Circulation Research

ORIGINAL RESEARCH

Social Bonds Retain Oxytocin-Mediated Brain-Liver Axis to Retard Atherosclerosis

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BACKGROUNDS: Social interaction with others is essential to life. Although social isolation and loneliness have been implicated as increased risks of cardiometabolic and cardiovascular diseases and all-cause mortality, the cellular and molecular mechanisms by which social connection maintains cardiometabolic and cardiovascular health remain largely unresolved.

METHODS: To investigate how social connection protects against cardiometabolic and cardiovascular diseases, atherosclerosis-prone, high-fat diet-fed *Apoe*$^{-/-}$ mouse siblings were randomly assigned to either individual or grouped housing for 12 weeks. Histological, flow cytometric, biochemical, gene, and protein analyses were performed to assess atherosclerotic lesions, systemic metabolism, inflammation, and stress response. The effects of oxytocin on hepatocytes and subsequent cardiometabolic and cardiovascular function were investigated by in vivo and in vitro approaches.

RESULTS: *Apoe*$^{-/-}$ mice housed individually developed larger vulnerable atherosclerotic lesions by disrupted lipid metabolism compared with those of mice in regular group housing, irrespective of body weight, eating behavior, feeding conditions, sympathetic nervous activity, glucocorticoid response, or systemic inflammation. Mechanistically, the chronic isolation reduced the hypothalamic production of oxytocin, which controls bile acid production and LPL (lipoprotein lipase) activity through the peripheral OXTR (oxytocin receptor) in hepatocytes, whose downstream targets include *Cyp7a1*, *Angptl4*, and *Angptl8*. While hepatocyte-specific OXTR-null mice and mice receiving adeno-associated virus targeting OXTR on hepatocytes led to severe dyslipidemia and aggravated atherosclerosis, oral oxytocin supplementation to socially isolated mice, but not to hepatocyte-specific OXTR conditional knockout mice, improved lipid profiles and retarded atherosclerosis development.

CONCLUSIONS: These results identify a novel brain-liver axis that links sociality to hepatic lipid metabolism, thus proposing a potential therapeutic strategy for loneliness-associated atherosclerosis progression.

Key Words: atherosclerosis ■ cardiovascular diseases ■ hepatocytes ■ lipid metabolism ■ neuropeptides ■ social isolation

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Accumulating evidence has shown that a lack of social connection is an increasingly important public health issue. Social isolation (SI)–induced chronic psychological stress and loneliness are associated with cardiometabolic disease and cardiovascular disease (CVD).[1–6] Moreover, various meta-analyses have consistently shown that the proportion of people suffering from SI and loneliness is steadily increasing worldwide.[7–10] Consequently, SI and loneliness emerge as critical determinants of CVD and all-cause mortality.

Many previous studies have tried to elucidate fundamental pathways and molecular links that connect social disruption to disease using many animal species and models. In the atherosclerosis research field, for instance, there are some studies showing that SI and psychological stress accelerate atherogenesis. Despite such phenotype, the cardiometabolic features and the putative mechanisms presented in the studies are considerably variable. On the one hand, male cynomolgus monkeys exposed to different conspecific

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Novelty and Significance

What Is Known?

- Social isolation and loneliness are global socioeconomic concerns that have been highlighted as increasing risks of cardiometabolic and cardiovascular diseases.
- Oxytocin is a hypothalamic neuropeptide essential to social bonding, emotional regulation, and mammalian reproduction.

What New Information Does This Article Contribute?

- Chronic isolation reduces oxytocin production in the hypothalamus, and hepatocytes predominantly express OXTR (oxytocin receptor) in the liver.
- Brain-derived oxytocin directly acts on the OXTR-expressing hepatocytes via circulation and maintains hepatic and systemic lipid homeostasis.
- The oxytocin-mediated lipid regulation is crucial for protecting against social isolation–induced atherosclerosis progression.

Several animal studies have shown that chronic social isolation accelerates atherosclerosis. However, the cardiometabolic features and the putative mechanisms presented in the studies are considerably variable. In the current study, we revisited the social isolation mouse model and revealed that hypothalamic oxytocin constitutes a brain-liver axis that links sociality to hepatic lipid metabolism. These findings suggest a potential novel therapeutic strategy for loneliness-associated atherosclerosis progression.

Nonstandard Abbreviations and Acronyms

AAV	adeno-associated virus
ANGPTL	angiopoietin-like protein
ANGPTL4	angiopoietin-like 4
ANGPTL8	angiopoietin-like 8
CVD	cardiovascular disease
CYP7A1	cholesterol 7 alpha-hydroxylase
GFP	green fluorescent protein
HFD	high-fat diet
HMGCR	3-hydroxy-3-methylglutaryl-coA reductase
LDL	low-density lipoprotein
LPL	lipoprotein lipase
OXTR	oxytocin receptor
SI	social isolation
V1aR	vasopressin receptor 1a
VLDL	very-low-density lipoprotein

stressors and fed a nonatherogenic diet developed atherosclerosis irrespective of blood pressure, serum lipid, or glucose levels, but, on the other hand, socially isolated hyperlipidemic animals exhibited increased plasma lipid levels, thereby developing severe atherosclerotic lesions.[11,12] Moreover, although sympathetic nervous activation, hypothalamic-pituitary-adrenal axis response, or immune-mediated inflammation have been suggested as potent underlying mechanisms that promote metabolic dysregulation in SI-induced chronic stress, it is not consistent among the studies.[13–17] Potential explanations for the inconsistency and variability include different housing, husbandry, and environmental conditions of the animals, extra stress from invasive experimental manipulations, and some social factors such as fighting and dynamic changes in social dominance hierarchy.[18,19] These data imply that an SI study with a consistent protocol to minimize unnecessary factors throughout the study is required to elucidate the underlying pathophysiological mechanisms of how SI disrupts cardiometabolic and cardiovascular health.

Here, we revisited the chronic SI model using hyperlipidemic *Apoe*$^{-/-}$ mice housed individually to ask how chronic psychological stress accelerates atherosclerosis. To strengthen and ensure the reliability and feasibility of the model, we used atherosclerosis-prone animals originally derived from the same parents, handled them minimally to avoid unnecessary stress, and gave them long-term exposure to isolation. We found that the reduction of oxytocin in the hypothalamus is responsible for the SI-related cardiometabolic and cardiovascular phenotype. Brain-derived oxytocin directly acts on OXTR (oxytocin receptor)-expressing hepatocytes via circulation, maintains hepatic and systemic lipid homeostasis, and suppresses the SI-induced atherosclerosis progression. Importantly, these effects were independent of body weight, eating behavior, feeding conditions, sympathetic nervous activity, glucocorticoid response, or systemic inflammation.

METHODS

Data Availability

The detailed methods used in this study and Major Resources Table are provided in the Supplemental Material. The data will be provided upon reasonable request.

RESULTS

Chronic Isolation Disrupts Hepatic Lipid Metabolism and Promotes Atherosclerosis

To investigate how social connection protects against cardiometabolic disease and CVD, we subjected 12-week-old atherosclerosis-prone, high-fat diet (HFD)-fed *Apoe*$^{-/-}$ mouse siblings to individual housing for 12 weeks, thereby mimicking chronic SI in humans (Figure 1A).[13] Although we found no statistically significant difference in body weight, amount of cumulative food intake, adipose tissue mass, or physical activity (Figure 1B; Figure S1A and S1B), mice subjected to SI developed larger atherosclerotic lesions, which contained thinner fibrous cap covering the larger necrotic core, with considerably higher serum total cholesterol and triglyceride levels than those of mice in regular group housing (Figure 1C through 1F). Specifically, SI mice had elevated serum levels of VLDL (very-low-density lipoprotein), intermediate-density lipoprotein, and LDL (low-density lipoprotein) but similar levels of high-density lipoprotein (Figure 1F and 1G). These results were consistent with previous clinical studies showing that SI and loneliness are adversely associated with cardiovascular risk factors including dyslipidemia.[6,20,21]

To uncover the molecular underpinnings of SI-induced increases in cholesterol and triglycerides, we performed gene profiling analysis in the liver and white adipose tissue. SI repressed *Cyp7a1* (cholesterol 7 alpha-hydroxylase) and *Angptl8* (angiopoietin-like 8) expressions and enhanced *Angptl4* (angiopoietin-like 4) expression in the liver but caused no statistically

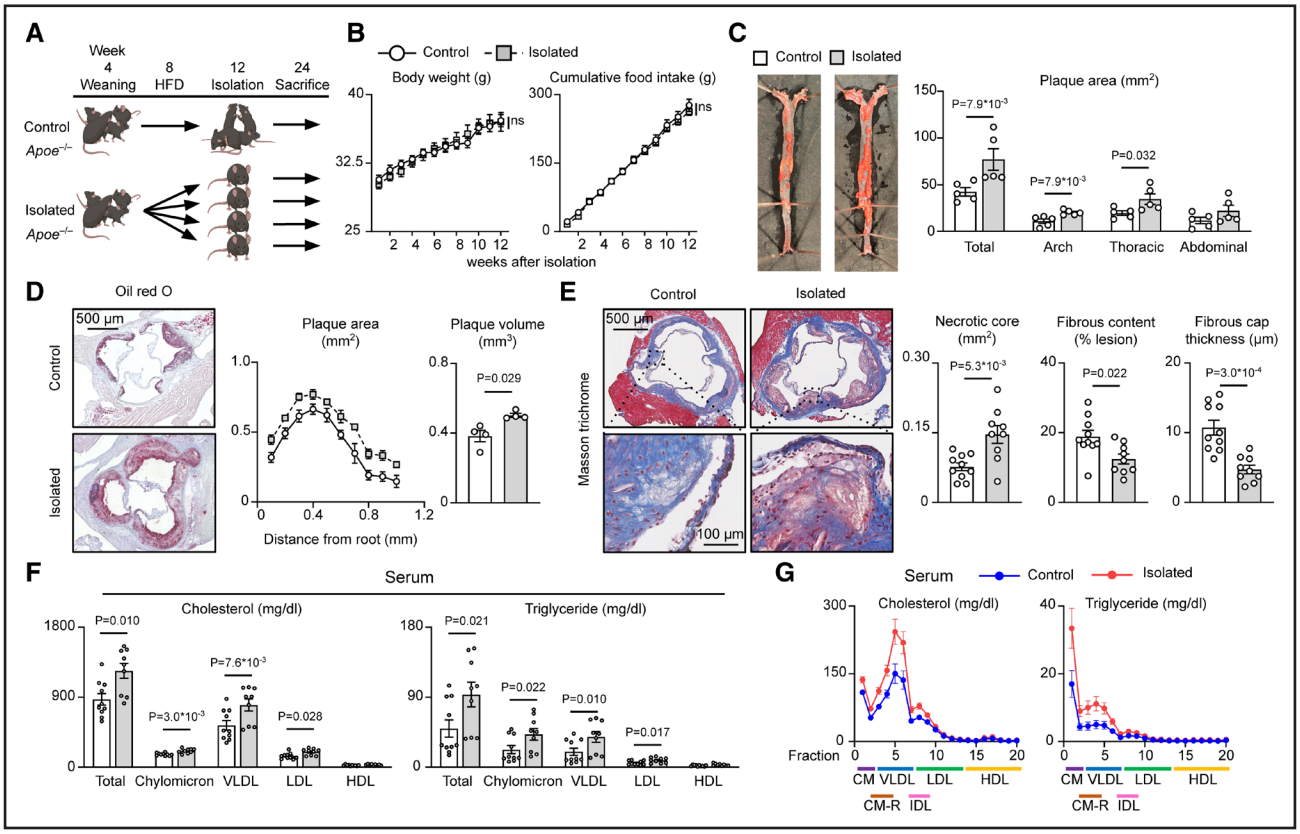

Figure 1. Chronic isolation promotes atherosclerosis and dyslipidemia.

A, Experimental outline. *Apoe*$^{-/-}$ mouse siblings fed a high-fat diet were subjected to chronic isolation for 12 weeks. **B**, Body weight and cumulative food intake (n=14 control *Apoe*$^{-/-}$ mice; n=11 isolated *Apoe*$^{-/-}$ mice). **C**, Representative en face atherosclerotic aorta images with oil red O staining and quantification of plaque areas after 12 weeks of chronic isolation (n=5 per group). **D**, Representative cross-sections of aortic roots stained with oil red O and measurement of lesion volume after 12 weeks of chronic isolation (n=4 per group). **E**, Representative cross-sections of aortic roots stained with Masson trichrome and quantification of necrotic core areas, fibrous contents, and fibrous cap thickness after 12 weeks of chronic isolation (n=10 control *Apoe*$^{-/-}$ mice; n=9 isolated *Apoe*$^{-/-}$ mice). **F** and **G**, Serum lipid profiles analyzed by high-performance liquid chromatography in the postprandial state after 12 weeks of chronic isolation (n=10 control *Apoe*$^{-/-}$ mice; n=9 isolated *Apoe*$^{-/-}$ mice). Data are mean±SEM; repeated measures ANOVA were performed in **B**. Mann-Whitney *U* tests were performed in **C** through **F** for statistical analysis. CM indicates chylomicron; CM-R, chylomicron remnant; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; and VLDL, very-low-density lipoprotein.

significant difference in gene expression related to lipid metabolism in white adipose tissue (Figure 2A; Figure S1C and S1D). The key transcriptomic changes in the SI liver were validated by protein measurement with Western blotting experiments, demonstrating that the liver subjected to SI had decreased CYP7A1 and ANGPTL8 and increased ANGPTL4 protein expressions, although expression of HMGCR (3-hydroxy-3-methylglutaryl-coA reductase) was comparable between the groups (Figure 2B). SI-induced decreased hepatic *Cyp7a1* expression was accompanied by diminished bile acid contents and more unconverted free cholesterol (Figure 2C). Accordingly, reduced bile acid production likely contributed to lower lipid absorption from the gut in the SI group compared with that in the control group, despite similar gut permeability (Figure 2D; Figure S1E). Furthermore, reduced activity with a comparable concentration of LPL (lipoprotein lipase) accompanied SI-induced *Angptl4* upregulation and *Angptl8* downregulation (Figure 2E; Figure S1F and S1G), which likely contributed to elevated serum chylomicron and triglyceride levels (Figure 1F and 1G). Although feeding can affect ANGPTL (angiopoietin-like protein) expression,[22] overnight fasting had minimal impact on *Angptl4* and *Angptl8* gene and protein expression patterns, serum cholesterol and triglyceride levels, and plasma LPL concentration and activity, indicating that SI effects on ANGPTL-associated lipid metabolism are independent of the feeding conditions (Figure S1H through S1L). *Srebf2* expression and genes governed by *Srebf2* such as *Hmgcr*, *Dhcr7*, and *Lss* were unaltered (Figure 2A; Figure S1C), suggesting that heightened de novo cholesterol synthesis is not a primary cause of SI-induced cholesterol accumulation in the liver.

Chronic Isolation Does Not Activate the Sympathetic Nervous System, Hypothalamus-Pituitary-Adrenal Axis, Glucose Metabolism, or Systemic Inflammation in *Apoe*$^{-/-}$ Mice

Though not consistent, sympathetic nervous activation, hypothalamic-pituitary-adrenal axis response, and immune-mediated systemic inflammation have been

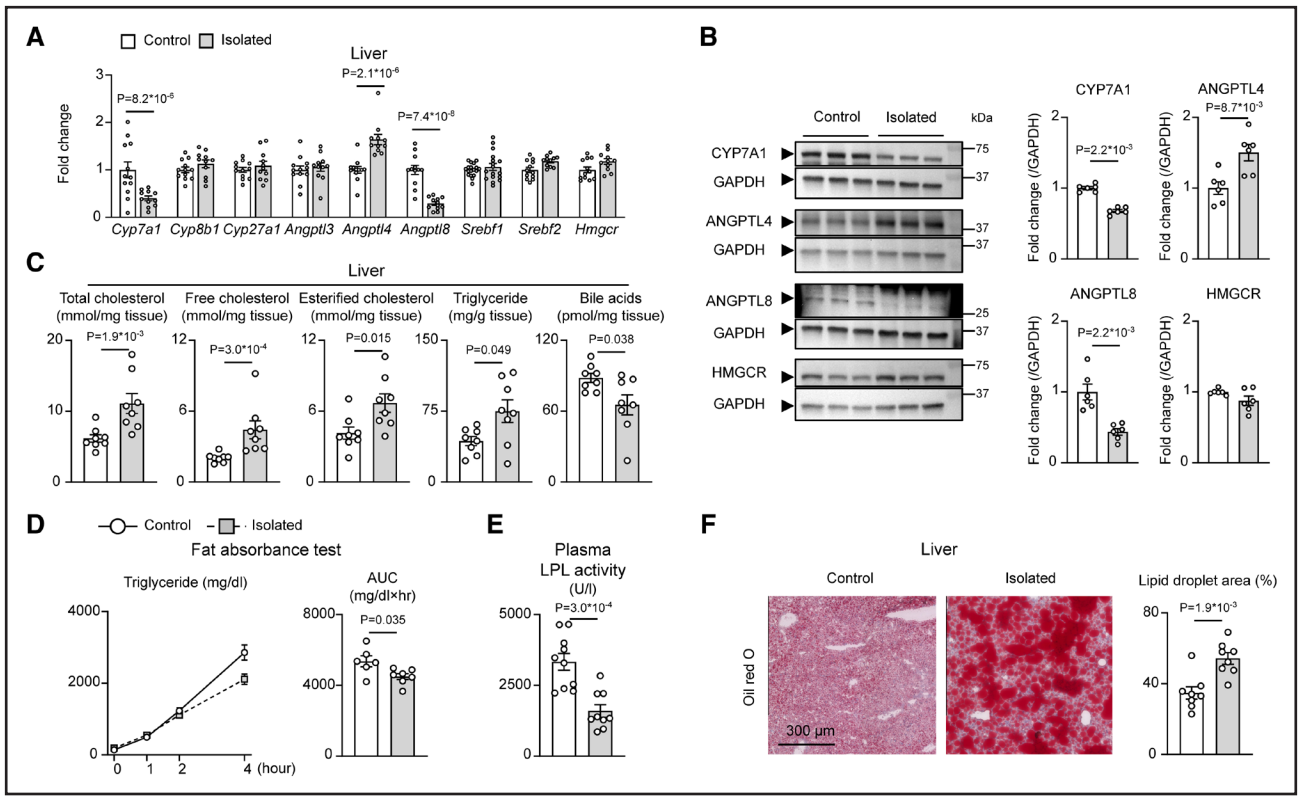

Figure 2. Chronic isolation disrupts hepatic lipid metabolism by decreasing bile acid production and plasma LPL (lipoprotein lipase) activity.

A, Transcription analysis of the liver in the postprandial state after 12 weeks of chronic isolation (n=12 control *Apoe*$^{-/-}$ mice; n=11 *Apoe*$^{-/-}$ isolated mice). **B**, Protein analysis of the liver in the postprandial state after 12 weeks of chronic isolation (n=6 control *Apoe*$^{-/-}$ mice; n=6 *Apoe*$^{-/-}$ isolated mice). **C**, Quantitative analysis of lipid components and bile acids in the liver after 12 weeks of chronic isolation (n=8 per group). **D**, Serum triglyceride concentrations on the fat absorbance test with Pluronic F-127 (n=6 control *Apoe*$^{-/-}$ mice; n=7 isolated *Apoe*$^{-/-}$ mice). **E**, Plasma LPL activity in the postprandial state after 12 weeks of chronic isolation (n=10 control *Apoe*$^{-/-}$ mice; n=9 isolated *Apoe*$^{-/-}$ mice). **F**, Representative cross-sections of livers stained with oil red O and quantification of lipid droplet areas after 12 weeks of chronic isolation (n=8 per group). Data are mean±SEM; multiple Student *t* tests with Benjamini-Hochberg tests were performed in **A**. Mann-Whitney *U* tests were performed in **B** through **F** for statistical analysis. ANGPTL4 indicates angiopoietin-like 4; ANGPTL8, angiopoietin-like 8; AUC, area under curve; CYP7A1, cholesterol 7 alpha-hydroxylase; and HMGCR, 3-hydroxy-3-methylglutaryl-coA reductase.

suggested as potent underlying mechanisms that promote metabolic dysregulation in SI-induced chronic stress.[13–17] Thus, we wondered whether SI-induced aberrant hepatic lipid metabolism similarly depended on the activation of such systemic factors. The blood pressure, pulse rate, urine catecholamine levels, adrenal gland weights, and adrenal gene expressions of *Th*, *Pnmt*, and *Dbh* (key enzymes responsible for catecholamine synthesis) were comparable between the groups (Figure S2A through S2G), indicating that chronic isolation does not drive systemic sympathetic activation. Moreover, tyrosine hydroxylase expression in the liver likewise showed no statistically significant difference between control and socially isolated *Apoe*$^{-/-}$ mice, which demonstrated the minimal contribution of local sympathetic activation to disrupted hepatic lipid metabolism in SI mice (Figure S2H). Nevertheless, SI mice exhibited slightly lower body temperatures, with increased expression of *Ucp1* and *Adrb3*, genes related to catecholamine-dependent heat production, in brown adipose tissue (Figure S2I and S2J), suggesting a local compensatory thermogenic response to SI-induced body temperature loss.[23] Regarding the hypothalamic-pituitary-adrenal axis, corticosterone levels in the blood and collected urine samples were similar between the groups (Figure S2K). Moreover, bone marrow hematopoiesis, hepatic immune cell numbers, expression of inflammatory genes in the liver and white adipose tissue, and plasma interleukin-6 concentrations remained unaltered (Figure S1C and S1D and S3A through S3H). Myeloid cell numbers and inflammatory gene expression were upregulated in the aorta but not in the periaortic fat of SI mice, suggesting that heightened inflammation is restricted within the atherosclerotic aorta after 12 weeks of chronic isolation in our model (Figure S3I through S3L). These data implied that both systemic and hepatic inflammation are unlikely to cause disrupted hepatic lipid homeostasis. Of note, SI neither impaired glucose tolerance nor compromised insulin sensitivity (Figure S2L through S2O), with comparable adipokine and GLP-1 (glucagon-like peptide-1) levels (Figures S1D and S2P). Collectively, these data indicate that SI lowered the hepatic bile acid production and impaired LPL-associated triglyceride-rich lipoprotein metabolism, thus contributing to the increased systemic atherogenic lipid contents, accelerated atherosclerosis, and hepatic steatosis (Figure 2F) independent of sympathetic nervous system activation, glucocorticoid response, or systemic inflammation.

Chronic Isolation Reduces Oxytocin Production in the Hypothalamus, and External Oxytocin Supplementation Improves Hepatic Lipid Metabolism and Retards Atherosclerosis in Chronically Isolated *Apoe*$^{-/-}$ Mice

Because the hypothalamus is a bioenergetic hub linking the central nervous system to systemic metabolism,[24] we sought to determine which hypothalamic hormones connect the state of social interaction to metabolic homeostasis. We performed gene expression analysis and surveyed the transcripts that encode metabolism-related proteins. We found that SI repressed the expression of *Oxt* that encodes oxytocin in the hypothalamus, which was supported by histological analysis demonstrating that hypothalamic oxytocin production was reduced in mice subjected to SI (Figure 3A and 3B; Figure S4A). Importantly, no statistically significant differences were observed in other genes potentially related to stress, behavior, appetite, and metabolism, including *Crh* (encoding corticotropin-releasing hormone) and *Pomc* (encoding pro-opiomelanocortin) in the hypothalamus (Figure S4A).

To test the long-term effects of oxytocin supplementation on SI-induced dyslipidemia and accelerated atherosclerosis, we administered oxytocin orally to mice via their drinking water (Figure 3C). Oral oxytocin supplementation did not affect the amount of food and water intake or body weight in *Apoe*$^{-/-}$ mice (Figure 3D; Figure S4B). Importantly, SI significantly decreased the urine and plasma oxytocin concentrations, which were restored to the control levels in response to oxytocin supplementation, while the urine vasopressin concentration remained unchanged (Figure 3E; Figure S4C). Likewise, oral oxytocin supplementation offset the elevated serum total cholesterol and triglyceride levels in SI mice (Figure 3F) and reverted the *Cyp7a1*, *Angptl4*, and *Angptl8* gene expression levels and their protein levels to those of the control group without statistically significant differences in other genes related to lipid and bile acid production (Figure 3G and 3H; Figure S4D). Accordingly, oxytocin-supplemented SI mice exhibited improved cholesterol contents and bile acid levels in their livers, feces, and blood (Figure 3I; Figure S4E and S4F), with recovered fat absorption efficacy and LPL activity and expression (Figure 3J and 3K; Figure S4D and S4G). Remarkably, oxytocin supplementation retarded atherosclerosis progression (Figure 4A and 4B), promoted atherosclerotic plaque stabilization (Figure 4C), improved aortic inflammation (Figure S4H), and alleviated hepatic steatosis even under SI (Figure 4D). Although the gut modulates fat absorption and excretion, and the gut-liver feedback loop of bile acids contributes to hepatic *Cyp7a1* suppression, expression of genes associated with such factors in the gut was unaltered following external oxytocin supplementation (Figure S4I). Importantly, although we detected oxytocin mRNA in the liver, the *Oxt* levels did not differ significantly regardless of chronic isolation or oxytocin supplementation (Figure S4D). Taken together, these data strongly suggest that the SI-induced accelerated atherosclerosis and disrupted lipid homeostasis were primarily due to decreased levels of brain-derived oxytocin, which facilitates bile acid

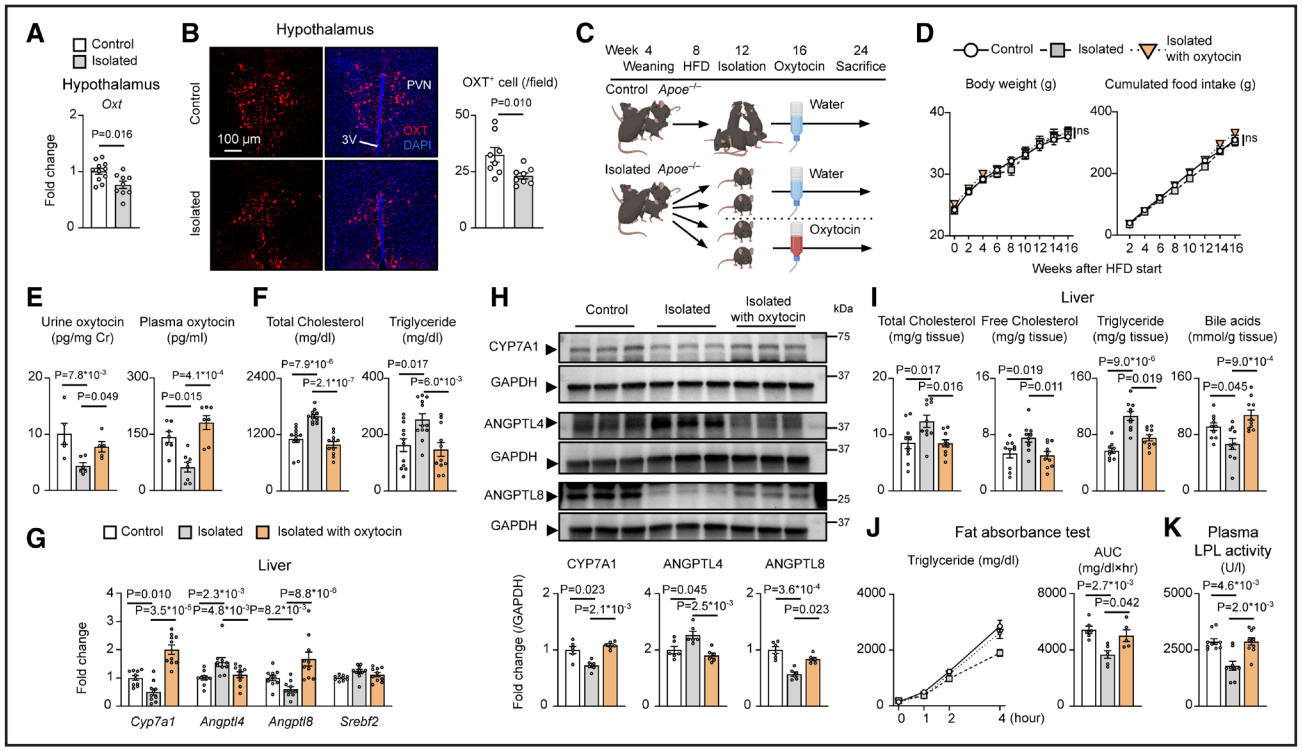

Figure 3. Chronic isolation reduces oxytocin production in the hypothalamus, and external oxytocin supplementation improves chronic isolation-induced hepatic lipid metabolism.

A, Oxytocin mRNA expression in the hypothalamus after 12 weeks of chronic isolation (n=11 control *Apoe*$^{-/-}$ mice; n=9 isolated *Apoe*$^{-/-}$ mice). **B**, Representative immunofluorescence images stained for oxytocin in the hypothalamus after 12 weeks of chronic isolation (n=8 per group). **C**, Experimental outline. Some of the isolated *Apoe*$^{-/-}$ mice were randomly assigned to receive oxytocin in their drinking water for 8 weeks. **D**, Body weights and cumulative food intake (n=23 control *Apoe*$^{-/-}$ mice; n=19 isolated *Apoe*$^{-/-}$ mice; and n=19 isolated *Apoe*$^{-/-}$ mice with oxytocin). **E**, Urine and plasma oxytocin concentrations after 8 weeks of oxytocin supplementation (n=7 control *Apoe*$^{-/-}$ mice; n=8 isolated *Apoe*$^{-/-}$ mice; and n=7 isolated *Apoe*$^{-/-}$ mice with oxytocin). **F**, Serum total cholesterol and triglyceride concentrations after 8 weeks of oxytocin supplementation (n=12 control *Apoe*$^{-/-}$ mice; n=12 isolated *Apoe*$^{-/-}$ mice; and n=11 isolated *Apoe*$^{-/-}$ mice with oxytocin). **G**, Transcription analysis in the liver after 8 weeks of oxytocin supplementation (n=10 per group). **H**, Protein analysis of the liver in the postprandial state after 8 weeks of oxytocin supplementation (n=6 per group). **I**, Quantitative analysis of lipid components and bile acids in the liver after 8 weeks of oxytocin supplementation (n=11 control *Apoe*$^{-/-}$ mice; n=11 isolated *Apoe*$^{-/-}$ mice; and n=10 isolated *Apoe*$^{-/-}$ mice with oxytocin). **J**, Serum triglyceride concentrations on the fat absorbance test with Pluronic F-127 (n=6 control *Apoe*$^{-/-}$ mice; n=6 isolated *Apoe*$^{-/-}$ mice; and n=5 isolated *Apoe*$^{-/-}$ mice with oxytocin). **K**, Plasma LPL (lipoprotein lipase) activity after 8 weeks of oxytocin supplementation (n=10 per group). Data are mean±SEM; Mann-Whitney *U* tests were performed in **A** and **B**; repeated measures ANOVA were performed in **D**; 1-way ANOVA with the Tukey post hoc tests were performed in **F**; and Kruskal-Wallis test with the Benjamini-Hochberg tests were performed in **E** and **G** through **K** for statistical analysis. 3V indicates third cerebroventricle; ANGPTL4, angiopoietin-like 4; ANGPTL8, angiopoietin-like 8; AUC, area under curve; CYP7A1, cholesterol 7 alpha-hydroxylase; DAPI, 4′,6-diamidino-2-phenylindole; HFD, high-fat diet; OXT, oxytocin; and PVN, paraventricular nucleus.

production by CYP7A1 upregulation and LPL activation through ANGPTL modulation in the liver.

OXTR, but Not V1a Receptor, Modulates Cyp7a1, Angptl4, and Angptl8 Expressions in the Hepatocytes

Next, we tested which cell and receptor types were responsible for the observed oxytocin-mediated phenotype in the liver. Oxytocin is known to signal via OXTR and V1aR (vasopressin receptor 1a). While V1aR is abundantly expressed on hepatocytes,[25] the cell types that express OXTR in the liver are largely unknown. Western blot analysis revealed that the livers in *Apoe*$^{-/-}$ mice consuming a standard chow diet exhibited OXTR proteins, whereas this OXTR expression was reduced in HFD-fed *Apoe*$^{-/-}$ mice irrespective of the presence or absence of chronic isolation or oxytocin treatment (Figure 5A; Figure S5A). Histological analysis demonstrated that OXTR was predominantly expressed on hepatocytes, particularly at the pericentral vein zone where most bile acid production and lipid metabolism occur[26] (Figure 5B). Moreover, *Oxtr*-Venus knock-in reporter mice showed that the degree of OXTR expression in cell types other than hepatocytes was negligible in the liver (Figure S6).

Having identified that OXTR is predominantly expressed in hepatocytes, we tested whether hepatocytes regulate *Cyp7a1*, *Angptl4*, and *Angptl8* expressions directly through oxytocin-OXTR signaling. We stimulated isolated hepatocytes with oxytocin in vitro and found that oxytocin, but not terlipressin, a V1aR-specific agonist, enhanced *Cyp7a1* and *Angptl8* and dampened *Angptl4*

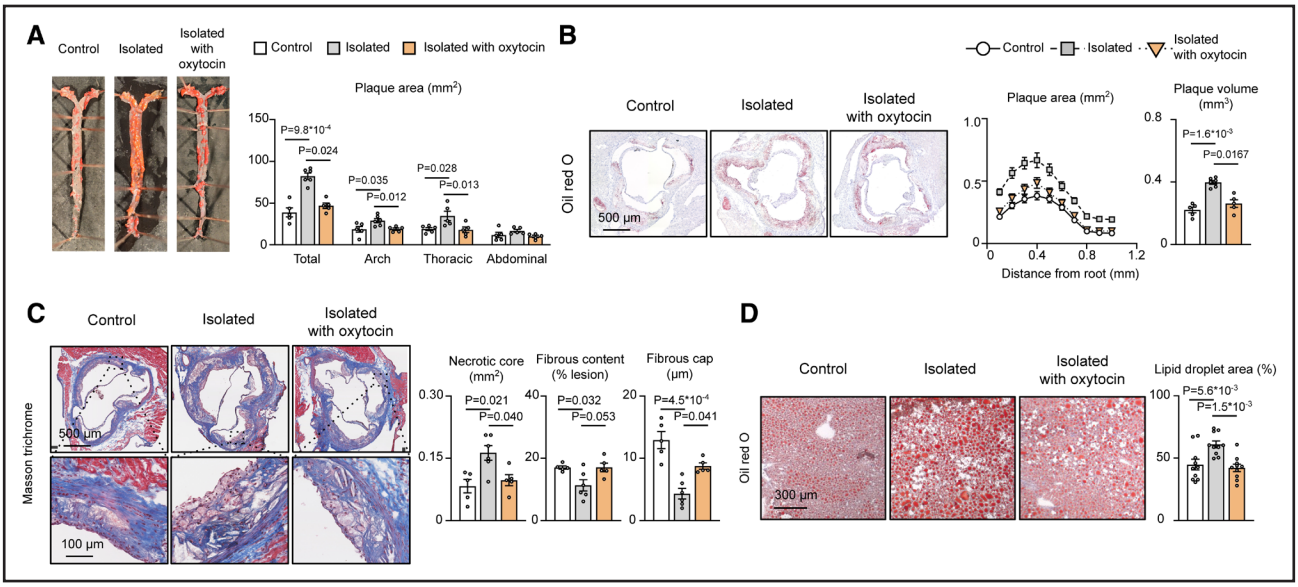

Figure 4. Oxytocin supplementation retards atherosclerosis and liver steatosis in chronically isolated mice.
A, Representative en face atherosclerotic aorta images with oil red O staining and quantification of plaque areas after 8 weeks of oxytocin supplementation (n=5 control *Apoe*$^{-/-}$ mice; n=6 isolated *Apoe*$^{-/-}$ mice; and n=5 isolated *Apoe*$^{-/-}$ mice with oxytocin). **B**, Representative cross-sections of aortic roots stained with oil red O and measurement of lesion volume after 8 weeks of oxytocin supplementation (n=5 control *Apoe*$^{-/-}$ mice; n=6 isolated *Apoe*$^{-/-}$ mice; and n=5 isolated *Apoe*$^{-/-}$ mice with oxytocin). **C**, Representative cross-sections of aortic roots stained with Masson trichrome and quantification of necrotic core areas, fibrous contents, and fibrous cap thickness after 8 weeks of oxytocin supplementation (n=5 control *Apoe*$^{-/-}$ mice; n=6 isolated *Apoe*$^{-/-}$ mice; and n=5 isolated *Apoe*$^{-/-}$ mice with oxytocin). **D**, Representative cross-sections of livers stained with oil red O and quantification of lipid droplet areas after 8 weeks of oxytocin supplementation (n=10 per group). Data are mean±SEM; the Kruskal-Wallis tests with Benjamini-Hochberg tests were performed for statistical analysis.

expression dose-dependently with negligible changes in other lipid metabolism-related genes (Figure 5C through 5E; Figure S7A and S7B). The addition of L368,899, an OXTR-selective antagonist, showed opposite effects, resulting in reduced *Cyp7a1* and *Angptl8* and augmented *Angptl4* expression, whereas SRX246, a V1aR-selective antagonist, did not affect those transcript levels (Figure 5F and 5G; Figure S7C and S7D). These key transcriptomic changes in vitro were validated by protein analyses (Figure S7E). Administering oxytocin or terlipressin to hepatocytes did not affect OXTR or V1aR expression (Figure S7A and S7B). By contrast, stimulation with palmitic acid or thapsigargin, the known inducers of endoplasmic reticulum stress, downregulated OXTR protein expression in hepatocytes, which were rescued with oleic acid supplementation (Figure S5B). These data indicated that brain-derived oxytocin acts remotely on hepatocytes specifically through OXTR to regulate *Cyp7a1*, *Angptl4*, and *Angptl8* and maintain lipid homeostasis in the liver and also suggested that HFD-induced endoplasmic reticulum stress could play a crucial role to regulate OXTR expression in hepatocytes under hyperlipidemic condition.

Hepatocyte-Specific Ablation of the OXTR Exacerbates Hepatic Lipid Metabolism and Atherosclerosis

To evidence the role of oxytocin in hepatic lipid metabolism and subsequent atherosclerosis progression in vivo, we injected recombinant AAV (adeno-associated virus)-DJ containing enhanced GFP (green fluorescent protein), albumin promoter, and short hairpin RNA, targeting mouse *Oxtr* into *Apoe*$^{-/-}$ mice to interfere with *Oxtr* gene expression on hepatocytes, followed by a HFD for 16 weeks (Figure S8A). GFP expression confined to the liver confirmed that the injected AAV was specifically incorporated into hepatocytes but not into other organs such as the hypothalamus and aorta (Figure S9A). AAV-treated *Apoe*$^{-/-}$ mice displayed reduced protein and gene levels of OXTR in the liver and elevated serum total cholesterol and triglyceride levels with negligible changes in the amount of food intake or body weight (Figures S8B and S8C and S9B and S9C). Consistent with observations in SI mice and our in vitro experiments, *Cyp7a1* and *Angptl8* expressions were downregulated, whereas *Angptl4* gene expression was upregulated, with no statistically significant differences in other genes related to lipid homeostasis (Figures S8D and S9C). Of note, protein levels of those key gene-derived products were consistent with their gene expression patterns (Figure S8E). Accordingly, disrupted lipid metabolism led to exaggerated atherosclerosis and liver steatosis resulting from hepatocyte OXTR knockdown (Figure S8F through S8K), which was consistent with our observations in SI mice.

Moreover, we further validated the key features of oxytocin's novel role via an additional in vivo model, in which we generated hepatocyte-specific OXTR-null *Apoe*$^{-/-}$ (*Oxtr*-cKO) mice by crossing *Apoe*$^{-/-}$ *Alb*-Cre

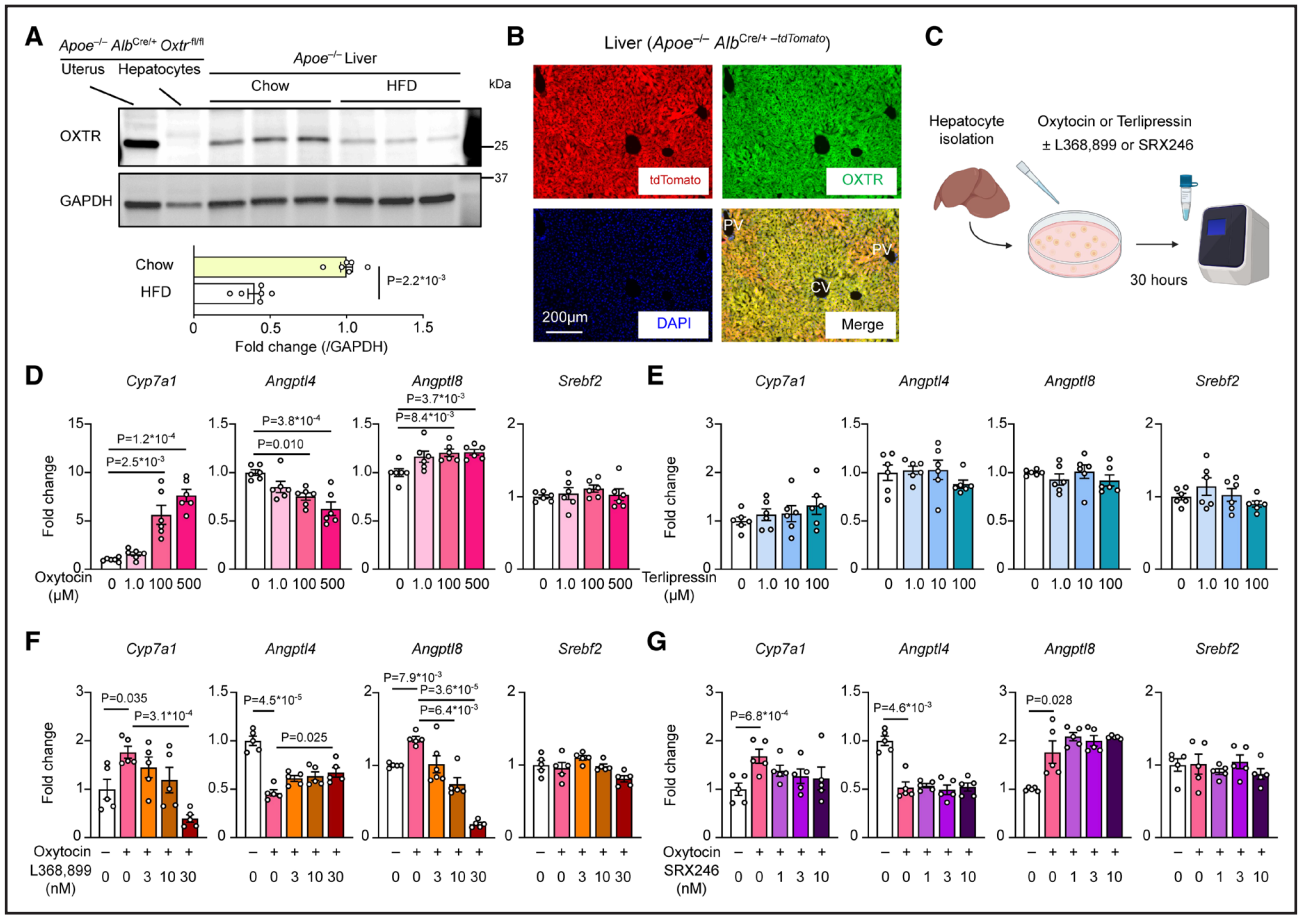

Figure 5. OXTR (oxytocin receptor), but not the V1a receptor, modulates *Cyp7a1*, *Angptl4*, and *Angptl8* expression in the hepatocytes.

A, OXTR protein expression in the liver. **B**, Representative immunofluorescence images of the liver from *Apoe*$^{-/-}$ *Alb*$^{Cre/+}$ tdTomato mice, stained for OXTR (green), tdTomato (red), and 4′,6-diamidino-2-phenylindole (DAPI; blue). **C**, Scheme for hepatocyte culture experiment. **D** through **G**, Transcription analysis of isolated hepatocytes cultured with (**D**) oxytocin (n=6 per group), (**E**) terlipressin (n=6 per group), (**F**) oxytocin and L368,899 (n=5 per group), and (**G**) oxytocin and SRX246 (n=5 per group). Data are mean±SEM; the Mann-Whitney *U* test was performed in **A**, and the Kruskal-Wallis tests with Benjamini-Hochberg tests were performed in **D** through **G** for statistical analysis. CV indicates central vein; HFD, high-fat diet; and PV, portal vein.

with *Apoe*$^{-/-}$ *Oxtr*-floxed mice, followed by HFD feeding for 16 weeks (Figure 6A). The *Oxtr*-cKO mice lost OXTR expression in hepatocytes (Figure 5A; Figure S9B) and recapitulated the phenotype of *Apoe*$^{-/-}$ mice subjected to AAV-mediated *Oxtr* knockdown in hepatocytes (Figure 6B through 6K; Figure S9D). Importantly, oral administration of oxytocin did not improve hepatic lipid metabolism and atherosclerosis development, as well as local inflammation in *Oxtr*-cKO mice fed an HFD, which likely excludes the extrahepatic effect of oxytocin (Figure 7). Collectively, these in vivo models clearly showed that oxytocin governs hepatic lipid homeostasis specifically via OXTR-expressing hepatocytes, thereby modulating atherosclerosis.

Oxytocin Regulates Hepatocyte *Cyp7a1*, *Angptl4*, and *Angptl8* Expression Through p38-FOXO1 Pathway

Finally, we tried to identify key signaling pathways downstream of OXTR, which simultaneously regulates *Cyp7a1*, *Angptl4*, and *Angptl8* expressions in hepatocytes. Previous studies reported that several molecules in the downstream signaling pathway under OXTR include p38 (p38 mitogen-activated protein kinases), ERK (extracellular signal-regulated kinase), AKT (protein kinase B), and PKC (protein kinase C).[27] Stimulation with oxytocin enhanced phosphorylation of p38 in hepatocytes with OXTR but not in the cells without OXTR (Figure S10A). We did not observe significant differences in phosphorylation of ERK1/2, AKT, or PKC in the presence or absence of OXTR in hepatocytes. Interestingly, there was a slight enhancement of ERK1/2 phosphorylation by oxytocin, which was diminished by SRX246, suggesting the importance of ERK1/2-related pathway under V1aR (Figure S10A). Previous reports demonstrated that p38 enhances FOXO1 (forkhead box protein O1) retention in the nucleus, which subsequently upregulates *Cyp7a1* and *Angptl8* and downregulates *Angptl4* expression in hepatocytes.[28,29] Consistently, our hepatocyte culture experiments demonstrated that oxytocin promoted nuclear localization of

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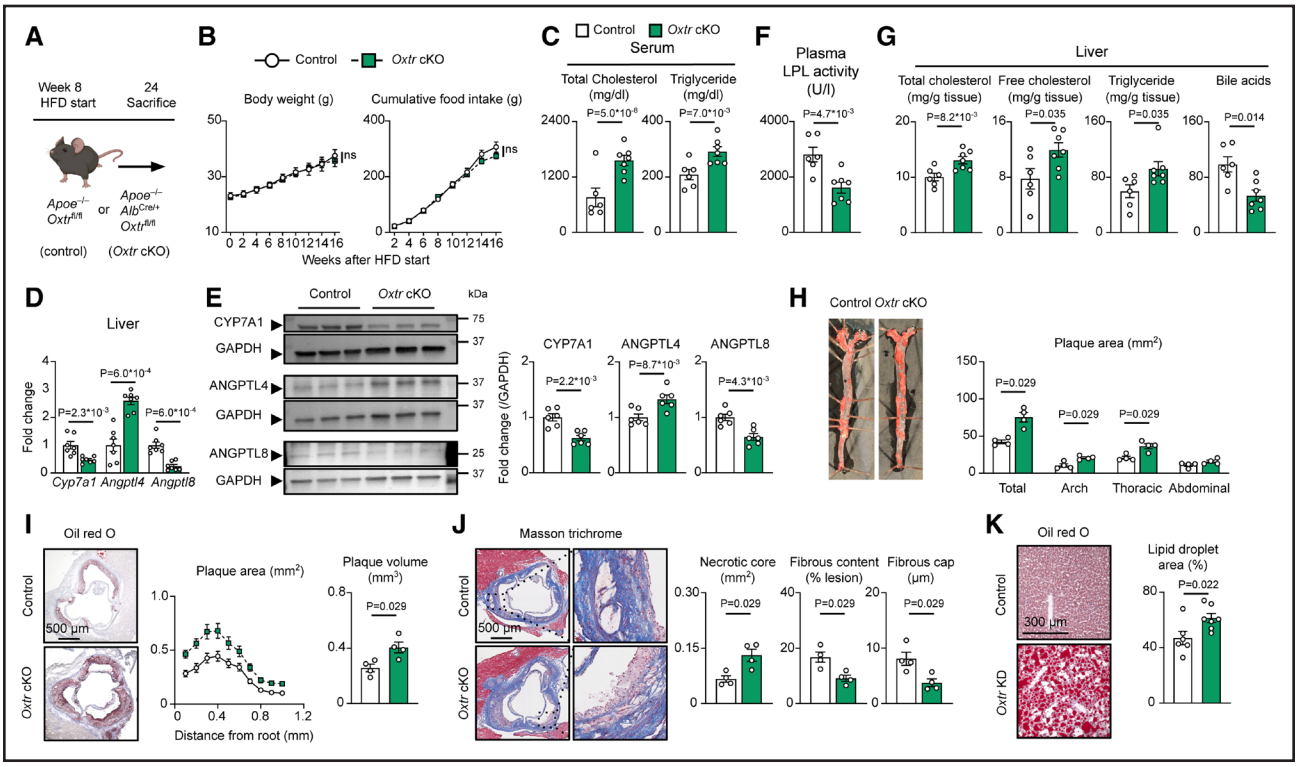

Figure 6. Hepatocyte-specific ablation of the oxytocin receptor exacerbates hepatic lipid metabolism and atherosclerosis.
A, Scheme of hepatocyte-specific conditional *Oxtr* knockout (cKO) model. *Apoe*$^{-/-}$ *Oxtr*$^{fl/fl}$ mice were defined as control, whereas *Apoe*$^{-/-}$ *Alb*$^{Cre/+}$ *Oxtr*$^{fl/fl}$ mice were defined as cKO mice. **B**, Body weight (n=6 control mice; n=7 cKO mice) and cumulative food intake (n=3 control mice; n=7 cKO mice). **C**, Serum total cholesterol and triglyceride concentrations (n=6 control mice; n=7 cKO mice). **D**, *Cyp7a1* (cholesterol 7 alpha-hydroxylase), *Angptl4* (angiopoietin-like 4), and *Angptl8* (angiopoietin-like 8) expressions in the liver (n=6 control mice; n=7 cKO mice). **E**, Protein analysis of the liver for CYP7A1, ANGPTL4, and ANGPTL8 (n=6 per group). **F**, Plasma LPL (lipoprotein lipase) activity (n=6 control mice; n=7 cKO mice) in the postprandial state. **G**, Quantitative analysis of lipid components and bile acids in the liver (n=6 control mice; n=7 cKO mice). **H**, Representative en face atherosclerotic aorta images with oil red O staining and quantification of plaque areas (n=4 per group). **I**, Representative cross-sections of aortic roots stained with oil red O and measurement of lesion volume (n=4 per group). **J**, Representative cross-sections of aortic roots stained with Masson trichrome and quantification of necrotic core areas, fibrous contents, and fibrous cap thickness (n=4 per group). **K**, Representative cross-sections of livers stained with oil red O and quantification of lipid droplet areas (n=6 control mice; n=7 cKO mice). Data are mean±SEM; repeated measures ANOVA were performed in **B**, and the Mann-Whitney *U* tests were used for statistical analysis in **C** through **K**.

FOXO1 in the presence of OXTR but not in the absence of the receptor (Figure S10B). Moreover, loss of OXTR resulted in the reduction of FOXO1 protein expression in hepatocytes (Figure S10B). Both SB202190, a p38 inhibitor, and AS1842856, an FOXO1 inhibitor, suppressed oxytocin-induced CYP7A1 and ANGTPL8 expressions and enhanced ANGPTL4 expression in the presence of oxytocin (Figure S10C). These data indicated that p38-FOXO1 is, at least in part, a key signaling pathway downstream of OXTR that regulates CYP7A1, ANGPTL4, and ANGPTL8 expressions in hepatocytes.

DISCUSSION

In this study, we identified a brain-liver axis, essential to maintaining systemic lipid homeostasis and modulating atherosclerosis progression, which depends on the hypothalamic neuropeptide oxytocin. We show that brain-derived oxytocin upregulates *Cyp7a1* to control bile acid production and modulates *Angptl4/8* to regulate LPL activity through peripheral OXTR in hepatocytes. We also demonstrate that oral oxytocin supplementation improves SI-induced atherosclerosis development by restoring dyslipidemia.

Oxytocin, which is predominantly produced in the hypothalamus and released into the systemic bloodstream through the posterior pituitary gland, is integral for mammalian reproduction and is also involved in a range of psychological and physiological processes, including social recognition, eating behavior, adipose tissue lipolysis, and glucose metabolism.[30–33] Our observation demonstrating that chronic SI reduced oxytocin production in the hypothalamus is consistent with previous reports, showing that physical contact increased oxytocin production in rodents,[34] whereas, in humans, solitary individuals produced less oxytocin than controls.[35] Although a recent study showed that the oxytocin-signaling pathway gene set in humans is correlated with SI-related loneliness and dyslipidemia,[36] no studies have mechanistically linked the neuropeptide to lipid metabolism in the development of CVD, and importantly, the underlying mechanisms of how oxytocin regulates cholesterol and triglyceride metabolism were unknown.

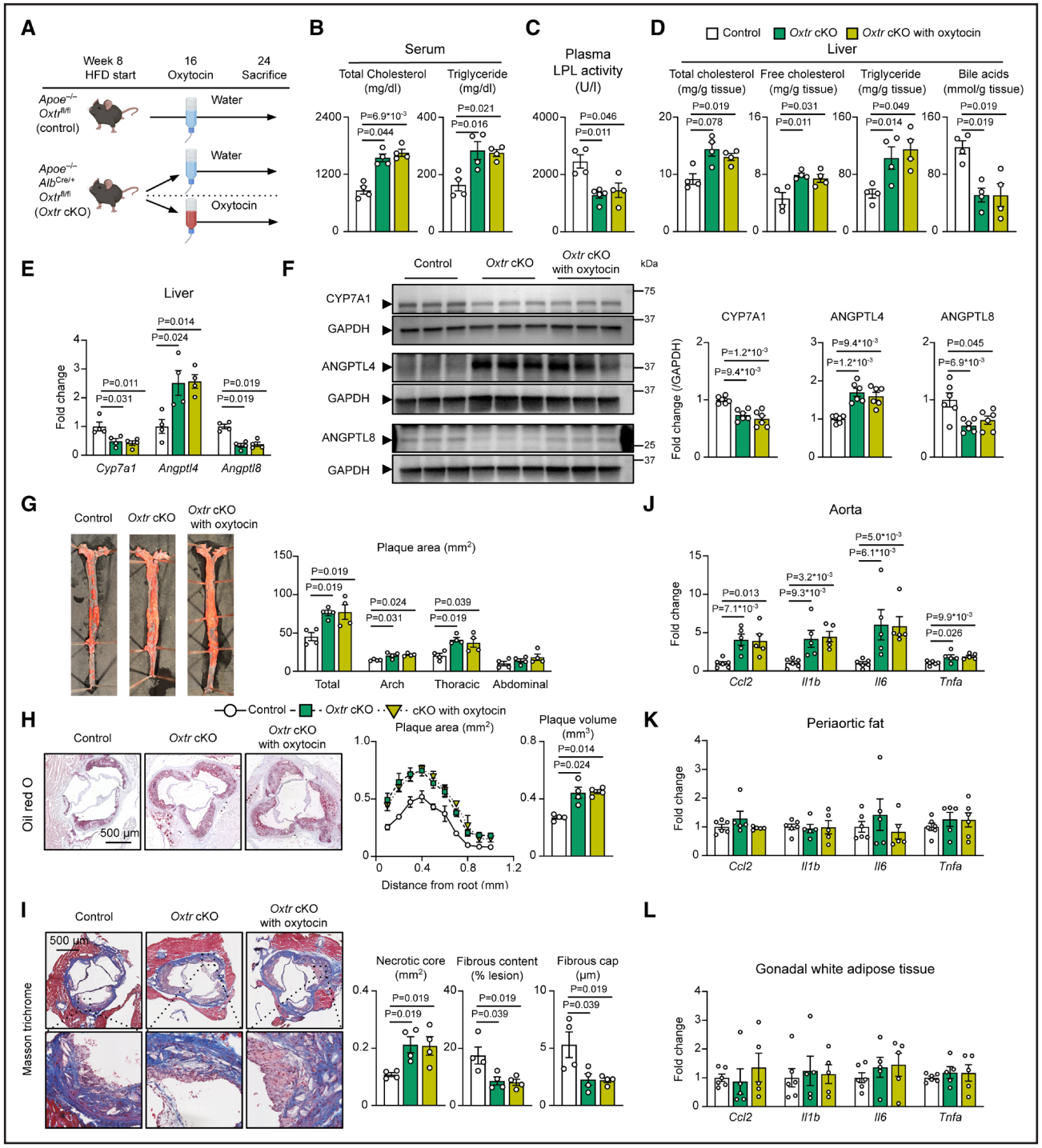

Figure 7. External oxytocin supplementation does not improve hepatic lipid metabolism and atherosclerosis development in hepatocyte-specific conditional *Oxtr* knockout (cKO) mice.

A, Experimental outline. Some cKO mice were randomly assigned to receive oxytocin in their drinking water for 8 weeks. **B**, Serum total cholesterol and triglyceride concentrations (n=4 control mice; n=4 cKO mice; and n=4 cKO mice with oxytocin). **C**, Plasma LPL (lipoprotein lipase) activity (n=4 per group) in the postprandial state. **D**, Quantitative analysis of lipid components and bile acids in the liver (n=4 per group). **E**, *Cyp7a1* (cholesterol 7 alpha-hydroxylase), *Angptl4* (angiopoietin-like 4), and *Angptl8* (angiopoietin-like 8) expressions in the liver (n=4 per group). **F**, Protein analysis of the liver for CYP7A1, ANGPTL4, and ANGPTL8 (n=6 per group). **G**, Representative en face atherosclerotic aorta images with oil red O staining and quantification of plaque areas (n=4 per group). **H**, Representative cross-sections of aortic roots stained with oil red O and measurement of lesion volume (n=4 per group). **I**, Representative cross-sections of aortic roots stained with Masson trichrome and quantification of necrotic core areas, fibrous contents, and fibrous cap thickness (n=4 per group). **J** through **L**, Gene expression of inflammatory mediators in (**J**) aorta (n=6 control mice; n=5 cKO mice; and n=5 cKO mice with oxytocin), (**K**) periaortic fat (n=6 control mice; n=5 cKO mice; and n=5 cKO mice with oxytocin), and (**L**) gonadal adipose tissue (n=6 control mice; n=5 cKO mice; and n=5 cKO mice with oxytocin). Data are mean±SEM; the Kruskal-Wallis tests with Benjamini-Hochberg tests were used for statistical analysis.

Our findings indicated that to maintain hepatic lipid homeostasis, oxytocin calibrates at least 2 pathways in the hepatocytes: CYP7A1-related bile acid production and ANGPTL4/8-related LPL modulation. *Cyp7a1* encodes a primary rate-limiting enzyme that converts free cholesterol to bile acids. In mice, *Cyp7a1* overexpression inhibits atherosclerosis progression through increased bile acids and decreased atherogenic lipids,[37] whereas, in humans, *CYP7A1* deficiency induces hyperlipidemia, enhanced hepatic cholesterol content, and loss of bile acid excretion, thereby accelerating atherosclerosis.[38] Furthermore, ANGPTL4 functions as a dimer to suppress LPL, thereby increasing triglyceride levels, which is counteracted by ANGPTL8.[22] In humans, inactivating *ANGPTL4* mutations reduces circulating triglyceride levels and confers a decreased risk of CVD. Likewise, inhibiting ANGPTL4 in animals reduces serum triglyceride levels.[39,40] Hepatocyte-specific suppression of ANGPTL4 abates triglyceride and triglyceride-rich cholesterol levels and alleviates atherosclerosis.[41] Consistent with these studies, our data demonstrated that SI-induced dyslipidemia was accompanied by decreased bile acid content, increased hepatic free and esterified cholesterol levels, and diminished LPL activity, all of which were the result of reduced production of oxytocin in the hypothalamus. Eventually, SI mice exhibited elevated serum levels of LDL, VLDL cholesterol, and triglyceride, leading to atherosclerosis progression.

Clinically, high circulating levels of LDL cholesterol are one of the established modifiable risk factors for CVD.[42,43] Recent pharmacological advances with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors combined with cholesterol transporter inhibitors and the anti-proprotein convertase subtilisin/kexin type 9 monoclonal antibodies have conferred effective LDL cholesterol–lowering strategies.[44] However, many studies have shown that LDL cholesterol goals are insufficiently achieved in a large proportion of patients.[45–47] Moreover, although numerous human observational and genetic studies have documented that elevated triglyceride-rich remnants or VLDLs are associated with increased risks of atherosclerotic CVD, the exact mechanisms and beneficial treatment approaches for this causal relationship are unknown.[48–51] To our knowledge, our study is the first to identify oxytocin as an effective therapeutics that simultaneously calibrates both the hepatic ANGPTL4/8- and CYP7A1-related metabolic pathways.

We also discovered that the hepatocytes functionally express OXTR. While oxytocin has a high affinity for the V1aR, which has been implicated in numerous oxytocin-dependent social behaviors,[52,53] our data indicated that oxytocin-mediated hepatic lipid modulation specifically relies on OXTR, not V1aR. Interestingly, hepatic OXTR expression was attenuated in response to HFD consumption, which could be a key mechanism to resist oxytocin-mediated protection against dyslipidemia not only under SI stress but also in the socially enriched environment. In this study, we suggest that enhanced endoplasmic reticulum stress triggered by HFD, often seen in nonalcoholic fatty liver disease,[54] could be one of the potential drivers for OXTR downregulation although further investigations will be required to elucidate how hepatocyte OXTR was downregulated under hyperlipidemic condition.

There are some discrepancies between previous studies and the present study. For example, on the one hand, one group previously published 2 papers with different animal species, demonstrating that continuous oxytocin administration by osmotic minipump improved atherosclerotic plaque development in the thoracic aorta but not in the aortic arch, but, on the other hand, oral oxytocin supplementation in our model improved not only plaque volume but also plaque stability throughout the aorta.[55,56] In addition, although 1 of the 2 papers shows via ex vivo approach that oxytocin attenuated adipose tissue inflammation, our study suggested that enhanced inflammation was not the main mechanism by which chronic SI promotes atherogenesis. Potential explanations for these discrepancies could include different routes for oxytocin administration, higher lipid concentrations in our study, different housing, husbandry, and environmental conditions for the animals, and different experimental approaches to elucidate the mechanisms. Nevertheless, further investigation will be needed to validate and reconcile the studies.

In conclusion, our study indicates that well-organized social connection maintains appropriate hypothalamic release of oxytocin, which modulates CYP7A1-mediated bile acid production and ANGPTL4/8-dependent LPL-associated lipid metabolism in an OXTR-specific manner in the hepatocytes, thereby attenuating dyslipidemia and atherosclerosis. This newly discovered brain-liver axis directly links sociality to metabolic function and CVD and provides a potential therapeutic avenue targeting the composite lipid metabolic pathways for loneliness-associated diseases (Figure S11).

ARTICLE INFORMATION

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Author Contributions

S. Ko conceived the project, designed and performed experiments, analyzed and interpreted data, made the figures, and drafted the article. A. Anzai conceived the project, designed experiments, interpreted data, and wrote the article. X. Liu, K. Kinouchi, K. Yamanoi, T. Torimitsu, G. Ichihara, H. Kitakata, K. Shirakawa, Y. Katsumata, and J. Endo performed experiments and analyzed and interpreted data. K. Hayashi, M. Yoshida, K. Nishimori, K.F. Tanaka, T. Onaka, M. Sano, and M. Ieda provided intellectual input and edited the article.

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Disclosures

None.

Supplemental Material

Expanded Materials & Methods
Table
Figures S1–S11
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