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

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Abstract

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 demonstrate EPO activity in non-hematopoietic tissues is mediated, in part, via 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 prior to 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 non-hematopoietic 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.

Graphical abstract

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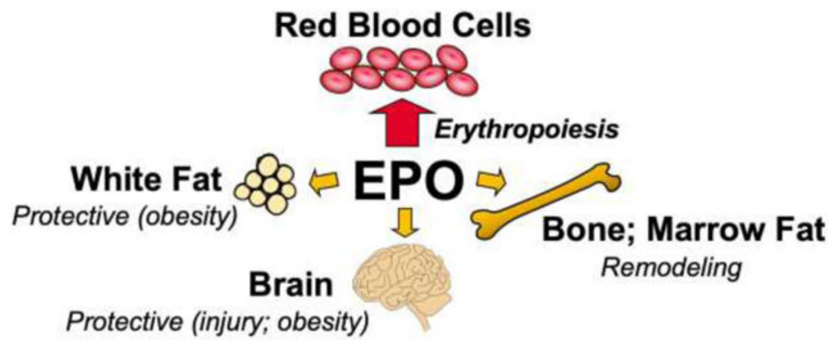

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Receptor mediated erythropoietin (EPO) response regulates fat mass and obesity related inflammation in a sex-dependent manner, and bone marrow adipogenesis/osteogenesis and EPO stimulated bone loss.

Keywords

Erythropoietin; erythropoietin receptor; metabolism; obesity; inflammation; adipose tissue; brain; sex-dimorphism; bone remodeling

Introduction

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 HIF2α for EPO) induces EPO by binding to the EPO gene hypoxic responsive element(4),(5),(6). HIF-α is stable and active under hypoxia and is targeted at normoxia by oxygen dependent prolyl-hydroxylase-domain enzymes (PHD) 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 non-hematopoietic response mediated via Epor expression beyond erythroid tissue and include protection against ischemic stress and injury in brain, vascular endothelium, heart, and skeletal muscle (15),(16). The non-hematopoietic 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 the adipogenic/osteogenic balance in bone maintenance(17),(18),(19),(20),(21).

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Erythropoietin 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 cytoplasmic 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 that 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),(27),(28),(29).

The GATA-motif and E-boxes also provide for Epor expression in select non-hematopoietic 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),(31),(32),(33). Endothelial cells expressing Epor induced by reduced oxygen and/or nitric oxide(34),(35),(36) exhibit EPO proliferative and chemotactic response(37),(38). In mice, Epor is required for vessel network development, and for EPO stimulated eNOS mediated cardioprotection(39),(40),(41),(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),(45),(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 and exogenous EPO contributes to skeletal muscle repair in mice(49, 51).

EPO activity in non-hematopoietic 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 ($EPOR_E$)(52). $EPOR_E$-mice have no gross morphological defects, demonstrating that non-hematopoietic Epor expression is not required for life(52). While food intake is comparable between $EPOR_E$ and wild-type mice on C57BL/6 background, $EPOR_E$-mice are glucose intolerant, become obese and insulin resistant with decreased metabolic rate and locomotor activity (Figure 1)(17). By age 8 months, female $EPOR_E$-mice exhibit 150% increase in fat mass and 55% increase in body weight and male $EPOR_E$-mice exhibit 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 $EPOR_E$ gonadal fat pads shifted to smaller cell size(17), indicating a disproportionate increase in adipocyte number with loss of non-hematopoietic Epor.

In wild-type mice, Epor expression in white adipose tissue is 60% the level of erythroid tissue (spleen) and in brown adipose tissue is an order of magnitude lower(17). Mice

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(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 show an increase of 20% in body weight due to 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). In adipocytes, EPO treatment also activates AKT but not in mice that lack Epor in adipocytes that also show reduced AKT phosphorylation compared with control mice(53). These mouse models demonstrate 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 on 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 showed increased erythropoiesis and reduced body weight and fat mass, improved glucose tolerance and increased fat metabolism(58). In contrast, $EPOR_E$-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 demonstrates that exogenous EPO regulation of body weight/fat mass is independent of EPO stimulated erythropoiesis and is mediated by EPO activity in non-hematopoietic tissue, especially in adipose tissue.

EPO treatment during high-fat diet feeding 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 expressions 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. Non-erythroid 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 non-shivering thermogenesis(59). The browning of white adipose tissue is characterized by increased uncoupling protein UCP1 that uncouples electron transport from oxidative phosphorylation to generate heat(59),(60). Increasing beige

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adipocytes in white adipose tissue is of particular interest with 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 show 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, both 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 increase in PPARα in white adipose tissue in cooperation with SIRT1 activity, an NAD-dependent class III histone deacetylase sirtuin(53). EPO induced PPARα his mediates the increase 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 that 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-depednent 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

$EPOR_E$-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 with high fat diet feeding only in males (Figure 1)(17), (19),(57). Only male mice show 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 that regulates glucose and lipid metabolism and obesity(69). Depletion of endogenous estrogen by ovariectomy in female mice results in increased fat mass accumulation during three weeks of high-fat diet feeding. 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

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effect of estrogen compared with EPO during diet induced obesity and the estrogen interference with EPO regulation of fat mass in female mice. 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 distinct origin and function from white and brown adipose tissue, increase with age and obesity, and at age 25 comprises 50% to 70% of human adult bone marrow volume and about 10% of total fat mass(70),(71),(72),(73),(74). Bone marrow adipose tissue negatively regulates hematopoiesis and, in mice, hematopoietic recovery after chemotherapy improved with inhibition of bone marrow adipocytes by PPARγ 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 non-hematopoietic 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 of bone marrow stromal cell derived adipogenesis and osteogenesis contribute 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). $EPOR_E$-mice with Epor restricted to erythroid tissue show an increase in adipocyte number in bone marrow by 2 to 3 fold 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 is reduced bone marrow adipocytes and bone loss in male and female mice, independent of change in fat mass in white adipose tissue(21),(79),(80),(81). PPARγ, 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 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 $EPOR_E$-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-

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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 due to 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),(90),(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 (ten days) increased hematocrit, did not affect body mass but decreased bone marrow adipocytes by 5 fold, reduced trabecular bone without further increase in osteoclast number and maintained cortical bone mineral density and volume(94). While EPO administration in non-obese 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 decreased 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),(99),(100),(101),(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),(104),(105). In a pilot study of patients with tibiofibular fractures, it was suggested that EPO injection at the fracture site two weeks after surgery promotes faster union by two weeks and lower rate of nonunion fracture(106).

EPO regulates bone marrow stromal cell differentiation

Mouse models of ectopic ossification demonstrated 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

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formation consisting of bone, adipocytes and stroma of donor origin and hematopoiesis from recipient(107). The bone ossicles mimicked the changes of 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 tenfold reduction in bone and lacking 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 more than two-fold 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 numbers of osteoblast, osteoclast and marrow adipocyte, and osteogenic cultures show reduced differentiation and mineralization(108). Like $\Delta EPOR_E$-mice, mice with osteoblast deletion of Epor show no additional bone loss with EPO treatment, indicating that bone loss requires 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 demonstrated 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),(112),(113),(114),(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, inflammatory cytokine production(119). This is characterized by the appearance of crown-like structures which are histological features of inflammatory adipose tissues of obese animals consisting of macrophages surrounding necrotic adipocytes(119).

In obese mice, two week 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, crown-like structures, expression of pro-inflammatory cytokines and production of TNFα and increases anti-inflammatory cytokine IL-10 production. Macrophages respond directly to EPO stimulation with increased STAT3 activation and reduced 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 high fat

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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 show a greater inflammatory response in adipose tissue(18). $\Delta EPOR_E$ white adipose tissue exhibits denser macrophage infiltration and increased crown-like 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 and improved insulin sensitivity. Other organs contributing to EPO activity during diet induced obesity include JAK2 dependent EPO protective effect on insulin producing pancreatic β-cells, inducing pancreatic islets 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 proopiomelanocortin

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 proopiomelanocortin (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 non-hematopoietic tissue are obese, exhibit reduced hypothalamus POMC production and 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).

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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 high fat diet feeding(20). Cerebral EPO exhibits a gender-specific response in high fat diet obesity and male but not female Tg21 mice exhibited 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 brain, and increased pro-inflammatory cytokines prior to overt obesity and inflammation in white adipose tissue(132). Transmembrane TNFα is expressed on activated macrophages, lymphocytes and other cell types (TNFα+ cells) and undergoes proteolytic cleavage to release the soluble form of TNFα(133). Male Tg21 mice on high fat diet show reduced hypothalamus activated microglial cells, TNFα+ cells, inflammatory cytokine gene expression and 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 high fat diet also showed 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 high fat diet feeding, were more glucose intolerant, and showed greater induction of hypothalamus TNFα, 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 that blocks the anti-obesity estrogen activity, female Tg21 mice exhibited the protective effect of cerebral EPO and only wild-type female mice showed increase fat mass and hypothalamus inflammation, microglial activation and inflammatory cytokine expression(Figure 2)(20).

Conclusion

Animal models demonstrate that both endogenous and exogenous EPO contribute to metabolic response. Epor expression in white adipose tissue, adipocytes and macrophages, and in brain, neurons and microglia, mediate 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 only observed in male mice. Secondary analysis of full-heritage Pima Indians from the Gila River Indian Community with high prevalence of obesity and type 2 diabetes(135),(136) show endogenous EPO level associated negatively with hemoglobin and in males a negative association with percent weight change per year while females showed a positive association(137). These gender specific relationships

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between EPO level and body weight are consistent with reduction of body weight with EPO treatment in 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),(139),(140). Assessment of elderly men with normal kidney function in Sweden showed high EPO level 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 methodology to selectively increase erythropoiesis while maintaining bone health or to promote a tissue specific non-hematopoietic response without increased erythropoiesis.

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Highlights

- Mouse models demonstrate erythropoietin metabolic response mediated by erythropoietin receptor expression in adipose tissue, brain and bone.
- Erythropoietin regulation of fat mass in white adipose tissue is gender dependent.
- Cerebral erythropoietin regulation of fat mass and hypothalamus inflammation during high-fat diet feeding is gender dependent.
- Erythropoietin regulates adipogenesis and osteogenesis in bone.
- Bone loss accompanying erythropoietin stimulated erythropoiesis is mediated by direct osteoblast response.

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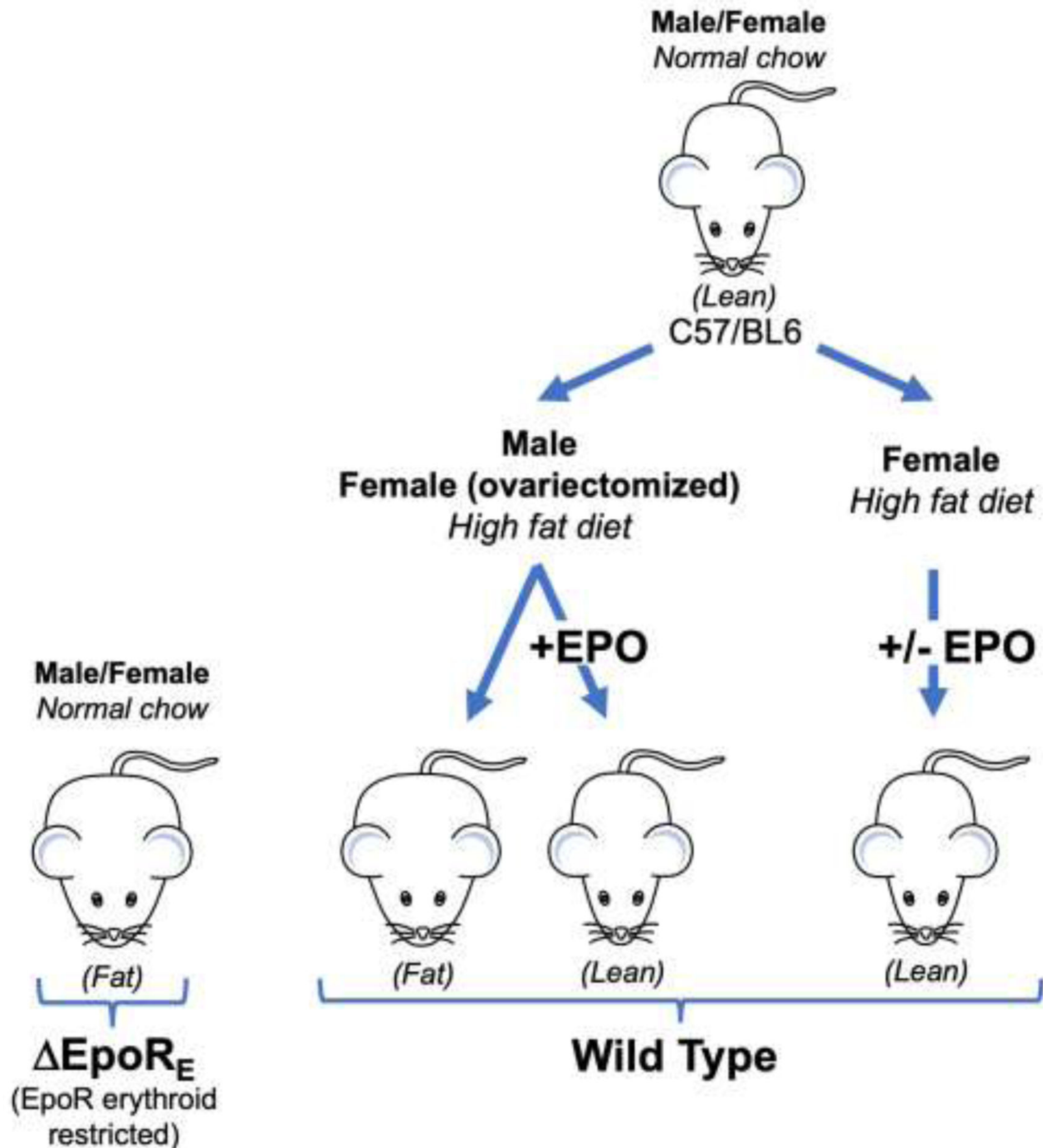

Figure 1. Erythropoietin (EPO) contributes to fat mass regulation.

$EPOR_E$-mice with Epor restricted to erythroid tissue exhibit accelerated body weight gain due to increased fat mass and become obese. Conversely, C57BL/6 male mice fed a high fat diet become obese while EPO treatment concomitant with high fat diet feeding 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 high fat

diet concomitant with EPO treatment exhibit increased hematocrit and protection against diet induced obesity.

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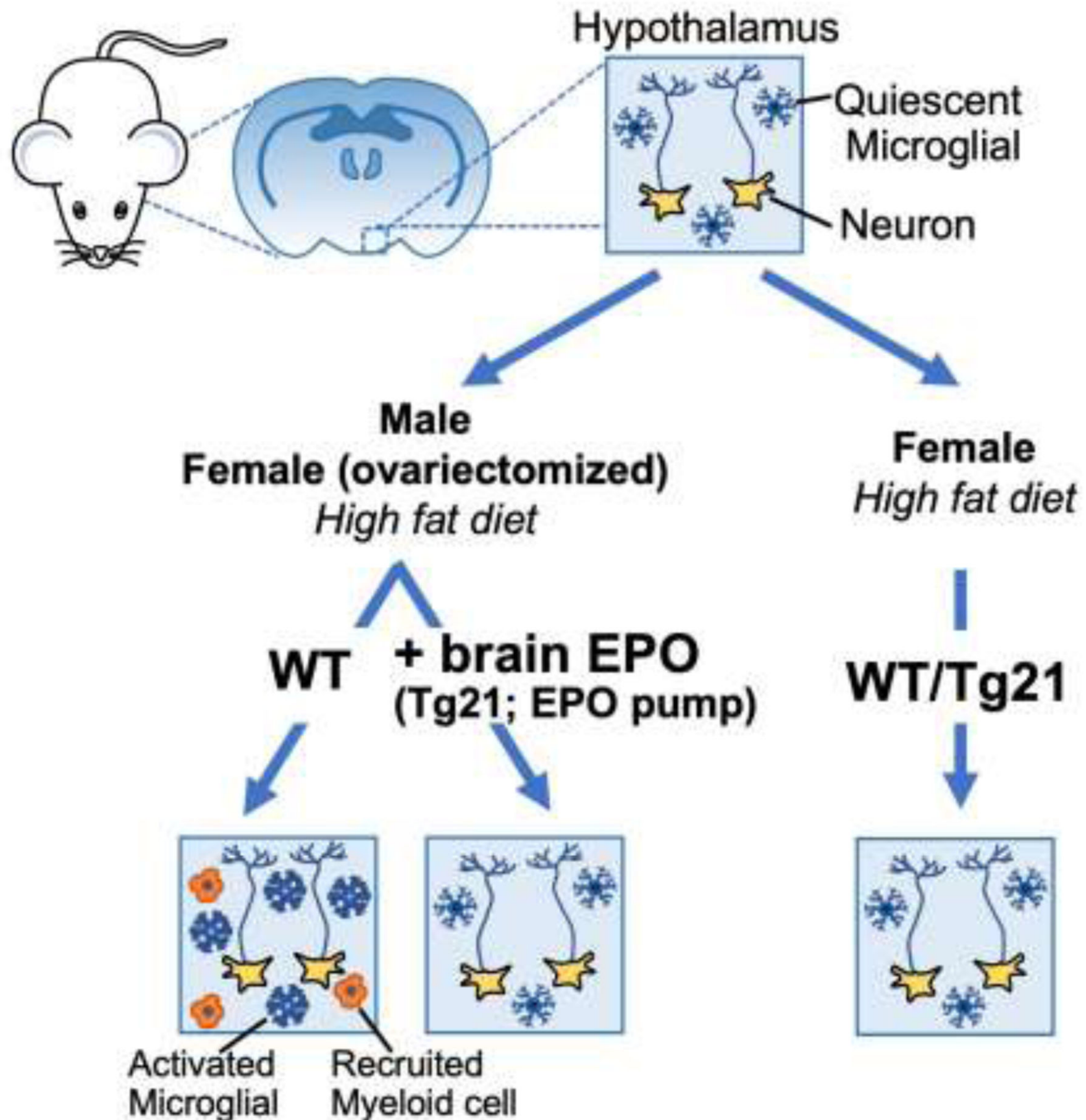

Figure 2. Cerebral erythropoietin (EPO) protects against high fat diet induced hypothalamus inflammation.

C57BL/6 male mice fed a high fat diet become obese 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 associate hypothalamic inflammation. Mice with implanted EPO intracerebroventricular pump and Tg21-mice exhibit normal hematocrit due to 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.

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