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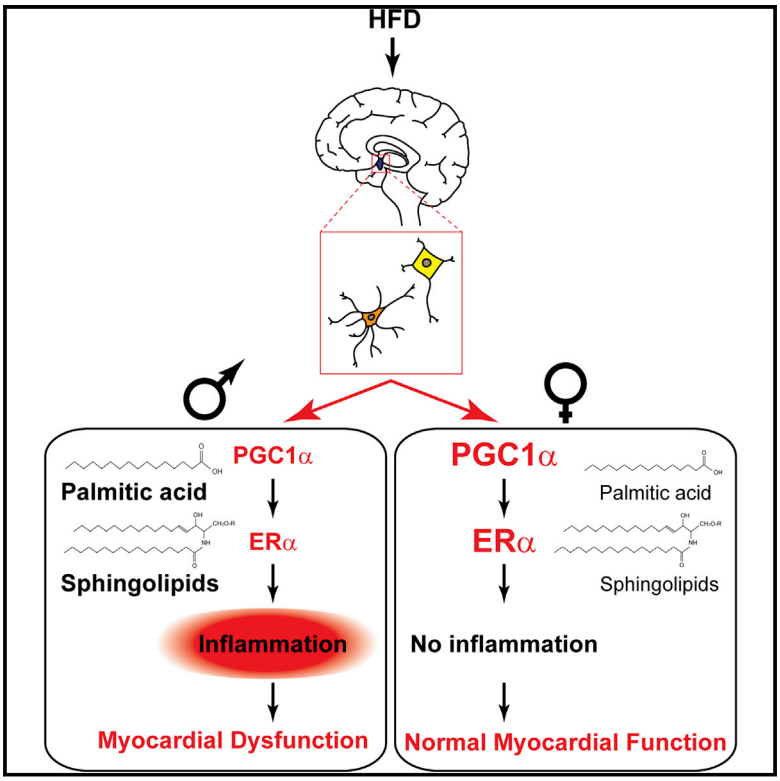
Hypothalamic PGC-1α Protects Against High-Fat Diet Exposure by Regulating ERα

Graphical Abstract



Highlights

Male and female mice differ in their metabolic response to fatty acids

Hypothalamic inflammation correlates with reductions in myocardial function

HFD-induced downregulation of ERα is permissive for increased inflammation

Fatty acids decrease PGC-1α in male, but not female, mice

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In Brief

In this study, Morselli et al. show that male and female mice differ in their inflammatory response to fatty acids via a mechanism involving the hypothalamic regulation of ERα by PGC-1α. These findings may provide insights into the reduced risk of obesity-related disease in premenopausal women.

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Article

Hypothalamic PGC-1α Protects Against High-Fat Diet Exposure by Regulating ERα

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SUMMARY

High-fat diets (HFDs) lead to obesity and inflammation in the central nervous system (CNS). Estrogens and estrogen receptor α (ERα) protect premenopausal females from the metabolic complications of inflammation and obesity-related disease. Here, we demonstrate that hypothalamic PGC-1α regulates ERα and inflammation in vivo. HFD significantly increased palmitic acid (PA) and sphingolipids in the CNS of male mice when compared to female mice. PA, in vitro, and HFD, in vivo, reduced PGC-1α and ERα in hypothalamic neurons and astrocytes of male mice and promoted inflammation. PGC-1α depletion with ERα overexpression significantly inhibited PA-induced inflammation, confirming that ERα is a critical determinant of the anti-inflammatory response. Physiologic relevance of ERα-regulated inflammation was demonstrated by reduced myocardial function in male, but not female, mice following chronic HFD exposure. Our findings show that HFD/PA reduces PGC-1α and ERα, promoting inflammation and decrements in myocardial function in a sex-specific way.

INTRODUCTION

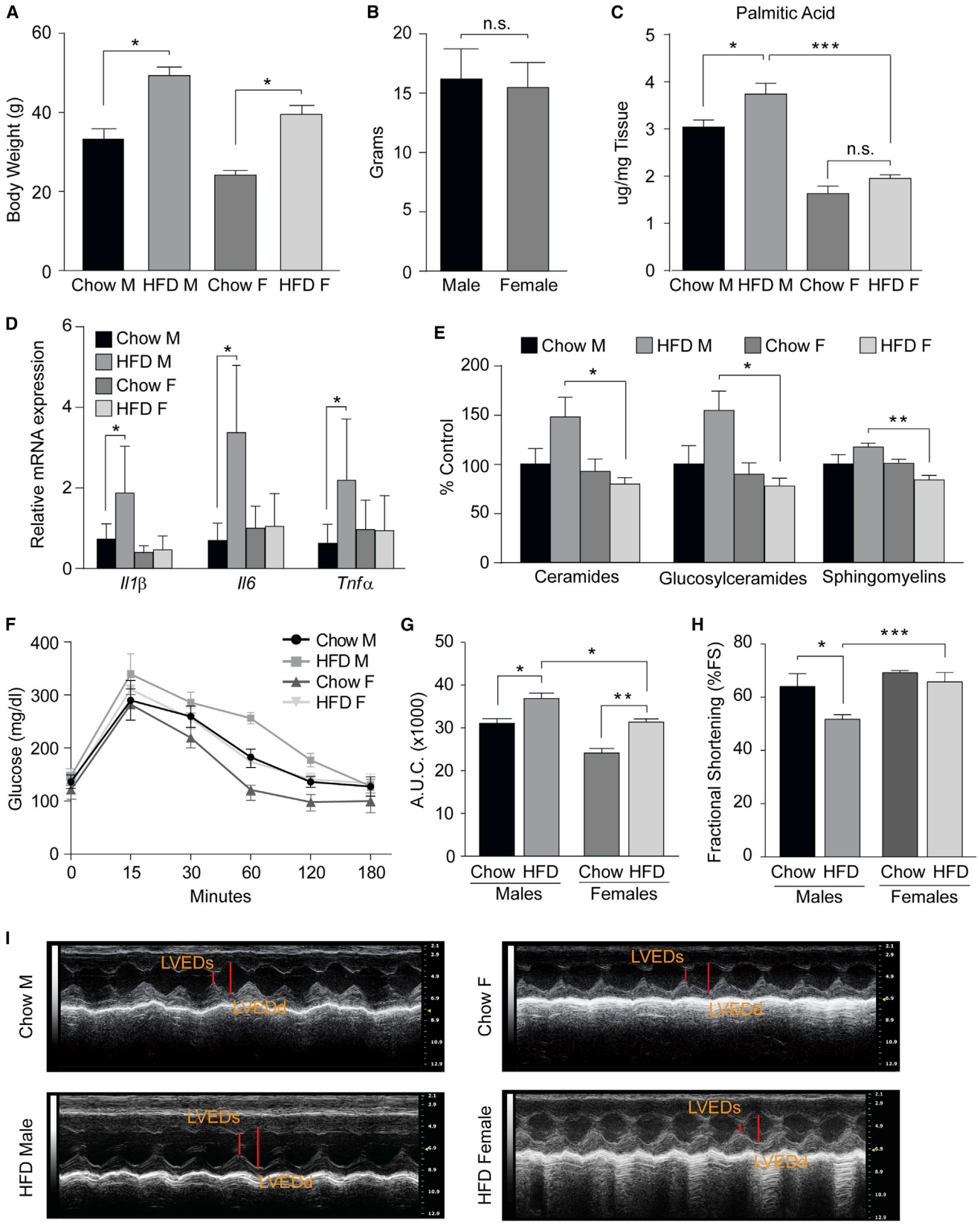
In the last 50 years, obesity has become a global epidemic. The World Health Organization estimates that more than half a billion adults worldwide are obese (Gregor and Hotamisligil, 2011). Obesity is associated with chronic, low-grade inflammation in adipose tissues and in the central nervous system (CNS) (Hotamisligil, 2006; Thaler et al., 2012). CNS inflammation, in turn, leads to insulin and leptin resistance and facilitates the onset of cardiovascular disease (Purkayastha et al., 2011; Thaler et al., 2012; Zhang et al., 2008); however, the mechanisms underlying CNS inflammation and accompanying comorbidities remain unclear.

The saturated long-chain fatty acid palmitic acid (PA) induces inflammation in the hypothalamus, and the plasma concentration of PA is significantly increased in obesity (Opie and Walfish, 1963; Reaven et al., 1988). Furthermore, PA crosses the blood-brain barrier (Dhopeshwarkar and Mead, 1973; Smith and Nagura, 2001), and high-fat diets (HFDs) promote CNS uptake of PA (Karmi et al., 2010; Wang et al., 1994). Intracerebroventricular infusion of PA activates proinflammatory responses in the hypothalamus and promotes insulin resistance (Posey et al., 2009). Thus, PA-induced hypothalamic inflammation could play a critical role in the development of obesity-related diseases such as cardiovascular disease.

Although obesity affects both males and females, there is a sexual dimorphism in the development of metabolic complications associated with obesity (Shi et al., 2009; Sugiyama and Agellon, 2012). Premenopausal women are protected from the adverse effects of obesity; however, the prevalence of metabolic disorders increases significantly after menopause (Ford, 2005). Interestingly, 17β-estradiol (E2) and its receptor, estrogen receptor α (ESR1/ERα), protect against obesity-related diseases (Musatov et al., 2007; Xu et al., 2011). Abnormal adiposity and glucose intolerance have been associated with polymorphisms and point mutations of the human *Erα* gene (Okura et al., 2003; Smith et al., 1994), and mice lacking ERα (ERKO) have increased adiposity and impaired glucose tolerance (Heine et al., 2000).

The peroxisome proliferator-activated receptor gamma, coactivator 1 α (PGC-1α) is a transcriptional coactivator involved in multiple metabolic pathways (Lin et al., 2005). PGC-1α is reduced in adipose tissue in genetically obese mice (*Lep^{ob}*/*Lep^{ob}*) as well as following HFD exposure (Crunkhorn et al., 2007). However, its role in the hypothalamus in the response to an HFD has not been characterized. Two studies have demonstrated that PGC-1α transcriptionally regulates ERα in vitro (Bourdoncle et al., 2005; Tcherepanova et al., 2000); however, it is unknown whether this regulation occurs in the hypothalamus in vivo.

In the present study, we evaluate the sexually dimorphic response to chronic HFD exposure. We demonstrate PA and



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sphingolipids are increased in the CNS of male when compared to female mice following consumption of an HFD. We show that chronic HFD exposure promotes hypothalamic inflammation and reduces ERα in male, but not female, mice. Further, we demonstrate that hypothalamic inflammation depresses myocardial function in male, but not female, mice. Treatment of neuronal and astrocyte cell cultures with PA, to mimic the in vivo effects of HFD exposure, increases inflammation and reduces ERα. PA-induced inflammation is enhanced in the absence of ERα, and E2 pretreatment protects against PA-induced inflammation only when ERα levels are restored. The mechanism by which fatty acids reduce ERα involves depletion of PGC-1α in neurons and astrocytes following PA treatment or HFD feeding in male, but not female, mice. Our data demonstrate a sexually dimorphic response to an HFD or PA: an HFD suppresses PGC-1α only in male mice, leading to downregulation of ERα and increased CNS inflammation, which is associated with decrements in myocardial function.

RESULTS

Long-Term Exposure to an HFD Promotes Hypothalamic Inflammation in Male Mice

Age-matched male and female mice were fed a chow diet or an HFD for 16 weeks. HFD males and females gained significantly more weight than controls (Figure 1A). Weight gain was matched in male and female mice (Figure 1B). HFD feeding significantly increased hypothalamic PA levels in males, but not in females (Figure 1C). Consistent with previous findings (De Souza et al., 2005; Thaler et al., 2012), HFD feeding increased proinflammatory cytokines (interleukin-1β [IL-1β], tumor necrosis factor α [TNF-α], *Il6*) and decreased the anti-inflammatory cytokine *Il10* in the hypothalamus of male, but not female, mice (Figure 1D; Figures S1A–S1C). Similar patterns of inflammation were found in the hippocampus and in the cortex (Figures S1D and S1E). No such changes were observed in females, despite similar weight gain following the HFD (Figure 1D; Figures S1A–S1E).

Sphingolipids are important signal transduction metabolites in immune-inflammatory responses and are antagonists of insulin signaling (Summers, 2006). Consumption of an HFD increases sphingolipid levels in plasma of rodents and humans (Haus et al., 2009; Holland et al., 2013; Samad et al., 2006). We found greater accumulation of ceramides in the hypothalamus of male HFD mice when compared to females (Figure 1E). Glucosylceramides were also significantly higher in HFD males than in females (Figure 1E), whereas the levels of sphingomyelins were lower in the HFD females. Collectively, these data suggest that decreased production of sphingolipids protects the hypothalamus of females from the proinflammatory effects of the HFD.

HFD Impairs Glucose Tolerance More in Male Than in Female Mice

To determine the metabolic consequences of HFD-induced inflammation, an oral glucose tolerance test (OGTT) was performed on male and female mice fed chow or HFD. Previous studies suggest female humans and rodents have improved glucose tolerance compared to males (Macotela et al., 2009; Yki-Järvinen, 1984). Consistent with this, chow females had lower glucose levels than males at every time point following the glucose gavage (Figure 1F). Although glucose homeostasis was impaired in both HFD male and female mice, there was a significant increase in glucose levels in HFD males when compared to females on the same diet (Figures 1F and 1G). Importantly, basal glucose levels and glucose clearance were similar between female mice exposed to the HFD and chow-fed males (neither of which had increased markers of inflammation in the hypothalamus); however, HFD exposure in males significantly impaired glucose homeostasis, and this correlated with increased inflammation in the CNS as well as in the periphery (Figure S1F). Notably, female mice showed higher plasma β-hydroxybutyrate levels (Figure S1G), consistent with reports that ketone bodies have anti-inflammatory effects (Gasior et al., 2006). Lastly, E2 is known for its anti-inflammatory role in the CNS (Barreto et al., 2009). E2 levels were significantly increased in females but unchanged in males following HFD exposure (Figure S1H), suggesting that E2 inhibits inflammation in HFD females.

Long-Term Exposure to an HFD Impairs Myocardial Function in Male Mice

Obesity is associated with comorbidities, including cardiovascular disease (Grundy et al., 2004), and the incidence of cardiovascular disease is sex dependent, with an increased risk in males when compared to premenopausal females (Czubryt et al., 2006; Ozbey et al., 2002). Further, work published by Purkayastha et al. demonstrates that hypothalamic inflammation induces hypertension (Purkayastha et al., 2011). To evaluate whether the sexual dimorphism in hypothalamic inflammation is associated with differences in cardiovascular function, we assessed myocardial function in male and female mice following HFD exposure. Consistent with previous reports (Battiprolu et al., 2012; Völkers et al., 2014), HFD feeding reduced myocardial function in male mice as demonstrated by decreased percent fractional shortening (%FS) (Figures 1H and 1I), but not in female mice (Figures 1H and 1I). This suggests that,

Figure 1. Chronic Exposure to a High-Fat Diet Promotes Inflammation

(A) Body weight at time of sacrifice. Chow male (M), n = 29; HFD M, n = 30; chow female (F), n = 30; HFD F, n = 32.

(B) Body-weight gain following HFD exposure.

(C) Palmitic acid amount in total brain. n = 4/group.

(D) mRNA levels of inflammatory markers in hypothalamic tissue following diet exposure. Chow M and F, n = 9; HFD M, n = 10; HFD F, n = 12.

(E) Hypothalamic sphingolipid content. n = 5/group.

(F and G) Oral glucose tolerance test (OGTT) (F) and area under the OGTT curve (G) of male and female mice fed chow or HFD for 14 weeks. Chow M and F, n = 4; HFD M and F, n = 5.

(H) Percent fractional shortening (%FS) following diet exposure. n = 7/group.

(I) Representative echocardiograms. LVEDd, left ventricular end-diastolic diameter; LVEDs, left ventricular end-systolic diameter.

Data are presented as mean ± SEM. *p < 0.05., **p < 0.01, and ***p < 0.001. See also Figure S1.

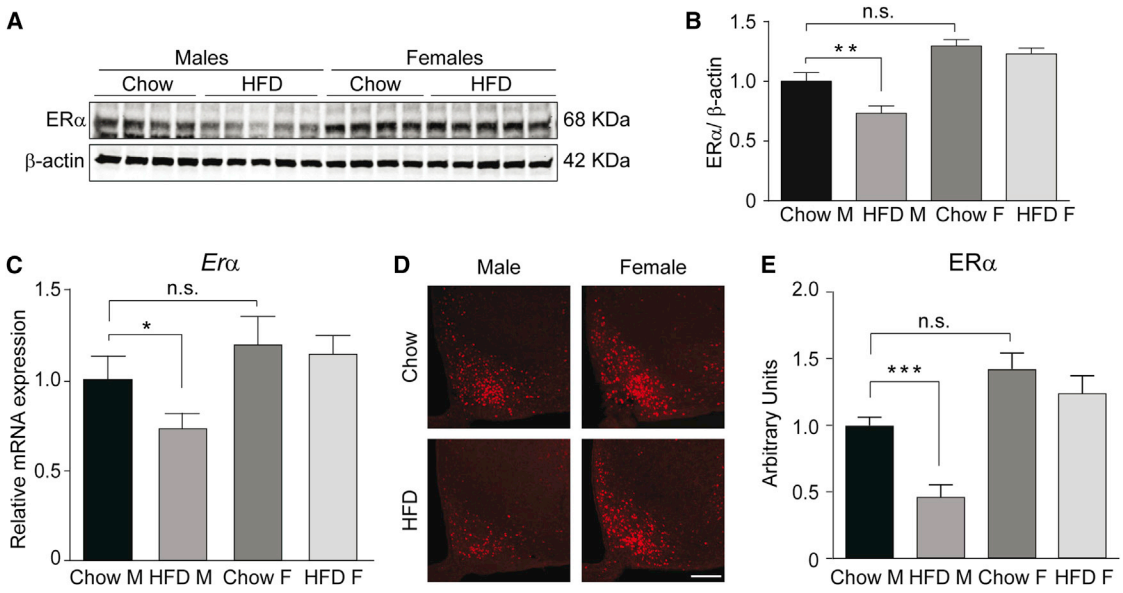


Figure 2. Chronic Exposure to a High-Fat Diet Decreases ERα

(A and B) Representative immunoblot (A) and quantification (B) of ERα in the hypothalamus. Chow M and F, n = 9; HFD M and F, n = 10.

(C) mRNA levels of *Erα* in hypothalamic tissue. Chow M and F, n = 9; HFD M, n = 10; HFD F, n = 12.

(D and E) Representative confocal images showing ERα immunoreactivity in the ARC (D) and relative quantification (E). n = 10/group. Scale bar, 125 μm.

Data are presented as mean ± SEM. *p < 0.05, **p < 0.01, and ***p < 0.001. See also Figure S2.

independent of similar body weight gain following HFD exposure, females are protected from the adverse effects of HFD. Our results are supported by Louwe et al., who showed a sex-dependent effect of HFD on cardiovascular function (Louwe et al., 2012). These data provide the physiological relevance of sexual dimorphisms in hypothalamic inflammation.

Long-Term Exposure to an HFD Decreases Hypothalamic ERα Expression in Male Mice

To investigate why females are protected from the adverse effects of the HFD, we measured ERα in hypothalamic tissue (Figure 2), since it has previously been shown that ERα protects against obesity-related diseases (Heine et al., 2000; Musatov et al., 2007). We found that ERα expression was significantly lower in the arcuate (ARC) of male, but not female, mice following HFD exposure (Figure 2). Additionally, *Erα*, and not *Erβ* (Figure S2F), levels were significantly decreased and inflammation increased in the hippocampus, cortex, and adipose tissue of HFD male mice, confirming a correlation between decreased ERα and higher tissue inflammation (Figures S2C and S2D). Collectively, these results indicate there is a sexually dimorphic response to HFD exposure, with greater PA, sphingolipids, inflammation, and glucose levels in HFD males when compared to females. These findings suggest reductions in myocardial function in male mice are correlated with reductions in ERα expression in the CNS.

E2 Pretreatment Protects against PA-Induced Inflammation in Neurons through ERα

Given the increased PA in the CNS of HFD males and the associated reductions in ERα, we addressed whether PA influences ERα in neurons. We exposed N43 cells (a hypothalamic cell line expressing ERα; Figure S3A) and primary hypothalamic neurons to PA, which significantly increased the expression of markers of inflammation (Figures 3A and 3D) and decreased ERα expression (Figures 3B, 3C, 3E, and 3F), supporting the hypothesis that reductions in ERα are permissive for PA-induced inflammation. To validate the sexually dimorphic response to PA-induced inflammation, we generated primary male and female hypothalamic neuronal cultures. PA increased inflammation and reduced ERα in male, but not female, primary neuronal cultures (Figure 3G). Importantly, only ERα was affected; PA treatment did not modulate *Erβ* expression (Figure S3B). Additionally, we determined that PA exposure significantly increased ceramide levels (Figure 3H), consistent with our findings in vivo. To determine if these results were specific to PA, we tested the inflammatory response to stearic and linoleic acid. Consistent with the literature (Arruda et al., 2011), stearic acid induced inflammation, while linoleic acid did not (Figure S3C). Importantly, ERα was significantly decreased following stearic acid, confirming that loss of ERα is permissive for increased inflammation (Figure S3C).

E2 acts through ERα to produce an anti-inflammatory effect in astrocytes (Barreto et al., 2009), and E2 is increased in female mice following HFD exposure (Figure S1H). To determine whether E2/ERα mediates a similar anti-inflammatory role in neurons, we cotreated neuronal cells with E2 and PA. Cotreatment with E2 did not decrease the PA-induced inflammation (Figure S3D); however, ERα expression was significantly decreased (Figures S3E and S3F). Importantly, when we pretreated the cells with E2 prior to PA treatment, ERα expression was not reduced (Figures 3B, 3C, 3E, and 3F) and the

PA-induced inflammation and increased ceramide levels were significantly inhibited (Figures 3A, 3D, and 3H). To assess the role of E2/ERα in mediating these protective effects, we transfected N43 cells with an ERα-specific small interfering RNA (siRNA) to downregulate ERα expression. Despite E2 pretreatment, lack of ERα abolished the E2-induced anti-inflammatory effect (Figure 3J), demonstrating that E2 requires ERα to decrease PA-induced inflammation.

ERα Overexpression Significantly Protects against PA-Induced Inflammation in Neurons

To determine whether ERα is sufficient to inhibit PA-mediated inflammation, we infected N43 cells with an adenoviral construct designed to overexpress ERα and treated with PA. ERα overexpression significantly decreased the proinflammatory effect of PA, suggesting that ERα protects against PA-induced inflammation (Figure 3K).

Absence of ERα in Microglia Is Associated with Inability of E2 to Protect against the Proinflammatory Effects of PA

More than 50% of the brain is composed of nonneuronal cells; microglia and astrocytes represent the most abundant cell types. Since it has been established that an HFD activates glial cells (Thaler et al., 2012), we evaluated the expression of ERα in microglia. To this end, we used CX3CR1$^{GFP/GFP}$ mice expressing the GFP construct in microglial cells and performed immunofluorescence with a previously validated ERα antibody (Figure S2B) to analyze the colocalization of ERα. Consistent with previously published reports (Saijo et al., 2011; Wu et al., 2013), analysis of the Pearson's coefficient confirmed ERα did not colocalize with microglial cells in the hypothalamus (Figures 4A and 4B). To determine whether the absence of ERα in microglia affects the ability of E2 to attenuate PA-induced inflammation, we treated BV2 cells, a microglial cell line lacking ERα, with E2 and PA. Despite E2 pretreatment, E2 anti-inflammatory activity was not observed (Figure 4C). These data confirm that E2 requires ERα to mediate its anti-inflammatory effects.

HFD Exposure Promotes Astrogliosis in Male, but Not Female, Mice

Chronic HFD exposure significantly activates astrocytes in rodents and humans (Lee et al., 2013; Thaler et al., 2012). Analysis of astrogliosis based on expression and staining of glial fibrillary acidic protein (GFAP) revealed an increase in the hypothalamus of male, but not female, mice (Figures 5A–5C). These data are consistent with enhanced expression of markers of inflammation in male, but not female, mice exposed to an HFD (Figure 1D).

To further elucidate the sexually dimorphic response to an HFD, we prepared primary hypothalamic astrocytes from male and female pups. Astrocyte activation was analyzed based on the expression of *Gfap* and markers of inflammation (*Il6* and *Il1β*). Both male and female astrocytes responded to PA treatment by increasing *Gfap, Il6*, and *Il1β* (Figures 5D and 5E). Interestingly, despite similar basal expression of *Gfap* and inflammatory genes in male and female astrocytes, there was significantly lower expression of these markers following PA in female astrocytes (Figures 5D and 5E). Consistent with previous studies (Spence et al., 2013), ERα was expressed in these cells (Figure S4). Increased markers of inflammation correlated with ERα protein levels, with males showing significant reductions in ERα following exposure to PA (Figures 5F and 5G). Pretreatment of astrocytes with E2 significantly decreased markers of inflammation in the presence of ERα (Figure 5E). These data further confirm the sexually dimorphic response to PA, with greater inflammation in male astrocytes correlating with a reduction in ERα.

PGC-1α Drives ERα Loss in the Hypothalamus of Male Mice

To address the mechanism by which HFD influences ERα within the ARC nucleus, we focused on PGC-1α, a key transcriptional coactivator involved in different metabolic pathways (Lin et al., 2005). PGC-1α transcriptionally regulates ERα in vitro (Bourdoncle et al., 2005; Tcherepanova et al., 2000); however, its role in vivo and in the hypothalamus is unclear.

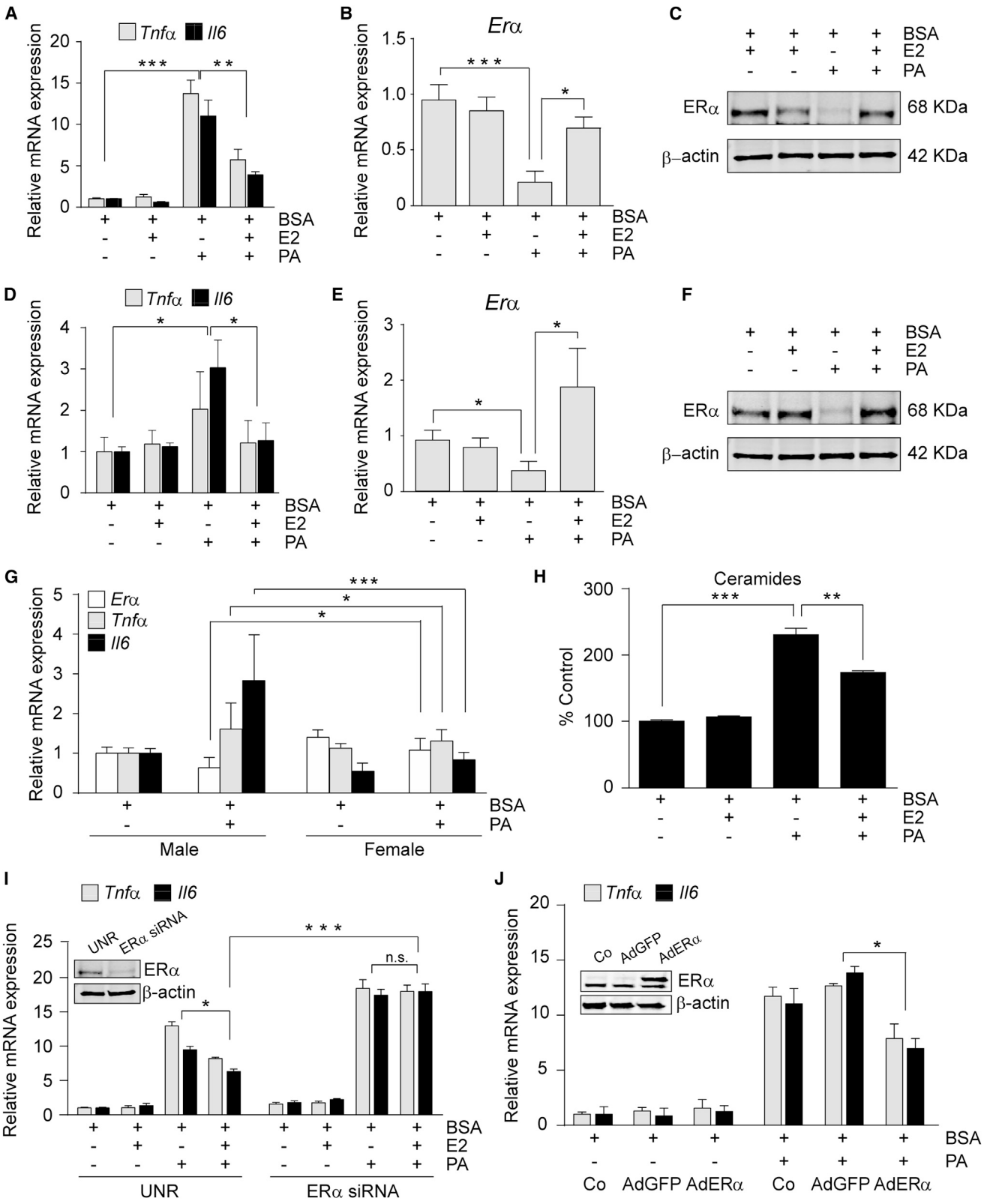
Consistent with a putative interaction between PGC-1α and ERα, we first demonstrated that they are colocalized in the hypothalamus (Figures 6A and 6B). To further demonstrate that PGC-1α transcriptionally regulates ERα, we evaluated ERα transcripts in PGC-1α$^{-/-}$ mice. Consistent with the hypothesis that exposure to an HFD reduces ERα expression in the CNS via effects on PGC-1α, we found that *Erα* mRNA levels were significantly reduced in PGC-1α$^{-/-}$ mice (Figure 6C). Importantly, in ERKO mice, *Pgc-1α* expression was unaltered (Figure 6D), suggesting that PGC-1α regulates ERα but ERα does not regulate PGC-1α. To confirm this finding, N43 cells were transfected with siRNA against PGC-1α. ERα mRNA and protein levels were significantly reduced following PGC-1α downregulation (Figures 6E–6G). Collectively, these results indicate that in vitro, as well as in vivo, PGC-1α transcriptionally regulates ERα.

HFD Exposure Decreases PGC-1α in the Hypothalamus of Male Mice

To evaluate whether an HFD influences PGC-1α in vivo, we analyzed PGC-1α expression following HFD exposure. The HFD reduced PGC-1 α in the hypothalamus, hippocampus, cortex, and visceral adipose tissue of male, but not female, mice (Figure 7C; Figures S5A–S5C). Western blot and immunofluorescence revealed reductions in PGC-1α in the hypothalamus of HFD male, but not female, mice (Figures 7A, 7B, 7D, and 7E). Treatment of N43 cells, primary neurons, and primary astrocytes with PA significantly reduced *PGC-1α* expression (Figures S5D–S5F). To confirm that these effects are specific to PGC-1α and not other nuclear transcription factors, we analyzed the expression of other nuclear transcription factors in the hypothalamus and found they were not altered by sex or an HFD (Figure S5G). Interestingly, *PGC-1α* is also decreased following stearic acid treatment, consistent with reductions in *PGC-1α* being associated with inflammation (Figure S5F). Thus, our findings demonstrate that HFD exposure and PA treatment reduce PGC-1α.

ERα Overexpression Is Sufficient to Significantly Inhibit PA-Induced Inflammation

To determine whether reductions in PGC-1α or changes in ERα are associated with PA-induced inflammation, we



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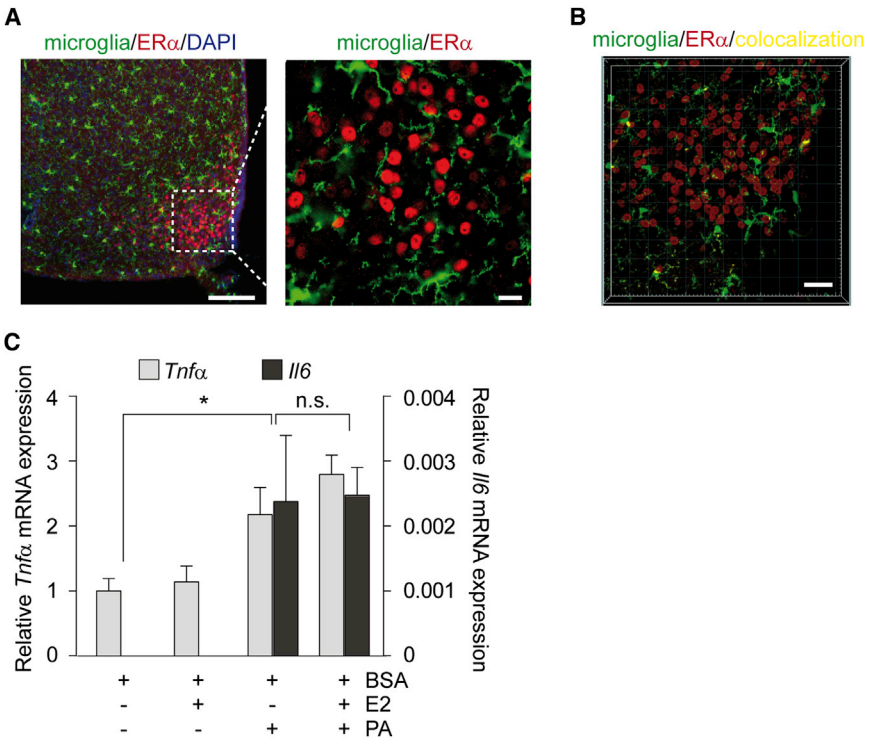

Figure 4. E2 Does Not Ameliorate PA-Induced Inflammation in Microglia due to Lack of ERα Expression

(A) Representative confocal images of ERα immunoreactivity in the ARC of $CX_3CR1^{GFP/GFP}$ male mice. Scale bar, 125 μm.

(B) Colocalization analysis of ERα and microglia in the ARC. Pearson's coefficient in data set volume and in region of interest (ROI) volume: −0.0175. Scale bar, 30 μm.

(C) BV2 cells were precultured for 1 hr with E2 and then treated for 8 hr as indicated. Data represent mRNA levels of inflammatory markers in BV2 cells. Results are presented as mean ± SEM. *p < 0.05. n = 3.

overexpressed ERα in cells transfected with siRNA targeting PGC-1α and treated with PA. PGC-1α downregulation enhanced the proinflammatory effect of PA (Figure 7F); however, ERα overexpression significantly inhibited PA-induced inflammation both in the presence and absence of PGC-1α (Figure 7F). These results demonstrate that ERα overexpression is sufficient to significantly inhibit PA-induced inflammation even in the absence of PGC-1α.

DISCUSSION

We demonstrate a sexually dimorphic response to HFD exposure, with HFD males having higher levels of PA and sphingolipids in the CNS than females. Our findings further demonstrate that males and not females have CNS and peripheral inflammation despite comparable weight gain following HFD consumption. Additionally, we show that reductions in PGC-1α and ERα facilitate inflammation. Lastly, our data demonstrate a physiologic relevance of ERα-regulated inflammation to metabolic homeostasis by demonstrating only in males a reduction in myocardial function.

ERα functions in the CNS to regulate food intake, glucose homeostasis, and energy expenditure (Musatov et al., 2007; Xu et al., 2011). HFD exposure induces hypothalamic expression of inflammatory cytokines such as TNF-α, IL-1β, and IL-6 in male mice (De Souza et al., 2005; Thaler et al., 2012). CNS inflammation has been associated with hypertension in male mice (Purkayastha et al., 2011). Epidemiological studies have characterized a sexual dimorphism in obesity-related metabolic complications, such as cardiovascular disease, with a higher prevalence in men and postmenopausal women than in premenopausal women (Shi et al., 2009; Sugiyama and Agellon, 2012). Our study extends these associations by characterizing HFD-induced increases in PA, sphingolipids, and inflammation and associated reductions in myocardial function in male, but not female, mice.

Our findings demonstrate that exposure of neurons and astrocytes to PA decreases ERα and leads to inflammation. These data are consistent with our previous study showing that ERα deletion induces inflammation in adipose tissues (Davis et al., 2013). Additionally, E2 pretreatment protects against PA-induced inflammation only if ERα is present, demonstrating E2, which is elevated in the HFD females, requires ERα to promote its anti-inflammatory effect. Importantly, these data

Figure 3. 17-β Estradiol, through Activation of ERα, Modulates PA-Induced Inflammation in Hypothalamic Neurons

(A–C) N43 cells were pretreated for 1 hr with E2 and then treated for 8 hr as indicated. (A) mRNA levels of *Tnfα* and *Il6* in N43 cells. mRNA (B) and protein (C) levels of ERα in N43 cells following treatments. n = 3.

(D–F) Primary hypothalamic neurons were pretreated for 1 hr with E2 followed by 8 hr of the indicated treatments. (D) mRNA levels of *Tnfα* and *Il6* in primary hypothalamic neurons following the aforementioned treatments. mRNA of *Erα* (E) and ERα protein levels (F) in primary neurons. n = 5.

(G) mRNA levels of the indicated genes following 8 hr treatment in primary male and female hypothalamic neurons. Males, n = 5; females n = 8.

(H) Ceramides content in N43 cells following pretreatment for 12 hr with E2, where indicated, and treated for 8 hr. n = 3.

(I) N43 cells were transfected with siRNAs, pretreated 48 hr later for 1 hr with E2, and cultured as indicated. mRNA levels of inflammatory markers following the treatments. The insert is a representative immunoblot of N43 cells transfected with control (UNR) siRNA or siRNA for ERα. n = 3.

(J) N43 cells were infected either with AdGFP (empty vector) or with AdGFP-ERα and then treated as indicated. Data represent mRNA levels of inflammatory markers following treatments. Insert shows representative immunoblots of N43 cells infected with the aforementioned viral constructs demonstrating ERα protein levels 48 hr after the adenoviral exposure. n = 3.

Data are presented as mean ± SEM. *p < 0.05, **p < 0.01, and ***p < 0.001. See also Figure S3.

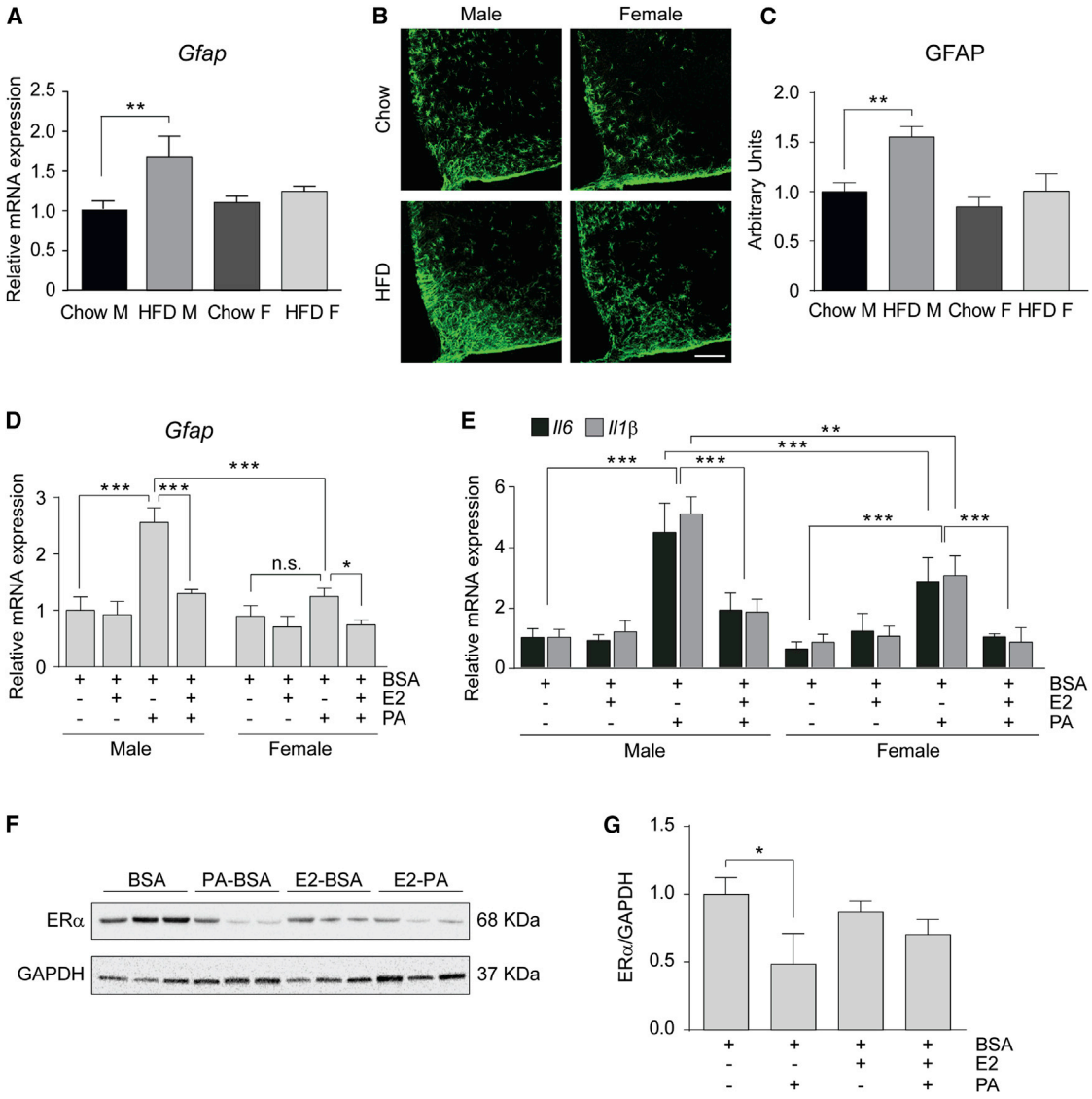

Figure 5. HFD Exposure Promotes Astrogliosis in Male Mice

(A) mRNA levels of *Gfap* in the hypothalamus. n = 12/group.

(B and C) Representative confocal images (B) and quantification (C) of GFAP immunoreactivity in the ARC. n = 10/group. Scale bar, 125 μm.

(D and E) mRNA levels of *Gfap* (D) and *Il6* and *Il1β* (E) in primary astrocytes. Primary astrocytes were cultured for 1 hr with E2 as indicated and then treated for 8 hr. n = 3–4.

(F and G) Representative immunoblot (F) and quantification (g) of ERα protein levels in primary astrocytes treated as above. n = 3/group.

Data are presented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001. See also Figure S4.

support previous studies indicating that E2 suppresses proinflammatory cytokines and increases the production of anti-inflammatory cytokines in an ERα-dependent manner in the CNS (Spence et al., 2013; Vegeto et al., 2003).

Our finding that, in the absence of ERα, E2 does not have protective effects against PA-induced inflammation is inconsistent with a previously proposed anti-inflammatory role for E2 acting through ERα in microglial cells (Arevalo et al., 2010; Barreto et al., 2009). This may be explained by the use of a microglial cell line lacking ERα in our study. Along with other studies, we show that microglia do not express ERα in vivo (Saijo et al., 2011; Wu et al., 2013); therefore, we suggest that E2 does not act through ERα in microglial cells. Saijo et al. show that ERβ in microglia has a protective role in modulating inflammation (Saijo et al., 2011). However, we did not see changes in *Erβ* expression in males or females following HFD exposure; nevertheless, our data do not rule out the possibility that ERβ, in other experimental settings, modulates inflammation in microglial cells.

Our data suggest that astrocytes and neurons are responsible for the sex-related differences that we see in vivo, since markers of inflammation are significantly increased following

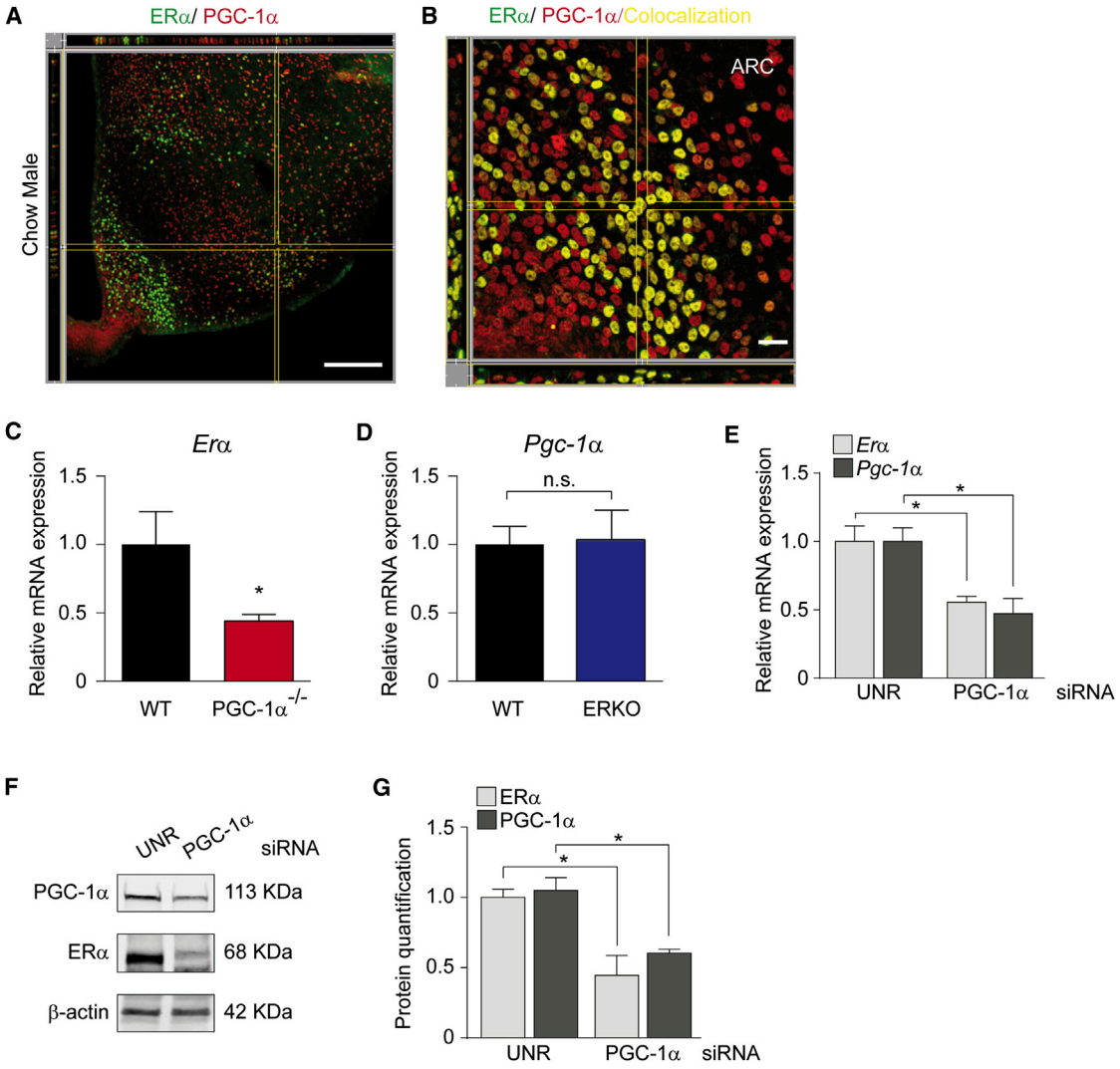

Figure 6. Reductions in Hypothalamic PGC-1α Are Associated with Reductions in ERα

(A and B) IMARIS imaging of colocalization between ERα (Alexa Fluor 488) and PGC-1α (Alexa Fluor 568) reconstructed using confocal images of the hypothalamus of a male mouse. Pearson's coefficient in ROI volume = 0.3893; Pearson's coefficient in colocalized volume = 0.1796. Scale bar, 250 μm. (B) IMARIS imaging of the colocalization (yellow areas) between ERα and PGC-1α. Pearson's coefficient in ROI volume = 0.4551; Pearson's coefficient in colocalized volume = 0.3167. Scale bar, 250 μm.

(C) mRNA levels of *Erα* in cortex from PGC-1α$^{-/-}$ male mice. n = 3 WT and n = 5 PGC-1α$^{-/-}$.

(D) mRNA levels of *Pgc-1α* in hypothalamus from ERKO male mice. n = 6 WT and n = 5 ERKO.

(E–G) N43 cells transfected with a control siRNA (UNR) or siRNA for PGC-1α. ERα levels were measured by quantitative PCR (E) and western blot (F) 48 hr later.

(F and G) Representative immunoblot (F) and quantification (G) of ERα and PGC-1α levels of N43 cells transfected with UNR or PGC-1α siRNA.

Data are presented as mean ± SEM, and *p < 0.05.

PA exposure in male neurons and astrocytes compared to females. Importantly, ERα is also decreased in male neurons and astrocytes following PA exposure and is partially restored following E2 pretreatment. These data, along with other studies (Spence et al., 2013), suggest that ERα is required in astrocytes and neurons to modulate the fatty acid-induced inflammatory response.

Our finding that PGC-1α regulates ERα expression in the hypothalamus shows that this transcriptional modulation occurs in vivo. These results extend previous reports showing that PGC-1α modulates ERα transcriptional activity in vitro (Bourdoncle et al., 2005; Tcherepanova et al., 2000). We hypothesize that PGC-1α and ERα constitute a hypothalamic signaling system involved in the response to HFD. This appears to be specific for PGC-1α–ERα, since neither HFD nor sex affects other nuclear transcription factors.

Depletion of ERα in different hypothalamic neuronal populations affects food intake, energy expenditure, and body weight (Xu et al., 2011). We demonstrate that in the presence of fatty acids, hypothalamic *Pgc-1α* expression is reduced in a sexually

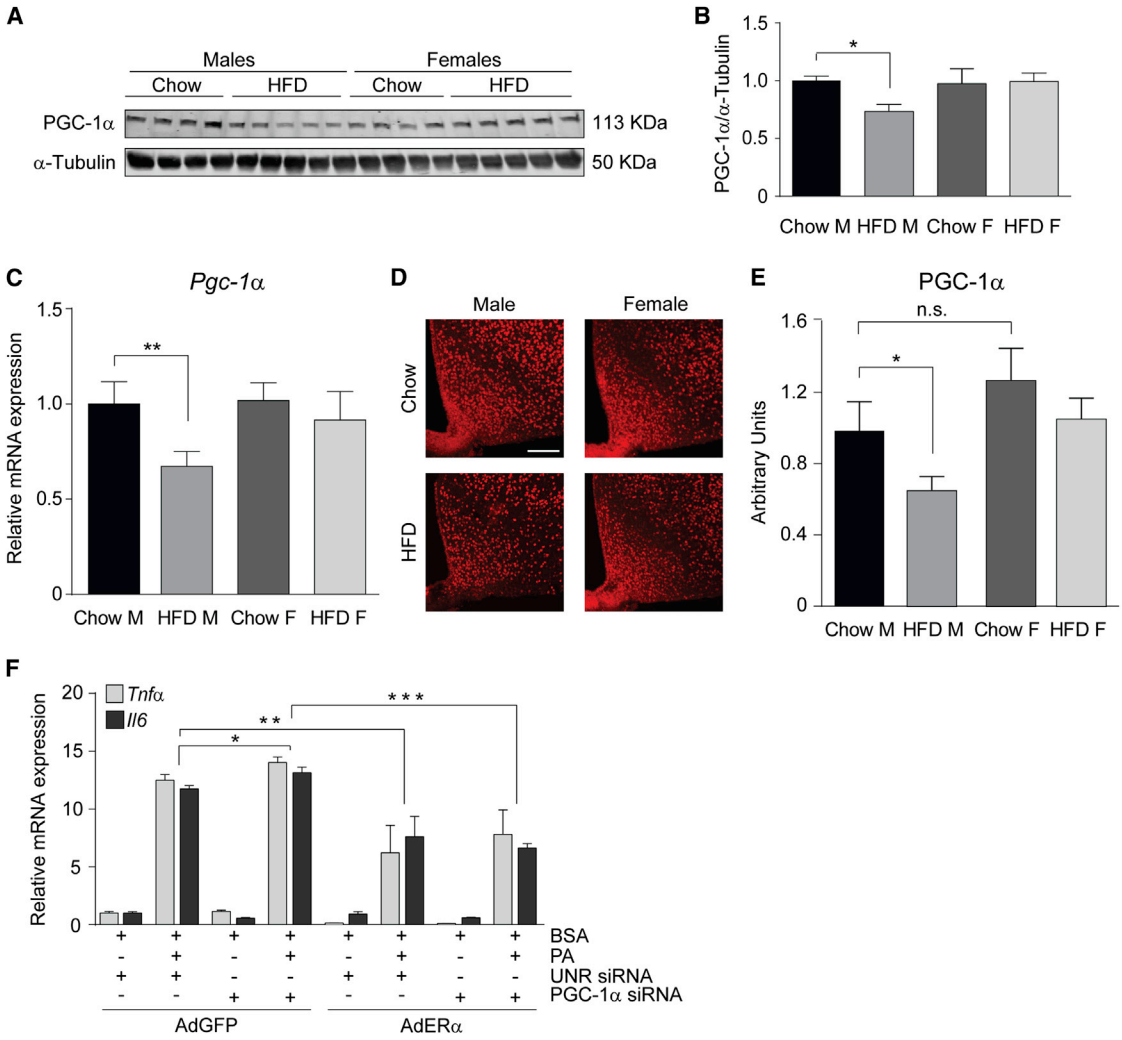

Figure 7. PGC-1α Is Decreased in the Hypothalamus of Male Mice Exposed to Chronic HFD

(A and B) Representative immunoblot (A) and quantification (B) of PGC-1α protein levels in the hypothalamus. n = 12/group.

(C) mRNA levels of PGC-1α in hypothalamus. n = 10/group.

(D and E) Representative confocal images showing PGC-1α immunoreactivity in the ARC (D) and its relative quantification (E). n = 10/group. Scale bar, 125 μm.

(F) mRNA expression of inflammatory markers from N43 cells transfected with a control siRNA (UNR) or siRNA for PGC-1α, followed by infection with AdGFP or AdGFP-ERα and treated as indicated. n = 3.

Data are presented as mean ± SEM. *p < 0.05, **p < 0.01, and ***p < 0.001. See also Figure S5.

dimorphic manner, implicating reduced hypothalamic PGC-1α in the development of obesity. This is consistent with reports that PGC-1α expression is reduced in the adipose tissue of insulin-resistant and morbidly obese individuals (Wilson-Fritch et al., 2004) and that polymorphisms of the PGC-1α gene correlate with increased risk of type 2 diabetes (Ek et al., 2001), findings that further indicate a critical role for PCG-1α in modulating metabolic function. These data, however, contradict previously published studies of total body PGC-1α knockout mice, as well as neuronal inactivation of PGC-1α, where reduced PGC-1α expression protected against diet-induced obesity (Lin et al., 2004; Ma et al., 2010). In those studies, PGC-1α deficiency led to degenerative lesions in the brain that worsened with age (Lin et al., 2004; Lucas et al., 2012; Ma et al., 2010). PGC-1α regulates a host of genes, and in both of these published models, PGC-1α was knocked down during development, likely provoking developmental compensations that could explain the lean phenotype. Importantly, our data demonstrate that an HFD/PA reduces PGC-1α in the hypothalamus and that this reduction correlates with impaired myocardial function. Further research using inducible knockdown of PGC-1α in adult mice is required to evaluate the role of PGC-1α in response to HFD-induced hypothalamic inflammation independent of developmental compensatory effects.

Loss of PGC-1α and ERα increases inflammation in the periphery as well as in conditions of nutrient excess and aging (Hsiao et al., 2013; Sczelecki et al., 2014). Here, we demonstrate PGC-1α is a determinant of PA-induced hypothalamic

inflammation. To confirm whether loss of PGC-1α or loss of ERα modulates the HFD/PA-induced inflammatory response, we knocked down PGC-1α in hypothalamic neurons and simultaneously overexpressed ERα following PA-induced inflammation. ERα ameliorated PA-induced inflammation, showing that it is a critical determinant of the anti-inflammatory pathway. Nevertheless, we are aware that ERα overexpression alone does not preclude the PA-induced inflammatory response. This suggests that other factors are necessary to completely inhibit the PA-induced inflammation.

In summary, HFD/PA-driven reductions in PGC-1α suppress ERα and cause hypothalamic inflammation, which correlates with impaired myocardial function. Our data reveal a sexually dimorphic response to the HFD, with males having increased CNS PA and sphingolipids when compared to females, and this is associated with increased inflammation. The inflammatory response to fatty acid exposure implicates the PGC-1α-ERα pathway as the molecular basis for sexually dimorphic HFD-induced hypothalamic inflammation. These critical insights into the sexually dimorphic response to HFD and its impact on myocardial function may provide a basis for future development of sex-specific treatments for obesity and its associated metabolic disorders.

EXPERIMENTAL PROCEDURES

Animals and Body Weight

Animal care and procedures were approved by the University of Texas Southwestern Medical Center. C57BL/6 mice purchased from The Jackson Laboratory, ERKO mice (gift from P. Chambon) (Dupont et al., 2000), $CX_3CR1^{GFP/GFP}$ mice (strain B6.129P-*Cx3cr1*^{tm1Litt}/J, The Jackson Laboratory), and PGC-1α$^{-/-}$ mice (gift from B. Spiegelman) were housed in a temperature-controlled environment in groups of two to five at 22°C–24°C using a 12 hr light/dark cycle. Mice were fed standard phytoestrogen-free chow (#2916, Harlan-Teklad) and exposed to a 42% HFD (#88137, Harlan-Teklad) at 8 weeks of age. Water was provided ad libitum.

Fatty Acids Analysis

Fatty acids were quantified using pentafluorobenzoyl bromide derivatization prior to electron capture negative ionization gas chromatography-mass spectrometry detection, as described previously (Quehenberger et al., 2011).

Sphingolipid Quantitation

Sphingolipids were quantified by liquid chromatography/electrospray ionization/tandem mass spectrometry with a TSQ Quantum Ultra Triple Stage Quadrupole Mass Spectrometer (Thermo Scientific) equipped with an electrospray ionization probe and interfaced with an Agilent 1100 HPLC (Agilent Technologies) (Holland et al., 2013).

Oral Glucose Tolerance Test

Mice were fasted for 3 hr (starting at 7 a.m.). Glucose (2.5 g/kg body weight) was gavaged; at the indicated time points, blood samples from the tail vein were collected. Glucose concentrations were measured using glucose strips and a glucometer (Bayer Contour). Mice did not have access to food throughout the experiment.

Echocardiography

Echocardiograms were performed on conscious mice using a Vevo 2100 system with a MS400C scanhead. Left ventricular end-diastolic diameter (LVEDd) and left ventricular end-systolic diameter (LVEDs) were measured from M-mode recordings. Fractional shortening (FS) was calculated as (LVEDd − LVEDs)/LVEDd and expressed as a percentage. Measurements were made from 2D parasternal short-axis views in diastole at the level of the papillary muscles (Battiprolu et al., 2012).

Cell Culture

N43 cells (Cellutions Biosystems) and BV2 cells (a gift from C.K. Glass) were cultured in HyClone Dulbecco's modified Eagle's medium (Thermo Scientific) containing 10% charcoal:dextran stripped fetal bovine serum (Gemini), 100 U/ml penicillin G sodium, 100 μg/ml streptomycin sulfate, and 100 mg/l sodium pyruvate. Cells were grown for 24 hr before treatments with medium containing 2% charcoal:dextran stripped fetal bovine serum. Cells were pretreated for the indicated time with 10^{-8} M E2 (Sigma) conjugated with fatty acid-free BSA (MP Biomedicals) and then treated for 8 hr with 10^{-8} M E2 or 100 μM PA (Matreya) conjugated with BSA alone or in combination.

RNAi in Hypothalamic Cell Cultures

Cells were cultured in six-well plates and transfected at 50% confluence with siRNAs targeting murine ERα (Thermo Scientific) or murine PGC-1α (Thermo Scientific) or with an unrelated control siRNA (UNR, Thermo Scientific) by means of Lipofectamine RNAiMAX transfection reagent (Life Technologies) according to the manufacturer's instructions. A total of 48 hr after siRNA transfection, cells were treated as indicated or directly lysed for protein or RNA extraction.

Adenoviral Constructs and Infections

Adenoviruses expressing FLAG-ERα (AdERα) and GFP were constructed as previously described (Luo et al., 2007). N43 cells were infected with the indicated adenoviral constructs in 1 ml of serum-free medium. A total of 4 hr after virus exposure, 1 ml of completely supplemented medium was added, and 48 hr later, cells were treated as indicated.

Quantitative PCR

For analysis of gene expression, mice were anesthetized and decapitated. Tissues were stored in RNA*later* (Ambion, Life Technologies) at 4°C, and 24 hr later, tissues were homogenized in 1 ml of TRIzol (Ambion, Life Technologies). For analysis of gene expression in cell cultures, cells were washed twice with cold PBS and lysed in 1 ml of TRIzol (Ambion, Life Technologies).

RNA from tissue or cells was extracted using the RNeasy Kit (QIAGEN) according to the manufacturer's instructions. Total RNA (1 μg) was reverse transcribed using the SuperScript III First-Strand Synthesis System (Invitrogen) according to the manufacturer's instructions. Quantitative PCR reactions were carried out on an ABI PRISM 7700 Sequence Detection System (Applied Biosystems). The $\Delta\Delta C_T$ method was used for relative quantification analysis.

Western Blot

Mouse hypothalamic tissue was dissected, immediately frozen in liquid nitrogen after extraction, and homogenized. Protein concentration in the supernatants was evaluated using the bicinchoninic acid technique (Pierce Biotechnology).

N43 cells and primary neuronal cultures were collected, washed with cold PBS and lysed using RIPA buffer supplemented with protease and phosphatase inhibitors (Roche Applied Science). Cell lysates were quantified as previously described.

Proteins (30 μg) from tissues or cell lysates were separated on Criterion TGX precast gels (Bio-Rad) and electrotransferred to membranes using the Trans-Blot Turbo transfer system (Bio-Rad). Nonspecific binding sites were saturated by incubating membranes for 1 hr in TBS supplemented with 5% nonfat powdered milk (w:v in 20 mM TBS) followed by overnight incubation with primary antibodies. Labeling was revealed with appropriate IRDye secondary antibodies (Li-Cor) using the Odyssey Quantitative Fluorescence Imaging Systems (Li-Cor).

Fluorescence Microscopy

Mice were anesthetized and perfused with 10% formalin. Brains were dissected and postfixed in 10% formalin for 24 hr followed by 2 hr treatment with 30% sucrose in PBS. Brain sections were cut at 30 μm using a Thermo Scientific HM 450 sliding microtome (Thermo Scientific). The sections were permeabilized in 0.01% Triton and blocked in 3% donkey serum (Jackson

Immuno Research) for 2 hr. Brain sections were incubated in the primary antibodies overnight (ERα, Santa Cruz; GFAP, Abcam; PGC-1α, Calbiochem) followed by the respective secondary antibodies (Alexa Fluor, Life Technologies) for 2 hr. Sections were then placed on gelatinized slides, mounted with VECTASHIELD anti-fading medium with DAPI (Vector Laboratories), and coverslipped. Slides were analyzed using a TCS SP2 confocal fluorescence microscope (Leica Microsystems GmbH). Five pictures containing the ARC region of the hypothalamus were taken from at least five mice per group, and staining was quantified using ImageJ software (http://rsb.info.nih.gov/ij/).

Statistical Analysis

Data are presented as mean ± SEM. Statistical analyses were performed with GraphPad Prism software (GraphPad Software) and Microsoft Office 2010 Excel. Comparisons between two conditions were made using the unpaired two-tailed Student's t test. A one-way ANOVA was used for comparison of more than two groups. Scheffe's F test was employed for post hoc analysis. $p < 0.05$ was considered to be statistically significant.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and five figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2014.09.025.

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