A fish oil diet produces different degrees of suppression of apoB and triglyceride secretion in human apoB transgenic mouse strains

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Abstract Human apolipoprotein B (apoB) transgenic (HuBTg) mouse strains were used to assess genetic effects on the response to fish oil (FO), a source of n-3 fatty acids. A congenic HuBTg strain of the C57BL/6 (B6) background and six F1 HuBTg strains were fed a FO for 2 weeks. Different responses of plasma lipid levels to FO were observed among these strains. In particular, plasma apoB levels changed minimally in FO-fed male B6 HuBTg mice, but increased markedly (~40%) in FO-fed male FVB/NJ (FVB) × B6 F1 HuBTg mice. These strain differences were determined mainly by hepatic apoB secretion rates and were likely regulated by posttranscriptional mechanisms. In addition, plasma triglyceride (TG) levels were reduced (14%) in FO-fed B6 mice, but not in FVB × B6 mice. These strain differences were determined mainly by TG secretion rates, but were not due to differences in hepatic lipogenesis. Hepatic mRNA levels of acyl-CoA oxidase, reflective of peroxisomal β-oxidation rate, were increased in FO-fed B6 but not in FVB × B6 mice, which could account for the difference in TG secretion rates. In summary, differential effects of FO on plasma apoB and TG levels in B6 and FVB × B6 HuBTg mice were associated with strain differences in hepatic apoB and TG secretion and in peroxisomal β-oxidation.— Ko, C., S. M. O'Rourke, and L-S. Huang. **A fish oil diet produces different degrees of suppression of apoB and triglyceride secretion in human apoB transgenic mouse strains. *J. Lipid Res.* 2003.** 44: **1946–1955.**

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Epidemiological studies have demonstrated an inverse correlation between fish consumption and the incidence of coronary heart disease (1–4). Dietary fish oil (FO) enriches hepatic plasma and microsomal membranes with n-3 fatty acids. This enrichment subsequently alters hormone binding to cell-surface receptors and affects intracellular signal transduction, which in turn modifies lipid metabolism [reviewed in ref. (5)]. These fatty acids also affect nuclear mechanisms that change the expression of various genes encoding enzymes involved in lipid metabolism (5). Dietary n-3 fatty acids exert pleiotrophic effects, including triglyceride (TG)-lowering action, which reduce many cardiovascular risk factors in humans (6–8). N-3 fatty acids reduce plasma TGs by inhibition of VLDL synthesis in the liver and/or stimulation of their catabolism (6, 9). N-3 fatty acids reduce microsomal fatty acid synthesis and increase peroxisomal and mitochondrial oxidation by altering the expression of genes involved in their biosynthesis (9). It is thought that these integrated mechanisms result in the reduction of hepatic VLDL TG synthesis.

In humans, a decrease of VLDL apoB flux by FO has been documented (10). The inhibition of apoB secretion by n-3 fatty acids has been shown to result from an increase in intracellular apoB degradation in cultured cells, including a human hepatoma cell line (HepG2) and rat and hamster primary hepatocytes (11–17). However, the effects of n-3 fatty acids on plasma LDL cholesterol levels in humans are less consistent (6). In some reports, increased plasma LDL levels produced by n-3 fatty acids have been documented (18). This unfavorable alteration in lipid profile may be due to a reduction of LDL receptor (LDLR)-mediated clearance and/or the increased conversion of VLDL to LDL particles (9, 19). Thus, plasma levels of LDL and apoB in subjects consuming FO could be affected both by VLDL apoB secretion and by LDL clearance rates.

We have previously shown that apoB and TG secretion rates are independently regulated in human apoB transgenic (HuBTg) mouse strains (20). We have also shown that hepatic apoB-100 secretion rates are genetically deter-

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Abbreviations: AOX, acyl-CoA oxidase; B6, C57BL/6; CPTI, carnitine palmitoyltransferase; FAS, fatty acid synthase; FVB, FVB/NJ; FO, fish oil; HuBTg, human apoB transgenic mouse; LDLR, LDL receptor; MTP, microsomal triglyceride transfer protein; PPAR, peroxisome proliferator-activated receptor; SREBP, sterol responsive element binding protein; TG, triglyceride; WTD, Western-type diet.

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mined in these strains (20, 21). In this report, we assess how genetic background affects plasma apoB and TG levels in response to FO feeding in various HuBTg mouse strains. We show that apoB and TG secretion rates are major determinants of the differential responses of plasma apoB and TG levels to FO feeding in two HuBTg mouse strains.

MATERIALS AND METHODS

Animals and diets

Congenic HuBTg mice of the C57BL/6 (B6) background were generated as described previously (20). Male B6 congenic HuBTg mice were crossed with female mice of various inbred strains to generate F1 mouse strains as described previously (20). Inbred strains used were 129/Sv (129), BALB/c (BALB), C3H/HeJ (C3H), CBA/J (CBA), DBA/2J (DBA), and FVB/NJ (FVB). The F1 offspring 129 × B6, BABL × B6, C3H × B6, and FVB × B6 were described previously (20). In this report, the new F1 mouse strains CBA × B6 and DBA × B6 were generated. Inbred mouse strains used were purchased from the Jackson Laboratory (Bar Harbor, ME). The insertion site of the human apoB transgene has been reported previously (21), and presence of the human apoB transgene in each mouse was determined by PCR as described (22).

Mice were maintained in a 12 h light/dark cycle (light cycle: 7 AM–7 PM). Mice were fed either a chow diet, a FO diet, or a Western-type diet (WTD) and had free access to water. Rodent chow (PicoLab Rodent Chow, No. 5001; Purina Lab Chows, St. Louis, MO) consisted of 4.5% (wt/wt) fat, 0.02% (wt/wt) cholesterol, and was free of casein and sodium cholate. The FO diet (ICN Biomedical, No. 960195) consisted of 21% fat (20% menhaden oil and 1% corn oil). The major n-3 fatty acids in the menhaden oil were C20:5 (16.03%) and C22:6 (10.85%). α-Tocopherol (0.12%) was included as an antioxidant in the diet. To minimize the oxidation of n-3 fatty acids, the FO diet was stored under nitrogen atmosphere. The WTD diet (No.88137; Teklad Premier Laboratory Diets, Madison, WI) consisted of 21% (wt/wt) fat (polyunsaturated/saturated = 0.07), 0.15% (wt/wt) cholesterol, and 19.5% casein similarly free of sodium cholate.

For fasting plasma samples and in vivo measurement of apoB and TG secretion experiments, mice were fasted for 4 h (10 AM–2 PM), retroorbitally bled, and/or subjected to experimental procedures immediately afterwards. For each experiment, age-matched male mice (12–20 weeks) were used unless otherwise indicated.

Measurement of plasma lipid and apolipoprotein concentrations

A colorimetric enzyme assay was used to measure plasma total TG levels (No. 339-10, Sigma, St. Louis, MO). For plasma human apoB levels, an antibody specific to human apoB was used in immunoturbidimetric assays as described previously (20).

Determination of in vivo apoB and TG secretion rates

Assessment of apoB and TG secretion rates in age-matched animals (n = 5–8/group) was performed as described previously (20). For the determination of apoB secretion rates, fasted mice were injected intravenously with a solution containing 200 μCi [^{35}S]methionine and 500 mg/kg Triton WR1339 (Sigma) in 0.9% NaCl. Blood was taken at 0 min (just prior to injection), 30 min, and 60 min after the injection. Plasma samples (10 μl) were subjected to 4% SDS-PAGE followed by fluorography. Both B-100 and B-48 bands were cut from dried gels and counted in a liquid scintillation counter. Both B-100 and B-48 protein counts were normalized by TCA-precipitable counts in the given plasma sample and expressed as protein count per 10 μl of plasma (cpm/10 μl), as previously described (20). ApoB secretion rates (cpm/10 μl plasma/0.5 h) were calculated by subtracting normalized protein counts at the 30 min time point from normalized protein counts at the 60 min time point.

The Triton WR1339 method described above was also employed to determine TG secretion rates with the exclusion of [^{35}S]methionine. Mice were bled at 0 min (before injection), 60 min, and 120 min after injection. Plasma samples from the 0 min, 60 min, and 120 min time points were measured for TG levels. The TG secretion rate was calculated by subtracting the TG level at the 60 min time point from the TG level at the 120 min time point and expressed as mg/dl/h.

RNA probe preparation and RNase protection assays

Total cellular RNA was isolated from the livers using the guanidinium thiocyanate method (23). The probes used for mouse LDLR (24) and microsomal TG transfer protein (MTP) (25) were described previously. The RNA probe for human apoB was derived from a cDNA clone, pB352, containing exon 26 sequences (640 bp) of the human apoB gene. A fragment of 274 bp was isolated for probe synthesis by digesting the pB352 clone with *Sca*I and *Nco*I enzymes. RNA probes for mouse apoB (26), acyl-CoA oxidase (AOX) (27), carnitine palmitoyltransferase I (CPTI) (GenBank Accession number AF017175) (28), fatty acid synthase (FAS) (29), sterol responsive element binding protein 1c (SREBP1c) (30), and peroxisome proliferator-activated receptor α (PPARα) (GenBank Accession number NM_011144) (31) were generated by amplification of the target gene from liver RNA (male B6 mice) by RT-PCR. PCR primers used and the size of amplified products for each probe are shown in **Table 1**. PCR products were cloned into a PCRII vector using a TA cloning kit obtained from Invitrogen (Carlsbad, CA). DNA sequences of each clone were verified by DNA sequencing using an ABI 377 automatic DNA sequencer (Perkin Elmer).

Antisense probes were synthesized using an in vitro transcription kit obtained from Promega (Madison, WI) and ^{32}P-αCTP (800 Ci/mmol). Mouse β-actin (a 100 bp *Hinf*I fragment), cyclophilin, or GADPH (Ambion Co., Austin, TX) were used as reference RNA to normalize for variation in RNA loading in RNase protection assays. RNase protection assays were carried out as described previously (25). Briefly, total cellular RNA (10 μg) was hybridized to a test riboprobe and a reference riboprobe in a hybridization buffer (30 μl) and incubated at 48°C overnight. For apoB probes, hybridization was carried out at 65°C to allow species-specific reactions with either a human or a mouse apoB probe. Following overnight hybridization, 20 units of RNase T2 (Life Technologies, Rockville, MD) was added to the mix. After incubation at 37°C for 2 h, RNase was removed by phenol extraction and protected RNA fragments were ethanol-precipitated, resuspended in 5 μl of loading buffer (95% formamide, 0.05% xylene cyanol, 0.05% bromphenol blue, 20 mM EDTA), and separated in 5% or 8% PAGE/7 M urea gels. Dried gels were exposed to X-ray film for 1 or 2 days at −80°C. For quantification, protected RNA fragments were cut and radioactivity was counted in a liquid scintillation counter.

RESULTS

Genetic background affects the response of plasma apoB levels to a FO-enriched diet in HuBTg mice

To determine genetic effects on the response to FO feeding, age-matched mice of the B6 and six F1 HuBTg strains were fed a low-fat chow diet and then switched to a high-fat diet enriched in FO for 2 weeks. Plasma samples

TABLE 1. PCR primer sequences for mouse RNA probes

Gene	Primer	Primer Sequence (5′ → 3′)	PCR Size (bp)
ApoB	Sense	AGT GCC TGC AGT GGA TCA AGT ACC TGC	223
	Antisense	TGG ACA GCT GAA GCT TAA GTT TTC CAG GAC	
AOX	Sense	TCA ACA GCC CAA CTG TGA CTT CCA TTA	227
	Antisense	TCA GGT AGC CAT TAT CCA TCT CTT CA	
CPTI	Sense	CCA GGC TAC AGT GGG ACA TT	209
	Antisense	GAA CTT GCC CAT GTC CTT GT	
FAS	Sense	TCA CCA CTG TGG GCT CTG CAG AGA AGC GAG	330
	Antisense	TGT CAT TGG CCT CCT CAA AAA GGG CGT CCA	
PPARα	Sense	GTG GCT GCT ATA ATT TGC TGT G	131
	Antisense	GAA GGT GTC ATC TGG ATG GTT	
SREPB1c	Sense	ATC GGC GCG GAA GCT GTC GGG GTA GCG TC	116
	Antisense	ACT GTC TTG GTT GTT GAT GAG CTG GAG CAT	

AOX, acyl-CoA oxidase; apoB, apolipoprotein B; CPTI, carnitine palmitoyltransferase; FAS, fatty acid synthase; PPARα, peroxisome proliferator-activated receptor α; SREBP1c, sterol responsive element binding protein 1c.

were collected from animals before and 2 weeks after starting FO feeding, and fasting plasma human apoB levels were then measured. As shown in **Table 2**, plasma apoB levels in chow-fed male animals varied among the seven mouse strains. The effects of the genetic background on plasma apoB levels in some of these F1 HuBTg mouse strains have been described previously (20). Table 2 also shows that the response of plasma apoB levels to FO feeding varied among the seven mouse strains tested. Plasma apoB levels were slightly increased (10%), but not significantly, in the parental B6 HuBTg strain after 2 weeks of FO feeding. However, plasma apoB levels in all six F1 mouse strains tested were significantly increased by FO. Increases ranged from 25% in the BALB × B6 strain to 60% in the 129 × B6 strain (Table 2). Plasma apoB levels were increased by FO by ~40% (38–46%) in the other four mouse strains (i.e., C3H × B6, CBA × B6, DBA × B6, and FVB × B6). Unlike levels in male B6 HuBTg mice, plasma apoB levels in female B6 HuBTg mice were increased significantly by FO (data not shown). The response to FO in female B6 HuBTg mice was similar to that in female mice of the F1 HuBTg strains. Therefore, only data derived from male mice are shown in this report.

The increases in plasma apoB levels by a FO-enriched diet in these mouse strains were likely due to the relatively high fat content (21%) compared with the chow diet, which has a low fat content (4.5%). In a separate set of experiments, male HuBTg mice were fed a WTD (21% fats, mainly saturated) for 2 weeks. As shown in Table 2, plasma apoB levels were lower in FO-fed B6 HuBTg mice compared with WTD-fed B6 HuBTg mice (17% reduction, $P = 0.02$). Similar results (16–26% reduction) were observed in 129 × B6, BALB × B6, CBA × B6, and DBA × B6 F1 strains (Table 2). However, in C3H × B6 and FVB × B6 HuBTg mouse strains, plasma apoB levels were not lower in FO-fed animals compared with WTD-fed animals.

Taken together, these data showed that genetic background affects the response of plasma apoB levels to FO feeding in HuBTg mouse strains. We intended to determine the genetic basis of strain differences in the response of apoB to FO feeding. However, differences in basal plasma apoB levels complicate metabolic and ge-

TABLE 2. ApoB responsiveness to fish oil feeding

HuBTg[a] Strain	N	ApoB[b] Chow[c]	ApoB[b] FO	ApoB[b] WTD	FO/Chow Ratio[g]	*P* Value[h] C versus FO	FO/WTD Ratio[g]	*P* Value[h] FO versus WTD
		mg/dl			%		%	
B6	10	78 ± 15	86 ± 11	103 ± 14	110	0.2	83	0.02
129 × B6	5	53 ± 5[f]	85 ± 6	106 ± 12	160	<0.001	80	0.008
BALB × B6	5	64 ± 4[d]	80 ± 9	99 ± 11	125	0.007	81	0.02
C3H × B6	5	57 ± 5[e]	83 ± 7	81 ± 7	146	<0.001	102	0.6
CBA × B6	3	64 ± 1[d]	88 ± 4	105 ± 6	138	<0.001	84	0.02
DBA × B6	3	79 ± 7	113 ± 9	152 ± 10	143	0.006	74	0.007
FVB × B6	5	85 ± 16	122 ± 9	122 ± 26	144	0.001	100	1

BALB, BALB/c; B6, C56BL/6; CBA, CBA/J; C3H, C3H/HeJ; DBA, DBA/2J; FVB, FVB/NJ; FO, fish oil HuBTg, human apoB transgenic mouse; WTD, Western-type diet; 129, 129/Sv.

[a] Age-matched male HuBTg mice were fed a chow diet and then switched to a FO diet or a WTD for 2 weeks. Blood samples were collected before and 2 weeks after high-fat-diet feeding.

[b] Fasting plasma samples were measured for human apoB and presented as means ± SD mg/dl.

[c] Comparisons were made between B6 and any given F1 HuBTg strain using Student's *t*-test.

[d] $P < 0.05$.

[e] $P < 0.01$.

[f] $P < 0.0001$.

[g] Ratio of plasma apoB levels in FO-fed versus chow-fed or FO-fed versus WTD-fed animals reflects percent differences in the two groups of animals.

[h] Plasma apoB levels between any two diet groups within each strain were compared using Student's *t*-test.

netic analyses on the strain differences in the response to FO. As shown in Table 2, four of the six F1 strains showed significant differences in basal plasma apoB levels compared with the parental B6 HuBTg strain. Therefore, we chose the FVB × B6, one of the two strains with similar basal plasma apoB levels compared with the parental B6 HuBTg strain, for further studies to assess possible mechanisms underlying strain differences in the response to FO. We note that FO feeding significantly increased plasma apoB levels in the FVB × B6 HuBTg strain (43% increase), but had a minimal effect on plasma apoB levels in the parental B6 HuBTg strain. On the other hand, saturated fat-enriched WTD increased plasma apoB levels in both B6 and FVB × B6 HuBTg strains by 32% and 43%, respectively. Interestingly, the mean body weight was not changed before and after FO feeding in the B6 HuBTg mice (29 ± 4 vs. 28 ± 3 g), whereas the body weight was significantly increased after FO feeding in FVB × B6 HuBTg mice (before vs. after = 38 ± 2 vs. 41 ± 1 g, $P = 0.006$). Similarly, the mean body weight was not changed before and after WTD feeding in B6 HuBTg mice (26 ± 2 vs. 27 ± 2 g), whereas the body weight was significantly increased after WTD feeding in FVB × B6 HuBTg mice (32 ± 2 vs. 37 ± 3 g, $P = 0.047$).

Hepatic apoB secretion rate is a major determinant for differential response of plasma apoB levels to FO between B6 and FVB × B6 mice

To determine whether the strain differences in the responsiveness of plasma apoB levels to FO are regulated by apoB secretion rates, clearance rates, or both, chow-fed and FO-fed mice (n = 6/group) were assessed for in vivo apoB secretion rates. Age-matched male HuBTg mice were injected with Triton WR1339 and [^{35}S]methionine as described in Materials and Methods. Plasma samples were collected at 30 min and 60 min time points followed by SDS-PAGE. Results are shown in **Fig. 1**. These results showed that the hepatic apoB-100 secretion rate in FO-fed B6 HuBTg mice did not statistically differ from those in chow-fed B6 HuBTg mice (Fig. 1A, left panels of 1C, 1D). Hepatic apoB-48 secretion rates were not significantly different between FO-fed and chow-fed B6 HuBTg mice either (Fig. 1A, left panels of 1C. 1D). In contrast, hepatic apoB-100 secretion was increased by 58% ($P = 0.003$) in the FO-fed FVB × B6 mice compared with chow-fed animals (Fig. 1B, right panels of 1C, 1D). Hepatic apoB-48 secretion rates were also increased by 49% ($P = 0.005$) in FO-fed FVB × B6 HuBTg mice compared with chow-fed animals (Fig. 1B, right panels of 1C, 1D). Overall, these results showed that FO feeding had a minimal effect on hepatic apoB secretion rates and on plasma apoB levels in B6 HuBTg mice. On the other hand, FO feeding markedly increased apoB secretion rates in FVB × B6 HuBTg mice. The increase of apoB secretion rates could account for the increase of plasma apoB levels observed in these animals. Thus, these data showed that differential hepatic apoB secretion rates were a major contributor to the strain differences in the response of plasma apoB to FO feeding in the B6 and the FVB × B6 HuBTg strains.

Decreased hepatic LDLR mRNA levels do not account for strain differences in the response of plasma apoB levels to FO feeding

To assess the contribution of LDL clearance rates to plasma apoB levels, we measured hepatic LDLR mRNA levels in both chow-fed and FO-fed animals. Total liver cellular RNA samples isolated from both FO-fed male B6 and FVB × B6 mice and their chow-fed controls (n = 5–6/diet/strain) were subjected to RNase protection assays. Representative samples are shown in **Fig. 2**. These results revealed an ~30% decrease in LDLR mRNA levels in FO-fed B6 HuBTg mice compared with chow-fed B6 HuBTg mice (FO vs. chow = 670 ± 85 vs. 916 ± 88 cpm, $P < 0.001$). These data indicate a likely decrease in LDL clearance in these animals and may explain the slight increase in plasma apoB in FO-fed B6 HuBTg mice.

Similar to the B6 strain, hepatic LDLR mRNA levels were also reduced (~20%) in the FO-fed FVB × B6 HuBTg mice compared with chow-fed FVB × B6 HuBTg mice (FO vs. chow = 644 ± 32 vs. 841 ± 121 cpm, $P = 0.02$). Taken together, these results demonstrated that both an increase in apoB-100 secretion and a reduction in LDL clearance contributed to the marked increase of plasma apoB levels in FO-fed FVB × B6 HuBTg mice. Since LDLR mRNA levels were similarly reduced (~20–30%) in both FO-fed B6 and FO-fed FVB × B6 HuBTg mouse strains, decreased clearance is unlikely to account for the strain differences in plasma apoB levels in FO-fed animals.

Strain differences in the response of plasma apoB levels to FO are not due to changes in hepatic apoB or MTP mRNA levels

To assess whether changes in hepatic apoB gene expression play a role in the strain differences in apoB secretion rates in FO-fed HuBTg mice, we measured hepatic mRNA levels of the human apoB transgene and of endogenous mouse apoB. In agreement with a large body of evidence in the literature, FO had no significant effect on either human apoB mRNA levels (B6: chow vs. FO = 42 ± 9 vs. 58 ± 13 cpm, $P = 0.5$; FVB: chow vs. FO = 40 ± 16 vs. 58 ± 16 cpm, $P = 0.1$) or mouse apoB mRNA levels (B6: chow vs. FO = 1,037 ± 99 vs. 1,118 ± 164, $P = 0.6$; FVB: chow vs. FO = 854 ± 222 vs. 784 ± 90, $P = 0.1$) (Fig. 2). These data showed that hepatic apoB mRNA levels do not play a role in the strain differences in hepatic apoB secretion rates between FO-fed B6 and FVB × B6 HuBTg mouse strains.

We have also assessed the expression of MTP, the large subunit of the MTP complex. MTP is mandatory for apoB secretion (32), and dietary fats have been shown to affect MTP mRNA levels (33). Figure 2 shows that FO feeding had no effect on MTP mRNA levels in either the B6 (chow vs. FO = 131 ± 67 vs. 173 ± 16, $P = 0.2$) or the FVB × B6 (chow vs. FO = 155 ± 42 vs. 235 ± 80, $P = 0.1$) strain. Therefore, the level of hepatic expression of the MTP gene is unlikely to play a role in the strain differences in apoB responsiveness to FO. Overall, these results showed that differential apoB responsiveness to FO is not due to changes in the expression of genes that are crucial to the assembly and secretion of apoB-containing lipoproteins.

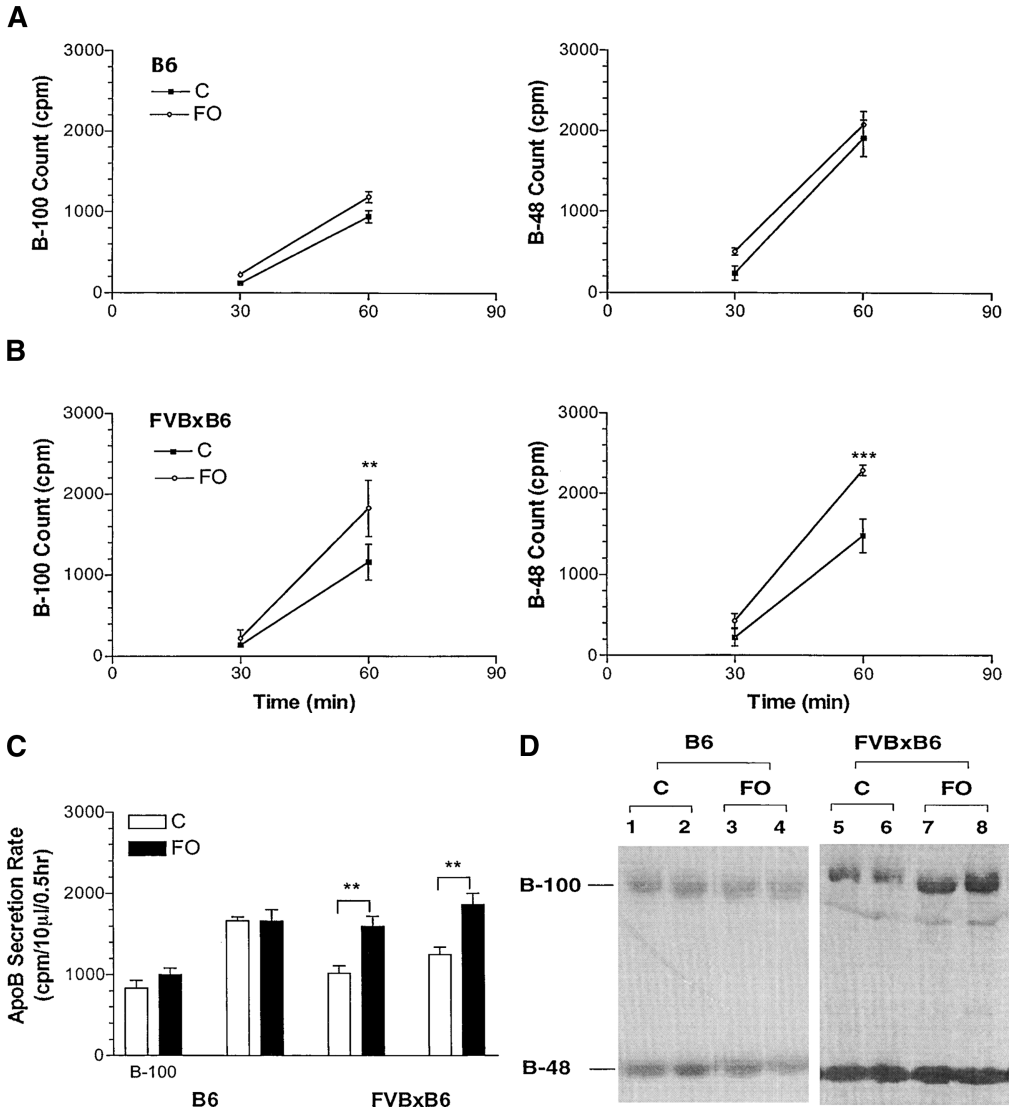

Fig. 1. In vivo apolipoprotein B (apoB) secretion in fish oil (FO)-fed human apoB transgenic (HuBTg) mice. Age-matched male mice (n = 6 or 7 per group) of either C56BL/6 (B6) (A) or FVB/NJ (FVB) × B6 (B) HuBTg strains were fed either a chow or a FO diet for 2 weeks. Mice were fasted for 4 h and then injected with Triton WR1339 and [^{35}S]methionine. Mice were subsequently bled at the 30 min and 60 min time points. Plasma samples (10 μl) from each time point were separated by 4% SDS-PAGE and followed by fluorography. ApoB protein bands from dried gels were cut, counted, and normalized against total TCA-precipitable counts. Mean values (cpm) for either B-100 or B-48 protein counts are shown on the *y* axis. Time (min) post injection is plotted on the *x* axis. The apoB secretion rate (cpm/10 μl/0.5 h) was calculated by subtracting normalized protein counts at the 30 min time point from normalized protein counts at the 60 min time point (C). Comparisons between two diet groups within each strain were performed using Student's *t*-test. *P* values are shown as follows: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. Representative samples from the 60 min time point are shown in the autoradiogram (D).

These data also suggested that the strain differences in hepatic apoB secretion rates and hence plasma apoB levels in FO-fed HuBTg mouse strains (B6 vs. FVB × B6) were likely to be due to differences in the posttranscriptional regulation of apoB secretion.

B6 and FVB × B6 HuBTg mice also differ in the response of plasma TG levels to FO feeding

To determine whether strain differences in the response to FO extend to TG metabolism, fasting plasma samples were assessed for TG levels. These results showed that strain differences were present in the response of plasma TG levels to FO. Compared with chow-fed animals, plasma TG levels were lower (14%) in FO-fed B6 HuBTg mice (chow vs. FO = 161 ± 36 mg/dl, n = 27 vs. 139 ± 36 mg/dl, n = 28, $P < 0.01$), but unchanged in FO-fed FVB × B6 HuBTg mice (chow vs. FO = 157 ± 25 mg/dl, n = 26 vs. 152 ± 18 mg/dl, n = 26). The strain differences in the response of plasma TG levels to FO did not parallel the response of plasma apoB. Therefore, these data showed a dissociation of plasma apoB and TG levels in response to FO in these two mouse strains. Unlike FO-fed animals,

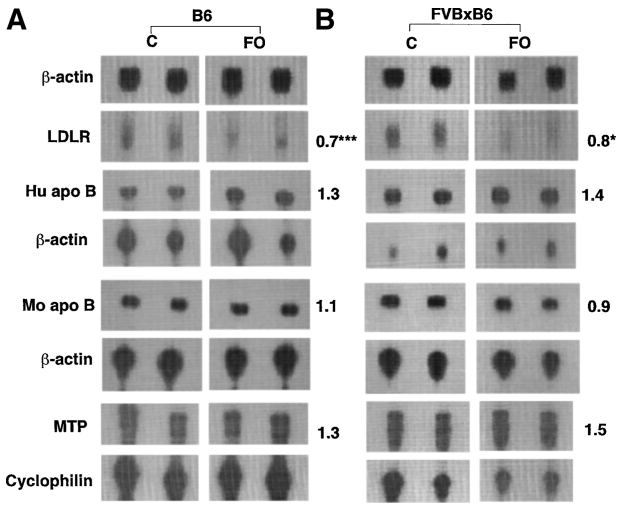

Fig. 2. Hepatic mRNA levels of genes involved in the clearance and secretion of apoB-containing lipoproteins. Total liver cellular RNAs from B6 (A) or FVB × B6 (B) HuBTg mice were subjected to RNase protection assays. In each assay, four or five age-matched male mice from each diet treatment (chow or FO) were used. Representative samples from each assay are shown. Target genes used were LDL receptor, human (Hu) apoB, mouse (Mo) apoB, and microsomal triglyceride (TG) transfer protein. Either β-actin or cyclophilin was used as an internal control. Protected fragments were cut and counted. After normalization, the ratios of RNA between FO-fed and chow-fed animals are shown next to the autoradiograms. Comparisons between two diet groups within each strain were performed using Student's *t*-test. *P* values are shown as follows: * $P < 0.05$ and *** $P < 0.001$.

plasma TG levels were significantly reduced in WTD-fed animals of both strains compared with chow-fed animals (B6: chow vs. WTD = 185 ± 24 vs. 139 ± 25 mg/dl, $P = 0.02$; FVB × B6: chow vs. WTD = 156 ± 10 vs. 113 ± 29 mg/dl, $P = 0.03$). Further studies were carried out to assess possible mechanisms underlying strain differences in the response of plasma TG levels to FO feeding in the B6 and FVB × B6 HuBTg strains.

Hepatic TG secretion rates contribute to strain differences in plasma TG levels in FO-fed B6 and FVB × B6 HuBTg mice

To determine whether strain differences in the response of plasma TG levels to FO are determined by changes in TG secretion, we employed the Triton WR1339 method to determine TG secretion rates in FO-fed animals. **Figure 3** shows that FO feeding reduced TG secretion rates by 36% ($P = 0.008$) in B6 HuBTg mice (chow vs. FO = 139 ± 60 vs. 89 ± 45 mg/dl/h). On the other hand, FO had no significant effect on TG secretion in FVB × B6 HuBTg mice (chow vs. FO = 184 ± 44 vs. 162 ± 43 mg/dl/h). These data showed that the described inhibitory effect of FO on TG secretion was present in B6, but not in FVB × B6, HuBTg mice. Taken together, these data showed that strain differences in plasma TG levels in FO-fed B6 and FVB × B6 HuBTg mice were mainly due to differential effects on TG secretion rates that were exerted by FO.

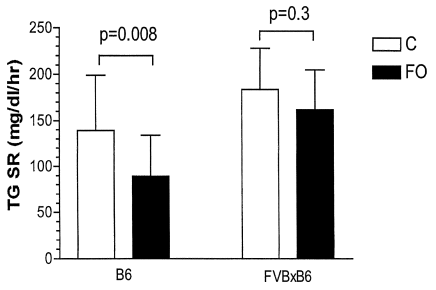

Fig. 3. In vivo TG secretion in FO-fed HuBTg mice. Age-matched male mice were fed either a chow (C, shown in open bars) or a FO (shown in solid bars) diet for 2 weeks. Mice were fasted for 4 h and then injected with Triton WR1339. Plasma samples were collected at 0 min (before injection), 60 min, and 120 min after injection. Plasma samples were measured for TG levels. TG secretion rates, shown on the *y* axis, were calculated by subtracting TG levels at the 60 min time point from the TG levels at the 120 min time point and expressed as mg/dl/h. Results from B6 HuBTg mice (chow: total n = 20; FO: total n = 16) were pooled from three separate experiments (n = 4 to 8/group), and results from FVB × B6 HuBTg mice (chow: total n = 13; FO: total n = 12) were pooled from two separate experiments (n = 5 to 8/group). Chow-fed animals are shown in open bars and FO-fed animals are shown in solid bars. *P* values were derived from Student's *t*-test.

Decreased hepatic lipogenesis does not account for strain differences in TG secretion rates in FO-Fed B6 and FVB × B6 mice

Decreased TG secretion rates in FO-fed animals could result from decreased lipogenesis or increased β-oxidation, either of which would decrease hepatic TG availability for TG secretion. To determine whether strain differences in TG secretion rates in FO-fed HuBTg mice were due to the differential response of hepatic lipogenesis to FO, we measured hepatic mRNA levels of FAS, and SREBP1c. FAS is a key enzyme in fatty acid biosynthesis and has been shown to be down-regulated by FO via a pathway mediated by the SREBP1c transcription factor (34). As shown in **Fig. 4**, FO markedly reduced mRNA levels of FAS (>90% decrease) with a concomitant reduction of SREBP1c (50–60% decrease) in both B6 and FVB × B6 HuBTg mouse strains. These data suggested that hepatic lipogenesis is likely down-regulated by FO in both mouse strains. These data also showed that FO-induced down-regulation of hepatic lipogenesis could not account for the strain differences in TG secretion rates between B6 and FVB × B6 HuBTg mouse strains.

Changes in peroxisomal β-oxidation contribute to strain differences in TG secretion rates in FO-fed B6 and FVB × B6 mice

To determine whether changes in fatty acid oxidation play a role in strain differences in TG secretion rates in FO-fed HuBTg mice, we measured hepatic mRNA levels of CPTI and AOX, which are key enzymes in the β-oxidation that takes place in mitochondria and peroxisomes, respectively. **Figure 5** shows that hepatic mRNA levels of CPTI were not affected by FO in either the B6 or the FVB × B6 HuBTg strain. On the other hand, a differential re-

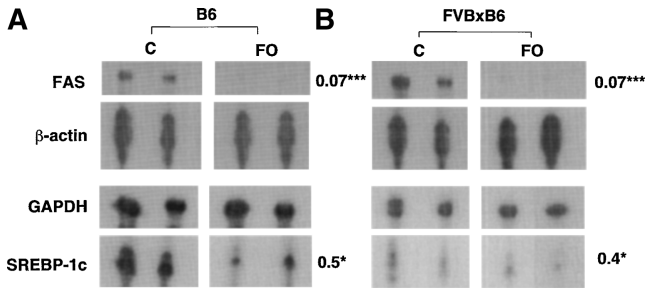

Fig. 4. Hepatic mRNA levels of genes involved in lipogenesis. Total liver cellular RNAs from B6 (A) or FVB × B6 (B) HuBTg mice were subjected to RNase protection assays. In each assay, 4 or 5 age-matched male mice from each diet treatment (chow or FO) were used. Representative samples from each assay are shown. Target genes used were fatty acid synthase and sterol responsive element binding protein 1c. Either β-actin or GADPH was used as an internal control. Protected fragments were cut and counted. After normalization, the ratios of RNA between FO-fed and chow-fed animals are shown next to the autoradiograms. Comparisons between two diet groups within each strain were performed using Student's *t*-test. *P* values are shown as follows: * $P < 0.05$ and *** $P < 0.001$.

sponse in the hepatic expression of AOX was observed. In the B6 HuBTg mice, hepatic AOX mRNA levels were increased (80%) by FO feeding (FO: chow = 2,781 ± 573 vs. 1,533 ± 130 cpm, $P = 0.02$), suggesting an increase in peroxisomal β-oxidation in FO-treated B6 HuBTg mice. An increase in AOX expression leading to increased peroxisomal β-oxidation could contribute to the lower TG secretion rates observed in FO-treated B6 HuBTg mice. In the FVB × B6 HuBTg mice, hepatic AOX mRNA levels were not affected by FO (FO: chow = 6,402 ± 555 vs. 6,012 ± 362 cpm, $P = 0.2$). These data showed that the lack of an increase in AOX mRNA levels in FO-fed FVB × B6 HuBTg mice was accompanied by a lack of change in the TG secretion rate. Overall, these data suggest that differential changes in peroxisomal β-oxidation may play a major role in strain differences of TG secretion rates and hence plasma TG levels in FO Fed HuBTg strains.

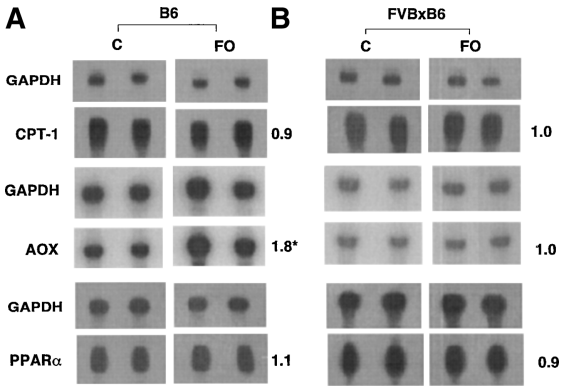

Fig. 5. Hepatic mRNA levels of genes involved in fatty acid oxidation. Total liver cellular RNAs from B6 (A) or FVB × B6 (B) HuBTg mice were subjected to RNase protection assays. In each assay, four or five age-matched male mice from each diet treatment (chow or FO) were used. Representative samples from each assay are shown. Target genes used were carnitine palmitoyltransferase I, acyl-CoA oxidase, and peroxisome proliferator-activated receptor α. GADPH was used as an internal control. Protected fragments were cut and counted. After normalization, the ratios of RNA between FO-fed and chow-fed animals are shown next to the autoradiograms. Comparisons between two diet groups within each strain were performed using Student's *t*-test. * $P < 0.05$.

The effect of FO on AOX gene expression has been shown to be absent in mice deficient in PPARα, a transcription factor known to be activated by polyunsaturated fatty acids, including n-3 fatty acids (35). Figure 5 shows that hepatic PPARα mRNA levels were not changed after FO feeding in B6 HuBTg mice despite the increase in AOX mRNA levels. Changes in the expression of AOX in FO-fed B6 HuBTg mice may have been mediated via ligand activation of the PPARα in these animals.

DISCUSSION

FO enriched with n-3 fatty acids exerts multiple beneficial effects on lipid metabolism, including TG-lowering effects that have been documented both in human and animal models (9). Using seven different HuBTg mouse strains, we show that differences in genetic background can affect the response of plasma apoB and TG levels to FO feeding. In particular, two of these mouse strains, a congenic B6 HuBTg strain and a FVB × B6 F1 strain derived from crosses between B6 HuBTg and FVB wild-type mice, displayed distinct responses to a 2 week feeding of a FO diet (21% fat). Plasma apoB levels were minimally changed in FO-fed male B6 HuBTg mice, but were markedly increased (~40%) in FO-fed male FVB × B6 F1 mice. However, in response to another high-fat diet (WTD, 21% fat) consisting mainly of saturated fats, plasma apoB levels were increased similarly (30–40%) in both strains compared with their controls fed a low-fat (4.5%) chow diet. These two mouse strains also differed in the response of plasma TG levels to FO feeding. Plasma TG levels were decreased (14%) in FO-fed B6, but were unchanged in FVB × B6, HuBTg mice. These two mouse strains thus provided tools for elucidating mechanisms underlying the response of lipid parameters to a diet enriched with n-3 fatty acids.

In the present study, we showed that both clearance and secretion rates contributed to changes in plasma apoB levels in FO-fed HuBTg mice. Hepatic LDLR mRNA levels were decreased in FO-fed B6 and FVB × B6 mice by 30% and 20%, respectively, suggesting a decrease in the clearance of apoB-containing lipoproteins in both strains. The extent of reduction in LDLR mRNA levels were similar in both strains and did not account for the strain differences in plasma apoB levels. On the other hand, hepatic apoB (both apoB-100 and apoB-48) secretion rates were unaffected in FO-fed B6 HuBTg mice, but markedly increased (57%) in FO-fed FVB × B6 HuBTg mice. The differences in hepatic apoB secretion rates between the two mouse strains could account for the different effects on plasma apoB produced by FO feeding.

Reduced LDL clearance by FO has been demonstrated in several animal models, including miniature pigs and monkeys (19, 36). Reduced LDLR activity has also been

documented in the livers of FO-fed rabbits (37). In vitro studies have shown that dietary n-3 fatty acids increase accumulation of apoB in cell medium by decreasing LDLR mRNA levels and LDLR proteins (38), or by decreasing the affinity of LDL from FO animals for binding LDLR (39). Indeed, hepatic mRNA levels of LDLR were reduced in both B6 and FVB × B6 mouse strains. Although reduced LDL clearance appeared to contribute to changes in plasma apoB levels after FO feeding, it did not contribute to the strain differences in the response of plasma apoB levels to FO in B6 and FVB × B6 HuTg mice. Instead, differences in their response to hepatic apoB secretion rates could account for the strain differences in plasma apoB levels. The differences in hepatic apoB secretion rates between B6 and FVB × B6 HuBTg mice were not due to changes in hepatic mRNA levels of either human apoB transgene or endogenous mouse apoB gene. Therefore, strain differences in apoB secretion rates are likely regulated by posttranscriptional mechanisms. These data agree with a large body of evidence that suggests that apoB secretion is regulated predominantly at the posttranscriptional levels [reviewed in refs. (40, 41)]. We also showed that hepatic mRNA levels of MTP, a necessary factor for apoB secretion, were unaffected by FO feeding in both B6 and FVB × B6 HuBTg mice and could not account for the strain difference in apoB secretion rates in FO-fed animals.

Dietary n-3 fatty acids have been shown to decrease VLDL apoB secretion in cultured cells (11–16). In rat primary hepatocytes, dietary n-3 fatty acids decrease apoB secretion by increasing intracellular apoB degradation (13, 14, 42). In hamster hepatocytes treated with n-3 fatty acids, the predominant site of intracellular degradation is in the endoplasmic reticulum (ER) (16), whereas the predominant site for apoB degradation in rat primary hepatocytes appears to be in a post-ER compartment (17). It has also been shown that n-3 fatty acids appear to preferentially reduce the secretion of large, assembled apoB-lipoprotein particles in rat primary hepatocytes (17). Studies have also suggested that n-3 fatty acids cause compositional changes of the phospholipids in the secretory compartments, which may contribute to impaired VLDL secretion (43). Overall, these studies suggest numerous potential sites of regulation by dietary n-3 fatty acids in hepatic cells. It is possible that B6 and FVB × B6 HuBTg mice differ in one of the steps that regulate the assembly and secretion of apoB-containing lipoproteins. Therefore, these two mouse strains will provide valuable tools to further study mechanisms underlying the regulation of apoB secretion by dietary n-3 fatty acids.

The current study showed that B6 and FVB × B6 HuBTg mice also differed in the response of their plasma TG levels to FO feeding. The TG-lowering effect of FO could result from increased lipolysis/clearance and/or decreased VLDL secretion. The differences between B6 and FVB × B6 HuBTg mice in the response of plasma TG levels to FO feeding were, in part, due to differences in TG secretion rates. The TG-lowering effect of FO, mediated via decreases in TG secretion rates, was observed in B6 but not in FVB × B6 HuBTg mice. Decreased VLDL TG secretion by FO could be due to a decrease in TG synthesis resulting from decreased lipogenesis and/or increased fatty acid oxidation. FO down-regulates hepatic lipogenesis by decreasing expression levels of certain enzymes, including FAS, involved in fatty acid biosynthesis (44, 45). This down-regulation is associated with decreases of SREBP1c mRNA levels and mature SREBP1c protein (34). In this report, we showed that hepatic FAS mRNA levels in FO-fed HuBTg mice of both B6 and FVB × B6 HuBTg mice were reduced to 7% of those in chow-fed controls. This decrease was accompanied by a 50% to 60% reduction of hepatic SREBP1c mRNA levels. Despite this marked decrease in factors involved in hepatic lipogenesis, TG secretion rates were not affected in FO-fed FVB × B6 HuBTg mice. These data suggest that changes in hepatic lipogenesis could not account for the differential responses of TG secretion between B6 and FVB × B6 HuBTg mice. These data also support findings that de novo lipogenesis is a minor source for the TGs that are destined for VLDL TG secretion (46, 47).

The current study shows that changes in peroxisomal β-oxidation, but not in mitochondrial β-oxidation, could account for the differences in TG secretion rates in FO-fed B6 and FVB × B6 HuBTg mice. Hepatic mRNA levels of CPTI, a key enzyme in mitochondrial β-oxidation, were not altered by FO in either the B6 or FVB × B6 strains. However, hepatic mRNA levels of AOX, a key enzyme for peroxisomal β-oxidation, were increased in B6 but not in FVB × B6 HuBTg mice. Therefore, it is likely that the increase of peroxisomal β-oxidation reduced the availability of TG substrates destined for secretion in FO-fed B6 HuBTg mice, and the unaffected TG secretion rates in FO-fed FVB × B6 HuBTg mice could result from a lack of change in peroxisomal β-oxidation. Increased fatty acid oxidation induced by FO feeding has been associated with decreased plasma TG levels in animal studies (9). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the two major n-3 fatty acid components of FO. Differential effects of EPA and DHA on fatty acids oxidation have been documented (48–50). EPA affects both mitochondrial and peroxisomal β-oxidation, whereas the effect of DHA is more limited to peroxisomal β-oxidation. Several studies in rats suggest that enhanced mitochondrial fatty acid oxidation by FO, rather than peroxisomal oxidation, was associated with decreased plasma TG levels (48, 49). However, increased peroxisomal oxidation alone has also been associated with decreased plasma TG levels in FO-fed rats (51, 52). Overall, these data suggest that the effects of dietary n-3 fatty acids on fatty acid oxidation may vary depending upon the nature of n-3 fatty acids.

The effect of FO on the upregulation of AOX is abolished in mice deficient in PPARα, a transcription factor (35). Dietary n-3 fatty acids and their derivatives activate PPARα by binding to the ligand domain of the protein (53). Activated PPARα binds to the PPAR responsive element in the target gene resulting in changes in transcription of the target gene (53). In B6 HuBTg mice, AOX mRNA levels were increased without changes in hepatic

PPARα mRNA levels, suggesting that up-regulation of AOX expression may be mediated via ligand activation of PPARα. Since activation of PPARα could result from binding to n-3 fatty acids or their derivatives, it is possible that B6 and FVB × B6 strains may differ in a factor (or factors) which generates an n-3 fatty acid derivative that is required for the transcriptional regulation of AOX mediated by PPARα.

In summary, we have shown that the responses of lipid metabolism to dietary n-3 fatty acids are genetically regulated. Genetic differences in factors regulating apoB and TG secretion in the B6 and the FVB × B6 HuBTg strains play a major role in the differential response of plasma apoB and TG levels to FO feeding in these two mouse strains. We speculate that similar mechanisms could account for the strain differences in FO response between B6 HuBTg and some, if not all, of the other F1 HuBTg strains. The B6 and FVB × B6 HuBTg strains will provide valuable models for understanding the mechanisms underlying the pleiotrophic effects exerted by dietary n-3 fatty acids. They are also suitable for genetic analysis to identify the genes regulating differential responses to FO feeding. Studies in our laboratory have identified chromosome intervals containing genes that are associated with the differential response of plasma apoB levels to FO feeding (Ko and Huang, unpublished observations).

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