The Mediation of Hepatic Lipogenesis Through Estrogens
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Abstract
Estrogens have been shown to protect against various diseases and disastrous metabolic consequences of poor diets. Although a large body of research demonstrates estrogen’s ability to control food intake, adipogenesis, and oxidative stress, research regarding the effects of estrogens on hepatic lipogenesis, steatosis, and non-alcoholic fatty liver disease is only now accumulating. Estrogen deficiency in both human and rodent models directly results in the upregulation of hepatic lipogenic signaling - in both serum and hepatic triglyceride content - which leads to the development of fatty liver. In all models, estrogen replacement completely reverses these outcomes. Similar to the endogenous estrogen hormone, plant-derived phytoestrogens also appear to have beneficial effects related to prevention of hepatic lipogenic signaling and steatosis in rodent models. Additionally, such compounds can completely overcome the hepatic consequences that result from estrogen deficiency. While published research strongly supports that estrogens, both endogenous and exogenous, can protect against hepatic lipogenic signaling that can contribute to the development of non-alcoholic fatty liver diseases and adverse weight gain, little research exists on elucidating the mechanism behind this protection. Various pathways have been suggested, including manipulation of both leptin and signal transducer and activator of transcription 3 (STAT3) signaling. However, the discovery of x-box protein 1 elicits the identification of another potential pathway through which estrogen may be working. While the supportive work is strong, further research is needed to determine the mechanism behind the protection by estrogens from hepatic lipogenesis and associated diseases. Keywords: Estrogens, Lipogenesis, adipogenesis, steatosis, phytoestrogens.

Introduction
Loss of estrogen dramatically increases the risk for many diseases because of estrogen’s pervasiveness in a variety of tissues. Weight gain associated with menopause can result in a secondary response, increasing the risk of weight-related diseases like diabetes, cardiovascular disease, and non-alcoholic fatty liver disease (Carr 2003; Matthews et al 1989). While the environmental causes of weight gain can be managed through dietary modification and physical activity, there are few options for preventing age-related and hormonal changes in body composition. As more women enter menopause already overweight or obese, any further increases in weight that are difficult to control will only result in increased risk of disease and an increased demand on the health care system (Heymsfield et al 1994; Poehlman 2002). In aged populations, BMI corresponds greatly to increased health care costs. Obese adults aged 65-years and older have increased yearly Medicare expenditures compared to those who are overweight; approximately $571 more in those with class I obesity and $1,271 more in those with class II/III obesity (Onwudiwe et al 2011). With the baby boomer population entering retirement age, such numbers are expected to rise in the coming years.

Hepatic lipogenesis contributes significantly to whole body fat accumulation and metabolic diseases (Glimcher and Lee 2009). Previous research indicates that hepatic lipogenesis plays a part in
these pathways can blunt weight gain (Birkenfeld et al 2011). Since today’s environment is one of caloric excess, in many individuals the conversion of carbohydrates to fat for storage is perpetually occurring. Because the liver is the first tissue in the body to come in contact with metabolites from much of what we eat, it is an important player in energy balance. While research investigating how estradiol impacts adiposity through controlling food intake and lipolysis is widespread, little research exists regarding its effects on lipogenesis in the liver. Preliminary research demonstrates that estrogens downregulate hepatic lipogenesis; thus they are currently used in the pharmacologic treatment in males with aromatase deficiencies suffering from hypertriglyceridemia and hepatic steatosis (Maffei et al 2004). The following review will focus on the published literature demonstrating the ability of endogenous and phytochemical estrogens to prevent obesity through downregulation of hepatic lipogenesis. Additionally, potential mechanisms behind this regulation will be discussed.

Sex differences in diet-induced obesity

It has long been established that in animal models of diet-induced obesity, females display some protection from the mal-effects of high-fat diets, whereas males do not. The protection is robust and includes increased glucose and cholesterol control, and in some studies females report reduced serum triglycerides more than males (Hwang et al 2010). Ovariectomy (OVX) in animal models produces similar effects, inducing a rapid weight gain that can be attenuated and reversed through estrogen replacement (Asarian and Geary 2002). Estrogen is a potent anti-obesity agent in a variety of body systems, both central and systemic. Estrogen reduces food intake, increases spontaneous physical activity and has potent lipolytic effects on adipocytes (Asarian and Geary 2002; Shi and Clegg hypothalamus and on the adipocyte are well established, limited research exists in other tissues such as the liver. A recent study shows that male rats given injections of 17β-estradiol (E2) had reduced levels of hepatic lipogenesis, suggesting that the anti-obesity mechanisms behind estrogen are farther reaching than previously demonstrated (Hewitt et al 2004).

Estrogen regulation of lipogenesis

Although the current knowledge base of estrogen regulation of hepatic lipogenesis is limited, some evidence suggests that the anti-obesity effects of estrogen include downregulation of hepatic lipogenesis. This has been demonstrated recently by Gao et al (2006) who found that 4 weeks of E2 supplementation in female ob/ob mice resulted in a significant downregulation of genes involved in hepatic lipogenesis. Microarray analysis indicated significant reductions in many genes, including fatty acid synthase (FAS), acetyl CoA carboxylase-1 (ACC1) and stearoyl CoA desaturase-1 (SCD1) (Gao et al 2006).

OVX in animal models removes the main site of production for the endogenous female sex hormones, allowing for a model of menopause but also for the investigation of the specific effects of both estrogen and progesterone in the female body. Various studies have demonstrated that ovariectomy increases the lipogenic capacity of the liver. Paquette et al in 2008 demonstrated that OVX in female rats resulted in increased gene expression of sterol regulatory binding protein-1c (SREBP-1c), SCD-1, and peroxisome proliferator-activated protein alpha (PPARα) compared to intact females. These increases were also accompanied by increased fat accumulation in the liver (Paquette et al 2008). In another study, pelleted E2 replacement (0.012 mg/d) in OVX rats provided a reduction in hepatic and adipose fat accumulation, SREBP1c, ACC1 and SCD1 gene expression, and reduction of proinflammatory markers,
nuclear factor kappa-B kinase unit beta (IKK beta) and nuclear concentrations of nuclear factor kappa-B (NFκB) (Pighon et al 2011). These results suggest a potential relationship between hepatic inflammation and lipogenesis in the E2-deficient female rat.

Estrogen sulfotransferase (EST), the enzyme responsible for estrogen inhibition, has been associated with hepatic lipogenesis. Tissue specific knockdown of the EST enzyme results in protection from lipogenic activity in the liver. This protection was due to maintenance of hepatic E2, as EST knockdown in male and OVX female rats did not result in reductions of hepatic lipogenic activity (Gao et al 2012).

Estrogen levels in males have also been shown to modulate hepatic lipogenesis. Deficiency in aromatase, an enzyme involved in the synthesis of E2 from androgens, causes increased hepatic lipogenesis and fat deposition. Two separate case studies in adult men have been published describing patients with a genetic mutation in the aromatase gene (Pura M 2003). Both patients reported metabolic syndrome accompanied by hepatic steatosis in addition to a variety of other complications. Treatment with testosterone in one patient resulted in furthering the severity of insulin resistance, whereas transdermal E2 treatment resulted in the reversal of both insulin resistance and the hepatic steatosis (Maffei et al 2004). Similar findings have been shown in animal models, attributing the loss of E2-mediated reductions in lipogenesis as a contributing factor to the complications of aromatase deficiency. The aromatase knockout (ArKO) mouse provides a good model of E2-deficiency in males which displays increased weight gain and changes in lipid metabolism, including increased post-prandial serum triglycerides and cholesterol, hepatic steatosis and insulin resistance (Takeda et al 2003). These results indicate an increased lipogenic capacity due to the ArKO mice display increased hepatic triglyceride concentrations and increased FAS expression. Additionally ACC gene expression may be elevated, however it did not reach significance in one study (p=0.096) (Hewitt et al 2004). Furthermore, treatment of ArKO mice via an estrogen receptor agonist ameliorates the observed hepatic lipid accumulation and normalizes lipogenic gene expression to comparable levels to wild-type mice (Chow et al 2011).

Previously proposed mechanisms

Several mechanisms behind the observed suppression of hepatic lipogenesis by E2 have been proposed. Microarray studies suggest two potential sites of regulation, direct upregulation of the leptin receptor and signal transducer and activator of transcription-3 (STAT3) gene expression. Estrogen supplementation was associated with reduced lipogenic gene expression along with the leptin receptor and STAT3, both of which have been previously demonstrated to be genomically regulated by estrogen receptor alpha. Lastly, an additional protein (x-box binding protein or XBP1) has also been shown to have connections with both the estrogen receptor and lipogenesis. However, further research on the direct mechanism of estrogen-regulated reductions in hepatic lipogenesis has yet to be published.

STAT3 directed mechanism

STAT3 is a transcription factor closely associated with receptor kinases that mediate cellular signaling in response to ligand binding at the receptor. Upon ligand binding, a conformation change occurs on the receptor resulting in the activation of its associated protein kinase. The kinase will then phosphorylate janus kinase (JAK) which then associates with and phosphorylates STAT3. The activated STAT3 protein then undergoes dimerization and can then translocate to the nucleus to mediate gene expression. The JAK-STAT
cytokine and growth factor receptors. Because of the diversity of associated receptors, STAT3 has the ability to modulate a vast number of genes involved with cell growth, survival, and death.

Liver-specific knockout of STAT3 in mice results in increased hepatic triglyceride content in addition to increased SREBP-1c gene expression, suggesting a STAT3-mediated mechanism in lipogenic regulation (Inoue et al 2004). Viral reintroduction of STAT3 was found to reverse these effects in mice. This purposed regulation of SREBP1c gene expression has later been attributed to a direct inhibitory role of STAT3 on the promoter of the SREBP1c gene (Ueki et al 2004). Lastly, estrogen receptor alpha has been shown to regulate STAT3 expression. The STAT3 promoter lacks an however estrogen receptor regulates gene expression through binding to regulatory STATs and the DNA bound activating protein-1 at their respective response elements (Gao et al 2006). By this roundabout mechanism, E2 is capable of inducing STAT3 gene expression, providing increased STAT3 levels that can bind to the SREBP1c promoter and prevent its transcription (Figure 1). This was recently demonstrated in pancreatic β-cells of mice with specific STAT3 deletion (Tiano and Mauvais-Jarvis 2012). In this study, the reduction of SREBP1c by estrogen receptor alpha was found to be dependent on STAT3; however authors also indicated the AMP-activated protein kinase pathway as another site of estrogen-mediated activity.

Figure 1: Estrogens may reduce hepatic lipogenesis by regulating STAT3 activity, which inhibits SREBP1c transcription.

Abbreviations: Acetyl CoA carboxylase 1 (ACC1), fatty acid synthase (FAS), steroyl CoA desaturase 1 (SCD-1), sterol regulatory binding protein-1c (SREBP-1c), signal transducer and
activator of transcription-3 (STAT3), DNA bound activating protein-1 (DBAP1), estrogen receptor alpha (ERα), leptin receptor (Ob-Rb). The encircled plus refers to transcriptional upregulation caused by the estrogen receptor.

Leptin receptor directed mechanism

The leptin receptor is one of the JAK-STAT associated receptor kinases that were previously mentioned. Leptin receptor activation results in the phosphorylation of STAT3, which is then capable of inhibiting SREBP1c gene expression, thereby reducing the lipogenic capacity of the liver. Leptin resistance, either by intracellular signaling or by reductions in receptor expression, thus has been associated with increased hepatic lipogenesis. Both the leptin receptor deficient mouse (db/db) and the leptin deficient mouse (ob/ob) present with increased hepatic lipogenesis, steatosis, and obesity.

It has been established that the estrogen receptor modulates leptin gene expression in various tissues. Estrogen receptor alpha knockout mice display reduced hepatic leptin receptor expression, which also corresponds with increased hepatic lipogenic gene expression (Bryzgalova et al 2006). Estrogen treatment in the ob/ob mouse produces opposing effects. A reversal of the increased lipogenic gene expression and steatosis are observed (Gao et al 2006). However, authors of this study suggest that the E2-mediated reductions in hepatic lipogenesis is most likely not due to impaired leptin signaling alone. In the ob/ob mouse, the leptin produced is a variant that cannot activate the receptor. While E2 does modulate leptin synthesis, the ob/ob mouse given E2 supplementation would still have impaired leptin signaling. Therefore, as authors suggested, a combination of both STAT3 and leptin modulation is most likely occurring (Figure 1).

A novel XBP-mediated mechanism

XBP1 is a novel protein that has recently been of interest in a variety of inflammatory-mediated diseases. In most tissues, XBP1 coordinates the unfolded protein response during endoplasmic reticulum stress and is necessary for cell survival. Deficiencies in XBP1 result in a variety of diseases including inflammatory bowel disease, Alzheimer’s and Parkinson’s Disease, and type 2 diabetes (Kaser et al 2008; Matus et al 2011; Ozcan et al 2004). However, prolonged activation of endoplasmic reticulum stress and XBP1 activity results in a shift to apoptosis of the cell.

XBP1 activation

Activation of XBP1 results from an unconventional splicing mechanism performed by inositol-requiring enzyme-1 (IRE-1). IRE-1 is an endoribonuclease that cleaves a 26 base pair fragment from the XBP1 mRNA between sites 531 to 556 (Yoshida et al 2001). The resultant mRNAs include the XBP1u (261 amino acids) and XBP1s (376 amino acids). XBP1 splicing results in a frameshift mutation that provides for a basic leucine zipper- DNA binding domain and a transactivation domain within the extended C-terminal region of the translated protein (Yoshida et al 2001). The splicing and subsequent mutation permits the transcriptional activity of the translated pXBP1s (p- delineates protein). The remaining pXBP1u translated protein is quickly degraded; however recent work suggests that it may serve as a negative regulator of the unfolded protein response (UPR) by sequestering pXBP1s and targeting it for proteasomal degradation (Yoshida et al 2006; Yoshida et al 2009).

IRE-1 activation normally occurs due to an accumulation of unfolded and misfolded proteins within the endoplasmic reticulum lumen. An accumulation of these proteins often suggests that the cell is undergoing stress, including viral infection, nutrient
degradation mechanism within the endoplasmic reticulum becomes overwhelmed, resulting in the need for increased machinery to handle the unfolded proteins (Zheng et al 2010). Such proteins bind directly to IRE-1, resulting in its oligomerization and transphosphorylation of its kinase domain. This then results in the subsequent activation of the endoribonuclease on the cytosolic region of IRE-1 (Cox et al 1993; Gardner and Walter 2011).

IRE-1 mediated XBP1 activation has been shown to be an important regulator of hepatic lipogenesis and potentially, adipogenesis (Glimcher and Lee 2009; Sha et al 2009). Some have suggested that saturated fat-induced activation of XBP1 is independent of endoplasmic reticulum stress (ER stress) and the UPR; however the consensus appears to be otherwise (Zheng et al 2010). Increased saturated fat consumption results in various changes to cell membranes, including that of the endoplasmic reticulum, affecting the overall composition and motility of the membranes. Saturated fatty acids are readily converted to cholesterols, which in large amounts can be incorporated within the endoplasmic reticulum membrane and lead to a depletion of ER-calcium stores (Feng et al 2003; Ron and Oyadomari 2004). As calcium is necessary for many cellular processes, including protein folding, reductions in calcium concentrations reduce the efficiency of protein folding within the endoplasmic reticulum lumen, thereby promoting ER stress (Di Jeso et al 2003; Hojmann Larsen et al 2001). Lastly, a recent study in macrophages found that through toll-like receptor-4 (TLR-4) activation, both ER stress and XBP1 activation can be induced (Martinon et al 2010). As saturated fat has demonstrated the ability to bind to TLR-4, this provides another site for saturated fat-induced XBP1 activation (Milanski et al 2009).

XBP1 and lipogenesis

necessary step for cell survival, in both adipose and hepatic tissue XBP1 has an alternative role in promoting lipogenesis (Glimcher and Lee 2009; Sha et al 2009). Pharmacologic activation of ER stress, and thus XBP1 activation, results in an upregulation of lipogenesis in hepatic cells, steatosis, and hepatic inflammatory signaling in mice (Lee et al 2012a; Lee et al 2012b). Mice with deficiencies in hepatic XBP1 had decreases in circulating triglycerides, free fatty acids, and cholesterol, in addition to reduced fat accumulation in the liver (Glimcher and Lee 2009). Additionally, pharmacologic inhibition of XBP1 activation in post-prandial environments resulted in a downregulation of lipogenic gene expression (Pfaffenbach et al 2010). Such positive metabolic outcomes in these mice are attributed to the transcriptional regulation of lipogenic genes by XBP1. XBP1 is a transcription factor for a variety of lipogenic genes including FAS, ACC, and SCD-1 (Ren et al 2012).

Estrogen regulation of XBP1

In 2004, an analysis of promoter regions for the estrogen receptors located a region on the XBP1 gene suggesting a potential relationship between E2 signaling and XBP1 expression (Wang et al 2004). Since then most studies have investigated the role of estrogen receptor alpha and XBP1 activity in E2-responsive breast cancers. However, two recent studies have shown positive outcomes in hepatic tissue after E2 treatment that appears to be dependent on regulating XBP1 activity. E2 treatment prior to an induced hypotension model of trauma-hemorrhage in male rats resulted in increased survival and reduced ER stress compared to vehicle-treated controls (Kozlov et al 2010). In OVX mice fed a high saturated fat diet, increased activation of hepatic XBP1 was observed compared to sham, chow-fed controls (Fukui et al 2011). Again, as demonstrated previously, E2 treatment significantly reduced XBP1 activation and ER stress in this study.
Lastly, unpublished work from the University of North Carolina at Greensboro found a significant reduction in total XBP1 mRNA in female rats compared to males fed a high fat diet for 72 hours (Miller 2011). This finding was accompanied by no increase in fat deposition in the female rats, whereas high fat diet resulted in increased fat gain in male rats. These findings will be further explored to determine if the female rats also had reduced hepatic lipogenic gene expression. Overall, early studies support a possible mechanism behind estrogen-induced reductions in lipogenesis that may be due in part to downregulation of XBP1 activity, which is outlined in figure 2.

Lastly, it is important to mention the potential of XBP1-based therapies in regulating hepatic lipogenic signaling. Silencing of XBP1 both in vivo and in vitro leads to reduced hepatic lipogenesis without any reports of toxicity (Ning et al 2011). However as XBP1 appears to be necessary for liver tumor cell survival, knockdown of this pathway may produce negative affects in regards to hepatocarcinoma treatment (Cusimano et al 2010). Therefore, the use of XBP1 drug therapy may be highly situational and would need to be addressed with caution.

Figure 2: Estrogens may be reducing hepatic lipogenesis by regulating the transcription of a potent transcription factor (XBP1) for various lipogenic genes. Abbreviations: Acetyl CoA carboxylase 1 (ACC1), fatty acid synthase (FAS), steroyl CoA desaturase 1 (SCD-1), diglyceride acyl-transferase (DGAT), diglyceride (DAG), triglyceride (TAG), endoplasmic reticulum (ER), inositol requiring enzyme 1 (IRE-1), x-box protein 1 (XBP1),
x-box protein 1 spliced variant (XBP1s), estrogen (E2), sterol regulatory binding protein-1c (SREBP-1c). The encircled plus refers to transcriptional upregulation caused by the estrogen receptor.

Phytoestrogens and lipogenesis

Phytoestrogens are plant-derived compounds that have demonstrated estrogenic activities by binding directly to the estrogen receptors. Because of the potential risks of hormone replacement therapy for use during menopause, much interest has emerged in investigating phytoestrogenic-compounds to manage the increase in disease-risk caused by the loss of ovarian hormones (Rossouw et al 2002). Phytoestrogens bind to the estrogen receptors at a lower affinity than E2, and thus are suggested to be safer than traditional hormone therapy (Kuiper et al 1998).

Much like E2, phytoestrogens have also emerged as potential regulators of hepatic lipogenic signaling, although research is much more limited in this area. Dietary genistein, a phytoestrogen derived from soy, reduced hepatic steatosis in male mice fed a high fat diet for 12 weeks (Kim et al 2010). These findings are further supported through observed downregulation of lipogenic genes in both human lung cancer cells and within the HepG2 cell line after treatment with genistein (Hess and Igal 2011; Shin et al 2007). An additional phytoestrogen, daidzein, has also been linked with reducing hepatic lipogenesis and steatosis in high fat diet-fed mice as observed by reduced lipogenic gene expression and lipid concentrations (Kim et al 2011).

Similar to the studies involving E2, the studies investigating phytoestrogens have yet to specifically target and study a mechanism behind their actions. Various pathways have been suggested, including inflammatory and insulin signaling, SREBP-1c processing, and regulation of the liver X receptor β (Kim et al 2010; Kim et al 2011; Shin et al 2007). Additionally, phytoestrogens, much like E2, have been demonstrated to affect XBP1 activity. In neuroblastoma cells, both genistein and daidzein reduced XBP1 expression and activity in an estrogen receptor-dependent fashion (Park et al 2009). This finding suggests that phytoestrogens might work in a similar fashion within the liver, but further research is necessary.

Conclusions

Women often gain weight during menopause because of the dramatic reduction of circulating E2, which may only exaggerate the rise in overweight and obesity particularly in older adults. Many of the anti-obesity effects of E2 have already been targeted for study, including its effects on appetite and on lipolysis in the adipocyte. However, the breadth of research on E2’s effects on hepatic lipogenesis pales in comparison. So far it has been suggested that E2 appears to downregulate hepatic lipogenesis in various animal models and in humans, while the mechanism has yet to be elucidated. Greater understanding of anti-obesity actions of E2 is needed. Further insight into the potential nutraceutical and pharmaceutical targets will not only help reduce the prevalence of excessive weight and obesity in older women, but also improve associated health outcomes.

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