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A high cholesterol diet elevates hippocampal cytokine expression in an age and estrogen-dependent manner in female rats

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Abstract

Background: While the effects of a proatherogenic diet have been widely studied in the context of systemic inflammation, much less is known about its effects on central or brain inflammation and its modulation with age. In this study, we examined the effect of a high cholesterol/choline diet in adult and older acyclic females to assess its impact on systemic and central inflammatory markers. Moreover, since the loss of ovarian hormones at menopause may predispose women to increased production of pro-inflammatory cytokines, we also tested the impact of estrogen replacement to adult and older females in diet-induced inflammation.

Methods: Ovariectomized adult female rats and older (reproductive senescent) female rats were replaced with estrogen or a control pellet and maintained thereafter on a diet containing either 4% cholesterol/1% choline or control chow for 10 weeks. Interleukin 1 β (IL-1 β) expression in the liver was used as a marker of systemic inflammation, while a panel of cytokine/chemokines were used to examine the effects of diet on the hippocampus.

Results: IL-1 β expression was elevated in the liver of adult and reproductive senescent females fed with the high cholesterol diet, although this was restricted to groups that were ovariectomized and not replaced with estrogen. Estrogen-treated animals of both ages did not have elevated IL-1 β levels when fed the high cholesterol diet. Diet-induced changes in cytokine/chemokine expression in the hippocampus however were critically age dependent and restricted to the reproductive senescent females. In this group, the high cholesterol diet led to an increase in interleukin (IL)-4, IL-6, IL-12p70, IL-13, RANTES (Regulated on Activation, Normal T Expressed and Secreted) and VEGF (vascular endothelial growth factor). Moreover, estrogen treatment to reproductive senescent females suppressed diet-induced expression of specific cytokines (RANTES, VEGF, IL-6) and attenuated the expression of others (IL-4, IL-12p70, IL-13).

Conclusions: These data indicate that a proatherogenic diet presents a significant risk for central inflammation in older females that are deprived of estrogen treatment.

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Keywords

high-cholesterol diet; inflammation; aging females; interleukin-1 beta; hippocampus; RANTES; VEGF; IL-4; IL-13

Background

Postmenopausal women have an increased risk for the development of osteoporosis, heart disease and neurodegenerative diseases. This increased risk, in part, may be due to the loss of ovarian hormones and a concomitant heightened inflammatory profile. Rodent and clinical studies show that surgical and/or natural estrogen deficiency selectively increases pro-inflammatory cytokines such as interleukin-1 β (IL-1 β), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α) from blood monocytes (Pacifci et al., 1989), granulocytes, macrophages (Jilka et al., 1992) and bone marrow (Bismar et al., 1995). Moreover, cytokine expression, such as IL-1 β , has been linked to cognitive decline through its actions on the hippocampus and related structures. Intercerebro-ventricular injections of IL-1 β (10-20 ng) impaired contextual fear conditioning (Goshen et al., 2007; Pugh et al., 1999) and overexpression of IL-1 β led to impairments in acquisition and retention on a spatial learning task in rodents (Moore et al., 2009). The pro-inflammatory cytokine IL-6 has been shown to exacerbate degeneration of forebrain GABAergic interneurons and lead to cognitive decline in three tests of spatial learning in mice (Dugan et al., 2009). Moreover, in hippocampal slices IL-1 β (Bellinger et al., 1993) and IL-18 (Cumiskey et al., 2007) inhibit long term potentiation (LTP).

Using acyclic female rats, which mimic salient aspects of the human menopause, our laboratory has shown that estrogen-replacement to these reproductive senescent females is pro-inflammatory, following excitotoxic injury to the olfactory bulb (Nordell et al., 2003) and neurotoxic in MCA-associated ischemic stroke (Selvamani and Sohrabji, 2008). Furthermore, reproductive senescent females display a constitutive increase in blood brain barrier permeability and loss of microvascular integrity as compared to their younger counterparts (Bake and Sohrabji, 2004). Thus, reproductive senescent females may be more susceptible to neural inflammation following a chronic peripheral or systemic inflammation due to passage of cytokines and inflammatory mediators via an impaired blood brain barrier. In an acute inflammation model using a low dose of LPS as an inflammagen, we reported that IL-1 β was increased in both the plasma and olfactory bulb of young adult and reproductive senescent females, while estrogen suppressed this cytokine in both ages (Johnson et al., 2006). In the present study we determined whether a chronic, systemic inflammation would differentially affect adult and senescent females and further assessed the impact of estrogen in this population.

Atherogenic diets including a Western style diet, high cholesterol or high fat diet have been shown to promote renal interstitial inflammatory disease (Eddy, 1996) and hepatic steatosis (Desai et al., 2008) as well as exacerbate the inflammatory response of the ischemic myocardium (Yaoita et al., 2005). In the brain, a high fat diet increased oxidative stress in the hippocampus (Wu et al., 2004) and cortex (Zhang et al., 2005). In addition, a high-fat diet resulted in impaired spatial learning (Pathan et al., 2008) while a high-fat sucrose diet impaired spatial learning following traumatic brain injury (Wu et al., 2003). The effects of a high fat diet in the context of aging are not very well studied. Cortés et al., (2002) reported that aortic atherosclerotic lesions are more prominent in young rabbits than in older ones following 2 months of a 1% cholesterol diet while atherosclerotic lesions in the aorta were comparable in young and aged mice following a 15% fat/ 1.25% cholesterol/ 0.5% cholate diet (Li et al., 2008). A high-fat diet also impairs working memory function in middle-aged rats (Granholm

et al., 2008). Few studies have examined the impact of a high fat diet in female rats and no studies have examined the effects of a high-fat diet on reproductive aging. In the present study, mature adults and reproductive senescent females were ovariectomized and replaced with either estrogen or control pellet, and then fed a high-fat diet (4% cholesterol) for 10 weeks. The mature adult and reproductive senescent groups were used to mimic two populations of females most likely to seek estrogen replacement, perimenopausal women and postmenopausal women.

Our data show that the high cholesterol/cholesterol diet increased IL-1 β expression in the liver of mature and senescent females but this was limited to the groups that did not receive estrogen treatment. In the hippocampus, a panel of cytokines and/or chemokines was examined. In mature adults, the high-cholesterol diet did not induce the expression of any hippocampal cytokine, while estrogen treatment suppressed RANTES expression. In contrast, in reproductive senescent females, diet-induced increases were observed for RANTES, vascular endothelial growth factor (VEGF), interleukin (IL)-4, IL-12p70 and IL-13. Interestingly, estrogen replacement suppressed the diet-induced elevation of RANTES and VEGF, and attenuated the diet-induced increases in IL-4, IL-12p70 and IL-13 expression in this older group.

These data indicate that reproductive age significantly modulates the effect of a high fat diet on brain cytokine levels, with older females more susceptible to elevated levels of inflammatory cytokines. Estrogen treatment is partially effective in attenuating this central inflammatory response.

Methods

Animals

Adult female Sprague Dawley rats were purchased from Harlan Laboratories (IN) as proven breeders (5 mos.; 250g) or retired breeders (9-11 mos.). The estrus cycle was determined by vaginal smears taken each morning for a period of 14-20 days. Vaginal smears were obtained with a cotton swab, placed on a slide and the cytology was examined (Olympus, BX2 microscope) for estrus cycle classification. Criteria used for mature adults were a normal but lengthened estrus cycle (approx. 10 day cycle). Rats that persisted in one stage for 7 days were considered acyclic and for these studies retired breeders were used once we had confirmed that they were in constant diestrus. All procedures were in accordance with NIH and institutional guidelines governing animal welfare. For tissue collection, the rats were deeply anesthetized (ketamine: 87 mg/kg; xylazine: 13 mg/kg) and decapitated.

Surgical procedures & experimental paradigm

Mature adults and reproductive senescent female rats were bilaterally ovariectomized as described previously (Jezierski and Sohrabji, 2000; Jezierski and Sohrabji, 2001; Johnson et al., 2006) at the start of the experiment and implanted with an estradiol or control pellet (Innovative Research of America, FL). The time-release pellets were identical to those used in previous studies (Jezierski and Sohrabji, 2001; Nordell et al., 2003) and were designed to maintain a plasma hormone level of 60-80 pg/ml. Previous studies have shown that a stable level of plasma estradiol is maintained at 3, 4 and 6 weeks, although a recent study reports a high supraphysiologic burst of hormone levels soon after pellet implantation (Singh et al., 2008). Controls received an empty pellet consisting of the same matrix material but devoid of hormone. Experimental procedures: Animals were fed *ad libitum* re-pelleted normal rat chow (Diet #8604, Harlan Teklad, WI) or normal rat chow (Diet #8604) enriched with 4% cholesterol and 1% cholic acid. Body weight was measured once a week and food was refilled every 3

days. Animals were terminated between 10 and 11 weeks following the initiation of the feeding regimen.

Tissue Collection

All animals were deeply anesthetized and transcardially perfused with cold phosphate buffered saline (pH = 7.4) after the blood was collected from the left ventricle of the heart using a heparin (500U/mL) coated syringe (16 gauge-needle). The rats were then decapitated and the brains rapidly dissected. Hippocampal and liver tissue was collected and stored at -80°C . Uterine weight was used to confirm ovariectomy and estrogen replacement. The brain and liver tissue was processed for protein extraction. Blood was centrifuged at 2500 rpm for 30 min. to collect the plasma.

Protein Collection

Proteins were isolated by homogenizing the tissue in previously described lysis buffer (Sohrabji et al., 2000). Protein concentrations were determined using the bicinchoninic acid method (Pierce, IL) with BSA as a standard. The plates were read at 560nm in an ELISA plate reader and concentrations of the samples were interpolated from a linear curve using KC3 software (Biotek, VT). Total protein content was determined as a product of protein concentration ($\mu\text{g}/\mu\text{L}$) x total fluid content of sample.

Analysis of Cholesterol, Glucose and Leptin Levels in Plasma

Non-fasting levels of plasma glucose from mature adults and reproductive senescent females were measured using the mutarotase-GOD enzymatic method (Wako, Richmond, VA). Total cholesterol (Cholesterol E) was measured also using a kit from Wako Diagnostics. Manufacturer's instructions were followed for both kits. The plates were read at 505nm in an ELISA plate reader and concentrations of the samples were interpolated from the standards using Magellan software (Tecan, Switzerland). Leptin levels were measured using the MilliplexTM MAP leptin kit (Millipore Corp., MA) using manufacturer's instructions. The Bio-Plex Suspension System was calibrated using CAL2 (High PMT, Bio-Plex Calibration Kit). A total of 10 μL of sample/standards/controls was used for this assay followed by addition of the beads. The plate containing the samples/standards/controls plus the premixed beads were incubated overnight at 4°C with shaking. The plate was washed twice, the washes were removed by vacuum filtration, the detection antibody (50 μL) was added and the plate incubated for 1 hr at room temperature. The plate was treated with streptavidin-phycoerythrin (50 μL) and incubated at room temperature for 30 min. After the final wash step, the beads were resuspended in 100 μL assay buffer and read using the Bio-Plex Suspension Array Systems with High-Throughput Fluidics.

Protein Expression of Cytokines/Chemokines

Peripheral inflammation in the liver was measured using the BioRad Rat IL-1 β X-plex assay (BioRad, CA) and examination of the cytokine/chemokine protein expression inflammatory profile in hippocampal cell lysates was performed using the rat MilliplexTM MAP kit (Millipore). The Bio-Plex Suspension System was calibrated as described above and standard/sample preparation was performed according to manufacturer's directions and as previously described (Lewis et al., 2008). The IL-1 β conjugated bead mixture (1X) was added to a pre-wet, 96-well filter plate followed by 50 μL of sample or standard which was added in duplicate and incubated (30 min., room temperature, with agitation). Following 3 washes, 25 μL of 1X Bio-Plex detection antibody was added and incubated for 30 min. at room temperature. The plate was washed 3 additional times and 1X BioRad Streptavidin-PE (50 μL) was added and incubated for 10 min. at room temperature. Following 3 final washes, the beads were resuspended in 125 μL of BioReady assay buffer and read by the BioPlex Suspension System.

For the Milliplex™ MAP rat cytokine/chemokine panel, 50 µL of sample/standard/controls were added to each well followed by addition of the beads. The plate containing the samples/standards/controls plus the premixed beads were incubated overnight at 4°C with shaking. The plate was washed twice, the washes were removed by vacuum filtration, the detection antibody (25µL) was added and the plate incubated for 2h at room temperature. Following the 2h incubation, streptavidin-phycoerythrin (25µL) was added and the plate incubated at room temperature for 30 min. The plate was washed twice and then the beads were resuspended in 150µL assay buffer and read. Cytokines and chemokine levels were normalized to total protein values.

Statistical Analysis

Statistical analysis was performed using a statistical software package (SPSS Inc., IL), and group differences were considered significant at $p \leq 0.05$. Data was analyzed using a two-way ANOVA [independent variables: hormone (estrogen-replaced vs placebo-replaced) and diet (high-fat and normal rat chow)] separately for mature adults and reproductive senescent females. Uterine weights were analyzed by an ANOVA to determine group differences. Since uterine weight was only affected by estrogen, the data are expressed as the mean weight $\pm$ SEM for estrogen- and placebo-replaced mature adult and reproductive senescent females for each study. All experimental groups had an $n = 5-7$, which is indicated in each figure legend.

Results

Uterine weight

Uterine weight was used as a cumulative indicator of ovariectomy and estrogen replacement. Uterine weights were significantly reduced in ovariectomized, placebo-replaced mature adults ($F_{(1,21)}: 102.1, p \leq 0.05$; main effect of hormone) and reproductive senescent females ($F_{(1,21)}: 41.7, p \leq 0.05$; main effect of hormone). The average uterine weight of ovariectomized, placebo-replaced mature adult females was 175.9 ± 19.4 mg while the uterine weight in reproductive senescent females was 230.5 ± 15.4 mg. The average uterine weight in estrogen-replaced mature adults was 666.8 ± 148.6 mg and 666.8 ± 4.3 mg in reproductive senescent females. No significant effects of diet were observed.

Weight gain

The high cholesterol diet had no significant effects on weight gain in the mature adult or reproductive senescent females over the 10 week period. However, hormone status did significantly affect weight gain. Starting weights for the mature adults was 258.09 ± 27.66 g for placebo-replaced females and 249.17 ± 13.96 g in estrogen-replaced females. Following the diet treatment period, the weights of the placebo-replaced females increased to 332.73 ± 30.39 g while the estrogen-replaced females had ending weights of 271.0 ± 12.99 g. Thus in placebo-replaced, mature adults there was a 26.38% and 28.01% increase in weight from their baseline weight when fed the control diet or high-cholesterol diet, respectively. Whereas, the weight of estrogen-replaced females increased by 4.31% from baseline in the absence of the high-fat diet and increased 7.02% from baseline when fed the high-fat diet ($F_{(1,19)}: 9.48, p < 0.05$, main effect of hormone, Fig. 1). A similar trend was observed for the reproductive senescent females. Starting weights for reproductive senescent females was 332.73 ± 21.85 g for placebo-replaced females and 298.67 ± 14.91 g in estrogen-replaced females. At termination of the experiment, the weights of the placebo-replaced females increased to 338.25 ± 22.21 g while the estrogen-replaced females had ending weights of 299.23 ± 18.19 g. Thus, in placebo-replaced females there was an 11.0 to 15.5% increase in weight from baseline when fed the normal or high-fat diet, respectively. Estrogen-replaced females decreased their weight by 2.57% in the absence of the high-fat diet and gained 1.54% from baseline when fed the high-fat diet ($F_{(1,21)}: 31.4526, p < 0.05$, main effect of hormone, Fig. 1). Given that reproductive senescent females were

heavier at the start of the experiment, their total % change in weight was not as dramatic as that observed for the mature adults, but hormone did significantly affect both groups. This is consistent with many previous studies that have shown that estrogen treatment decreases body weight and food intake (Butera and Czaja, 1984; Dubuc, 1985; Węgorzewska et al., 2008).

Plasma cholesterol, glucose and leptin analysis

Cholesterol—The high-fat diet increased total cholesterol levels in mature adults and reproductive senescent females. A 2.36-fold increase in total cholesterol was observed in placebo-replaced mature adults fed the high-fat diet and a 3.21-fold increase in total cholesterol was observed in estrogen-replaced mature adults fed the high-fat diet as compared to the normal diet ($F_{(1,16)}: 3.45$, $p \leq 0.05$, interaction effect hormone and diet, Fig. 2). In reproductive senescent females, a 1.62-fold increase was observed for ovariectomized, placebo-replaced females as compared to placebo-replaced females fed the control diet ($F_{(1,16)}: 26.2$, $p \leq 0.05$, main effect of diet, Fig. 2) while a 2.39-fold increase was observed in the estrogen-replaced females fed the high-fat diet as compared to those females fed the control diet. **Glucose:** Examination of plasma glucose levels showed no significant differences between females fed the normal rat chow and the high-fat diet in mature adults or reproductive senescent females (data not shown). **Leptin:** Leptin was significantly decreased with diet in both mature adults and reproductive senescent females. In placebo-replaced, mature adult females fed the high-fat diet, leptin was reduced by 62% and a 38% reduction was observed in estrogen-treated females ($F_{(1,14)}: 8.11$, $p < 0.05$, main effect diet, Fig. 3). In placebo-replaced, reproductive senescent females fed the high-fat diet, there was a 75% reduction and in estrogen-treated females a 47% reduction was observed ($F_{(1,14)}: 20.09$, $p < 0.05$, main effect diet, Fig. 3). Decreased leptin levels may be indicative of increased satiety in response to the high-fat diet as compared to the control diet which has been previously noted in another short-term, high-fat feeding regimen study (Ainslie et al., 2000).

Effect of the high-fat diet on liver inflammation

The impact of high fat diet on liver inflammation was assessed by measuring IL-1 β . In both mature adult females and reproductive senescent females, the high-cholesterol diet increased IL-1 β expression, while estrogen attenuated the diet-induced increase in this cytokine (Fig. 4). In mature adult females, IL-1 β increased 4.4-fold in the liver of placebo-replaced females fed the high-fat diet as compared to females fed the normal diet ($F_{(1,19)}: 12.5078$, $p \leq 0.05$, interaction effect of hormone and diet, Fig. 4). Estrogen attenuated the high-cholesterol diet-induced increase in IL-1 β to basal levels. In reproductive senescent females, IL-1 β increased 7.8-fold in placebo-replaced females fed the high-fat diet as compared to females fed the normal diet ($F_{(1,19)}: 46.3247$, $p \leq 0.05$, interaction effect of hormone and diet, Fig. 4). Similar to the mature adults, estrogen attenuated the diet-induced increase in IL-1 β in senescent females.

Impact of high fat diet on hippocampal cytokine levels

Chemokine/Cytokine Analysis—Hippocampal tissue from mature adult and reproductive senescent females was subject to expression profiling of a panel of cytokine/chemokines. The chemokine ligand 10 (IP-10), IL-1 β , IL-5, IL-9, IL-10, granulocyte-colony stimulating factor (CSF) and granulocyte macrophage-CSF were not detectable in the hippocampus of mature adult or reproductive senescent females. The cytokines IL-1 α , IL-2, IL-18, IL-17, tumor necrosis factor alpha (TNF α) and interferon gamma (IFN γ) as well as the chemokines eotaxin, monocyte chemoattractant protein-1 (MCP-1) and growth related oncogene (GRO-KC; aka: CXCL1) were detectable but not significantly regulated in mature adults or reproductive senescent females.

Mature adult females: The cytokines IL-4, IL-6, IL-12p70, IL-13 and the chemokine VEGF were detected in the mature adult hippocampus, but were not regulated by diet or hormone in this group. In the case of the pro-inflammatory cytokine RANTES, estrogen treatment to mature adult suppressed cytokine expression by approximately 13-22% ($F_{(1,14)}:18.17$, $p<0.05$, main effect of hormone, Fig. 5).

Reproductive senescent females: In the reproductive senescent group, the high-cholesterol diet elevated hippocampal expression of RANTES, VEGF, IL-4, IL-12p70 and IL-13. RANTES was significantly increased 1.89-fold by diet in the absence of estrogen, but not in comparably fed estrogen-replaced females ($F_{(1,13)}:4.69$, $p<0.05$, interaction effect of diet and hormone, Fig. 5). Similarly, diet-induced increases in (2.18-fold) VEGF were observed in placebo-replaced reproductive senescent females ($F_{(1,13)}:9.65$, $p<0.05$, main effect of diet, Fig. 5). In the case of IL-4 and IL-12p70, high-fat diet increased these pro-inflammatory cytokines, while estrogen attenuated but did not eliminate their expression [(IL-4: $F_{(1,12)}:25.96$, $p<0.05$, main effect of hormone; $F_{(1,12)}:12.83$, $p<0.05$, main effect diet)/(IL-12p70: $F_{(1,12)}:5.02$, $p<0.05$, main effect of hormone; $F_{(1,12)}:9.07$, $p<0.05$, main effect diet; Fig. 6). IL-13 was increased by diet in both placebo- and estrogen-replaced reproductive senescent females by 206-fold and 113-fold, respectively ($F_{(1,14)}:39.17$, $p<0.05$, main effect of diet, Fig. 6). Lastly, hormone but not diet, reduced IL-6 expression by 32% ($F_{(1,12)}:11.75$, $p<0.05$, main effect of hormone, Fig. 7).

Discussion

The present study shows that reproductive age significantly impacts the effect of a high-cholesterol diet on hippocampal expression of cytokines. A high fat diet elevated liver expression of IL-1 β in both mature and reproductive senescent females, but only elevated proinflammatory mediators in the senescent hippocampus. Furthermore, estrogen treatment to reproductive senescent females either suppressed or attenuated diet-induced cytokine expression. These data are consistent with our previous studies showing that older acyclic females have a more permeable blood brain barrier (Bake et al., 2009; Bake and Sohrabji, 2004), and sustain greater cell death following ischemic stroke injury (Selvamani and Sohrabji, 2008). However the present data diverge from our other studies in that estrogen treatment exerts a protective effect on diet-induced cytokine expression. Specifically, we have shown that estrogen treatment is detrimental when administered to ovariectomized reproductive senescent females in neuroinflammatory and stroke models (Nordell et al., 2003; Selvamani and Sohrabji, 2008). The present study underscores the importance of reproductive age as a risk factor for neural inflammatory disease and further suggests that, depending on the type of inflammatory stimulus, estrogen treatment to this older group may be pro- or anti-inflammatory.

These data have important implications for both cardiovascular disease and cognition. Poor dietary choices are often described as risk factors for vascular disease and intake of high-fat diets following the menopause, may put women at risk for metabolic syndrome [reviewed in (Lobo, 2008)]. This syndrome is associated with a higher proportion of centrally localized body fat, increased insulin resistance, dyslipidemia, increased inflammatory biomarkers in the blood and increased hypercoagulation. Increases in adipose tissue can lead to heightened atherosclerosis and contribute to pro-inflammatory cytokines such as TNF α and IL-6 [reviewed in (Eder et al., 2009; Greenberg and McDaniel, 2002)]. High-fat diet feeding regimens have been shown to increase IL-1 β (Alexaki et al., 2004; Thirumangalakudi et al., 2008), RANTES (El Seweid et al., 2005; Wu et al., 2007), TNF α , IFN- γ , GMC-SF, IL-1 α , IL-2 and IL-12 (Naura et al., 2009) and VEGF (Gomez-Ambrosi et al., 2009) peripherally.

Diet regimens can also influence central nervous system (CNS)-specific inflammatory processes. High-fat feeding regimens are reported to promote inflammation and oxidative

stress in the cortex, hypothalamus and the hippocampus (Milanski et al., 2009; Pistell et al., 2009). Similar to young animals, in middle-aged mice, microglial activation as well as increased expression of TNF α , IL-6, and MCP-1 was observed in the cortex following an 8-week high-fat (60% fat) feeding regimen (Pistell et al., 2009). In the hippocampus, which mediates memory tasks, a high-fat diet resulted in microglial activation, morphological changes (Granholt et al., 2008) and memory impairments in young (Pathan et al., 2008) and 16 month-old male rats (Granholt et al., 2008). Few studies have focused on the effect of diet on brain inflammation in females and virtually none have focused on the reproductive transition in females. In the current study, a relatively modest increase in dietary fat (4% cholesterol) induced proinflammatory cytokines in the reproductive senescent female hippocampus, but not in the mature adult, indicating that this older population is more susceptible to systemic inflammagens.

The cluster of cytokines induced by diet in the hippocampus of older females mediate a wide variety of actions. At least two of these cytokines, RANTES (Wong and Fish, 2003) and VEGF (Senger et al., 1993) are chemotactic for T-cells and macrophages respectively. IL-4, and to a lesser extent IL-13, stimulate the proliferation of T-cells and their differentiation to Th2 cells (Paul, 2010). Additionally, increases in IL-12p70, the bioactive form of IL-12 suggest that Th1 type cells may also be expanded (Gee et al., 2009) although the classic Th1 cytokine interferon-gamma is not regulated by high-fat diet. Collectively, the diet-induced cytokine profile observed in the reproductive senescent female suggests a shift to a Th2-type response, which may be a protective tissue response. This is consistent with a report that a hypercholesterolemic diet causes a shift from Th1 to a Th2 profile in atherosclerotic plaques and in the spleen (Zhou et al., 1998).

While these data are consistent with a T-cell type recruitment and activation in the hippocampus of older females, the mechanism underlying this recruitment may differ in estrogen-replaced and estrogen-deficient animals. In the ovariectomized, estrogen-deficient senescent female, RANTES, presumably from activated local microglia, may provide a potent recruitment signal to T cells. However, estrogen treatment to senescent females suppresses diet-induced RANTES expression, and attenuates (but does not suppress) other Th2 markers (IL-4, IL-13). While other hitherto not tested inflammatory mediators may explain this discrepancy one important consideration is the health and integrity of the blood brain barrier in this population. Our previous studies have shown that the blood brain barrier is more permeable in reproductive senescent females as compared to younger females, and estrogen treatment to this older group specifically increases permeability in the hippocampus. Hence it is possible that a T-cell population is already resident in this group and is expanded and differentiated into either Th1 or Th2 profiles.

RANTES and VEGF, both upregulated by diet in the aging female hippocampus, play a significant role in blood brain barrier function. RANTES is reported to increase migration of peripheral blood mononuclear cells in an in vitro blood brain barrier model (Ubogu et al., 2006). Moreover, in RANTES^{-/-} mice, blood brain barrier permeability, trafficking of leukocytes and platelet adhesion in cerebral venules and infarct size were reduced as compared to wild type mice (Terao et al., 2008). VEGF also influences blood brain barrier function in the presence and absence of injury. In a rat focal ischemia model, early administration (1h) of human VEGF following ischemia increased blood brain barrier permeability, infarct size and hemorrhaging within the ischemic core. In mice, intracerebral infusion of VEGF in the absence of injury led to a transient increase in the number of microvascular profiles leaking albumin (Dobrogowska et al., 1998) while chronic infusion of VEGF for 6 days led to significant deterioration of the blood brain barrier (Proescholdt et al., 1999). Our data suggests that in the absence of estrogen, reproductive senescent females may be more susceptible to diet-induced dysregulation of the blood brain barrier.

Estrogen deprivation itself has the potential to promote chronic inflammation. In bone and circulating monocytes, estrogen deficiency increases pro-inflammatory cytokines and their receptors [reviewed in (Pfeilschifter et al., 2002)] and in post-menopausal women, estrogen treatment reduces cytokine expression in blood cultures (Rogers and Eastell, 2001), bone marrow (Cheleuitte et al., 1998) and peripheral blood mononuclear cells (Rachon et al., 2002). In the current study, estrogen reduced diet-induced cytokines in the senescent females, which is consistent with the actions ascribed for this hormone. However, since the pattern of cytokine activation suggests a Th2 profile, estrogen suppression of this profile cannot strictly be considered anti-inflammatory.

Collectively, our data shows that the high-fat diet-induced inflammatory response is regulated differently with ovarian age and estrogen. Specifically, younger females are protected from the neural effects of an atherogenic diet, even though the diet increased IL-1 β in the liver and elevated plasma cholesterol at both ages. A central inflammatory response was only seen in the reproductive senescent females, which was partially attenuated by estrogen treatment to this group. Our data suggest that with ovarian aging, a poor diet may be more detrimental to brain health and increase risk factors for cognitive decline.

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Figure 1.

Cumulative percent weight change in mature adult females and reproductive senescent females fed a normal or high-fat diet for 10 weeks. Placebo-replaced mature adults and reproductive senescent females showed a significant increase in weight as compared to their estrogen-replaced counterparts irrespective of the diet. Thus, hormone replacement but not diet significantly affected weight gain. Data points above the red dotted line indicate weight gain while data points below the line indicate weight loss. A total of 5-7 replicates were used for statistical analysis. Statistical significance at $p \leq 0.05$ is indicated by (*).

Figure 2.

Plasma cholesterol from mature adults and reproductive senescent females fed a normal or high-fat diet for 10 weeks. Total cholesterol significantly increased in both mature and reproductive senescent females fed the high-fat diet. A total of 5-7 replicates were used for statistical analysis. Main effect of diet indicated by (a).

Figure 3.

Plasma leptin from mature adults and reproductive senescent females fed a normal or high-fat diet for 10 weeks. Leptin expression significantly decreased in both mature and reproductive senescent females fed the high-fat diet. A total of 5-7 replicates were used for statistical analysis. Main effect of diet indicated by (a).

Figure 4.

IL-1 β protein expression in liver homogenates from mature adults and reproductive senescent females fed a normal or high-fat diet for 10 weeks. In the absence of estrogen replacement, IL-1 β expression was significantly increased by diet in mature adult and reproductive senescent females. A total of 5-7 replicates were used for statistical analysis and data was normalized to ovariectomized, placebo-replaced females fed the control diet. An interaction effect of diet and hormone was observed in both mature adult and reproductive senescent females and is indicated by (c). Statistical significance at $p \leq 0.05$ is indicated by (*).

Figure 5.

RANTES and VEGF expression in hippocampal homogenates from mature adults and reproductive senescent females fed a normal or high-fat diet for 10 weeks. In mature adults RANTES was suppressed by estrogen replacement. In reproductive senescent females, diet-induced increases in RANTES AND VEGF were suppressed by estrogen-replacement. A total of 5-7 replicates were used for statistical analysis and data was normalized to ovariectomized, placebo-replaced females fed the control diet. Main effect of hormone indicated by (b), interaction of diet and hormone indicated by (c) and statistical significance at $p \leq 0.05$ is indicated by (*).

Figure 6.

Interleukin protein expression in hippocampal homogenates from mature adults and reproductive senescent females fed a normal or high-fat diet for 10 weeks. In reproductive senescent females, diet-induced increases in IL-4, IL-12p70 and IL-13 were attenuated but not eliminated by estrogen-replacement. These cytokines were not significantly regulated in mature adults. A total of 5-7 replicates were used for statistical analysis and data was normalized to ovariectomized, placebo-replaced females fed the control diet. Main effect of diet is indicated by (a), main effect of hormone is indicated by (b).

Figure 7.

IL-6 protein expression in hippocampal homogenates from mature adults and reproductive senescent females fed a normal or high-fat diet for 10 weeks. In mature adult, IL-6 expression was not significantly regulated. In reproductive senescent females estrogen suppressed IL-6 expression in the presence or absence of the high-fat diet feeding regimen. A total of 5-7 replicates were used for statistical analysis and data was normalized to ovariectomized, placebo-replaced females fed the control diet. Main effect of hormone is indicated by (b).