The relationship between post-prandial bile acid concentration, GLP-1, PYY and ghrelin.

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The relationship between post-prandial bile acid concentration, GLP-1, PYY and ghrelin.

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Full title: The relationship between post-prandial bile acid concentration, GLP-1, PYY and ghrelin.

Short title: Bile acid and gut hormone responses.

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Abstract

Background: Gut hormones peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) play an integral role in appetite control and energy homeostasis. Entero-endocrine L-cells can be stimulated by nutrients and or bile acids to co-secrete PYY and GLP-1. The aim of this study was to determine the response of bile acids, PYY, GLP-1 and ghrelin after a test meal.

Design: Twelve subjects with a BMI of 22.8 (0.52) kg/m² (mean (SEM)) received a 400 kcal test meal after which blood samples were taken every 30 minutes from 0 to 180 minutes. PYY, GLP-1 and ghrelin were measured by radioimmunoassays. Fractionated bile acids were measured by HPLC-MSMS.

Results: PYY positively correlated with glycochenodeoxycholic acid (GCDCA) ($r_s = 0.23$, $p = 0.03$) and taurochenodeoxycholic acid (TCDCA) ($r_s = 0.26$, $p = 0.02$). GLP-1 positively correlated with GCDCA ($r_s = 0.22$, $p = 0.047$) and glycodeoxycholic acid (GDCA) ($r_s = 0.3$, $p = 0.005$). Ghrelin negatively correlated with GDCA ($r_s = -0.45$, $p = < 0.0001$), TCDCA ($r_s = -0.23$, $p = 0.034$) and taurodeoxycholic acid (TDCA) ($r_s = -0.44$, $p = < 0.0001$).

Conclusion: PYY and GLP-1 responses correlated with chenodeoxycholic acid (CDCA) counterparts, whereas ghrelin negatively correlated with deoxycholic acid (DCA) counterparts. Specific bile acids may thus differentially affect entero-endocrine cells.

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Introduction

Bile acids have several endocrine effects and are involved in three main signalling pathways; mitogen-activated protein kinase (MAPK) pathways (1,2); via the G-protein coupled receptor TGR5 (3,4), and via nuclear hormone receptors such as farnesoid X receptor α (FXR α) (5-7). Administration of bile acids to mice increases energy expenditure in brown adipose tissue via induction of the cyclic-AMP-dependent thyroid hormone activating enzyme type 2 iodothyronine deiodinase (D2) (8). *In vitro* treatment of brown adipocytes and human skeletal myocytes with bile acids increases D2 activity and oxygen consumption (8). In addition bile acid stimulation of FXR α activates a signalling pathway culminating in increased energy expenditure and decreased adiposity (9-11). In humans circulating bile acid concentrations correlate with measures of insulin sensitivity (12) and administration of bile acids has been found to prevent high fat diet-induced obesity and development of insulin resistance in mice (13). Modulation of bile acid homeostasis using bile acid sequestrants such as cholestyramine and colesevelam have been shown to improve glycaemic control in patients with type 2 diabetes mellitus (14-16). The mechanisms by which bile acids improve glycaemic control are largely thought to be FXR α -independent and are instead mediated by binding to TGR5, leading to cAMP generation and activation of the intracellular type 2 thyroid hormone deiodinase (17). Bile acids also act via the phosphoinositide 3-kinase (PI3 kinase)/AKT pathway, directly promoting insulin signalling and glycogen synthase activation, thus aiding insulin-dependent control of glucose metabolism in the liver (18). In addition bile acid activation of TGR5 has been found to stimulate GLP-1 production *in vitro*, which promotes insulin secretion and thus improves glucose metabolism.

Bile acids constitute a large family of molecules composed of a steroid structure with four rings and a five or eight carbon side-chain terminating in a carboxylic acid. Each bile acid has a specific number and orientation of hydroxyl groups (-OH). The principal primary bile acids in humans are cholic acid (CA) and chenodeoxycholic acid (CDCA). Ursodeoxycholic acid (UDCA) is the 7 β epimer of CDCA. Bile acids can be conjugated to either glycine or taurine, forming bile salts. Glycochenodeoxycholic acid (GCDCA) and taurochenodeoxycholic acid (TCDCA) are the glycine and taurine conjugates of CDCA, respectively and glycocholic acid (GCA) and taurocholic acid (TCA) are the glycine and taurine conjugates of CA, respectively. Bile acids can also be conjugated in phase II metabolic reactions such as N-acetylglucosaminidation at the C7 position with a beta-hydroxyl group (19). Bacteria in the intestinal tract can convert primary bile acids into secondary bile acids by removing a hydroxyl group, for example CDCA becomes lithocholic acid (LA) and CA becomes deoxycholic acid (DCA) (Figure. 1.).

Gut hormones peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) play an integral role in appetite control and energy homeostasis. PYY and GLP-1 are secreted by L-cells of the distal small intestine in response to nutrients and bile acids (20-22). Exaggerated responses of PYY and GLP-1 may contribute to the weight loss and improvement in glycaemic control following gastric bypass surgery for the treatment of obesity. The mechanisms for increased concentrations of PYY and GLP-1 after gastric bypass are not entirely understood. The 'hindgut hypothesis', described by Mason (23), proposes that altered anatomy following bariatric surgery accelerates the delivery of nutrient-dense chyme to the distal intestine which stimulates increased gut hormones secretion. In addition plasma bile acid concentrations are doubled in individuals after gastric bypass surgery compared to obese individuals who have not had bariatric surgery (24) and it is possible that co-delivery of these bile acids within the chyme enhances the stimulation of gut hormone release post-gastric bypass.

A role of bile acids in appetite suppression was proposed as early as 1968 (25). Several studies indicate that bile acids deoxycholic acid and taurocholic acid combined with fatty acids are more significant than fatty acids alone in stimulating PYY release in the ileum (21,26-29). Bile acid infusions of taurochenodeoxycholic acid and deoxycholic acid have been shown to increase plasma PYY in rabbits (27,30) and deoxycholic acid infusion in the human colon stimulates PYY release in a dose-dependent manner (21). Importantly the concentrations of bile acids used in these studies are consistent with the physiological concentrations of bile acids found in the intestinal lumen post-prandially (31), supporting a physiological role of bile acids in the stimulation of PYY release. However, not all bile acids have been found to stimulate PYY release with hyodeoxycholic acid and ursodeoxycholic acid having no effect on PYY release (27,32). The reason why PYY would be stimulated by specific bile acids is not clear. The post-prandial peak concentrations of GLP-1 and total bile acid concentrations inversely correlate with fasting and post-prandial glucose after gastric bypass (24).

Ghrelin is secreted from X/A-like cells of the fundus of the stomach (33,34) and may play a role in regulating pre-meal hunger, meal initiation and long-term energy balance (35,36). Circulating ghrelin concentrations increase during fasting and decrease after eating (37) while anticipation of meals may contribute to its secretion (38). The mechanisms by which ghrelin promotes hunger and increased energy intake are not entirely clear. There is some evidence to suggest that ghrelin may exert its effects by acting on the central nervous system (39). Obese subjects have lower fasting plasma ghrelin concentrations than lean controls (40), although appetite is commonly increased, suggesting that higher body mass is associated with

1 increased gastric responsiveness to ghrelin (41). A relationship between ghrelin and bile acids has not been
2 described.
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7 The aim of this study was to investigate the associations between the responses of bile acids, PYY, GLP-1
8 and ghrelin after a standard 400 kcal test meal.
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11 **Methods**
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16 All studies were performed according to the principles of the Declaration of Helsinki. The Local Research and
17 Ethics Committee at King's College Hospital approved the study (05/Q0703/4). Written informed consent was
18 obtained.
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23 *Subjects and Sample Collection*
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25 We studied 12 healthy male subjects (age 21.2 (0.46) years, BMI 22.8 (0.52) kg/m² (mean (SEM))).
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27 Subjects fasted overnight from 23:00 before ingesting a 400 kcal test meal (46.6g carbohydrate, 28.5 g fat
28 and 10.4 g protein) at 09:00. EDTA blood samples were taken every 30 minutes from 0 to 180 minutes.
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33 *Bile acid and hormone measurement*
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35 Fractionated bile acids were measured using HPLC-MSMS as previously described by Tagliacozzi *et al*
36 (42). Bile acids analysed were chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), cholic acid (CA),
37 ursodeoxycholic acid (UDCA), lithocholic acid (LA) and their respective glycine (G-) and taurine (T-)
38 conjugates. The method was linear between 0.1-10 µmol/L for all bile acids and their conjugates with intra-
39 assay coefficient of variations (CV) ranging from 3.6 - 8.0 % and inter-assay CVs ranging from 1.5 - 6.8 %.
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41 The lower limit of quantitation was 0.1 µmol/L. LCA and UDCA and their conjugates and CDCA were below
42 the detection limit. Glucose was measured using the glucose oxidase method on the Advia 2400 analyser
43 (Siemens Healthcare Diagnostics, Frimley, UK). Insulin was measured using a two site sandwich
44 chemiluminescent immunoassay on the Advia Centaur analyser (Siemens Healthcare). The intra-assay CV
45 was 3.3% and inter-assay CV was 4.8%. PYY was measured by an established in-house radioimmunoassay
46 as previously described by Adrian *et al* (43). The assay measured the biologically active components, both
47 the full length (PYY₁₋₃₆) and the fragment (PYY₃₋₃₆). The detection limit was 5 pmol/L and the intra- and inter-
48 assay CVs were 5.8% and 9.8%, respectively. GLP-1 was measured by an established in-house
49 radioimmunoassay as previously described by Kreymann *et al* (44). The detection limit was 7.5 pmol/L and
50 the intra-assay CV was 6.1%. Ghrelin was measured by the commercially available Phoenix Pharmaceutical
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assay kit as previously described by Patterson *et al* (45). The assay measured total human ghrelin (acylated and des-acylated) and the intra-assay CV was 8.8%

Statistical Analysis

Results are reported as mean (SEM) and correlation co-efficient (rs). Statistical analysis was performed using Analyse-It for Excel (Analyse-It, Leeds, UK). Data were normally distributed and a Spearman correlation was used. Significance is shown by a $P < 0.05$.

Results

None of the unconjugated bile acids changed significantly throughout the study period but all glycine and taurine conjugated bile acids and total bile acids increased significantly (Table. 1.). The predominant bile acid measured was GCDCA, followed by GCA and GDCA. All other bile acids had peak concentrations of $< 1.00 \mu\text{mol/L}$. Table. 2. and Figure. 2. show the changes in plasma hormones and glucose before and after the standardised 400 kcal meal.

Correlations were sought between individual types of bile acids, PYY, GLP-1 and ghrelin. Bile acids which significantly correlated with PYY were GCDCA ($r_s = 0.23$, $p = 0.03$), total glycine conjugated bile acids ($r_s = 0.23$, $p = 0.03$), TCDCA ($r_s = 0.26$, $p = 0.02$) (Figure. 3.), total taurine conjugated bile acids ($r_s = 0.24$, $p = 0.03$) and total bile acids ($r_s = 0.26$, $p = 0.02$). Bile acids which significantly correlated with GLP-1 were GCDCA ($r_s = 0.22$, $p = 0.047$), GDCA ($r_s = 0.3$, $p = 0.005$), total glycine conjugated bile acids ($r_s = 0.26$, $p = 0.02$) and total bile acids ($r_s = 0.26$, $p = 0.02$) (Figure. 3.). Bile acids which significantly correlated with ghrelin were GDCA ($r_s = -0.45$, $p = < 0.0001$) (Figure. 3.), TCDCA ($r_s = -0.23$, $p = 0.03$), TDCA ($r_s = -0.44$, $p = < 0.0001$), total taurine conjugated bile acids ($r_s = -0.23$, $p = 0.038$) and total bile acids ($r_s = -0.22$, $p = 0.044$).

Discussion

The CDCA group of bile acids GCDCA and TCDCA correlated with PYY. GCDCA also correlated with GLP-1. GCDCA and TCDCA share a common chemical structure with -OH hydroxylation at C7 and a single hydrogen ion at C12 (Figure. 1.). The positive correlation found between GCDCA and PYY and GLP-1 and TCDCA and PYY suggests that the specific chemical structure of bile acids may play a role in stimulating L-cells. UDCA and its conjugates and CDCA also share this chemical structure but concentrations were below

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2 the detection limit of our assay and therefore we are unable to comment whether a similar correlation is
3 possible with PYY and GLP-1.
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7 The DCA group of bile acids GDCA, TDCA and TCDCA correlated negatively with ghrelin. DCA counterparts
8 are hydroxylated at both C7 and C12. Bile acids may contribute through an endocrine mechanism to the
9 inhibition of ghrelin secretion from P/D1 cells of the fundus of the stomach post-prandially. Ghrelin and
10 cholecystokinin (CCK) are negatively correlated (46). CCK is a hormone released from the I-cells of the small
11 intestine in response to lipid ingestion which stimulates the release of bile from gallbladder, and also acts as
12 a satiety signal. It is possible that increased CCK levels post-prandially stimulate increased bile acid
13 secretion which may in turn inhibit ghrelin synthesis through neural or endocrine mechanisms.
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22 Limitations of our study included the homogenous nature of our volunteers and therefore the data can not be
23 extrapolated to females or older people. The detection limit of the assay was insufficient for determination of
24 a correlation with UDCA, its conjugates and CDCA. The number of subjects were too small to draw firm
25 conclusions, but the data suggested that the total bile acid and GCDCA response may have been biphasic in
26 9 of 12 subjects (Figure. 4.). It is possible that with 30 minute sampling the peaks and troughs may have
27 been missed in all subjects. The biphasic response of bile acids in some of our subjects may also have
28 accounted for the low rs values of our correlations.
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38 **Conclusion**

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40 This study suggests that post-prandial conjugated bile acids correlate with appetite regulating gut peptides
41 PYY, GLP-1 and ghrelin. The post-prandial responses of the CDCA counterparts primarily correlated with
42 PYY and GLP-1 responses, whereas the post-prandial DCA counterparts correlated with changes in ghrelin.
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44 Specific bile acids may play a part in entero-endocrine cell stimulation or inhibition. Targeted experiments,
45 specifically with bile acids hydroxylated at C7 may now be considered to determine the therapeutic value of
46 these bile acids in reducing weight through stimulation of PYY and GLP-1 and inhibition of ghrelin.
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Table. 1. Baseline and peak values (mean (SEM)) of glycine and taurine conjugated bile acids in plasma following a 400 kcal test meal.

Bile Acid	Baseline ($\mu\text{mol/L}$)	Peak ($\mu\text{mol/L}$)	P value
GCDCA	0.38 (0.05)	3.82 (0.39)	< 0.0001
GDCA	0.11 (0.02)	1.13 (0.22)	0.0006
GCA	0.15 (0.02)	1.45 (0.19)	< 0.0001
Total glycine conjugated bile acids	0.77 (0.08)	6.97 (0.77)	< 0.0001
TCDCA	0.03 (0.01)	0.34 (0.14)	0.048
TDCA	0.02 (0.01)	0.16 (0.04)	0.007
TCA	0.01 (0.01)	0.14 (0.05)	0.022
Total taurine conjugated bile acids	0.16 (0.09)	0.66 (0.21)	0.012
Total bile acids	1.41 (0.14)	8.33 (0.85)	< 0.0001

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Table. 2. Baseline and peak values (mean (SEM)) of plasma hormones and glucose following a 400 kcal test meal.

	Baseline	Peak	P value
PYY (pmol/L)	21.3 (1.98)	34.9 (2.54)	< 0.001
GLP-1 (pmol/L)	52.1 (4.63)	72.5 (4.54)	< 0.001
Ghrelin (pmol/L)	1026 (133.5)	550 (75.3)	< 0.001
Glucose (mmol/L)	4.6 (0.08)	5.6 (0.10)	< 0.001
Insulin (mU/L)	5.7 (1.57)	48.8 (6.42)	< 0.001

Figure. 1. The structure of bile acids.

Bile acid	R1 (C3)	R2 (C7)	R3 (C12)
CA	OH	OH	OH
CDCA	OH	OH (α)	H
DCA	OH	H	OH
LCA	OH	H	H
UDCA	OH	OH (β)	H

Legend:

X unconjugated - OH, X tauro- conjugated - NHCH₂CH₂CH₂SO₃H, X glyco- conjugated - NHCH₂COOH, CA - cholic acid, CDCA - chenodeoxycholic acid, DCA - deoxycholic acid, LCA - lithocholic acid, UDCA - ursodeoxycholic acid

Figure. 2. Changes in plasma levels of gut hormones following a 400 kcal test meal in 12 subjects.
Expressed as mean (SEM).
a) PYY, b) GLP-1 and c) ghrelin
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Figure. 3. Scatter graph demonstrating the correlation between a) taurochenodeoxycholic acid (TCDCA) and PYY; b) total bile acids and GLP-1 and c) glycochenodeoxycholic acid (GDCA) and ghrelin.

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Figure. 4. Line graphs demonstrating the response of a) glycochenodeoxycholic acid (GCDCA) and b) total bile acids following a 400 kcal test meal in 9 subjects. Expressed as mean (SEM).

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- (1) Gupta. S., Stravitz. R.T. & Dent. P. *et al.* (2001) Down-regulation of cholesterol 7 α -hydroxylase (CYP7A1) gene expression by bile acids in primary rat hepatocytes is mediated by the c-Jun N-terminal kinase pathway. *J Biol Chem*, **276**, 15816-15822.
- (2) Qiao. L., Han. S. I. & Fang. Y. *et al.* (2003) Bile acid regulation of C/EBP β , CREB, and c-Jun function, via the extracellular signal-regulated kinase and c-Jun NH₂-terminal kinase pathways, modulates the apoptotic response of hepatocytes. *Mol Cell Biol*, **23**, 3052-3066.
- (3) Maruyama. T., Miyamoto. Y. & Nakamura. T. *et al.* (2002) Identification of membrane-type receptor for bile acids (M-BAR). *Biochem Biophys Res Commun*, **298**, 714-719.
- (4) Kawamata. Y., Fujii. R. & Hosoya. M. *et al.* (2003) A G protein-coupled receptor responsive to bile acids. *J Biol Chem*, **278**, 9435-9440.
- (5) Parks. D. J., Blanchard. S. G. & Bledsoe. R. K. *et al.* (1999) Bile acids: natural ligands for an orphan nuclear receptor. *Science*, **284**, 1365-1368
- (6) Makishima. M., Okamoto. A. Y. & Repa. J. J. *et al.* (1999) Identification of a nuclear receptor for bile acids. *Science*, **284**, 1362-1365.
- (7) Mangelsdorf. D. J., Thummel. C. & Beato. M. *et al.* (1995) The nuclear receptor superfamily: the second decade. *Cell*, **83**, 835-839.
- (8) Watanabe. M., Houten. S. M. & Matak. C. *et al.* (2006) Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. *Nature*, **439**, 484-489.
- (9) Holt. J. A., Luo. G. & Billin. A. N. *et al.* (2003) Definition of a novel growth factor-dependent signal cascade for the suppression of bile acid biosynthesis. *Genes Dev*, **17**, 1581-1591.
- (10) Inagaki. T., Choi. M. & Moschetta. A. *et al.* (2005) Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. *Cell Metab*, **2**, 217-225.

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(11) Tomlinson. E., Fu. L. & John. L. *et al.* (2002) Transgenic mice expressing human fibroblast growth factor-19 display increased metabolic rate and decreased adiposity. *Endocrinology*, **143**, 1741-1747

(12) Shaham. O., Wei. R. & Wang. T. J. *et al.* (2008) Metabolic profiling of the human response to a glucose challenge reveals distinct axes of insulin sensitivity. *Mol Syst Biol*, **4**, 214.

(13) Ikemoto. S., Takahashi. M. & Tsunoda. N. *et al.* (1997) Cholate inhibits high-fat diet-induced hyperglycemia and obesity with acyl-CoA synthetase mRNA decrease. *Am J Physiol*, **273**, E37-E45.

(14) Garg. A., Grundy. S.M. (1994) Cholestyramine therapy for dyslipidemia in non-insulin-dependent diabetes mellitus. A short-term, double-blind, crossover trial. *Ann Intern Med*, **121**, 416-422.

(15) Goldberg. R. B., Fonseca. V. A. & Truitt. K. E. *et al.* (2008) Efficacy and safety of colesevelam in patients with type 2 diabetes mellitus and inadequate glycemic control receiving insulin-based therapy. *Arch Intern Med*, **168**, 1531–1540.

(16) Fonseca. V. A., Rosenstock. J. & Wang. A. C. *et al.* (2008) Colesevelam HCl improves glycemic control and reduces LDL cholesterol in patients with inadequately controlled type 2 diabetes on sulfonylurea-based therapy. *Diabetes Care*, **31**, 1479–1484.

(17) Yamagata. K., Daitoku. H. & Shimamoto. Y. *et al.* (2004) Bile acids regulate gluconeogenic gene expression via small heterodimer partner-mediated repression of hepatocyte nuclear factor 4 and Foxo1. *J Biol Chem*, **279**, 23158-62315.

(18) Han. S. I., Studer. E. & Gupta. S. *et al.* (2004) Bile acids enhance the activity of the insulin receptor and glycogen synthase in primary rodent hepatocytes. *Hepatology*, **39**, 456-463,

(19) Trottier. J., Milkiewicz. P. & Kaeding. J. *et al.* (2006) Coordinate regulation of hepatic bile acid oxidation and conjugation by nuclear receptors. *Mol Pharm*, **3**, 212-222.

(20) Anini. Y., Fu-Cheng. X. & Cuber. J. C. *et al.* (1999) Comparison of the postprandial release of peptide YY and proglucagon-derived peptides in the rat. *Pflugers Arch*, **438**, 299-306.

- (21) Adrian. T. E., Ballantyne. G. H. & Longo. W. E. *et al.* (1993) Deoxycholate is an important releaser of peptide YY and enteroglucagon from the human colon. *Gut*, **34**, 1219-1224.
- (22) Katsuma. S., Hirasawa. A. & Tsujimoto. G. (2005) Bile acids promote glucagon-like peptide-1 secretion through TGR5 in a murine enteroendocrine cell line STC-1. *Biochem Biophys Res Commun*, **329**, 386-390.
- (23) Mason. E. E. (1999) Ileal [correction of ilial] transposition and enteroglucagon/GLP-1 in obesity (and diabetic?) surgery. *Obes Surg*, **9**, 223-228.
- (24) Patti. M. E., Houten. S. M. & Bianco. A. C. *et al.* (2009) Serum bile acids are higher in humans with prior gastric bypass: potential contribution to improved glucose and lipid metabolism. *Obesity*, **17**, 1671-1677.
- (25) Bray. G. A., Gallagher. T. F. J. (1968) Suppression of appetite by bile acids. *Lancet*, **1**, 1066-1067.
- (26) Soper. N. J., Chapman. N. J. & Kelly. K. A. *et al.* (1990) The 'ileal brake' after ileal pouch-anal anastomosis. *Gastroenterology*, **98**, 111-116.
- (27) Ballantyne. G. H., Longo. W. E. & Savoca. P. E. *et al.* (1989) Deoxycholate-stimulated release of peptide YY from the isolated perfused rabbit left colon. *Am J Physiol*, **257**, G715-G724.
- (28) Wen. J., Phillips. S. F. & Sarr. M. G. *et al.* (1995) PYY and GLP-1 contribute to feedback inhibition from the canine ileum and colon. *Am J Physiol Gastrointest Liver Physiol*, **269**, G945-G952.
- (29) Aponte. G. W., Fink. A. S. & Meyer. J. H. *et al.* (1985) Regional distribution and release of peptide YY with fatty acids of different chain length. *Am J Physiol*, **249**, G745-G750.
- (30) Armstrong. D. N., Krenz. H. K. & Modlin. I. M. *et al.* (1993) Bile salt inhibition of motility in the isolated perfused rabbit terminal ileum. *Gut*, **34**, 483-488.
- (31) Dietschy. J. M. (1968) Mechanisms for the intestinal absorption of bile acids. *J Lipid Res*, **9**, 297-309.

1
2 (32) Plaisancié. P., Dumoulin. V. & Chayvialle. J. A. *et al.* (1996) Luminal peptide YY-releasing factors in the
3 isolated vascularly perfused rat colon. *J Endocrinol*, **151**, 421-429.
4
5
6
7
8 (33) Cummings. D. E., Weigle. D. S. & Frayo. R. S. *et al.* (2002) Plasma ghrelin levels after diet-induced
9 weight loss or gastric bypass surgery. *N Engl J Med*, **346**, 1623–1630.
10
11
12
13 (34) Date. Y., Kojima. M. & Hosoda. H. *et al.* (2000) Ghrelin, a novel growth-hormone-releasing acylated
14 peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans.
15
16
17 *Endocrinology*, **141**, 4255-4261.
18
19
20
21 (35) Cummings. D. E., Purnell. J. Q. & Frayo. R. S. *et al.* (2001) A preprandial rise in plasma ghrelin levels
22 suggests a role in meal initiation in humans. *Diabetes*, **50**, 1714–1719.
23
24
25
26
27 (36) Cummings. D. E., Shannon. M. H. (2003) Roles for ghrelin in the regulation of appetite and body weight.
28
29 *Arch Surg*, **138**, 389-396.
30
31
32
33 (37) Tschöp. M., Wawarta. R. & Riepl. R. L. *et al.* (2001) Post-prandial decrease of circulating human ghrelin
34 levels. *J Endocrinol Invest*, **24**, RC19-RC21.
35
36
37
38
39 (38) Drazen. D. L., Vahl. T. P. & D'Alessio. D. A. *et al.* (2006) Effects of a fixed meal pattern on ghrelin
40 secretion: evidence for a learned response independent of nutrient status. *Endocrinology*, **147**, 23–30.
41
42
43
44
45 (39) Theander-Carrillo. C., Wiedmer. P. & Cettour-Rose. P. *et al.* (2006) Ghrelin action in the brain controls
46 adipocyte metabolism. *J Clin Invest*, **116**, 1983-1993.
47
48
49
50
51 (40) Tschöp. M., Weyer. C. & Tataranni. P. A. *et al.* (2001) Circulating ghrelin levels are decreased in human
52 obesity. *Diabetes*, **50**, 707-709.
53
54
55
56
57 (41) Cremonini. F., Camilleri. M. & Vazquez Roque. M. *et al.* (2006) Obesity does not increase effects of
58 synthetic ghrelin on human gastric motor functions. *Gastroenterology*, **131**, 1431-1439.
59
60

- (42) Tagliacozzi. D., Mozzi. A. F. & Casetta. B. *et al.* (2003) Quantitative analysis of bile acids in human plasma by liquid chromatography-electrospray tandem mass spectrometry: a simple and rapid one-step method. *Clin Chem Lab Med*, **41**, 1633-1641.
- (43) Adrian. T. E., Ferri. G. L. & Bacarese-Hamilton. A. J. *et al.* (1985) Human distribution and release of a putative new gut hormone, peptide YY. *Gastroenterology*, **89**, 1070-1077.
- (44) Kreymann. B., Williams. G. & Ghatei. M. A. *et al.* (1987) Glucagon-like peptide-1 7-36: a physiological incretin in man. *Lancet*, **330**, 1300-1304.
- (45) Patterson. M., Murphy. K. G. & le Roux. C. W. *et al.* (2005) Characterization of ghrelin-like immunoreactivity in human plasma. *J Clin Endocrinol Metab*, **90**, 2205-2211.
- (46) Date. Y., Toshinai. K. & Koda. S. *et al.* (2005) Peripheral interaction of ghrelin with cholecystokinin on feeding regulation. *Endocrinology*, **146**, 3518-3525.