adipose tissue but not from brown adipose tissue or from adipose tissue with adipocyte deletion of Epor. Hence, endogenous EPO and EPO administration contribute to white adipose tissue metabolism including direct adipocyte EPO response. In white adipose tissue, the nuclear receptor protein peroxisome proliferator-activated receptor (PPAR)-α reduced obesity-related inflammation and enhanced expression of brown fat-associated gene expression, including the thermogenesis effector UCP1 and transcription factor PRDM16 [61,62]. EPO stimulated an increase in PPAR-α in white adipose tissue in cooperation with SIRT1 activity, an NAD-dependent class III histone deacetylase sirtuin [53]. EPO induced PPAR-α mediates the increases in brown fat-associated gene and mitochondrial gene expression, oxygen consumption rate, and fatty acid oxidation [53].

In brown fat of young male mice, EPO treatment increased PRDM16, which regulates brown adipocyte differentiation, UCP1 expression, STAT3 activation, and secretion of fibroblast growth factor 21 (FGF21), and

improved glucose tolerance and insulin sensitivity [63]. In liver, EPO regulated lipid metabolism, increased lipolysis, decreased lipogenesis, activated STAT3 signaling, and also increased FGF21 in a SIRT1-dependent manner [64,65], suggesting that EPO can suppress obesity and hepatic steatosis. In obese male ob/ob mice, EPO treatment provided protection against obesity, reduced body weight, and hemoglobin A1c [17,57]. EPO-stimulated metabolic response is dependent on EPO dose and duration of treatment [66]. EPO induction at high altitude and the potential for EPO regulation of fat mass may contribute to the lower prevalence of obesity at high altitude [67,68].

Gender-specific response to EPO regulation of fat mass

$\Delta EPOR_E$ mice with Epor restricted to erythroid tissue are glucose intolerant and become obese and insulin resistant with age, indicating that endogenous EPO regulates fat mass [17]. Females exhibit an earlier onset of obesity and insulin resistance with a greater proportionate increase in fat mass. In wild-type mice, EPO-stimulated erythropoiesis is accompanied by loss of fat mass and body weight on normal chow and reduced fat mass accumulation and protection against obesity on a high-fat diet only in males (Figure 1) [17,19,57]. Only male mice manifest EPO-stimulated expression of mitochondrial oxidative genes in white adipose tissue. This sex-dimorphic EPO regulation of fat mass is related to estrogen production in female mice, which regulates glucose and lipid metabolism and obesity [69]. Depletion of endogenous estrogen by ovariectomy in female mice results in increased fat mass accumulation during 3 weeks of a high-fat-diet. Fat mass is reduced by EPO treatment and even more with estradiol supplementation, which was not further enhanced by the combination of EPO and estradiol (Figure 1) [19]. This indicates the greater protective effect of estrogen compared with EPO during diet-induced obesity and the interference of estrogen with EPO regulation of fat mass in female mice. The EPO-stimulated increase in hematocrit was comparable with and without ovariectomy, adding evidence that EPO regulation of fat mass is independent of EPO erythropoietic activity.

EPO regulation of bone marrow adipocytes and bone

Bone marrow adipocytes have an origin and function distinct from those of white and brown adipose tissue, increase with age and obesity, and at age 25 constitute 50% to 70% of human adult bone marrow volume and about 10% of total fat mass [70–74]. Bone marrow adipose tissue negatively regulates hematopoiesis, and in mice, hematopoietic recovery after chemotherapy improved with inhibition of bone marrow adipocytes by the PPAR-γ inhibitor [75,76]. Bone marrow stromal cells contribute to maintenance of the hematopoietic microenvironment and regulate differentiation of bone-resorbing osteoclasts [77]. Bone marrow stromal cells also include nonhematopoietic progenitors for bone growth and remodeling that can differentiate into bone marrow adipocytes or bone-forming osteoblasts. Pathologies of bone loss are often associated with fatty marrow, and dysregulation of the balance between bone marrow stromal cell-derived adipogenesis and osteogenesis contributes to aging and osteoporosis [78]. Epor is expressed on a variety of cells in bone marrow: erythroid/hematopoietic cells, bone remodeling osteoclasts and osteoblasts, bone marrow adipocytes, and bone marrow stromal cells that differentiate into osteoblasts, bone marrow adipocytes, and chondrocytes. Endogenous EPO regulates bone marrow adipocytes as well as white adipose tissue, and during bone development, EPO signaling maintains the normal balance between osteogenesis and adipogenesis in the bone marrow [17,21]. $\Delta EPOR_E$ mice with Epor restricted to erythroid tissue exhibit a two- to threefold increase in adipocyte number in bone marrow and concomitant reduction in trabecular bone, indicating a shift in bone marrow stromal cell differentiation toward adipogenesis and reduced osteogenesis [21].

With EPO treatment, accompanying the increase in EPO-stimulated erythropoiesis are reductions in bone marrow adipocytes and bone loss in male and female mice, independent of change in fat mass in white adipose tissue [21,79–81]. PPAR-γ, expressed predominantly in adipose tissue, is central to regulation of adipocyte gene expression and differentiation [82]. EPO treatment reduces PPAR-γ expression in bone marrow stromal cells, which contributes to reduced bone marrow adipogenesis [21]. Transgenic mice expressing high human EPO levels also exhibit reduced bone marrow adipocytes and trabecular and cortical bone with increased numbers of bone-resorbing osteoclasts [21,81,83]. These mice yield osteoblasts and osteoclasts that produce human EPO with increased differentiation potential, consistent with premature differentiation reducing endogenous trabecular bone, and increased alkaline phosphatase expression and mineralization [21]. Conversely, osteoblasts from $\Delta EPOR_E$ mice that lack endogenous EPO signaling exhibit reduced alkaline phosphatase expression and mineralization [21]. Osteoblasts exhibit EPO producing potential, raising the possibility for autocrine-regulated EPO response [84]. EPO treatment of mesenchymal stem cell cultures increased bone mineralization in cells from young healthy human donors but not in cultures from older healthy donors, suggesting an age-dependent response [85]. EPO activity to increase osteoblast differentiation may contribute to bone loss and affect bone health by limiting osteogenic expansion. Elevated levels of the phosphate-regulating hormone fibroblast

growth factor 23 (FGF23) have been linked to greater risk of fractures in elderly men, especially among individuals with chronic kidney disease [86,87]. EPO-stimulated FGF23 production in hematopoietic stem cells was associated with an increase in serum FGF23 and reduced serum phosphate, suggesting a possible mechanism of EPO-induced bone reduction caused by disrupted mineralization [88].

Although increased bone mineral density in postmenopausal obese women initially suggested obesity as a protective factor for osteoporosis, obesity was also associated with reduced bone strength and increased fracture risk [89–91]. Increased visceral and bone marrow fat in obese men was associated with impaired bone microarchitecture and mechanical properties [92]. Obese mice with increased bone marrow adiposity exhibited increased inflammatory cytokine production, osteoclast number, and bone resorption, linking increased inflammation in response to increased marrow adiposity with osteoclastogenesis and bone resorption [93]. Beyond simply filling marrow space, bone marrow adipocytes negatively regulate hematopoiesis, raising the possibility that reducing marrow adipogenesis may promote hematopoietic transplant recovery [75]. In obese mice, short-term EPO treatment (10 days) increased hematocrit; did not affect body mass but decreased bone marrow adipocytes fivefold; reduced trabecular bone without further increase in osteoclast number; and maintained cortical bone mineral density and volume [94]. While EPO administration in nonobese mice reduced bone marrow cellularity, decreased hematopoietic CD45+ cells, and increased the percentage of bone marrow erythroid cells, these parameters remained unchanged with EPO treatment in obese mice. EPO did not affect cortical bone or the increased bone marrow stromal cells in obese mice [94,95], perhaps in support of the need for maintenance of cortical bone to accommodate the increased body weight and resultant mechanical stress. In bone, osteoblast precursors reach bone formation sites by moving through proximal blood vessels, and the decrease in bone marrow endothelial cells in obese individuals is proposed to reduce vasculature [96,97]. The reduction in bone marrow endothelial cells in obese mice is reversed with EPO treatment [94], and may contribute to increased vasculature and bone repair.

EPO-stimulated bone remodeling is context dependent. In rodent models of bone fracture repair, EPO stimulated early endochondral ossification and bone mineralization, accelerated bone healing, inhibited bone resorption and reduced osteoclasts, increased endosteal vascularization, and reduced NFκB expression [98–102]. Animal models of bone injury suggest the potential for EPO to recruit bone marrow stromal cells with bone-repairing ability to enhance bone regeneration or accelerate bone morphogenic protein 2 healing activity [103–105]. In a pilot study of patients with tibiofibular fractures, it was suggested that EPO injection at the fracture site 2 weeks after surgery promotes faster union by 2 weeks and a lower rate of nonunion fracture [106].

EPO regulates bone marrow stromal cell differentiation

Mouse models of ectopic ossification illustrated the potential for EPO to regulate bone marrow stromal cell differentiation to osteoblastic or adipogenic lineages and to recapitulate endogenous formation of bone and bone marrow adipocytes [21]. Transplantation of collagen sponges containing bone marrow stromal cells into immunodeficient mice resulted in ossicle formation consisting of bone, adipocytes, and stroma of donor origin and hematopoiesis from the recipient [107]. The bone ossicles mimicked the changes in endogenous bone and bone marrow adipocyte formation of donor mice with altered EPO signaling. For bone marrow stromal cells from transgenic mice expressing high EPO, ossicle formation was significantly attenuated with a marked decrease in marrow adipocytes and greater than 10-fold reduction in bone and a lack of well-defined trabecular and cortical bone [21]. Bone marrow stromal cells from $\Delta EPOR_E$ mice that lack EPO signaling produced ossicles with reduced bone formation and a greater than twofold increase in marrow adipocytes.

In mice with targeted deletion of Epor in osteoblasts, trabecular bone is reduced by more than 20% by 12 weeks of age without change in the numbers of osteoblasts, osteoclasts, and marrow adipocytes, and osteogenic cultures exhibited reduced differentiation and mineralization [108]. Like $\Delta EPOR_E$ mice, mice with osteoblast deletion of Epor manifested no additional bone loss with EPO treatment, indicating that bone loss requires a direct osteoblast EPO response and is not related to EPO-stimulated erythropoiesis [21,108]. Receptor activator of nuclear factor κB ligand (RANKL) made in osteoblasts, bone marrow stromal cells, and B and T lymphocytes contributes to bone remodeling by activating osteoclasts via binding to its receptor (RANK) to promote bone resorption [109]. In bone marrow B cells, EPO increased RANKL expression, and knockdown of Epor increased trabecular and cortical bone mass and decreased trabecular bone loss with EPO treatment [110].

EPO reduces inflammation in white adipose tissue in obese mice

EPO protection against inflammation reduces proinflammatory cytokine response and macrophage infiltration and has been observed in animal models of tissue injury, including adult and preterm brain, acute and chronic heart injury, and chemical-induced colitis mediated in part by JAK2, STAT, and AKT activation [111–115]. In mouse models, EPO decreased hypoxic

and inflammatory response in sepsis-induced acute kidney injury and suppressed macrophage foam cell formation in cardiovascular disease [116,117]. In white adipose tissue, macrophages in the stromal vascular fraction contribute to metabolic homeostasis [118]. White adipose tissue in obese mice shifts toward a pro-inflammatory state with increased macrophage infiltration, M2-like pro-inflammatory subtype, and inflammatory cytokine production [119]. This is characterized by the appearance of crownlike structures, which are histological features of inflammatory adipose tissues of obese animals consisting of macrophages surrounding necrotic adipocytes [119].

In obese mice, 2 weeks of EPO treatment increases hematocrit without change in fat mass, but improves glucose tolerance and insulin sensitivity and shifts obesity-associated white adipose tissue inflammation toward an anti-inflammatory state [18,120]. EPO administration reduces white adipose tissue macrophage infiltration, crownlike structures, expression of pro-inflammatory cytokines, and production of tumor necrosis factor (TNF)-α, and increases anti-inflammatory cytokine interleukin (IL)-10 production. Macrophages respond directly to EPO stimulation with increased STAT3 activation and reduced inducible nitric oxide synthase (iNOS) and IL-1β expression. EPO treatment shifts the macrophage population toward an anti-inflammatory subtype that requires IL-4 and STAT6 activity, indicating that EPO contributes to local macrophage subtype polarization [18]. Endogenous EPO also provides immune modulatory activity. On a high-fat diet, weight gain and obesity are comparable in $\Delta EPOR_E$ mice with Epor restricted to erythroid tissue and control mice, but $\Delta EPOR_E$ mice exhibit a greater inflammatory response in adipose tissue [18]. $\Delta EPOR_E$ white adipose tissue exhibits denser macrophage infiltration and increased crownlike structures, inflammatory chemokine expression in the stromal vascular fraction, TNF-α production, and circulating inflammatory monocytes. These mice have greater glucose intolerance and insulin resistance that are unchanged with EPO treatment [18].

In addition to adipocyte response to EPO [121], macrophage inflammatory response in white adipose tissue during obesity influences insulin resistance [122,123], further linking erythropoietin metabolic response with improved insulin sensitivity. Other organs contributing to EPO activity during diet-induced obesity include a JAK2-dependent EPO-protective effect on insulin-producing pancreatic β cells, inducing pancreatic islets and proliferative anti-inflammatory and angiogenic activity in diabetic mouse models [124]. In liver, EPO enhances AKT activation and reduces obesity-associated gluconeogenesis and liver inflammation in obese mice [125]. EPO also exerts a neuroendocrine response in mice affecting metabolic homeostasis [17,126].

EPO regulates hypothalamus production of pro-opiomelanocortin

EPO treatment in male mice increases locomotor activity and decreases food intake to promote a lean phenotype, decreasing body weight and fat mass [17]. Regulation of appetite by the hypothalamus is mediated by neurons in the arcuate nucleus that sense changes in nutrient status. Stimulation of neurons that produce neuropeptide Y and agouti-related protein increase appetite, while activation of neurons that produce pro-opiomelanocortin (POMC) suppresses appetite. Hypothalamus Epor expression localizes to POMC neurons, and EPO administration increases POMC in the hypothalamus and in primary hypothalamus neural cell cultures, but not expression of neuropeptide Y or agouti-related protein [17,126]. EPO stimulates STAT3 activation in the hypothalamus and POMC neuron cultures, and $\Delta EPOR_E$ mice exhibit decreased hypothalamus STAT3 activation and POMC production [17,126].

The hypothalamic–pituitary axis contributes importantly to the balance between energy intake and energy expenditure to maintain metabolic homeostasis through secretion of endocrine hormones [127,128]. In the hypothalamus, EPO increases POMC production, while in the pituitary, EPO decreases cytosolic calcium-dependent POMC-derived adrenocorticotropic hormone (ACTH) secretion [129,130]. In contrast, $\Delta EPOR_E$ mice that lack EPO signaling in nonhematopoietic tissue are obese and exhibit reduced hypothalamus POMC production and an elevated plasma concentration of ACTH [17,129]. The metabolic changes observed in $\Delta EPOR_E$ mice provide evidence that the activity of endogenous EPO in the hypothalamic–pituitary axis contributes to neuroendocrine regulation of metabolism and obesity [128].

Cerebral EPO protects against diet-induced obesity

Transgenic mice overexpressing brain-specific human EPO without affecting hematocrit (Tg21 mice) [131] have improved glucose tolerance on normal chow and high-fat diet and increased insulin sensitivity during a high-fat diet [20]. Cerebral EPO exhibits a gender-specific response in high-fat-diet obesity, and male but not female Tg21 mice exhibit resistance to obesity, reduced fat mass accumulation, and higher energy expenditure [20]. Overnutrition promotes hypothalamus inflammation with activation of microglial cells, specialized macrophage cells in the brain, and increased pro-inflammatory cytokines before overt obesity and inflammation in white adipose tissue [132]. Transmembrane TNF-α is expressed on activated macrophages, lymphocytes, and other cell types (TNF-α+ cells) and undergoes proteolytic cleavage to release the soluble form of TNF-α [133]. Male Tg21 mice on a high-fat diet exhibit reduced levels of hypothalamus-activated

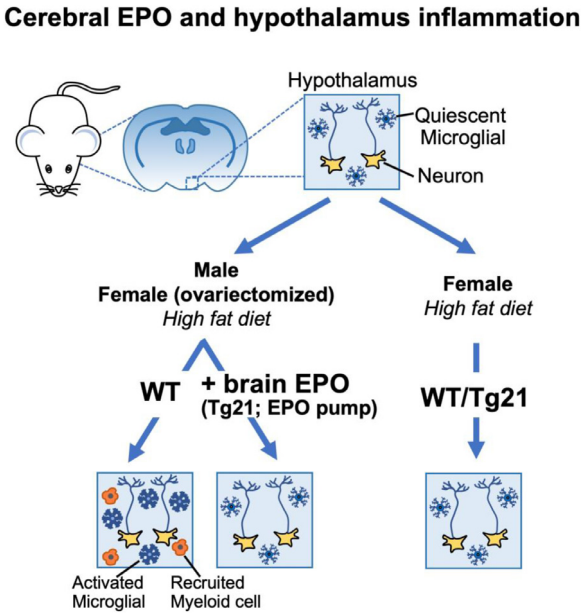

Figure 2. Cerebral EPO protects against high fat diet-induced hypothalamus inflammation. C57BL/6 male mice fed a high-fat diet become obese, and the obesity is accompanied by hypothalamic inflammation and associated microglial cell activation. With implantation of an EPO-secreting intracerebroventricular pump or by generation of transgenic Tg21 mice that express human EPO in brain, elevated cerebral EPO decreased susceptibility to diet-induced obesity and protected against obesity-associated hypothalamic inflammation. Mice with an implanted EPO intracerebroventricular pump and Tg21 mice exhibit a normal hematocrit because of limited transport of secreted or transgenic EPO across the blood–brain barrier. Estrogen provides protection against diet-induced obesity and associated hypothalamic inflammation in female wild-type and Tg21 mice. Ovariectomy in female mice abrogates the estrogen anti-obesity activity, and ovariectomized mice fed a high-fat diet become obese with concomitant hypothalamic inflammation. Ovariectomized Tg21 mice with elevated cerebral EPO exhibit reduced susceptibility to diet-induced obesity and protection against hypothalamic inflammation.

microglial cells, TNF-α+ cells, inflammatory cytokine gene expression, recruitment of blood myeloid monocyte-derived cells, and reduced serum ACTH and corticosterone (Figure 2) [20]. Increased cerebral EPO via an intracerebroventricular pump in male wild-type mice on a high-fat diet also exhibited decrease weight gain and reduced fat mass accumulation and, in the hypothalamus, reduced inflammatory cytokine expression and increased anti-inflammatory IL-10 expression (Figure 2) [20]. In contrast, male mice with targeted deletion of Epor in neural cells gained more weight on a high-fat diet, were more glucose intolerant, and exhibited greater induction of hypothalamus TNF-α, activated microglial cells, and recruitment of peripheral myeloid cells [20].

The sex-dimorphic response of Tg21 mice to high fat diet-induced obesity provides another illustration of estrogen protective activity against diet-induced obesity in female mice that suppresses EPO metabolic activity in fat mass regulation as well as associated hypothalamus inflammation. With ovariectomy, which blocks anti-obesity estrogen activity, female Tg21 mice exhibited the protective effect of cerebral EPO, and only wild-type female mice exhibited increased fat mass and hypothalamus inflammation, microglial activation, and inflammatory cytokine expression (Figure 2) [20].

Conclusions

Animal models illustrate that both endogenous EPO and exogenous EPO contribute to metabolic response. Epor expression in white adipose tissue, adipocytes, and macrophages, and in brain, neurons, and microglia, mediates EPO regulation of glucose metabolism, insulin sensitivity, fat mass, and obesity-related inflammation. A demonstrated gender-specific ventilatory response in mice with hypoxia induction of EPO is sensitive to ovarian steroids [134]. Similarly, estrogen anti-obesity activity in female mice contributes to the EPO sex-dimorphic metabolic response and EPO activity in adipose tissue and brain to regulate fat mass, and obesity-related inflammation is observed only in male mice. Secondary analysis of full-heritage Pima Indians from the Gila River Indian Community with a high prevalence of obesity and type 2 diabetes [135,136] reveals endogenous EPO levels associated negatively with hemoglobin and, in males, negatively with percentage weight change per year compared with the positive association observed in females [137]. These gender-specific relationships between EPO level and body weight are consistent with reduction of body weight with EPO treatment only in male mice and ovariectomized female mice [19].

EPO regulation of bone marrow adipocytes and skeletal bone formation is not gender specific and is mediated by Epor in bone marrow stromal cells, osteoblasts, adipocytes, and osteoclasts [21,108]. In mice, endogenous EPO is required for normal bone development and regulation of bone marrow adipocytes, while continuous EPO treatment to stimulate erythropoiesis decreases bone formation and marrow adiposity, providing implications for bone health in erythropoietic pathologies with elevated EPO such as thalassemia, sickle cell disease, and polycythemia vera [138–140]. Assessment of elderly men with normal kidney function in Sweden revealed that a high EPO level was associated with higher fracture risk independent of hemoglobin and age [141]. New pharmacological approaches to stimulate EPO activity, such as the prolyl hydroxylase inhibitors [12,13], may provide the methodology to selectively increase erythropoiesis while maintaining bone health or to promote a tissue-specific nonhematopoietic response without increased erythropoiesis.

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