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Chronic infusion of tauroolithocholate into the brain increases fat oxidation in mice

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Abstract

Bile acids can function in the postprandial state as circulating signaling molecules in the regulation of glucose and lipid metabolism via the transmembrane receptor TGR5 and nuclear receptor FXR. Both receptors are present in the central nervous system, but their function in the brain is unclear. Therefore, we investigated the effects of intracerebroventricular (i.c.v.) administration of tauroolithocholate (tLCA), a strong TGR5 agonist, and GW4064, a synthetic FXR agonist, on energy metabolism. We determined the effects of chronic i.c.v. infusion of tLCA, GW4064, or vehicle on energy expenditure, body weight and composition as well as tissue specific fatty acid uptake in mice equipped with osmotic minipumps. We found that i.c.v. administration of tLCA (final concentration in cerebrospinal fluid: 1 μ M) increased fat oxidation (tLCA group: 0.083 ± 0.006 vs control group: 0.036 ± 0.023 kcal/h, $F = 5.46$, $P = 0.04$) and decreased fat mass (after 9 days of tLCA infusion: 1.35 ± 0.13 vs controls: 1.96 ± 0.23 g, $P = 0.03$). These changes were associated with enhanced uptake of triglyceride-derived fatty acids by brown adipose tissue and with browning of subcutaneous white adipose tissue. I.c.v. administration of GW4064 (final concentration in cerebrospinal fluid: 10 μ M) did not affect energy metabolism, body composition nor bile acid levels, negating a role of FXR in the central nervous system in metabolic control. In conclusion, bile acids such as tLCA may exert metabolic effects on fat metabolism via the brain.

Key Words

- ▶ brain
- ▶ bile salts/bile acids
- ▶ adipose tissue
- ▶ energy metabolism

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Introduction

Bile acids (BAs) are intestinal emulgators, but also serve as circulating signaling molecules in various organs including liver, muscle and adipose tissue (Kuipers *et al.* 2014).

Modulation of metabolic transcriptional programs and signaling cascades by BAs are mediated by the Takeda G protein-coupled receptor 5 (TGR5) and

nuclear farnesoid X receptor (FXR), which are widely expressed throughout the body in rodents and humans (Parks *et al.* 1999, Makishima *et al.* 2002, Maruyama *et al.* 2002, Kawamata *et al.* 2003, Keitel *et al.* 2010). Activation of TGR5 in intestinal L cells and pancreas induces glucagon-like peptide (GLP) 1 and insulin secretion, respectively (Thomas *et al.* 2009, Kumar *et al.* 2012). In brown adipose tissue (BAT), TGR5 increases energy expenditure by promoting thyroid hormone signaling (Watanabe *et al.* 2006). One of the main functions of FXR is to provide negative feedback for *de novo* BA synthesis in the liver (Lefebvre *et al.* 2009, Kuipers *et al.* 2014), but FXR has also been implicated in the regulation of metabolic processes outside of the enterohepatic circulation including pancreatic insulin secretion and adipocyte differentiation (Al-Shukaili *et al.* 2006, Rizzo *et al.* 2006, Abdelkarim *et al.* 2010). Hence, bile acids and their receptors may play an important role in the postprandial state facilitating nutrient trafficking and anabolic responses (Trauner *et al.* 2010, van Nierop *et al.* 2017).

Interestingly, BAs and these two receptors are also present in the central nervous system (Mano *et al.* 2004a, McMillin *et al.* 2016, Zheng *et al.* 2016). More specifically, TGR5 protein was detected in neurons and glia cells in rat and human brains using immunohistochemistry (Keitel *et al.* 2010) and FXR was present and active in mouse brain (Huang *et al.* 2016). The presence of these receptors and their agonists suggest a functional role for BAs in the brain. Considering the well-described effects of BAs in lipid and carbohydrate metabolism, we hypothesized that central effects of BA contribute to the regulation of energy metabolism.

Therefore, the aim of the present study was to determine the effects of central bile acid signaling on energy metabolism and/or BA biosynthesis in mice. We used the secondary bile acid tauroolithocholate (tLCA) to modulate bile acid receptors, in particular to target TGR5. TLCA is the strongest natural TGR5 agonist (Sato *et al.* 2008). We used the synthetic FXR agonist GW4064 to target the FXR receptor. We show that central tLCA infusion enhances fat oxidation and results in lower fat mass in mice.

Materials and methods

Animals

Male C57Bl/6J mice (Charles River) were housed under a 12 h/12 h light/darkness cycle in temperature (21–22°C) and humidity controlled rooms. Standard chow or high fat

diet (HFD, energetic content: 45% fat, 35% carbohydrates, 20% protein) (D12451, Research Diet Services, Wijk bij Duurstede, The Netherlands) and water were available *ad libitum* two weeks prior and during the study. The animal protocol was approved by the Institutional Ethics Committee for Animal Procedures, Leiden University Medical Center, The Netherlands. The following experiments were carried out: Mice (15 weeks old) received chronic intracerebroventricular (i.c.v.) administration of the natural bile acid and TGR5 agonist tLCA (sodium tauroolithocholate, Sigma Aldrich) or vehicle (control group). This experiment was repeated with mice on a HFD. In another experiment mice received i.c.v. administration of the synthetic FXR agonist GW4064 (Sigma Aldrich) or vehicle (control group). In an additional experiment, mice received the same dose of tLCA or vehicle intraperitoneally (i.p.) via an osmotic minipump to control for systemic effects of the compounds in the i.c.v. experiment. Final group sizes were $n = 5–7$ for experiments on chow diet and $n = 9–10$ for experiments on HFD.

Surgery and treatment

Mice were randomised based on body weight, anaesthetised using a mixture of dexmedetomidine (0.5 mg/kg), midazolam (5 mg/kg), and fentanyl (0.05 mg/kg), and stereotactically equipped with a cannula (Brain Infusion Kit 3, ALZET Cupertino) targeting the left lateral ventricle of the brain (–0.45 mm anteroposterior, –1.00 mm lateral and 2.50 mm dorsoventral of bregma). The cannula was secured on the skull with glue (Loctite 454, The Netherlands) and connected via a catheter to a subcutaneously placed osmotic minipump (Model 1004, ALZET). The catheter was filled with saline to delay drug delivery by ~5 days. To reach a final concentration in cerebrospinal fluid of 10 μ M GW4064 or 1 μ M tLCA, concentrations of the compounds in the minipump were calculated assuming a total cerebrospinal fluid volume of 35 μ L and a refresh rate of 18 μ L/h. Vehicles were 80% dimethyl sulfoxide (DMSO)/saline for GW4064 and 20% DMSO/saline solution for tLCA. To control for leakage into the periphery, mice received the same dose of tLCA intraperitoneally using osmotic minipumps that were placed into the abdominal cavity via a small incision in the abdominal wall. All mice received appropriate stitches. The sedation was antagonized with a mixture of antiparnezol (2.5 mg/kg), flumazenil (0.5 mg/kg), and naloxone (1.2 mg/kg) and mice received postoperative analgesics (0.03 mg/kg buprenorphine). During treatment the mice were single housed in metabolic

cages (LabMaster System, TSE Systems, Germany/for the HFD experiment: Promethion System, Sable Systems, USA). At day 9 of treatment for chow-fed mice or day 23 of treatment for HFD-fed mice, a triglyceride clearance test was performed (see below) and the mice were killed by cervical dislocation. Various organs and tissues were collected that were immediately snap frozen, fixated in 4% formalin or prepared for counting radioactivity. The brains were frozen on dry ice and subsequently sliced to control correct placement of the i.c.v. cannula.

Body composition and indirect calorimetry

Body composition was determined in conscious mice using an EchoMRI-100 (EchoMRI, Houston, TX, USA). The metabolic cages allowed measuring food intake, oxygen ($V\cdot O_2$) consumption, carbon dioxide ($V\cdot CO_2$) production and ambulatory physical activity (semi quantitative by beam breaks). Based on these values, we calculated the respiratory exchange ratio (RER, the ratio of carbon dioxide produced by the body to oxygen consumed by the body), energy expenditure, carbohydrate and fat oxidation rates for the last 4 days of treatment (Van Klinken *et al.* 2012).

Triglyceride clearance test

We prepared an emulsion of very low density lipoprotein (VLDL)-like triglyceride-rich lipoprotein emulsion particles from 100 mg of total lipid including glycerol trioleate (triolein; 70 mg), egg yolk phosphatidylcholine (22.7 mg), lysophosphatidylcholine (2.3 mg), cholesteryl oleate (3.0 mg) and cholesterol (2.0 mg) and added glycerol tri 3 H]oleate (3 H]TO) (100 μ Ci). The emulsion was sonicated and fractionated using successive density gradient ultracentrifugation steps (Rensen *et al.* 1997) and the fraction containing TRL-like particles with an average size of 80 nm was isolated. The isolated particles were stored at 4°C under argon and used within 5 days after preparation.

To study the uptake of triglyceride-derived fatty acids, mice were fasted for 4 h and blood was drawn via the tail vein to determine BA levels (for the complete protocol, [Supplementary methods](#), see section on [supplementary data](#) given at the end of this article). Subsequently, at $t = 0$, the mice received an iv injection of the prepared 3 H]TO-labeled VLDL-like emulsion particles (1 mg TG). After 15 min, mice were killed and perfused with ice-cold saline. We calculated the uptake of 3 H]TO-derived radioactivity by metabolic organs and expressed it as a percentage of injected dose per gram wet tissue weight.

Bile acid analysis

Plasma BA concentrations were determined using liquid chromatography–tandem mass spectrometry (sample preparation and analysis are detailed in [Supplementary methods](#)). The assay included 22 different types of BAs, but not all BA types were present in murine plasma including unconjugated and conjugated forms of lithocholic acid (LCA). Therefore, total BA concentration consisted of ursodeoxycholic acid (UDCA), cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), β -muricholic acid (β -MCA) and taurine (t) conjugated forms tCA, tDCA, α -MCA and t β -MCA. If one of these BA did not reach the lower limit of quantification (LOQ), the value was set to 50% (i.e. 0.025 μ M).

RNA isolation, cDNA synthesis and qPCR analysis

RNA was isolated and purified using TRI Reagent (Ambion) and a Total RNA isolation kit (Macherey Nagel) according to the manufacturers' protocols. The RNA yield was determined with a spectrophotometer (DenoVix) and cDNA was synthesized with equal RNA input using the AMV first-strand cDNA synthesis kit with oligo d(T) primers (Roche Molecular Biochemicals). We performed cDNA reactions without reverse transcriptase in order to check for genomic DNA contamination. Quantitative real-time PCR was done using the SensiFAST SYBR No-ROX kit (Bioline) and the Lightcycler 480 (Roche). Quantification was done with LinReg software. The expression value of a gene of interest was normalized to the geometric mean of three reference genes according to the Minimum Information for Publication of Quantitative Real-Time PCR Experiments guidelines. The expression of the reference genes did not significantly differ between groups. [Supplementary Table 1](#) shows a list of primers we tested.

Histological analysis of fat depots

Subcutaneous and gonadal white adipose tissue (sWAT and gWAT, respectively) and interscapular and subscapular brown adipose tissue (iBAT and sBAT, respectively) were formalin-fixed and paraffin embedded. Five micrometer sections were sliced and stained for hematoxylin eosin (HE), tyrosine hydroxylase (TH) (Ab112, Abcam) or uncoupling protein 1 (UCP1) (Ab10983, Abcam) as described previously (Kooijman *et al.* 2015b). Pictures were taken using an Axioskop (Zeiss) microscope with neofluar objectives (Zeiss), MicroPublisher 5.0 RTV

camera (Q imaging) and ImagePro Plus 6.3 software (Media Cybernetics). All images were inspected while blind for the experimental condition. We quantified WAT adipocyte size in HE-stained sections by running particle analysis in ImageJ. In BAT the percentage of area occupied with intracellular lipid vacuoles in HE-stained sections and percentage of area positive for TH staining or the relative intensity of UCP1 staining was determined by threshold analysis in ImageJ software.

Statistics

The main outcome parameters of this study were respiratory exchange ratio (RER), energy expenditure (EE) in kilocalories per hour (kcal/h), physical activity in number of beam breaks (bb), fat oxidation in kcal/h and carbohydrate oxidation in kcal/h from the metabolic cages, and the body weight and body composition in grams (g) at the end of treatment. Metabolic cage data were analysed using a repeated measures two-way ANOVA with independent variables *Time* and *Group*. Statistics are presented with *F* (Dfn, DFd) value and *P*. For other outcomes, the differences between treatment and control group were analysed using a two-tailed *t*-test. Statistics are presented with *t* (*df*) value and *P*. These data analyses were conducted using GraphPad Prism 6.0 statistical software. Mean day and night outcomes for the metabolic cages were analysed using SPSS Statistics 23 (IBM). All data are presented as mean $\pm$ standard error of the mean (S.E.M.). Differences with *P* < 0.05 were considered statistically significant, *P* < 0.1 were named as trend.

Results

Central tLCA infusion enhances fat oxidation and decreases fat mass

Central chronic infusion of tLCA in mice fed a chow diet decreased respiratory exchange ratio (RER) during the dark period ($t = 2.62$, $P = 0.03$; Fig. 1A) although total energy expenditure in the dark did not significantly change ($t = -1.89$, $P = 0.09$; Fig. 1B). This was reflected by increased overall fat oxidation rate compared to control mice ($F = 5.46$, $P = 0.04$) (Fig. 1C), whereas carbohydrate oxidation rate remained unaffected (Fig. 1D). This effect was more prominent during the dark phase ($t = -2.73$, $P = 0.02$) than during the light phase. I.c.v. tLCA did not affect locomotor activity (Fig. 1E), suggesting that tLCA increases resting energy expenditure.

In line with these data, we observed that tLCA decreased body fat mass in mice by 26% (1.35 ± 0.13 vs 1.96 ± 0.23 g, $P = 0.03$), although this was not reflected in changes in the total body weight (Fig. 2A and Supplementary Table 2). There was no difference between the groups in dietary intake. Central administration of tLCA did not alter total or individual plasma BA concentrations, including tLCA levels (Fig. 2B). tLCA could not be detected in plasma as was to be expected since concentrations of this secondary bile acid are already low in the enterohepatic circulation. In addition, intraperitoneal administration of tLCA did not affect energy expenditure (Supplementary Fig. 1), indicating that the stimulation of fat oxidation was not due leakage of tLCA into the circulation and subsequent peripheral TGR5 signaling.

Central GW4064 infusion does not affect energy expenditure

In contrast to i.c.v. administration of tLCA, central administration of the FXR agonist GW4064 did not affect RER, energy expenditure, substrate oxidation or physical activity (Fig. 3A, B, C, D and E and Supplementary Table 3). This observation was supported by the lack of differences in body weight and composition between GW4064 and vehicle treated mice (Fig. 4A and Supplementary Table 2). The treatment was well tolerated and there was no effect on dietary intake. In addition, GW4064 did not alter plasma total BA concentrations or BA profile (Fig. 4B), indicating the absence of leakage of GW4064 from the cerebrospinal fluid into the circulation or regulation of hepatic BA production through central pathways.

Central tLCA infusion induces browning in white adipose tissue

Since we observed that tLCA enhanced fatty acid oxidation and reduced total fat mass, we next investigated the effects of i.c.v. administration of tLCA on adipose tissue morphology, notably sWAT and gWAT. Consistent with lower fat mass in tLCA-treated animals, we observed smaller sWAT adipocytes in these mice compared to controls (i.c.v. tLCA: $416 \pm 74 \mu\text{m}^2$, control: $676 \pm 67 \mu\text{m}^2$; $P = 0.03$) (Fig. 5A). Remarkably, we identified regions of subcutaneous adipocytes containing multilocular lipid droplets in five out of seven mice treated with tLCA but not in controls, hinting towards browning of sWAT (Fig. 5B). Indeed, immunohistochemical staining

Figure 1

Calorimetrics during central tLCA administration. Mice received intracerebroventricular (i.c.v.) infusion of tauroolithocholic acid (tLCA, grey) or vehicle (control) for 8 days. (A, B, C, D and E) Calorimetric outcomes of the metabolic cages of the last 4 days (grey background: lights off/darkness period) and mean of the light and darkness period. (A) Respiratory exchange ratio (RER), the animals that received i.c.v. tLCA had significantly lower RER during the darkness period. (B) Energy expenditure (EE), during the darkness period EE tended to be increased in i.c.v. tLCA mice. (C) Fat oxidation, mice that received i.c.v. tLCA showed overall significantly increased fat oxidation rates compared to vehicle treated controls. This effect was most pronounced during the darkness period. (D) Carbohydrate oxidation. (E) Physical activity. Data are presented as mean $\pm$ s.e.m., * $P < 0.05$, # $P < 0.1$.

confirmed the presence of uncoupling protein 1 (UCP1) within these regions (Fig. 5C) and increased presence of tyrosine hydroxylase (TH), which is the rate-limiting enzyme in norepinephrine production (Fig. 5D). No effects of i.c.v. infusion of tLCA on the hypothalamic–pituitary–thyroid (HPT) axis were found as determined by *TSHb* mRNA expression in the pituitary (Bianco *et al.* 2014) (not shown). Together, these data suggest enhanced sympathetic signaling toward sWAT. TLCA treatment did not alter mRNA expression of thermogenic genes including *Ucp1*, *Pgc1a*, *Fas*, *Fxr* and *Hsl* in whole tissue gene expression analysis of sWAT (Fig. 5E). However, it must be noted that the mice that showed browning of

the sWAT had the highest *Ucp1* mRNA expression. Central administration of tLCA did not alter gWAT adipocyte size (Fig. 5A) or weight, nor the uptake of triglyceride-derived fatty acids by the WAT depots (Fig. 5F). Thus, central infusion of tLCA appears to induce browning of sWAT, resulting in smaller adipocyte cell size.

Brown adipose tissue analysis after i.c.v. tLCA administration

Browning of WAT often coincides with increased BAT activity. Therefore, we performed additional analyses for two main brown adipose tissue (sBAT

Figure 2

Body composition and plasma bile acids after chronic central tLCA administration. Mice received intracerebroventricular (i.c.v.) infusion of tauroolithocholic acid (tLCA, grey) or vehicle (control) for 8 days after which body composition and plasma bile acid profile were measured. (A) There were no differences between the groups in total body weight or lean mass. However, mice that received tLCA had lower fat mass than their controls. (B) The plasma total bile acid concentration and bile acid profile did not differ between mice that received i.c.v. tLCA and their vehicle treated controls. If a bile acid was not detected, its concentration was set to 50% of the lower limit of detection (dotted line), i.e. 0.025 μM. UDCA, ursodeoxycholic acid; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; MCA, muricholic acid; t-: taurine conjugated form. Data are presented as mean ± S.E.M., * $P < 0.05$.

and iBAT) depots. I.c.v. tLCA only tended to increase the uptake of triglyceride-derived fatty acids in sBAT (i.c.v. tLCA: $27.5 \pm 5.0\%$ dose/g, control: $12.9 \pm 4.1\%$ dose/g; $P = 0.06$), but in iBAT there was no difference between the groups (i.c.v. tLCA: $34.6 \pm 8.9\%$ dose/g, control: $15.9 \pm 5.1\%$ dose/g; $P = 0.12$), possibly related to lack of power (Fig. 6A). Histological analysis of both BAT depots revealed no significant differences between the groups in brown adipocyte lipid content (Fig. 6B). I.c.v. tLCA only tended to increase protein expression of tyrosine hydroxylase (TH) and UCP1 in iBAT, but not in sBAT (Fig. 6C and D). There were no differences between the i.c.v. tLCA group and control group in mRNA expression of genes involved in lipid oxidation and the previously reported BA-induced TGR5-D2-UCP1 pathway (Fig. 6E). Together these data seem to suggest that tLCA regulates energy expenditure through central bile acid signaling by increasing the sympathetic outflow towards the white and possibly brown fat depots, thereby enhancing the thermogenic capacity of the tissues, although it must be noted that no statistically significant effects were found in the brown adipose tissue.

White and brown adipose tissue analysis in HFD-fed mice after i.c.v. tLCA administration

Next, we investigated whether tLCA treatment can protect against the metabolic consequences of HFD feeding. Similar to lean mice on chow diet, i.c.v. administration of tLCA did not promote the uptake of triglyceride-derived fatty acids by the WAT depots (Fig. 7A). We also did not observe significant differences in sWAT mRNA expression of metabolically relevant markers between treatment groups, although again *Ucp1* tended to be increased (Fig. 7B). In addition, we observed that tLCA treatment enhanced uptake of triglyceride-derived fatty acids by BAT depots (Fig. 7C). These results were accompanied by a marked increase in mRNA expression of thermogenic genes including *Lpl*, *Tgr5*, *Dio2* and *Ucp1*, indicating increased BAT thermogenic activity (Fig. 7D).

tLCA treatment did not affect energy expenditure when mice were fed a HFD, resulting in unchanged body weight or gWAT weight (Supplementary Fig. 2). Taken together, these findings indicate that chronic central tLCA treatment is not able to overcome the metabolic consequences of HFD feeding, despite brown adipose tissue activation.

Figure 3

Calorimetrics during central FXR agonist administration. Mice received intracerebroventricular (i.c.v.) infusion of the synthetic FXR agonist GW4064 (dark grey) or vehicle (control) for 8 days. (A, B, C, D and E) Calorimetric outcomes of the metabolic cages of the last 4 days (grey background: lights off/darkness period) and means for the light and darkness period. (A, B, C, D and E) There were no differences between the i.c.v. GW4064 and control group in (A) respiratory exchange ratio, (B) energy expenditure (control group $n=4$), (C) fat oxidation, (D) carbohydrate oxidation or (E) physical activity. Data are presented as mean $\pm$ S.E.M.

Discussion

In this study, we showed that chronic central infusion of the bile acid tLCA in chow fed mice increases fat oxidation and reduces fat mass associated with increased browning of subcutaneous white adipose tissue (sWAT). These effects of i.c.v. administration of tLCA are not explained by leakage of tLCA into the systemic circulation, since infusion of the same dose of tLCA into the peritoneal cavity did not influence metabolic parameters. Central infusion of the synthetic FXR agonist, GW4064, did not influence any of the metabolic parameters, indicating that the effects of i.c.v. administration of tLCA are not explained by activation of FXR.

Chronic i.c.v. infusion of tLCA reduces adiposity

Chronic central infusion of tLCA increased fat oxidation and lowered fat mass, but did not affect body weight. Most likely a longer treatment period will be needed for these metabolic changes to translate in changes in body weight. The sWAT of mice that received i.c.v. tLCA administration, showed characteristics of beige/brite fat such as multilocular lipid droplets and UCP1 expression. The beige/brite adipocytes are an intermediate form of brown and white adipocytes, and possess the capacity to dissipate stored energy in the form of heat (Harms & Seale 2013, Rosenwald & Wolfrum 2014). Thereby, induction of browning can increase energy expenditure.

Figure 4

Body composition and plasma bile acids after chronic central FXR agonist administration. Mice received intracerebroventricular (i.c.v.) infusion of the synthetic FXR agonist GW4064 (dark grey) or vehicle (control) for 8 days after which body composition and plasma bile acid profile were measured. (A) There were no differences between the groups in total body weight, lean mass or fat mass. (B) The plasma total bile acid concentration and bile acid profile did not differ between mice that received i.c.v. GW4064 and their vehicle treated controls. If a bile acid was not detected, its concentration was set to 50% of the lower limit of detection (dotted line), i.e. 0.025 μM. UDCA, ursodeoxycholic acid; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; βMCA, β-muricholic acid; t-, taurine conjugated form. Data are presented as mean ± S.E.M.

Since browning in WAT is stimulated by increased sympathetic outflow as demonstrated upon cold exposure or β3-adrenergic receptor agonism (Berbee *et al.* 2015), we measured neural input to adipose tissue using TH staining. Indeed, we found increased TH expression at sites of browning in sWAT. Increased sympathetic innervation of sWAT is most often preceded by stimulation of BAT depots (Nedergaard & Cannon 2014). Despite some trends, we did not observe robust effects on BAT activity in the i.c.v. tLCA group, although this is likely due to a high biological variation. When we metabolically challenged the mice by a HFD, we observed increased activity of BAT after i.c.v. tLCA treatment. However, we could not detect characteristics of browning in sWAT of the i.c.v. tLCA group. Additionally, central tLCA administration did not promote energy expenditure. Taken together our data point to a minor role for central bile acid signaling in peripheral fat metabolism.

The mechanisms by which i.c.v. administered tLCA increased fat oxidation and lowered fat mass remain speculative. Direct peripheral effects of BAs on energy expenditure have been described in both mice (Watanabe *et al.* 2006) and humans (Broeders *et al.* 2015) and explain the interest for bile acids and their receptors in the field of obesity and T2DM (Trauner *et al.* 2010, Prawitt *et al.* 2011,

van Nierop *et al.* 2017). However, the involvement of the central nervous system in BA signaling has not been investigated before. The most important brain region that regulates and integrates metabolic signals is the hypothalamus. There is a relation between increased sympathetic outflow due to hypothalamic activation and browning of WAT and thermogenesis in BAT (reviewed in Kooijman *et al.* 2015a, Contreras *et al.* 2016). Browning of white adipose tissue occurs after selective intestinal FXR agonism via fexaramine in diet-induced obese, but not in TGR5^{-/-} mice (Fang *et al.* 2015). In that study, the BA pool shifted from a predominantly 'tβ-MCA and tCA' pool towards a 'tβ-MCA and LCA' pool pointing at a role for TGR5 in the browning of WAT and increased thermogenic capacity. Remarkably, the contribution of LCA to the BA pool increased ~4 fold whereas total BA levels decreased. Our results suggest that central LCA signaling may have contributed to these results.

Central FXR does not affect bile acid or energy metabolism

Central infusion of GW4064, a synthetic and specific agonist of the nuclear hormone bile acid receptor FXR, did not affect energy metabolism. The main function of FXR

Figure 5

Changes in white adipose tissue after central tLCA administration. Mice received intracerebroventricular (i.c.v.) infusion of tauroolithocholic acid (tLCA, grey) or vehicle (control, white) for 8 days. At the end of treatment, mice were injected intravenously with [3 H]TO-labeled VLDL-like emulsion particles and were killed for analysis of two white adipose tissue (WAT) depots: gonadal WAT (gWAT) and subcutaneous WAT (sWAT). (A) I.c.v. tLCA-treated mice had significantly smaller adipocytes in sWAT compared to their controls. (B) Hematoxylin eosin (HE) staining showing typical examples of smaller adipocytes in sWAT of an i.c.v. tLCA-treated mouse and not in gWAT or a control mouse. (C) Immunohistochemical staining for uncoupling protein 1 (UCP1) revealed sites of browning in sWAT in the i.c.v. tLCA group and not in the control group or gWAT. (D) Also tyrosine hydroxylase (TH) expression was increased at sites of browning in sWAT. (E) mRNA expression of metabolic relevant genes in sWAT did not show significant differences between i.c.v. tLCA and control mice. However, variation in *Ucp1* mRNA expression in the i.c.v. tLCA group was high and mice that showed browning of sWAT had highest *Ucp1* mRNA expression. (F) There was no difference between the groups in uptake of triglyceride-derived fatty acids by gWAT or sWAT. Data are presented as mean $\pm$ s.e.m., * $P < 0.05$, pictures show representative examples of their group. *Pcg1α*, peroxisome proliferator-activated receptor gamma coactivator 1- α ; *Fas*, fatty acid synthase; *Fxr*, farnesoid X receptor; *Hsl*, hormone-sensitive lipase.

in peripheral tissues is to regulate bile acid biosynthesis (Lefebvre *et al.* 2009). The present study did not detect a role for FXR in central metabolic control. Neither did we find any effects of chronic i.c.v. administration of a FXR agonist on plasma BA profiles or total BA concentrations. Some, but not all, studies have found FXR to be present in the brain (Huang *et al.* 2016, McMillin *et al.* 2016). Therefore, the question remains to be answered whether FXR has a functional role in the brain. We conclude that central FXR agonist administration does not influence metabolism or BA biosynthesis.

Bile acids, bile acid receptors and the brain

The role of central BAs and their receptor TGR5 is controversial. It is still unclear where BAs in the brain originate from and what their functions are (Quinn & DeMorrow 2012). Mano and colleagues (Mano *et al.* 2004a) found unconjugated forms of chenodeoxycholic

acid (CDCA), cholic acid (CA) and deoxycholic acid (DCA) in rat brains in a protein-bound form. Other types of BAs or unbound BAs were not detected, also not in healthy human CSF (Ogundare *et al.* 2010). However, many intermediates of the cholesterol-to-bile acid bioconversion have been detected in rodent brain (cells) (Zhang *et al.* 1997, Mano *et al.* 2004b) and human CSF (Ogundare *et al.* 2010, Saeed *et al.* 2014). Various enzymes are necessary for the multiple-step conversion of cholesterol into a type of bile acid. Some of these enzymes have also been detected in brain and *in vitro* studies demonstrated the synthesis of LCA and CDCA by brain cultures (Naqvi & Nicholas 1970, Mano *et al.* 2004b). Therefore, there are indications for the presence of metabolic pathways for central BA synthesis. Another possibility is that BAs cross the blood-brain barrier. An early study in guinea pigs demonstrated fast efflux of LCA after intracerebral injection, indicating that BAs cross the blood-brain barrier (Naqvi *et al.* 1970). However, more recent human studies have shown that

Figure 6

Changes in brown adipose tissue after central tLCA administration. Mice received intracerebroventricular (i.c.v.) infusion of taurochenodeoxycholic acid (tLCA, grey) or vehicle (control, white) for 8 days. At the end of treatment, mice were injected intravenously with [^3H]TO-labeled VLDL-like emulsion particles and were killed for further analysis of two brown adipose tissue (BAT) depots: subscapular BAT (sBAT) and interscapular BAT (iBAT). (A) i.c.v. tLCA-treated mice showed a trend for increased triglyceride-derived fatty acids uptake in sBAT ($P=0.06$). (B) There was no statistical difference between the groups in BAT lipid content. (C and D) Immunohistochemical staining showed a trend for increased protein expression in iBAT in i.c.v. tLCA-treated mice of TH ($P=0.06$) and UCP1 ($P=0.09$). (E) The mRNA expression of metabolic relevant genes in sBAT was not increased. Data are presented as mean $\pm$ S.E.M., * $P < 0.1$. TH, tyrosine hydroxylase; UCP1, uncoupling protein 1; *Lpl*, lipoprotein lipase; *Tgr5*, Takeda G protein-coupled receptor 5; *Dio2*, deiodinase 2; *Pgc1α*, peroxisome proliferator-activated receptor gamma coactivator 1-alpha.

Figure 7

Changes in white and brown adipose tissue of HFD-fed mice after central tLCA administration. Mice fed a high fat diet (HFD) received intracerebroventricular (i.c.v.) infusion of taurochenodeoxycholic acid (tLCA, grey) or vehicle (control, white) for 23 days. At the end of treatment, mice were injected intravenously with [^3H]TO-labeled VLDL-like emulsion particles and white adipose tissue (WAT) and brown adipose tissue (BAT) depots were collected for analysis. (A) Uptake of triglyceride-derived fatty acids by gonadal WAT (gWAT) and subcutaneous WAT (sWAT) did not differ between i.c.v. tLCA-treated mice and their controls. (B) Although mRNA expression of metabolically relevant markers in sWAT did not exhibit significant differences between groups, *Ucp1* expression showed a trend for increase in the i.c.v. tLCA group. (C) Uptake of triglyceride-derived fatty acids by interscapular BAT (iBAT) was slightly increased and significantly increased in subscapular BAT (sBAT) in i.c.v. tLCA-treated compared to their controls. (D) mRNA expression of metabolic relevant genes was increased in iBAT of i.c.v. tLCA-treated mice. Data are presented as mean $\pm$ S.E.M., * $P < 0.05$. *Pgc1α*, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; *Fas*, fatty acid synthase; *Fxr*, farnesoid X receptor; *Pparg*, peroxisome proliferator-activated receptor-gamma; *Ucp1*, uncoupling protein-1; *Lpl*, lipoprotein lipase; *Tgr5*, Takeda G protein-coupled receptor 5; *Dio2*, deiodinase 2.

BAs can in fact cross from the peripheral circulation into the brain (Parry *et al.* 2010). We did not detect any tLCA in plasma, therefore, we deem it unlikely that the low and slowly release dose of tLCA in our study crossed the blood brain barrier in the i.c.v. infused mice.

A limitation of our study was that we were not able to use either a very specific TGR5 agonist or a TGR5^{-/-} mouse model and this may have had two consequences. Firstly, we cannot exclude the possibility that tLCA did not stimulate FXR. However, central infusion of the FXR agonist, did not increase fat oxidation and lowers fat mass. Indeed, the affinity of tLCA for FXR is relatively low, whereas it is very high for TGR5. In a comparative activation assay Sato and coworkers showed that the EC₅₀ of tLCA is 20 µM for FXR and 0.3 µM for TGR5 (Sato *et al.* 2008). Furthermore, tLCA is a conjugated bile acid that has lower affinity for FXR compared to true FXR agonists such as CDCA (Parks *et al.* 1999). Conjugated bile acids such as tLCA need active transport across the cellular membrane to activate the nuclear FXR. Although bile acid receptors, such as the apical sodium–bile acid transporter are present in the brain, their abundance is much less compared to the ileum where active bile acid uptake occurs (Mertens *et al.* 2017). Finally, we are not aware of a deconjugation mechanism in the brain that may have facilitated passive uptake. All this suggests that the actions of tLCA may be mediated via TGR5 and not FXR. Secondly, tLCA may have activated other receptors than TGR5 in the brain. *In vitro* tLCA can interact with the muscarinic receptor M3 in Chinese hamster ovary cells, although effective concentrations were much higher than the dose we used (Raufman *et al.* 2002). Further investigations will be needed to dissect the function of central BAs and central TGR5, for example using a highly specific synthetic TGR5 agonist.

Conclusion

Central administration of the natural bile acid tLCA, but not a specific FXR agonist, alters peripheral fat metabolism. Therefore, central mechanisms may be involved in the metabolic effects of circulating bile acids.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/JOE-17-0503>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

The study was designed by H M E, P C N R and M R S. Data was acquired by H M E, L L T, S K, M K, R v d B, I M M and J v d H. Data analysis and interpretation were performed by H M E, L L T, S K, A K G, A B, A K, J A R, P C N R and M R S. All authors critically reviewed and have approved the final manuscript.

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