Expanded Material and Methods

Production of Human PON3 Transgenic Mice

A clone of the human *PON3* gene in a P1-derived artificial chromosome (PAC) vector was purchased from Invitrogen. PCR and Southern blot analysis confirmed that this clone contained the intact human *PON3* gene. This PAC clone was purified by Qiagen Large-Construct kit according to manufacturer's protocols. The PAC DNA was then digested with Pac I and fractionated on a 0.3% agarose gel to separate a 40.6 kb DNA fragment containing the human *PON3* gene with 4 kb of 5'-flanking and 0.4 kb of 3'-flanking sequences from the rest of the vector and insert sequences. The 40.6 kb DNA fragment was isolated from the gel, diluted to a concentration of 0.6 μg/ml, and microinjected into fertilized C57BL/6J (B6) mouse eggs to produce transgenic mice.

Mice, Diets, Adiposity and Atherosclerotic Lesion Analysis

Mice on the B6 background were maintained either on a 6% fat chow diet or, for the induction of atherosclerotic lesions, on an atherogenic diet (Teklad, Madison, WI) containing 15.75% fat, 1.25% cholesterol, and 0.5% sodium cholate for 15 weeks. PON3 Tg-1 mice on the B6 background were also backcrossed twice with LDLRKO mice on a B6 background (The Jackson Laboratory, Bar Harbor, ME) to obtain PON3 Tg-1/LDLRKO mice and LDLRKO littermates. These were maintained on a 6% fat chow diet until 3 months of age and then switched to a Western diet containing 42% fat and 0.15 % cholesterol without cholate (Teklad, Madison, WI) for another 8 weeks at which time the mice were sacrificed. Atherosclerotic lesion areas in the aortic root region were measured as described [1]. Immunostaining was performed on aortic lesion cryostat sections using a rat anti-mouse MOMA-2 monoclonal antibody (Accurate Chemical &

Scientific Corporation, NY) as previously decribed [2]. The weight, fat mass, and lean mass of mice were recorded using quantitative nuclear magnetic resonance [3] at 3 months of age and at sacrifice.

Southern Blot and PCR Analyses

A ^{32}P-labeled human PON3 cDNA fragment was used as a probe for Southern hybridization, and bands were visualized and quantified using a PhosphorImager 445SI (Molecular Dynamics). For routine identification of PON3 Tg mice, PCR analysis was performed using the primers 5'- AGAATGTTTTGTCTGAGAAGCCCAGG -3' and 5'-ATCCATAAGAAAAGCCACACTCACTG -3', yielding a 274 bp product.

Quantitative-RT-PCR Analysis

Total RNA was isolated from various tissues using Trizol reagent (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. One μg of total RNA from each sample was treated with 1 unit of DNase I, amplification grade (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. The DNase I-treated total RNA was then reverse-transcribed into first strand cDNA using the ThermoScript RT-PCR system (Invitrogen, Carlsbad, CA) according to manufacturer's protocol. Real-time RT-PCR was then performed using the QuantiTect SYBR Green PCR kit (Qiagen, Valencia, CA) in an ABI Prism 7700 cycler. The primers used for specific cDNA synthesis were: MCP-1: 5'-CAG CCA GAT GCA GTT AAC GCC-3' and 5'-TAG GGC AGA TGC AGT TTT AAA TAA-3', Acta2: 5'-TCC GAC ACT GCT GAC AGA GGC-3' and 5'-CCT CAT AGA TAG GCA CGT TGT GA-3', CD68: 5'-CTT CCC ACA GGC AGC ACA G-3' and 5'-AAT GAT GAG AGG CAG CAA GAG G-3', Adiponectin: 5'-GTG ATG GCA GAG ATG GCA CTC CT-3' and 5'-CTT GCC AGT GCT GCC GTC AT-3', mouse

PON3: 5'-GAT CTG AAT GAG CAA AAC CCA GAG GC-3' and 5'-GAG TCC ATG TTG GGG TGA TTC ACG AC-3', human PON3: 5'-GCC ACC AGA GAC CAC TAT TTT ACC A-3' and 5'- ATC ACC TTC AGT TGA GTT AAA TCC-3', and 36B4: 5'-CAC TGG TCT AGG ACC CGA GAA G-3' and 5'-GGT GCC TCT GGA GAT TTT CG-3'. The PCR conditions were: 95 ^{o}C 15 min, 40 cycles of 94 ^{o}C 15 sec, 60 ^{o}C 30 sec and 72 ^{o}C 30 sec, and 4 ^{o}C hold.

Immunoblotting and Lactonase Assay

Membrane protein extracts containing PON3 were prepared from livers and adipose tissues as described by Draganov et al. [4], fractionated by SDS-PAGE and transferred onto a nitrocellulose membrane. The membrane was then incubated with a rabbit anti-human PON3 antiserum (1:1000 dilution), washed, incubated with a secondary antibody, and detected using electrochemiluminescence (Amersham). Five μg of liver membrane protein extract per sample was used for determination of lovastatin lactone hydrolysis activity, using HPLC, as described by Draganov et al. [5].

Assays and LDL Oxidation

Mice were fasted for 16 hours before bleeding. Plasma lipids were determined by enzymatic colormetric assays [1]. Plasma glucose and insulin levels were determined as previously described [6]. Plasma leptin, adiponectin and MCP-1 levels were determined by enzyme-linked immunosorbent assay (ELISA) using kits from R&D Systems**.** Mouse HDL was isolated in the absence of EDTA by ultracentrifugation as described [1]. Human LDL was isolated by ultracentrifugation as described [1]. For the LDL oxidation assay, human LDL (1 mg/ml in PBS) was incubated with 5 μM $CuSO_4$, with or without the presence of 0.5 mg/ml of mouse HDL, for 3 hours at 37 ^{o}C. After the incubation, BHT

was added to a final concentration of 20 μM to stop the reaction. Lipid hydroperoxide contents of samples were then determined using the Fox assay [7].

Cholesterol efflux assay

Cellular cholesterol efflux was measured as previously described with some modifications [8]. Briefly, RAW 264.7 macrophages at $5x10^5$ cells/mL were incubated overnight with medium containing 0.8mg/ml lipoprotein-deficient serum before washing and incubating the cells with 1μCi/mL ^{3}H-labeled cholesterol and 50μg/mL of acetylated-LDL. After 48 hours, cells were washed and equilibrated in DMEM containing 1% essential fatty acid-free albumin. Cholesterol efflux potential of HDL was determined by incubating 25 μg/ml HDL cholesterol each from PON3 Tg and non-Tg mice with labeled cells for four hours at 37 ^{o}C. Cholesterol efflux was expressed as the percentage of total radioactive counts released to the medium.

Studies of F2 Intercross Derived from Inbred Mouse Strains B6 and C3H/HeJ on an ApoE KO Background

Approximately 160 male and 160 female F2 mice derived from the intercross between the parental strains B6 apoE KO and C3H/HeJ (C3H) apoE KO mice were maintained on a low fat chow diet until 8 wk of age and then switched to a "Western diet" for 16 wk before sacrifice. After a 4 hr fast, body weight was recorded, and plasma samples were collected for determination of insulin levels and MCP-1 levels using ELISA. Various fat pads were extracted and recorded for weight. Total RNA from various organs was isolated using Trizol reagent. The adipose expression levels of 23,574 transcripts of each mouse were profiled in duplicate using oligonucleotide microarrays manufactured by Agilent Technologies (Palo Alto, CA, USA), as previously described [9]. Gene expression

is reported as the mean log10 ratio (mlratio) relative to pool derived from 150 mice randomly selected from the F2 population.

References

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Supplemental Table 1 Plasma leptin levels in female PON3Tg and non-Tg mice maintained on various diets

Genotype	Sample size	Diet	Leptin (ng/ml)
non-Tg	4	Chow	2.8 ± 0.9
PON3 Tg-1	6	Chow	3.0 ± 0.9
non-Tg	9	Chow	3.0 ± 0.4
PON3 Tg-2	5	Chow	3.7 ± 1.4
non-Tg	9	Atherogenic	3.6 ± 1.2
PON3 Tg-2	11	Atherogenic	3.8 ± 0.5
LDLRKO	15	Western	9.5 ± 1.9
PON3 Tg-1 /LDLRKO	9	Western	15.5 ± 3.3

Supplemental Table 2 Plasma lipids and glucose levels of PON3 Tg mice maintained on chow or atherogenic diets

Sex	Genotype	Diet	Triglycerides	Total Cholesterol	HDL Cholesterol	VLDL/LDL Cholesterol	Glucose
			mg/dl	mg/dl	mg/dl	mg/dl	mg/dl
Male	non-Tg	Chow	58 ± 12	97 ± 5	86 ± 4	11 ± 2	127 ± 6
Male	PON3 Tg-1	Chow	74 ± 18	103 ± 6	93 ± 4	10 ± 2	132 ± 12
Female	non-Tg	Chow	29 ± 3	79 ± 4	69 ± 4	10 ± 1	104 ± 5
Female	PON3 Tg-1	Chow	26 ± 3	76 ± 3	62 ± 2	14 ± 1*	110 ± 6
Male	non-Tg	Atherogenic	21 ± 3	302 ± 13	63 ± 4	240 ± 12	119 ± 6
Male	PON3 Tg-1	Atherogenic	18 ± 2	303 ± 16	62 ± 2	240 ± 15	133 ± 6
Female	non-Tg	Atherogenic	7 ± 1	249 ± 43	47 ± 4	202 ± 44	113 ± 6
Female	PON3 Tg-1	Atherogenic	7 ± 1	268 ± 25	51 ± 6	217 ± 27	109 ± 5
Male	non-Tg	Chow	73 ± 10	93 ± 3	77 ± 3	16 ± 1	110 ± 4
Male	PON3 Tg-2	Chow	71 ± 9	94 ± 2	80 ± 2	14 ± 2	103 ± 6
Female	non-Tg	Chow	53 ± 8	73 ± 3	58 ± 2	15 ± 2	106 ± 8
Female	PON3 Tg-2	Chow	67 ± 11	70 ± 4	53 ± 3	17 ± 3	100 ± 11
Male	non-Tg	Atherogenic	26 ± 7	301 ± 16	76 ± 8	225 ± 15	108 ± 11
Male	PON3 Tg-2	Atherogenic	20 ± 5	285 ± 9	71 ± 7	214 ± 15	101 ± 7
Female	non-Tg	Atherogenic	6 ± 1	274 ± 50	57 ± 5	217 ± 53	90 ± 6
Female	PON3 Tg-2	Atherogenic	7 ± 1	268 ± 38	52 ± 6	217 ± 40	93 ± 6

*: $p < 0.05$, vs. sex- and diet-matched non-Tg littermates.

Supplemental Table 3 Plasma lipids, glucose, and insulin levels of PON3 Tg-1/LDLRKO mice maintained on either chow or Western diets

Sex	Genotype	Diet	Triglycerides	Total Cholesterol	HDL Cholesterol	VLDL/LDL Cholesterol	Glucose	Insulin
			mg/dl	mg/dl	mg/dl	mg/dl	mg/dl	μg/l
Male	LDLRKO	Chow	122 ± 6	335 ± 9	105 ± 3	230 ± 9	144 ± 5	0.46 ± 0.08
Male	PON3 Tg-1 /LDLRKO	Chow	125 ± 7	314 ± 11	103 ± 4	211 ± 13	141 ± 9	0.37 ± 0.05
Female	LDLRKO	Chow	102 ± 10	272 ± 6	78 ± 2	195 ± 5	124 ± 6	0.52 ± 0.19
Female	PON3 Tg-1 /LDLRKO	Chow	97 ± 8	290 ± 5*	81 ± 2	209 ± 4*	122 ± 5	0.39 ± 0.07
Male	LDLRKO	Western	263 ± 13	1550 ± 82	84 ± 3	1472 ± 87	173 ± 7	1.70 ± 0.22
Male	PON3 Tg-1 /LDLRKO	Western	281 ± 17	1445 ± 91	86 ± 3	1234 ± 97	162 ± 11	1.40 ± 0.13
Female	LDLRKO	Western	103 ± 17	1180 ± 37	68 ± 3	1113 ± 37	126 ± 5	0.49 ± 0.15
Female	PON3 Tg-1 /LDLRKO	Western	110 ± 15	1114 ± 49	83 ± 3*	1031 ± 48	154 ± 20	0.41 ± 0.08

*: $p < 0.05$, vs. sex- and diet-matched non-Tg littermates.