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Stearoyl-CoA desaturase 1, elongase 6 and their fatty acid products and precursors are altered in ovariectomized rats with 17β-estradiol and progesterone treatment

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ABSTRACT

Sex differences in monounsaturated fatty acid (MUFA) levels suggest ovarian hormones may affect MUFA biosynthesis. Sprague-Dawley rats (8 weeks of age) were ovariectomized or sham operated with ovariectomized rats implanted with a constant-release hormone pellet providing 17β-estradiol, progesterone, both or neither at 10 weeks of age. After 14 days, rats were fasted overnight and sacrificed to collect plasma and livers for analysis. Hepatic stearoyl-CoA desaturase (SCD1) expression was unchanged between ovariectomized and sham controls, as determined by microarray and immunoblotting. However, SCD1 protein was increased in rats treated with estradiol plus progesterone. Elongase 6 protein levels were increased with 17β-estradiol treatment compared with sham. Rats treated with 17β-estradiol and 17β-estradiol plus progesterone had increased 16:0, 18:0, 16:1n-7 and 18:1n-7 in hepatic and plasma phospholipids. Ovarian hormones appear to be involved with MUFA biosynthesis, but the relationship appears complex and involves elongase 6 and SCD1.

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1. Introduction

Monounsaturated fatty acids (MUFA) have traditionally been considered beneficial fatty acids with respect to health [1,2], and there is increasing evidence that MUFA and their metabolites can affect cell signalling and gene expression [3–6]. Interestingly, recent evidence suggests that certain MUFA species and the enzymes involved in MUFA biosynthesis may contribute to obesity and cardiovascular disease [7–9], indicating that a more complete understanding of the regulation of MUFA biosynthesis is required.

MUFA are either obtained from the diet or are produced *de novo* from the delta-9 desaturation of saturated fats, catalyzed by stearoyl-CoA desaturase (SCD), the rate-limiting step in MUFA biosynthesis [10]. While several isoforms of SCD have been identified, the predominant isoform in the liver is SCD1 [11]. The preferred substrate of SCD1 is 18:0, which is desaturated to 18:1n-9 [11]; however, SCD1 can also convert 16:0 into 16:1n-7, although to a much lesser extent [12]. Due to their role in synthesizing 18:0, the preferred substrate for SCD1 from *de novo* or dietary 16:0, fatty acid elongases need to be considered when examining MUFA synthesis. Elongase 6 preferentially elongates saturated fatty acids (SFA) [13], suggesting that this enzyme plays an important role in determining hepatic MUFA composition. The regulation of the expression and activities of these enzymes, however, is unclear.

Ovarian hormones appear to play an important role in determining MUFA composition. Postmenopausal women have a lower percentage of 16:0 and 16:1n-7 in serum phospholipids as compared with premenopausal women and postmenopausal women receiving hormone therapy [14]. Furthermore, changes in the relative percentage of 16:0, 18:0, 16:1n-7 and 18:1n-9 in plasma and erythrocyte fatty acids are observed at 24 weeks gestation, delivery, and postpartum [15], suggesting changes in hormone concentrations influence MUFA composition.

Therefore, the purpose of this study was to examine the effects of ovarian hormones on both the enzymes involved in MUFA biosynthesis using ovariectomized rats treated with 17β-estradiol, progesterone or 17β-estradiol plus progesterone, and plasma and hepatic MUFA composition. Previous studies suggest that estrogen negatively regulates SCD1 expression, as mRNA expression of SCD1 is increased in ovariectomized rats, with expression levels returning to sham controls following estradiol treatment [16,17]; however, protein content was not determined. In addition, the effect of hormones on other enzymes involved in MUFA biosynthesis and a comprehensive examination of MUFA concentrations has not been reported, and only 17β-estradiol but not progesterone has been examined. Finally, given lipogenesis, lipoprotein synthesis, fatty acid beta-oxidation, phospholipid and triacylglycerol synthesis, and fatty acid transporters may affect MUFA tissue

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levels, a full transcriptome microarray was used to compare changes in hepatic gene expression between ovariectomized and sham operated rats.

2. Materials and methods

2.1. Animals

All animal experiments were carried out according to guidelines of the University of Waterloo Animal Care Committee and Canadian Council on Animal Care. Eight week old rats were ovariectomized or sham-operated prior to arrival at the University of Waterloo Kinesiology Department animal housing facility and were housed under standard conditions and fed a standard rat chow (Teklad AIN-93G, Harlan, Mississauga, ON), as described previously [18]. At ten weeks of age, a constant release hormone pellet was implanted in the ovariectomized rats. Rats were anaesthetized with isoflurane and pellets inserted into a small incision in the rear of the neck, which was closed using surgical staples. Hormone pellets (Innovative Research of America, Sarasota, FL) that have been used previously in similar studies [19,20] were used for the 14 day hormonal treatments. The pellets contained 0.5 mg 17β-estradiol (E-121), 0.5 mg 17β-estradiol plus 15 mg progesterone (HH-115), or 25 mg progesterone (P-131) designed to be released over a 21-days. The experimental groups were; sham-operated ($n=6$), ovariectomized without hormone treatment (OVX, $n=6$), OVX with 17β-estradiol (OVX+E, $n=6$), OVX with progesterone (OVX+P, $n=5$), and OVX with 17β-estradiol plus progesterone (OVX+EP, $n=7$). Two weeks after pellet implantation, all animals were fasted overnight, sedated by intraperitoneal sodium pentobarbital injection and sacrificed by heart removal after cardiocentesis. The estrus cycle of the sham rats was not controlled for at the time of sacrifice. Blood was collected by cardiocentesis into an EDTA-containing syringe and plasma was isolated by centrifugation at 1500 g, and stored at −80 °C. Livers were quickly excised, washed in saline (0.9% NaCl, w/v), and snap-frozen in liquid nitrogen, prior to storage at −80 °C.

2.2. Plasma hormone concentrations

Plasma 17β-estradiol and progesterone concentrations of all rats were determined using ELISA kits (Estradiol EIA Kit 582251, Progesterone EIA Kit 582601, Cayman Chemical, Ann Arbor, MI). Plasma samples were diluted or concentrated as needed, to ensure the resulting hormone concentrations would be in the range of the assay. For each assay, samples and standards were loaded to the 96-well plate in duplicate. Two wells were designated each for blanks, total activity, non-specific binding and maximum binding. Plates were read at a wavelength of 420 nm, and the plasma concentrations of each hormone were determined by comparison to standard curves.

2.3. Whole genome microarray analysis

RNA was extracted from livers of sham operated and ovariectomized rats ($n=4$/group) using Trizol reagent, as described previously [21]. Purity of extracted RNA was quantified using the 260/280 ratio on a Nanodrop 2000c. Integrity of the RNA was examined with an Agilent BioAnalyzer (Agilent, Mississauga, ON). Once checked for integrity, 100 ng total RNA was prepared for hybridization to Affymetrix Rat Gene 1.1 ST Array Strips (part number 901627, Affymetrix, Fremont, CA), according to the manufacturer's instructions. Briefly, total RNA was used to synthesize cDNA, followed by cRNA. Second cycle cDNA was synthesized and fragmented, followed by labelling with biotin and hybridization to microarrays. Strips were washed, stained and scanned on the Affymetrix Gene Atlas platform.

Microarray data was corrected for background noise, quantile normalized using RMA, and summarized by median polish (JMP Genomics Version 5, SAS, Cary, NC, USA). Principal component analysis was used to assess within and between group variability. Variance component analysis was used to determine the effect of treatment, peg position, and array number on the variance in the data set.

FunNet was used to identify biological pathways that were differentially regulated in our gene expression list [22]. In order to obtain a more global appreciation of the biological pathways that were regulated between groups, our gene expression list included all genes with a $p < 0.05$ (i.e. not adjusted for multiple testing). The list of differentially expressed genes between sham and OVX were characterized into biological themes using pathways established from the Kyoto Encyclopedia of Genes and Genomes (KEGG). A list of specific biological processes that were differentially regulated between sham and OVX were then identified.

2.4. Immunoblotting

Protein was extracted from livers of all animals in a buffer containing 0.25 M sucrose, 0.01 M Tris-HCl, 0.01 M $MgCl_2$, 2.5 M DTT, and complete protease inhibitor tablets. Protein content was determined by using a bicinchoninic acid procedure. 20 μg of protein was separated on a 7.5% polyacrylamide gel, and transferred to a polyvinylidene difluoride membrane (Bio-Rad Laboratories). The membranes were blocked with 5% BSA or 5% non-fat milk powder as appropriate in TBS with 0.5% (v:v) Tween-20 (TBS-T) at room temperature for 1 h. Membranes were then incubated for 1 h at room temperature with a primary antibody for SCD1 (Abcam, ab19862, diluted 1:1500 in 5% milk in TBS-T), Elongase 6 (Abcam, ab69857, 1:1000 in 5% milk in TBS-T), full-length endoplasmic reticulum SREBP-1c (Santa Cruz Biotechnology, sc-8984, 1:500 in 5% BSA in TBS-T) and β-actin (Santa Cruz Biotechnology, Sc-130657, 1:1000 in 5% BSA in TBS-T). Membranes were washed with TBS-T, and incubated for 1 h with an appropriate horseradish peroxidise-conjugated secondary antibody and washed again. Enhanced Chemiluminescence Western Blotting Detection Reagents were applied to the membrane (GE Healthcare, Mississauga, ON) to allow visualization of bands on a Chemigenius2 Bioimaging system (Syngene inc., Frederick, MD). Quantification of luminescence was done using Genesnap software v. 7.07 (Syngene). Values were normalized to β-actin.

2.5. Fatty acid analyses

Lipids were extracted from plasma and liver using 2:1 chloroform:methanol (v:v) [23] containing butylated hydroxytoluene as an antioxidant and ethyl docosatrienoate (22:3*n*-3 ethyl ester, Nu-Chek Prep Inc, Elysian, MN) as an internal standard. Triheptadecanoate (T-155 Nu-Chek Prep) and 1,2-diheptadecanoyl-*sn*-glycerol-3-phosphocholine (850360 P, Avanti Polar Lipids Inc, Alabaster, AL) were included as internal standards for triacylglycerols and phospholipids, respectively. Triacylglycerols and phospholipids were isolated from total lipid extracts by thin layer chromatography using 20 × 20 cm plates with a 60Å silica gel layer (Whatman International LTD, Maidstone, England) and 60:40:2 heptane:diethyl ether: acetic acid (v:v:v) as the mobile phase [24]. Bands were visualized under UV light with 2,7-dichlorofluorescein (Sigma-Aldrich, Oakville, ON), identified by comparison to a reference standard and collected by scraping the band from the plate. Triacylglycerols and phospholipids were extracted off the silica by using 2:1 chloroform:methanol.

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