DIFFERENTIAL EFFECTS OF BILE ACIDS ON THE POST-PRANDIAL SECRETION OF GUT HORMONES: A RANDOMISED CROSSOVER STUDY

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Running head
Bile acids modify gut hormone secretion

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ABSTRACT

AIMS

Bile acids (BA) regulate post-prandial metabolism directly and indirectly by affecting the secretion of gut hormones like glucagon-like peptide-1 (GLP-1). The post-prandial effects of BA on the secretion of other metabolically active hormones are not well understood. The objective of this study was to investigate the effect of oral ursodeoxycholic acid (UDCA) and chenodeoxycholic acid (CDCA) on post-prandial secretion of GLP-1, oxyntomodulin (OXM), peptide YY (PYY), glucose-dependent insulinotropic peptide (GIP), glucagon and ghrelin.

METHODS

Twelve healthy volunteers underwent a mixed meal test 60 minutes after ingestion of UDCA (12-16 mg/kg), CDCA (13-16 mg/kg) or no BA in a randomised cross-over study. Glucose, insulin, GLP-1, OXM, PYY, GIP, glucagon, ghrelin and fibroblast growth factor 19 were measured prior to BA administration at -60, 0 (just prior to mixed meal) and 15, 30, 60, 120, 180 and 240 minutes after the meal.

RESULTS

UDCA and CDCA provoked differential gut hormone responses: UDCA did not have any significant effects, but CDCA provoked significant increases in GLP-1 and OXM and a profound reduction in GIP. CDCA increased fasting GLP-1 and OXM secretion in parallel with an increase in insulin. On the other hand, CDCA reduced post-prandial secretion of GIP, with an associated reduction in post-prandial insulin secretion.

CONCLUSIONS

Exogenous CDCA can exert multiple salutary effects on the secretion of gut hormones; if these effects are confirmed in obesity and type 2 diabetes, CDCA may be a potential therapy for these conditions.

KEY WORDS:

Bile acid; Glucagon-like Peptides; Gut Hormones; Neuroendocrine Cells

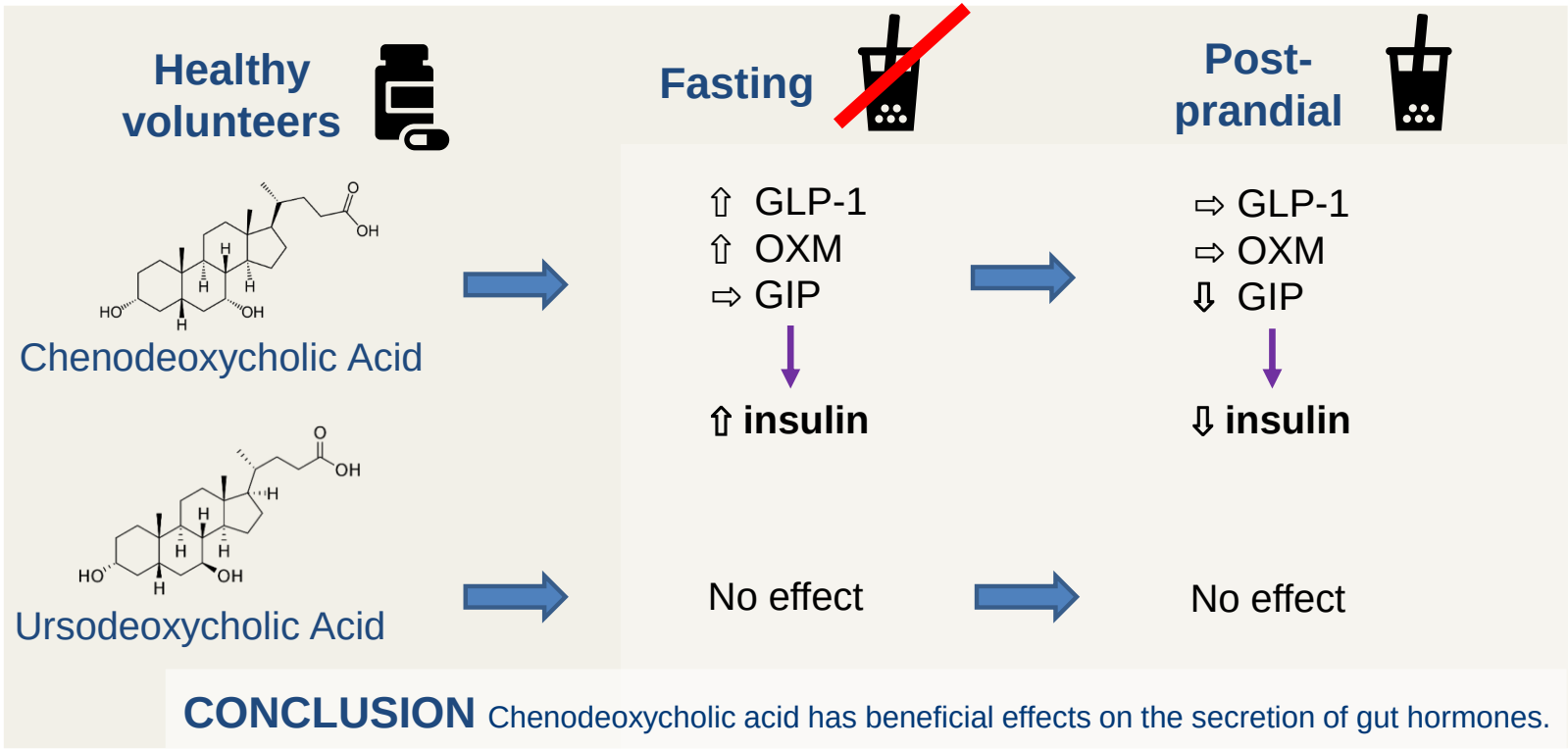
ABBREVIATIONS:

BA: bile acid; CDCA: chenodeoxycholic acid; FGF19: fibroblast growth factor 19; FXR: farnesoid X receptor; GIP: glucose-dependent insulinotropic peptide; GLP-1: glucagon-like peptide 1; OXM: oxyntomodulin; PYY: peptide tyrosine tyrosine; RYGB: Roux-en-Y gastric bypass; TGR5/GPBAR1: Takeda G-protein-receptor 5/G-protein coupled bile acid receptor 1; UDCA: ursodeoxycholic acid

NEW AND NOTEWORTHY

- Oral CDCA and UDCA have different effects on gut and pancreatic hormone secretion.
- A single dose of CDCA increased fasting secretion of the hormones GLP-1 and OXM with an accompanying increase in insulin secretion.
- CDCA also reduced post-prandial GIP secretion which was associated with reduced insulin.
- In contrast, UDCA did not change gut hormone secretion fasting and post-prandially.
- Oral CDCA could be beneficial to patients with obesity and diabetes.

Differential effects of bile acids on the post-prandial secretion of gut hormones: a randomised crossover study



CONCLUSION Chenodeoxycholic acid has beneficial effects on the secretion of gut hormones.

INTRODUCTION

Bile acids (BA) are thought to possess both gut hormone-mediated and gut hormone-independent actions that regulate appetite and assist with post-prandial nutrient metabolism by acting on the nuclear farnesoid X receptor (FXR) and the G-protein coupled cell membrane receptor Takeda G-protein-receptor 5/G-protein coupled bile acid receptor 1 (TGR5/GPBAR1) (18). Key gut hormones that regulate metabolism include glucagon-like peptide 1 (GLP-1), an incretin hormone that stimulates post-prandial insulin secretion from the pancreas, suppresses appetite and reduces body weight (3); oxyntomodulin (OXM), a dual agonist of both GLP-1 and glucagon receptors, which causes suppression of appetite and loss of body weight (12); peptide tyrosine tyrosine (PYY), a satiety hormone that acts through neuropeptide Y2 receptors to suppress appetite and reduce body weight (4); glucose-dependent insulinotropic peptide (GIP), a major incretin that may have greater effects on post-prandial insulinotropy than GLP-1 (21) and which promotes triglyceride uptake in adipocytes (3); and ghrelin, which stimulates appetite and suppresses insulin secretion (6).

In animal models, BA stimulate secretion of GLP-1 and PYY from enteroendocrine L-cells in the small bowel and the colon through basolateral TGR5/GPBAR1 (16). Through FXR, the BA chenodeoxycholic acid (CDCA) potently stimulates fibroblast growth factor 19 (FGF19) secretion from ileal enterocytes (36): this hormone also has regulatory effects on hepatic carbohydrate (23) and protein metabolism (14). In the liver, FXR activation decreases lipogenesis, increases fatty acid oxidation and decreases glycolysis (8, 32). Interestingly, FXR activation has been reported to decrease proglucagon expression and GLP-1 secretion from L-cells (28). Exogenous BA have been demonstrated to stimulate GLP-1 secretion when fasting (9, 19, 22, 35). Intraduodenally infused CDCA, however, did not lead to any significant difference in the oral glucose tolerance test (OGTT)-induced secretion of GLP-1, PYY and cholecystokinin (19). Very few studies have examined the actions of oral BA in the context of a meal stimulus. One small study investigating the effect of a single dose of oral ursodeoxycholic acid (UDCA)

showed a small increase in early post-prandial GLP-1 secretion in healthy volunteers (20); a randomised, open-label study of UDCA treatment over 12 weeks showed a similar short-lived increase in early post-prandial GLP-1 secretion accompanying improvements in HbA1c and body weight in people with type 2 diabetes and chronic liver disease (25). After treatment of people with obesity and type 2 diabetes with a mixture of BA over 28 days, there was a small increase in GLP-1 secretion in response to a meal stimulus (7).

Few of the abovementioned studies have comprehensively interrogated the effects of oral BA on gut hormones other than GLP-1. Although GLP-1 is of importance, the combination of GLP-1 with other gut hormones such as OXM and PYY has synergistic metabolic effects (5). Moreover, there are potentially diverse effects of BA on metabolism via the hormones GIP, glucagon, and ghrelin. Therefore, the objective of this study was to comprehensively investigate the effects of a single dose of oral BA on metabolically active gut and pancreatic hormone levels, measured using high sensitivity and high specificity assays, following a standardised meal in healthy volunteers. We compared the effects of two species of BA: UDCA and CDCA. These BA were chosen for their contrasting pharmacological properties on the major BA receptors. CDCA is a potent FXR agonist and also stimulates TGR5/GPBAR1, whereas UDCA has weak or absent activity at TGR5/GPBAR1 (16) and may act as a competitive antagonist at FXR (26), hence theoretically increasing GLP-1 secretion (28).

METHODS

STUDY DESIGN AND PARTICIPANTS

Healthy volunteers were recruited for this mechanistic physiological study, which took place at the UK National Institute for Health Research (NIHR) Imperial Clinical Research Unit Facility (CRF) at Hammersmith Hospital, London, between January and March 2018. The study was randomised, crossover and open label, with each participant attending for three visits spaced approximately one week apart. Participants were recruited from the CRF's healthy volunteer database. Included participants were normoglycaemic (HbA1c <39mmol/mol (5.7%) and fasting glucose <5.6 mmol/L); exclusion criteria included pregnancy and known sensitivity to BA treatment. The primary outcomes were GLP-1, OXM and PYY secretion following ingestion of the mixed meal as measured by the area-under-curve (AUC) for concentration vs time. Secondary outcomes included AUCs for glucose, insulin, ghrelin, GIP and FGF19.

MIXED MEAL TEST

During each visit, participants underwent a mixed meal test) 60 minutes following ingestion of no BA (negative control: Nil), UDCA or CDCA. BA were administered in the form of tablets (250 mg or 500 mg). Participants consumed 12-16 mg/kg of UDCA (Advanz Pharma) or 13-16 mg/kg of CDCA (Leadiant Pharmaceuticals) with water, one hour before the test meal. The timing of BA administration before mixed meal consumption was based on previous studies that suggested stimulation of GLP-1 secretion within 30-60 min (9, 20, 22). The doses in this study were based on established daily doses used in clinical practice for gallstone dissolution and given to the nearest multiple of 250 mg. The order of treatments was randomised using a computerised random number generator (www.random.org). All mixed meal tests were performed in the morning. Prior to commencement study investigators confirmed that subjects had fasted for at least 10 hours and avoided heavy exercise and alcohol the previous day. Bloods were sampled from an indwelling venous cannula at the following time points:

prior to ingestion of bile acid (-60 min), immediately prior to consumption of the meal (0 min), and then at intervals after commencement of the meal (15, 30, 60, 120, 180, 240 min). The standardised meal consisted of two vanilla flavour Ensure Plus milkshakes (440 ml, Abbott Nutrition), served in a glass, equivalent to 700 kilocalories (22 g fat, 26 g protein and 100 g carbohydrate). Participants were directed to consume the entire meal in no more than 10 minutes.

BLOOD SAMPLING AND ASSAYS

Blood samples for gut hormone analysis were collected in lithium heparin tubes containing 0.1 mL of aprotinin (1000 KIU/4 mL of blood, Nordic Pharma Ltd) and a dipeptidyl peptidase 4 (DPP-4) inhibitor Diprotin A (20 µg/mL blood, Enzo Life Sciences (UK) Ltd) and set on ice immediately after collection. After centrifugation at 4°C, plasma was separated and stored at -80°C until analysis. Total GLP-1 and glucagon were measured using validated, enzyme linked immunosorbent assays (ELISA; Mercodia 10-1278-01 and 10-1271-01), with lowest limits of quantification (LLOQ) of 0.65 pmol/L and 1.5 pmol/L respectively. Total GLP-1 was measured as this is regarded as the best measure of L-cell production, taking into account the local degradation of GLP-1 by dipeptidyl peptidase-IV in the capillaries draining the gut mucosa (15). The high-stringency 'Alternative' protocol was used for the glucagon assay to minimise cross-reactivity with other proglucagon derived peptides (1). Total PYY was measured using an in-house radioimmunoassay with LLOQ of 8.7 pmol/L. Plasma OXM was measured using a specific and sensitive mass-spectrometry validated immunoassay with LLOQ of 0.5 pmol/L (34). GIP and ghrelin were measured using a Milliplex human metabolic hormone magnetic bead panel (Millipore HMHEMAG-34K) customised to measure these two hormones with LLOQs of 0.3 pmol/L and 4 pmol/L respectively. The intra- and inter-assay coefficient of variation (CV) for total GLP-1, glucagon, PYY and OXM were <10%. The intra-assay and inter-assay CVs for GIP and ghrelin were 10% and 15% respectively. Blood for insulin and FGF19 was collected in plain tubes. Samples were allowed to clot at room temperature for 15 minutes before centrifugation, the serum was separated and then stored at -80°C until analysis. FGF19 was measured using ELISA (Biovendor RD191107200R), with an LLOQ of

4.8 pg/ml, and intra-assay and inter-assay CVs of <10%. Glucose, HbA1c and insulin were measured by North West London Pathology: Abbott Architect hexokinase assay for glucose, chemiluminescent microparticle immunoassay for insulin and Tosoh G8 HPLC assay for HbA1c, with CVs for precision of <5%, <5% and <10% respectively. BA were measured from plasma using HPLC-tandem mass spectrometry (27). This assay measures 15 fractions of BA with intra-assay CV of 1.5-6.8% and inter-assay CV of 3.6-8.0%.

STATISTICAL ANALYSIS

Estimates of hepatic insulin sensitivity (HOMA2 %S) were derived from fasting glucose and fasting insulin using the interactive, 24-variable homeostasis modelling assessment using default values (11). Matsuda's Insulin Sensitivity Index (MISI) (17) was calculated using the following formula in $pmol^{-1} \cdot mmol^{-1}$: 10000/(fasting glucose [mmol/L] × fasting insulin [pmol/L] × mean glucose during mixed meal from 0 to +120 min × mean insulin during mixed meal from 0 to +120 min)$^{0.5}$. The insulinogenic index (Δins15/Δglu15) was calculated as ($insulin_{15\ min}$ – fasting insulin / $glucose_{15\ min}$ – fasting glucose) in mU/mmol; this measure has been validated using a liquid mixed meal and shown to correlate well with insulin secretion during a hyperglycaemic clamp (29). Using the trapezoid rule, total AUC was calculated from -60 to 240 min and incremental AUCs from -60 to 0 min (fasting phase) and from time of ingestion of the mixed meal at 0 min to 240 min (post-prandial phase). A power calculation for a repeated measures one-way ANOVA, using the GLP-1 AUCs and standard deviation (SD) reported by Murakami et al. (20), showed that with 12 participants we would have 80% power to determine a statistically and physiologically significant difference in AUC with an alpha of 0.05. Statistical analysis was performed using GraphPad Prism 8.1.2 (GraphPad Software) and STATA15 (STATACorp LLC). The effects of each treatment on fasting values at -60 and 0 minutes were analysed using a repeated measures linear mixed model incorporating the baseline value at -60 minutes, age, sex, BMI and HbA1c as covariates. Glucose, insulin and gut hormone AUCs were compared between treatments using a repeated measures linear mixed model, incorporating age, sex, BMI and HbA1c as covariates.

Adjusted p-values with the Bonferroni correction are reported. P-values <0.05 were considered statistically significant.

STUDY APPROVAL

The study was performed in accordance with Good Clinical Practice principles and all participants gave written consent prior to inclusion in the study. Ethical approval was obtained from the UK National Health Service Health Research Authority West London Research Ethics Committee (reference 17/LO/0126). As this was a physiological study (where the BA were used as a tool to provoke a physiological response), it was not considered a clinical trial of an investigational medicinal product in conformance with Regulation (EU) No 536/2014.

RESULTS

PARTICIPANT CHARACTERISTICS

Thirteen volunteers were recruited and began study visits; one dropped out after one visit due to a change in employment circumstances. Twelve completed all three visits and were included in the analysis. Baseline demographics are listed in Table 1. No volunteers were taking any medications, and none had had previous bowel resection or cholecystectomy. There were no adverse events during the study.

ORAL CDCA AND UDCA ENRICHED CIRCULATING BA LEVELS DURING THE MIXED MEAL

In the Nil control arm, the mixed meal led to a small increase in total CDCA detected in the blood reflecting endogenous secretion and enterohepatic recirculation. After ingestion of exogenous unconjugated UDCA or CDCA, we detected a large rise in the corresponding unconjugated BA in blood samples as well as a rise in the conjugates resulting from hepatic metabolism, especially the glycine conjugates (Supplementary Table S1 https://figshare.com/s/d38e15d3725307801769). The pharmacological dose of CDCA employed led to an approximately 5.5-fold increase in mean total CDCA (unconjugated+conjugated) exposure, as judged by the AUC over the Nil control arm; the oral UDCA led to around a 35-fold increase in mean total UDCA exposure over the Nil control arm. Total UDCA and CDCA peaked at 30 min after the mixed meal ingestion (90 min after BA ingestion) (Supplementary Figure S1 https://figshare.com/s/010ed85de9ca9e4dcfcc).

CDCA ATTENUATES THE POST-PRANDIAL RISE IN INSULIN WITHOUT A CHANGE IN GLUCOSE EXCURSION

After BA administration, the total AUC ($tAUC_{-60\ to\ 240}$) for glucose was not significantly altered in comparison to Nil control (Table 2). Neither UDCA nor CDCA changed fasting glucose levels significantly after ingestion based on the incremental area-under curve between -60 min and 0 min ($iAUC_{-60\ to\ 0}$), nor was there a significant change between the interventions in the glucose excursion

after mixed meal as judged by the incremental area-under-curve from 0 to 240 min ($iAUC_{0\ to\ 240}$) in these healthy volunteers (Figure 1A, Table 2). After CDCA, there was a 36% reduction in insulin $tAUC_{0\ to\ 240,}$ with a smaller reduction noted with UDCA. Assessment of the response of fasting insulin levels to CDCA using $iAUC_{-60\ to\ 0}$ showed a significant rise in insulin secretion, followed by an attenuation of post-prandial insulin $iAUC_{0\ to\ 240}$ in comparison to Nil control by 58%; a similar analysis with UDCA showed no significant changes in the fasting and post-prandial phases (Figure 1B-D and Table 2). Calculation of the insulinogenic index ($insulin_{15\ min}$ – fasting insulin / $glucose_{15\ min}$ – fasting glucose) as a measure of acute insulin secretion in response to the mixed meal (2) showed this to be significantly lower after ingestion of CDCA when compared to Nil control (Figure 1E). Calculation of Matsuda's Insulin Sensitivity Index (MISI) as a measure of whole-body insulin sensitivity did not show a significant difference between treatment arms (Figure 1F).

CDCA INCREASES FASTING GLP-1 AND OXM SECRETION

The overall secretion of GLP-1 and OXM ($tAUC_{-60\ to\ 240}$) was significantly increased in response to CDCA compared to Nil control. There were no significant changes in $tAUC_{-60\ to\ 240}$ of PYY, ghrelin nor glucagon. In parallel to the rise in fasting insulin $iAUC_{-60\ to\ 0}$, CDCA increased GLP-1 and OXM $iAUC_{-60\ to\ 0}$ in comparison to Nil control (Table 2). In the Nil control arm, as expected, we saw post-prandial increases in GLP-1, OXM, PYY and glucagon as measured by $iAUC_{0\ to\ 240,}$ with a suppression of ghrelin. Assessing the differences in post-prandial secretion after CDCA using $iAUC_{0\ to\ 240,}$ there was no significant difference for GLP-1, OXM, PYY, glucagon nor ghrelin (Figure 2, Table 2).

CDCA SUPPRESSES POST-PRANDIAL GIP SECRETION

There was a reduction in GIP $tAUC_{0\text{-}240}$ after CDCA in comparison with Nil. This was driven by a marked post-prandial suppression of $iAUC_{0\ to\ 240}$ by approximately 33%; there was no difference in $iAUC_{-60\ to\ 0}$ (Table 2). There was also a delay in the median Tmax (time of peak of GIP secretion) from 60 to 120 min with CDCA (Figure 2D), which is likely related to the pharmacokinetic Tmax of total CDCA at 30 min (Supplementary Figure S1 https://figshare.com/s/010ed85de9ca9e4dcfcc).

UDCA DOES NOT ACUTELY AFFECT THE RELEASE OF GUT HORMONES

Unlike CDCA, UDCA treatment did not lead to significant changes in GLP-1 and OXM, nor were there any changes in PYY, GIP and ghrelin $tAUC_{-60\ to\ 240}$. Although there was a statistically significant reduction in glucagon $tAUC_{-60\ to\ 240}$ driven by a slightly lower basal glucagon level pre UDCA administration, we did not see any significant change in $iAUC_{-60\ to\ 0}$ and $iAUC_{0\ to\ 240}$ (Figure 2A-E and Table 2) and overall we considered that UDCA did not significantly affect the release of the measured gut hormones over the time of the study.

EFFECTS OF UDCA AND CDCA ON FGF19 SECRETION

CDCA, but not UDCA, was associated with >2× increase in FGF19 $tAUC_{-60\ to\ 240}$, principally driven by an increase after 120 min (180 minutes after CDCA administration), consistent with the time course of enterocyte FGF19 production following FXR activation (36) (Figure 2F, Table 2).

DISCUSSION

We show that CDCA increases the secretion of insulinotropic L-cell gut hormones GLP-1 and OXM, primarily by increasing fasting levels, and this occurs in parallel to an increase in insulin. Although CDCA is an FXR agonist which could theoretically inhibit GLP-1 secretion (28), the TGR5/GPBAR1-mediated positive effect appears to be dominant within the first 60 minutes of administration, noting that FXR activation is a relatively slow process. We observed that even with maximal FXR activation at 120 to 240 min (as indicated by the FGF19 biomarker) there was no indication of suppression of GLP-1 or OXM secretion. We did not see any increase in fasting PYY secretion, and there was no increase in post-prandial secretion of GLP-1, OXM, PYY nor glucagon. We compare our findings with the study of Meyer-Gerspach et al. who found an increase in fasting GLP-1 and no change in response to an oral glucose tolerance test with intraduodenal instillation of CDCA. Notably, they found an increase in fasting PYY with CDCA which we did not observe (19). Calderon et al. (7) showed that ileo-colonic delivery of a mixture of BA (including conjugated and unconjugated cholic acid, deoxycholic acid and CDCA) for 28 days to people with obesity and diabetes led to a small enhancement of GLP-1 secretion in response to a mixed meal stimulus but they did not study any other metabolically influential gut hormones. It is possible that the difference in this case was related to their use of a mixture of BA. Our findings are consistent with those of Nielsen et al. (22) who found that CDCA led to increased fasting secretion of GLP-1 in patients who had undergone Roux-en-Y gastric bypass, although they also documented increased PYY secretion, possibly due to direct delivery of BA to the L-cells via the bypass.

In contrast to GLP-1 and OXM, we saw no change in fasting GIP, but a 33% fall in post-prandial GIP secretion following CDCA. The pre-existing evidence of BA effects on the K-cell secretion of GIP is scanty: in animal models, BA independently stimulate secretion of both GIP and GLP-1 (16). In people with type 2 diabetes there was no effect of UDCA treatment on GIP secretion in response to a high-fat meal (25). In patients who have undergone gastric bypass, oral CDCA did not significantly influence fasting GIP levels (22), similar to our observations. Our finding that post-prandial GIP secretion was

suppressed by CDCA is connected to our observation that post-prandial insulin levels were attenuated. A similar finding was reported by Meyer-Gerspach et al., showing that intraduodenal infusion of CDCA prior to an oral glucose tolerance test resulted in no difference in glucose levels, but an attenuated release of insulin and C-peptide in response to the glucose load (19). Both GLP-1 and GIP contribute equally to the incretin effect in healthy humans (30); with no enhancement of GLP-1 secretion post-prandially, the reduction in post-prandial GIP secretion with CDCA is a plausible explanation for the reduced insulin secretion. An alternative hypothesis is that enterocyte FXR activation and secretion of FGF19 in turn suppresses hepatic gluconeogenesis (23) and activates glycogen and protein synthesis (14). FGF19 may have an 'insulin sparing' effect on glucose disposal via these metabolic mechanisms. Noting that the relative reduction in post-prandial insulin with CDCA (58%) was larger than the reduction in GIP (33%), we believe that both mechanisms may explain the reduced insulin secretion in the face of unchanged glucose tolerance.

We did not see any significant change in gut hormone secretion (fasting or post-prandial) with UDCA. Murakami et al. observed a small stimulatory effect on post-prandial GLP-1 secretion with a low dose of UDCA (200 mg) in healthy volunteers although with no significant effect on insulin levels (20). Shima et al. observed a similar increase in post-prandial GLP-1 in patients with type 2 diabetes that took UDCA treatment for 12 weeks (25). These studies only reported a statistically significant change in GLP-1 AUC measured in the first 60 min and the first 30 min respectively (20, 25) and not for the entire duration of the mixed meal study. The Linco assay for GLP-1 used in the Japanese studies does not perform well in comparison with validated assays (10). In contrast, we have used a well characterised and validated assay for GLP-1 (33). We also note that Nielsen et al. (22) did not document any changes in GLP-1, PYY, glucagon nor GIP secretion with UDCA in their cohort of Roux-en-Y gastric bypass patients, consistent with our findings. Our findings with respect to UDCA are likely to be more robust.

Limitations of the study include that it examined the acute effect of a single dose of BA, and it only included healthy volunteers with normal glucose tolerance. We have also not examined any interaction that the exogenous BA might have with gut microbiota, although we note that there was

no evidence of conversion of CDCA to detectable lithocholic acid, nor of CDCA to UDCA during the short study period. The conversion of CDCA to UDCA by microbiotal epimerization is likely to require a longer time span of 8-16 hours to manifest (24). It is possible that chronic BA treatment might have differing metabolic effects due to the longer time afforded to allow microbiotal conversion of the exogenous BA.

Taken in total, CDCA may have salutary effects on metabolic physiology by enhancing secretion of GLP-1 and OXM which can act in concert to reduce appetite (5). In contrast UDCA does not appear to have a significant effect on gut hormone secretion. We make the novel observation that CDCA suppresses post-prandial GIP secretion: this may abrogate its deleterious effects on adipose tissue, which include increased lipid accumulation and increased inflammation (13). The impact of CDCA may differ in the context of type 2 diabetes. The secretion and action of gut hormones is altered in this context: for example post-prandial secretion of GLP-1 is attenuated (29), and GIP loses its insulinotropic effect (31). Therefore, further research is required to determine if CDCA has similar acute effects on gut hormone secretion and insulin levels in patients with obesity and type 2 diabetes and whether these effects occur with chronic treatment.

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DATA AVAILABILITY

The data sets generated during and/or analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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CONTRIBUTION STATEMENT

TT, ERMcG, KM, BK, JRFW and SRB contributed to study design, data collection, statistical analysis, data interpretation, and writing of the manuscript. ERMcG and KM contributed to the running of the

study, sample analysis, data collection and data interpretation. OK and AA contributed to study design. JC, CL and RV contributed to sample analysis. SV facilitated supply of CDCA. JJH and NJWA contributed to the analysis of samples and data interpretation. All authors contributed to critical review of the manuscript. TT is the guarantor of this work and had full access to all the data in the study; she takes responsibility for the integrity of the data and the accuracy of the data analysis.

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TABLES

Table 1: Baseline characteristics of study subjects (n=12). Mean (standard deviation) is listed for each parameter except for categorical data.

Parameter	**Mean (standard deviation)**
Age (years)	38.5 (14.7)
Female: male	8:4
BMI (kg/m^2)	24.8 (3.9)
Ethnicity White: Asian: Black: Other	 7: 2: 1: 2
Fasting plasma glucose (mmol/L)	4.7 (0.4)
Fasting plasma insulin (mU/L)	5.7 (2.8)
HbA1c (mmol/mol)	32.7 (2.8)
Hepatic insulin resistance (iHOMA2 %S)	162.4 (60)

Table 2: Glucose, insulin, gut and pancreatic hormone total (tAUC) and incremental (iAUC) area-under-curve values after mixed meal following ingestion of Nil, UDCA or CDCA. Repeated measures linear mixed model with adjustment for age, sex and HbA1c: estimated marginal means and [95% confidence interval] are presented to 3 significant figures. Bonferroni-adjusted p-values are reported for the indicated contrasts. GLP-1, glucagon-like peptide-1; OXM, oxyntomodulin; PYY, peptide YY; GIP, glucose-dependent insulinotropic peptide.

	Parameter	Nil	UDCA	Nil vs UDCA p-value	CDCA	Nil vs CDCA p-value
Glucose mmol·min/L	$tAUC_{-60\ to\ 240}$	1490 [1420, 1550]	1460 [1390, 1520]	0.818	1450 [1390, 1520]	0.722
	$iAUC_{-60\ to\ 0}$	-1.75 [-7.34, 3.84]	-2.25 [-7.84, 3.34]	1.000	0.00 [-3.94, 3.94]	1.000
	$iAUC_{0\ to\ 240}$	47.4 [-17.0, 112]	18.2 [-46.3, 82.7]	0.708	5.07 [-59.6, 69.7]	0.363
Insulin mU·min/L	$tAUC_{-60\ to\ 240}$	9680 [7270, 12100]	7280 [4870, 9690]	0.032	6170 [3760, 8590]	0.001
	$iAUC_{-60\ to\ 0}$	-30.8 [-58.3, -3.16]	-33.3 [-60.8, -5.66]	1.000	22.0 [-5.59, 49.6]	<0.001
	$iAUC_{0\ to\ 240}$	8440 [6680, 10200]	6930 [5100, 8770]	0.175	3530 [1660, 5390]	<0.001
GLP-1 pmol·min/L	$tAUC_{-60\ to\ 240}$	1530 [921, 2150]	1600 [983, 2210]	1.000	2170 [1550, 2780]	<0.001
	$iAUC_{-60\ to\ 0}$	-36.0 [-80.5, 8.54]	12.6 [-31.9, 57.2]	0.108	80.3 [36.1, 125]	<0.001
	$iAUC_{0\ to\ 240}$	1040 [545, 1540]	1040 [547, 1540]	1.000	1020 [527, 1520]	1.000
OXM pmol·min/L	$tAUC_{-60\ to\ 240}$	1520 [1120, 1920]	1690 [1290, 2090]	0.872	2260 [1860, 2660]	0.001
	$iAUC_{-60\ to\ 0}$	-78.4 [-167, 10.7]	-41.5 [-131, 47.6]	0.965	49.8 [-39.2, 139]	0.030
	$iAUC_{0\ to\ 240}$	903 [596, 1210]	948 [641, 1255]	1.000	1080 [775, 1390]	0.515
PYY pmol·min/L	$tAUC_{-60\ to\ 240}$	8720 [4536, 12900]	10400 [6180, 14600]	1.000	14000 [9850, 18200]	0.061
	$iAUC_{-60\ to\ 0}$	-100 [-395, 195]	-19.2 [-301, 262]	1.000	145 [-136, 428]	0.273
	$iAUC_{0\ to\ 240}$	3340 [-36.1, 6720]	5880 [2500, 9260]	0.406	5550 [2170, 8920]	0.539
GIP pmol·min/L	$tAUC_{-60\ to\ 240}$	33200 [20000, 46500]	32300 [19000, 45500]	1.000	23200 [9950, 36500]	0.011
	$iAUC_{-60\ to\ 0}$	-118 [-210, -26.2]	-51.7 [-144, 40.4]	0.330	-76.1 [-168, 15.9]	0.760
	$iAUC_{0\ to\ 240}$	30500 [17600, 43400]	29500 [16600, 42400]	1.000	20400 [7520, 33300]	0.010
Ghrelin pmol·min/L	$tAUC_{-60\ to\ 240}$	5260 [3040, 7470]	5300 [3090, 7520]	1.000	5410 [3190, 7620]	1.000
	$iAUC_{-60\ to\ 0}$	115 [-198, 428]	220 [-93.6, 533]	1.000	218 [-95.5, 531]	1.000
	$iAUC_{0\ to\ 240}$	--1370 [-2790, 47.0]	-1170 [-2590, -246]	1.000	-1455 [-2870, -37.0]	1.000
Glucagon	$tAUC_{-60\ to\ 240}$	2960 [1880, 4040]	2310 [1230, 3390]	0.012	2750 [1670, 3820]	0.725

pmol·min/L	$iAUC_{-60\ to\ 0}$	-29.1 [-77.8, 19.5]	-11.8 [-60.5, 36.8]	1.000	33.0 [-15.6, 81.6]	0.050
	$iAUC_{0\ to\ 240}$	767 [465, 1070]	758 [456, 1060]	1.000	801 [499, 1100]	1.000
FGF19 pg·min/ml	$tAUC_{-60\ to\ 240}$	99500 [67500, 131000]	118000 [86800, 151000]	0.466	217000 [185000, 249000]	<0.001
	$iAUC_{-60\ to\ 0}$	-387 [-1840, 1070]	-2050 [-3500, -595]	0.106	-233 [-3780, -877]	0.047
	$iAUC_{0\ to\ 240}$	18800 [-10000, 47500]	26300 [2510, 55100]	1.000	73600 [44800, 102000]	0.003

FIGURES

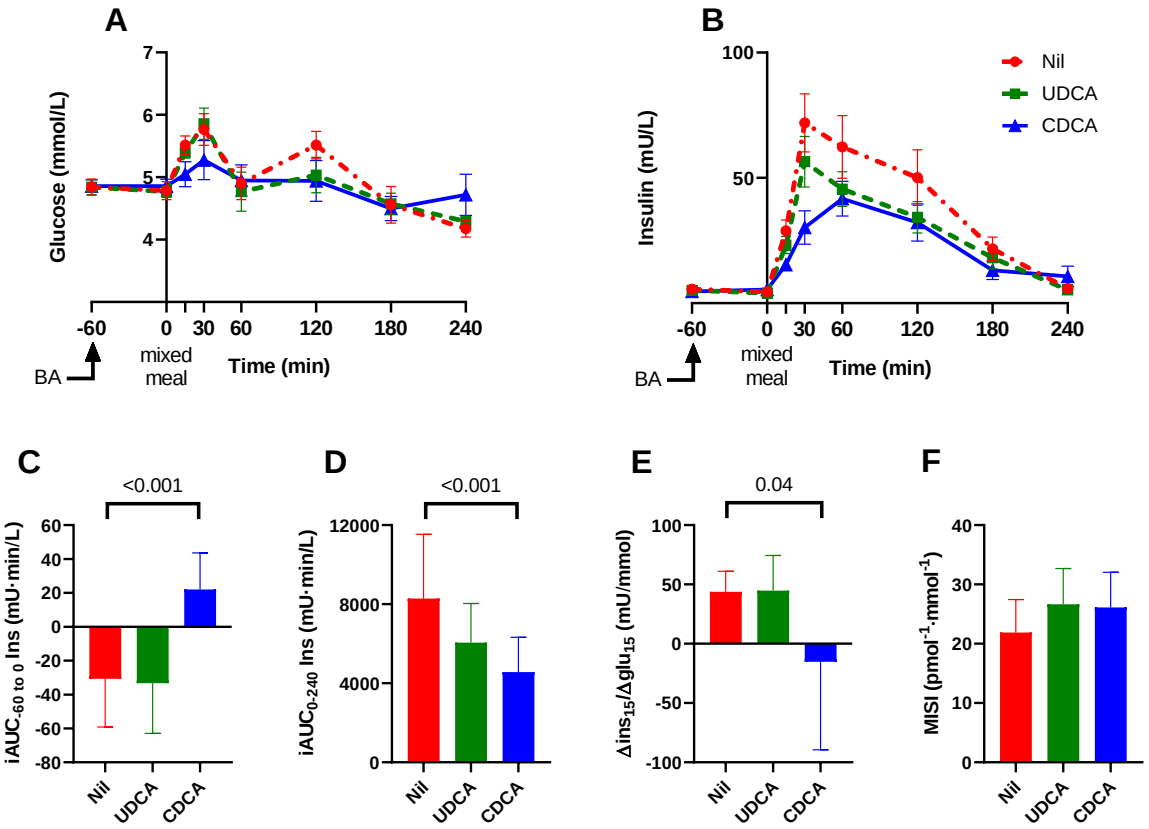

Figure 1: Glucose and insulin responses to bile acid intervention. Nil: dot-dashed lines, filled circles, red lines/bars; UDCA: dashed lines, filled squares, green lines/bars; CDCA: solid lines, filled triangles, blue lines/bars. Dynamics of glucose (A) and insulin (B), means and SEM plotted. Incremental area-under-curve (iAUC) for insulin from -60 to 0 min (C) and 0 to 240 min (D), after BA ingestion at -60 min and mixed meal ingestion at 0 min plotted as means and 95% confidence intervals. Bonferroni adjusted p-values for contrast of Nil vs CDCA indicated (repeated measures linear mixed model). (E) Insulinogenic index ($insulin_{15\ min}$ – fasting insulin / $glucose_{15\ min}$ – fasting glucose) in mU/mmol plotted as means and 95% confidence intervals, Bonferroni adjusted p-value reported for contrast of Nil vs CDCA (repeated measures linear mixed model). (F) Matsuda whole-body insulin sensitivity index (MISI) in $pmol^{-1}{\cdot}mmol^{-1}$, means and SEM plotted.

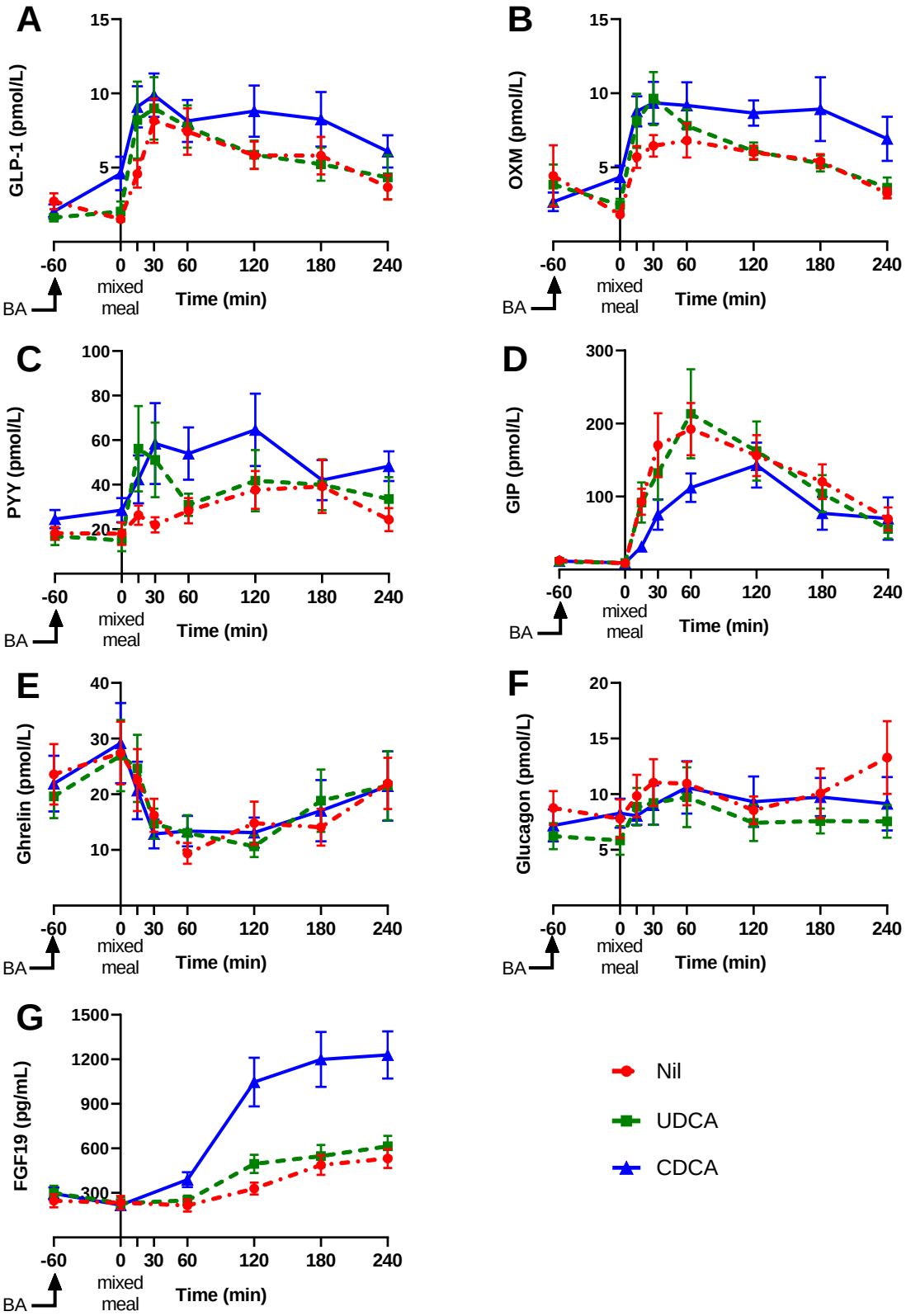

Figure 2: Gut hormone and FGF19 responses to bile acid intervention at -60 min and mixed meal at 0 min. Nil: dot-dashed lines, filled circles, red lines; UDCA: dashed lines, filled squares, green lines;

492 CDCA: solid lines, filled triangles, blue lines. Dynamics of GLP-1 (A), oxyntomodulin (OXM – B), peptide
493 YY (PYY – C), glucose-dependent insulinotropic peptide (GIP – D), Ghrelin (E), Glucagon (F), FGF19 (G)
494 after BA ingestion at -60 min and mixed meal ingestion at 0 min plotted as means and SEM.