SUPPLEMENTAL MATERIAL

The presence of XX versus XY sex chromosomes is associated with increased HDL cholesterol levels in the mouse

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MATERIALS AND METHODS

Mice

Four Core Genotypes (FCG) C57BL/6 mice were bred and genotyped as described previously.[1] Briefly, XX female mice were mated with XY–(Sry+) male mice to generate XX, XX(Sry+), XY–, and XY–(Sry+) offspring, and genotyping was performed by PCR to detect presence of the Sry transgene (forward: AGCCCTACAGCCACATGATA; reverse: GTCTTGCCTGTATGTGATGG) and Y-chromosome–specific sequence (forward: CTGGAGCTCTACAGTGATGA; reverse: CAGTTACCAATCAACACATCAC).[1] Where indicated, gonadectomy was performed at 75 days of age, as previously described.[1] XY* mice, backcrossed to strain C57BL/6EiJ for >10 generations, were bred as described previously.[1,2] XY* males have the Y* chromosome that recombines aberrantly with the X chromosome. Mating XY* males with XX females generates the three genotypes included in this study: XX, XX^{Y*}, and XY*, which are similar to XX, XXY, and XY mice, respectively. Progeny of XY* mice were gonadectomized at 75 days of age, as previously described.[1]

Gonadal males and females were housed in separate cages and maintained at 23°C with a 12:12 hour light:dark cycle. All mice were initially fed Purina mouse chow diet containing 5% fat (Purina 5001; PMI Nutrition International, St. Louis, MO). Where specified, mice were fed a chow diet until age 3.5 months of age (4 weeks after gonadectomy), and then fed an atherogenic diet for 16 weeks (diet TG90221 containing 7.5% cocoa butter, 1.25% cholesterol, 0.5% sodium cholate; Teklad Research Diets, Madison, WI).

For all studies, blood samples were obtained at 7.5 months of age (FCG mice) or 14 months of age (XY* mice) after fasting 0800–1300. Plasma was collected after centrifugation of whole blood at 3,400 x g for 10 minutes at 4°C. Mouse studies were conducted in accordance with and approved by the Institutional Animal Research Committee of the University of California, Los Angeles.

Measurement of plasma lipid

Total cholesterol, HDL cholesterol, free cholesterol, triglycerides, and free fatty acid levels were determined by enzymatic colorimetric assays.[3] Combined LDL cholesterol and VLDL cholesterol concentration was determined by subtracting HDL cholesterol values from total cholesterol values. Lipoproteins were fractionated from 150 µL of plasma pooled from 3 mice of each genotype by fast protein liquid chromatography at the Mouse Metabolic Phenotyping Center (Vanderbilt University, Nashville, TN).

HDL-ApoA-I Exchange Assay

The HDL-ApoA-I exchange assay was performed on freshly thawed plasma using site-directed spin-label electron paramagnetic resonance (EPR) as described by Borja

et al.[4] Briefly, plasma samples (in triplicate) were diluted by a factor of 4 in PBS and PEG 6000 was added to a final concentration of 4%. ApoB-containing lipoproteins were removed by centrifugation (13,000 rpm, 10 min, 4°C), and clarified plasma was combined with spin-labeled apoA-I. EPR measurements were performed on each sample at 6°C and again after 15 min at 37°C using a Bruker eScan EPR spectrometer with temperature controller (Noxygen). HDL-apoA-I exchange activity was defined as the value obtained at 6°C (normalized to an internal standard) from value obtained at 37°C (normalized to same internal standard) followed by subtracting the baseline spectra for spin-labeled apoA-I in PBS. Additional calculations were performed as described.[4]

Immunoblotting

Plasma aliquots (0.5 uL) were fractionated by SDS-PAGE in a 4-20% Tris-glycine gel and transferred onto a nitrocellulose membrane. Rabbit anti-mouse antibodies against ApoA-I, ApoA-II, and ApoA-IV were described previously[5,6] and used at 1:4000 dilution. Rabbit anti-mouse antibody against ApoE (Cat. K23100R, Meridian Life Science, Memphis, TN) was used at 1:2000. A mouse monoclonal antibody against ApoB[7] was used at 1:1000. HRP-conjugated rabbit anti-mouse antibody against IgG or HRP-conjugated goat anti-rabbit antibody against IgG was used at 1:10,000 (Cat. Sc-2030, Santa Cruz Biotechnology, Santa Cruz, CA). Chemiluminescence (ECL2, Cat. 80196, Thermo Fisher, Rockford, IL) was detected using ChemiDoc XRS+ and quantified by ImageLab 4.0.1 (Bio-Rad, Hercules, CA).

Quantitative RT-PCR

Mouse livers were dissected, flash frozen in liquid nitrogen, and stored at –80°C. RNA was isolated from tissues using Ribozol (Cat. N580, Amresco, Solon, OH). First strand cDNA was generated by reverse transcription with iScript (Cat. 170-8840, Bio-Rad). Quantitative RT-PCR was performed with a Bio-Rad CFX Connect Real-Time PCR Detection System using SsoAdvanced SYBR Green Supermix (Bio-Rad). β2 microglobulin and TATA box-binding protein mRNA were amplified in each sample as normalization controls. All primer sequences are shown in Supplemental Table II.

Statistical Analysis

Groups were compared using two-way ANOVA (NCSS 2001; Number Cruncher Statistical Systems, Kaysville, UT) with main factors of sex (gonadal male vs. gonadal female) and sex chromosome complement (XX vs. XY). In the XY* study, the three groups were compared using one-way ANOVA with Duncan's multiple comparison test. Statistically significant comparisons or interactions are presented ($p<0.05$). All error bars represent one standard deviation.

Methods References

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