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Intracellular lipid dysregulation interferes with leukocyte function in the ovaries of meat-type hens under unrestricted feed intake

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ABSTRACT

Meat-type Red-feather country hens fed *ad libitum* (AD-hens) exhibit obesity-associated morbidities and a number of ovarian irregularities. Leukocyte participations in ovarian activities are unstudied in AD-hens. In contrast to feed-restricted hens (R-hens), ovulatory process of the F1 follicle appeared delayed in AD-hens in association with reduced F1 follicle progesterone content, gelatinase A (MMP-2) and collagenase-3 (MMP-13) activities coincident with elevated IL-1β and no production ($P < 0.05$), and increased leukocyte infiltration of inflamed necrotic follicle walls. Extracts of AD-hen F1 follicle walls induced greater leukocyte migration than extracts from F1 follicle wall extracts of R-hens ($P < 0.05$). Co-cultures of granulosa cells with increasing numbers of leukocytes from either AD-hens or R-hens exhibited dose dependent reductions in progesterone production and increases in cell death. AD-hen leukocytes were less proapoptotic than their R counterparts ($P < 0.05$). Granulosa MMP-13 and MMP-2 activities were also suppressed in the co-cultures with heterophils or monocytes in a dose-dependent manner ($P < 0.05$). AD heterophils and R monocytes had a greater inhibitory effect on MMP activities in the co-cultures than their respective counterparts ($P < 0.05$). Both basal and LPS-induced IL-1β secretion and MMP-22 or MMP-2 activities in freshly isolated AD-hen leukocytes were reduced ($P < 0.05$). Exposure of AD or R leukocytes to 0.5 mM palmitate impaired IL-1β secretion and MMP-22 or MMP-2 activity. Inhibition of ceramide synthesis with FB1 and ROS production with *n*-MPG scavenging rescued MMP activity and IL-1β production in palmitate treated heterophils, but exacerbated monocyte suppression. These latter findings suggest that intracellular lipid dysregulation in leukocytes contributes to ovarian dysfunction in AD-hens.

1. Introduction

Egg production is impaired in several strains of meat-type hens when provided with *ad libitum* access to feed (AD-hens) (Hocking et al., 1989; Yu and Robinson, 1992; Pan et al., 2014). Poultry meat from country chickens is

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preferred in many Asian markets and interestingly Red Feather hens of meat-type country chickens manifest dyslipidemia and systemic inflammation in association with impaired egg production as occurs in commercial meat-type hens derived from Cornish x Plymouth Rock broiler strains (Chen et al., 2006; Pan et al., 2012, 2014) In both cases, systemic inflammation was due to a condition termed lipotoxicity (Unger, 2002; Prieur et al., 2010) that develops when cellular fatty acid availability exceeds cellular ability to dispose of fatty acids via β-oxidation or store them as neutral lipids. Under these conditions, cells dysfunction can undergo apoptosis and further induce chronic *in situ* inflammation. Cellular lipid dysregulation was evident in lipotoxic broiler AD-hens as elevated tissue saturated fatty acid concentrations and ceramide accumulation (Chen et al., 2006; Pan et al., 2012; Xie et al., 2012). Changes in tissue lipid composition were related to pronounced granulosa cell apoptosis, increased follicle interleukin-1β (IL-1β) production and leukocyte infiltration in the prevoulatory F1 follicle (Pan et al., 2012, 2014; Xie et al., 2012; Walzem and Chen, 2014). However, effects within individual cell types were not studied.

In mammals, it is well documented that leukocytes contribute to the tissue remodeling during the ovulatory process (Brannstrom and Enskog, 2002; Bukulmez and Arici, 2000). Activation of leukocytes recruited into the follicles and following release of regulatory factors such as inflammatory cytokines, proteases, and reactive oxygen species act as *in situ* modulators of follicle maturation and ovulation. Evidence now supports the notion that macrophages are activated by lipid metabolites and further modulation by lipid signaling is a common pathology in atherosclerosis, obesity associated insulin resistance and inflammation arising from metabolic syndrome such as liver steatosis (Prieur et al., 2010), which meat-type AD-hens also manifest (Chen et al., 2006; Pan et al., 2012). However, less is known about the effect of lipid overload on leukocytes in avian species and subsequent impact on ovarian functions.

We previously found that MMP (matrix metalloproteinase) activity and cytokine production were impaired in conjunction with lipotoxic development from peripheral leukocytes in broiler AD-hens (Liu et al., 2014; Pan et al., 2012; Walzem and Chen, 2014). The present study thus sought to determine whether exogenous palmitic acid, a fatty acid shown active in lipotoxic mechanisms (Walzem and Chen, 2014) also induces lipotoxic development in leukocytes of meat-type country chickens. A second aim was to assess subsequent impacts of feed intake on leukocytes and their ability to act in granulosa cell functions related to ovulatory process.

2. Materials and methods

2.1. Animal management

Two hundred commercially reared meat-type Red Feather (RF) Taiwan country chickens breeder pullets at age 14 week were purchased from a local breeder farm. All birds were allowed free access to a soy and corn-based breeder pre-lay mash (11.6 MJ/kg metabolizable energy; 15% crude protein). After 2 weeks of adaptation to experimental conditions half of the 16 week old pullets were continued with *ad libitum* feed intake (AD-hens) with a regular layer mash (11.6 MJ/kg metabolizable energy; 17% crude protein) and the other half were fed the same layer mash but in amounts equivalent to 85% level of the average feed intake of AD flock on the previous day (R-hens). Details of animal management and performance were described previously (Pan et al., 2014).

2.2. Necropsy, ovarian morphology, and tissue collection

At age 37 week, 9 hens from both the AD and R groups were randomly selected for necropsy from those hens with average clutch length (successive egg laying day) greater than 3 days and having laid an egg on the preceding day to ensure preovulatory F1 follicle collection. To further insure enough retention of the largest follicle entering the F1 position before ovulation, necropsy was performed immediately after oviposition because ovulation typically occurs within 1 h post-ovipositon (Onagbesan et al., 2006). Hens were anesthetized with isoflurane prior to tissue collection. Of the 9 F1 follicles collected from each feeding group, 6 were used to measure progesterone production, nitric oxide (NO) and interleukin-1β (IL-1β) content, and matrix metalloproteinase (MMP) activity. The remaining 3 follicles were used for histological examination including assessment of leukocyte infiltration.

2.3. Histochemistry and immunohistochemistry

Fixation, embedding and hematoxylin and eosin (H&E) staining of F1 follicles were conducted by the Histology Service in the National Chung Hsing University, Taiwan. Tissue sections from the midline area of the stigma strip were imaged following H&E staining using a microscope (DMIRB, LEICA, Germany). Leukocyte infiltration into the follicle walls was visualized by antigen retrieval using an avian-specific mouse monoclonal antibody conjugated with FITC (KUL01, Abcam, Cambridge, UK) to identify monocytes and macrophages (Liu et al., 2014).

2.4. Isolation of peripheral heterophils and monocytes

Blood from hens (36–40 weeks) were collected every 5–7 days and used for peripheral leukocyte isolation by discontinuous gradient centrifugation. Commercial Histopaque® 1077 and 1119 (Sigma, St. Louis, MO) was used to isolate monocytes and heterophils, respectively (Kogut et al., 2001; Liu et al., 2014). In each round of culture studies, isolated cells from two hens per treatment group were pooled and used in the analysis. Five rounds of leukocyte collection were used for migration analysis and 4 rounds were used for analyses of co-cultures with granulosa cells or with LPS and palmitic acid.

2.5. Co-culture of granulosa cells with leukocytes

Granulosa cells were isolated exactly as described by Gilbert et al. (1977). In each round of co-culture study,