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► **To cite this version:**

Rachel E Roberts, Clare Glicksman, J Alaghband-Zadeh, Roy Sherwood, Naeem Akuji, et al.. The relationship between post-prandial bile acid concentration, GLP-1, PYY and ghrelin.. *Clinical Endocrinology*, 2010, 74 (1), pp.67. 10.1111/j.1365-2265.2010.03886.x . hal-00600463

**HAL Id: hal-00600463**

**<https://hal.science/hal-00600463>**

Submitted on 15 Jun 2011

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

Journal:	<i>Clinical Endocrinology</i>
Manuscript ID:	CEN-2010-000396
Manuscript Type/Office:	1 Original Article - UK/Europe
Date Submitted by the Author:	16-Jun-2010
Complete List of Authors:	Roberts, Rachel; King's College London, Medical School Glicksman, Clare; King's College London, Biochemistry Alaghband-Zadeh, J; King's College London, Biochemistry Sherwood, Roy; King's College London, Biochemistry Akuji, Naeem; King's College London, Medical School le Roux, Carel; Imperial College London, Imperial Weight Centre
Key Words:	Bile acids, Peptide YY (PYY), Glucagon-like peptide 1 < Investigations & Rx: < Pancreas, Ghrelin < Hormones/related: < Obesity/Lipids/Nutrition

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

3  
4 **Short title:** Bile acid and gut hormone responses.

5  
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21  
22 **Key terms:** Bile acids, peptide YY (PYY), glucagon-like peptide-1 (GLP-1), ghrelin

23  
24 **Funding:** Local R&D

25  
26 **Disclosure statement:** The authors have nothing to declare.

27  
28 **Ethical approval:** The study was approved by KCH Research Ethics Committee (Reference Number:

29  
30 05/Q0703/4)

31  
32 **Word count:** 1, 992

**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/m<sup>2</sup> (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.

## 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  $\alpha$  (FXR $\alpha$ ) (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 $\alpha$  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 $\alpha$ -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 $\beta$  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.).

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2 Gut hormones peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) play an integral role in appetite control  
3 and energy homeostasis. PYY and GLP-1 are secreted by L-cells of the distal small intestine in response to  
4 nutrients and bile acids (20-22). Exaggerated responses of PYY and GLP-1 may contribute to the weight  
5 loss and improvement in glycaemic control following gastric bypass surgery for the treatment of obesity. The  
6 mechanisms for increased concentrations of PYY and GLP-1 after gastric bypass are not entirely understood.  
7 The 'hindgut hypothesis', described by Mason (23), proposes that altered anatomy following bariatric surgery  
8 accelerates the delivery of nutrient-dense chyme to the distal intestine which stimulates increased gut  
9 hormones secretion. In addition plasma bile acid concentrations are doubled in individuals after gastric  
10 bypass surgery compared to obese individuals who have not had bariatric surgery (24) and it is possible that  
11 co-delivery of these bile acids within the chyme enhances the stimulation of gut hormone release post-  
12 gastric bypass.  
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24 A role of bile acids in appetite suppression was proposed as early as 1968 (25). Several studies indicate that  
25 bile acids deoxycholic acid and taurocholic acid combined with fatty acids are more significant than fatty  
26 acids alone in stimulating PYY release in the ileum (21,26-29). Bile acid infusions of taurochenodeoxycholic  
27 acid and deoxycholic acid have been shown to increase plasma PYY in rabbits (27,30) and deoxycholic acid  
28 infusion in the human colon stimulates PYY release in a dose-dependent manner (21). Importantly the  
29 concentrations of bile acids used in these studies are consistent with the physiological concentrations of bile  
30 acids found in the intestinal lumen post-prandially (31), supporting a physiological role of bile acids in the  
31 stimulation of PYY release. However, not all bile acids have been found to stimulate PYY release with  
32 hyodeoxycholic acid and ursodeoxycholic acid having no effect on PYY release (27,32). The reason why  
33 PYY would be stimulated by specific bile acids is not clear. The post-prandial peak concentrations of GLP-1  
34 and total bile acid concentrations inversely correlate with fasting and post-prandial glucose after gastric  
35 bypass (24).  
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49 Ghrelin is secreted from X/A-like cells of the fundus of the stomach (33,34) and may play a role in regulating  
50 pre-meal hunger, meal initiation and long-term energy balance (35,36). Circulating ghrelin concentrations  
51 increase during fasting and decrease after eating (37) while anticipation of meals may contribute to its  
52 secretion (38). The mechanisms by which ghrelin promotes hunger and increased energy intake are not  
53 entirely clear. There is some evidence to suggest that ghrelin may exert its effects by acting on the central  
54 nervous system (39). Obese subjects have lower fasting plasma ghrelin concentrations than lean controls  
55 (40), although appetite is commonly increased, suggesting that higher body mass is associated with  
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2 increased gastric responsiveness to ghrelin (41). A relationship between ghrelin and bile acids has not been  
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4 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  
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9 and ghrelin after a standard 400 kcal test meal.

## 10 11 **Methods**

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16 All studies were performed according to the principles of the Declaration of Helsinki. The Local Research and  
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18 Ethics Committee at King's College Hospital approved the study (05/Q0703/4). Written informed consent was  
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20 obtained.

### 21 22 *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/m<sup>2</sup> (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  
29 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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### 32 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*  
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37 (42). Bile acids analysed were chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), cholic acid (CA),  
38  
39 ursodeoxycholic acid (UDCA), lithocholic acid (LA) and their respective glycine (G-) and taurine (T-)  
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41 conjugates. The method was linear between 0.1-10 µmol/L for all bile acids and their conjugates with intra-  
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43 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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45 The lower limit of quantitation was 0.1 µmol/L. LCA and UDCA and their conjugates and CDCA were below  
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47 the detection limit. Glucose was measured using the glucose oxidase method on the Advia 2400 analyser  
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49 (Siemens Healthcare Diagnostics, Frimley, UK). Insulin was measured using a two site sandwich  
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51 chemiluminescent immunoassay on the Advia Centaur analyser (Siemens Healthcare). The intra-assay CV  
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53 was 3.3% and inter-assay CV was 4.8%. PYY was measured by an established in-house radioimmunoassay  
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55 as previously described by Adrian *et al* (43). The assay measured the biologically active components, both  
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57 the full length (PYY<sub>1-36</sub>) and the fragment (PYY<sub>3-36</sub>). The detection limit was 5 pmol/L and the intra- and inter-  
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59 assay CVs were 5.8% and 9.8%, respectively. GLP-1 was measured by an established in-house  
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radioimmunoassay as previously described by Kreymann *et al* (44). The detection limit was 7.5 pmol/L and  
the intra-assay CV was 6.1%. Ghrelin was measured by the commercially available Phoenix Pharmaceutical

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2 assay kit as previously described by Patterson *et al* (45). The assay measured total human ghrelin (acylated  
3 and des-acylated) and the intra-assay CV was 8.8%  
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### 5 6 7 *Statistical Analysis*

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9 Results are reported as mean (SEM) and correlation co-efficient (rs). Statistical analysis was performed  
10 using Analyse-It for Excel (Analyse-It, Leeds, UK). Data were normally distributed and a Spearman  
11 correlation was used. Significance is shown by a  $P < 0.05$ .  
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### 14 15 16 17 **Results**

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19 None of the unconjugated bile acids changed significantly throughout the study period but all glycine and  
20 taurine conjugated bile acids and total bile acids increased significantly (Table. 1.). The predominant bile  
21 acid measured was GCDCA, followed by GCA and GDCA. All other bile acids had peak concentrations of  $<$   
22 1.00  $\mu\text{mol/L}$ . Table. 2. and Figure. 2. show the changes in plasma hormones and glucose before and after  
23 the standardised 400 kcal meal.  
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26  
27 Correlations were sought between individual types of bile acids, PYY, GLP-1 and ghrelin. Bile acids which  
28 significantly correlated with PYY were GCDCA ( $rs = 0.23$ ,  $p = 0.03$ ), total glycine conjugated bile acids ( $rs =$   
29  $0.23$ ,  $p = 0.03$ ), TCDCA ( $rs = 0.26$ ,  $p = 0.02$ ) (Figure. 3.), total taurine conjugated bile acids ( $rs = 0.24$ ,  $p =$   
30  $0.03$ ) and total bile acids ( $rs = 0.26$ ,  $p = 0.02$ ). Bile acids which significantly correlated with GLP-1 were  
31 GCDCA ( $rs = 0.22$ ,  $p = 0.047$ ), GDCA ( $rs = 0.3$ ,  $p = 0.005$ ), total glycine conjugated bile acids ( $rs = 0.26$ ,  $p =$   
32  $0.02$ ) and total bile acids ( $rs = 0.26$ ,  $p = 0.02$ ) (Figure. 3.). Bile acids which significantly correlated with  
33 ghrelin were GDCA ( $rs = - 0.45$ ,  $p = < 0.0001$ ) (Figure. 3.), TCDCA ( $rs = - 0.23$ ,  $p = 0.03$ ), TDCA ( $rs = - 0.44$ ,  
34  $p = < 0.0001$ ), total taurine conjugated bile acids ( $rs = - 0.23$ ,  $p = 0.038$ ) and total bile acids ( $rs = - 0.22$ ,  $p =$   
35  $0.044$ ).  
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### 50 51 **Discussion**

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53 The CDCA group of bile acids GCDCA and TCDCA correlated with PYY. GCDCA also correlated with GLP-  
54 1. GCDCA and TCDCA share a common chemical structure with -OH hydroxylation at C7 and a single  
55 hydrogen ion at C12 (Figure. 1.). The positive correlation found between GCDCA and PYY and GLP-1 and  
56 TCDCA and PYY suggests that the specific chemical structure of bile acids may play a role in stimulating L-  
57 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

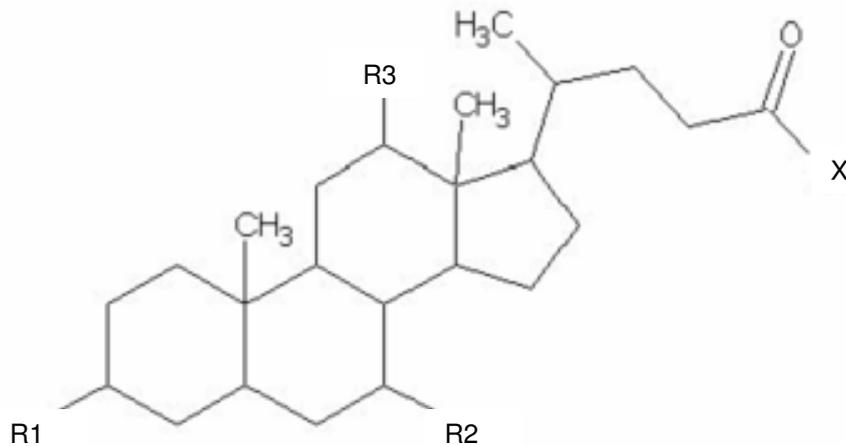
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Table. 2. Baseline and peak values (mean (SEM)) of plasma hormones and glucose following a 400 kcal test meal.

	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

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Figure. 1. The structure of bile acids.



Bile acid	R1 (C3)	R2 (C7)	R3 (C12)
CA	OH	OH	OH
CDCA	OH	OH ( $\alpha$ )	H
DCA	OH	H	OH
LCA	OH	H	H
UDCA	OH	OH ( $\beta$ )	H

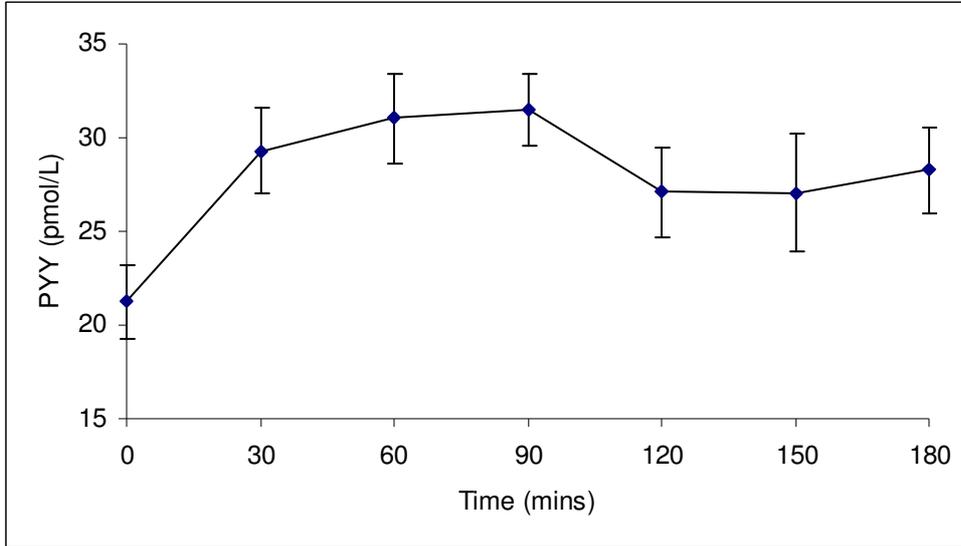
Legend:

X unconjugated - OH, X tauro- conjugated -  $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{SO}_3\text{H}$ , X glyco- conjugated -  $\text{NHCH}_2\text{COOH}$ , CA - cholic acid, CDCA - chenodeoxycholic acid, DCA - deoxycholic acid, LCA - lithocholic acid, UDCA - ursodeoxycholic acid

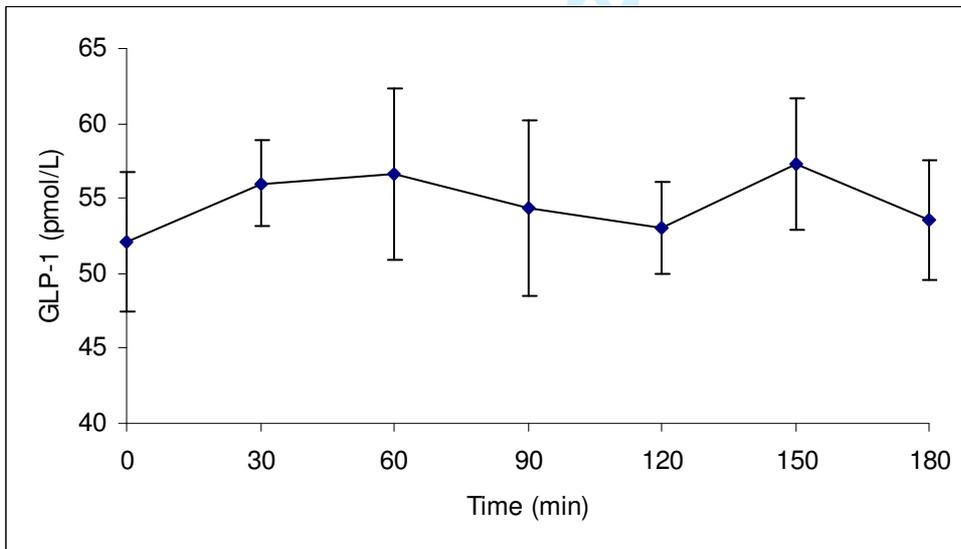
Figure. 2. Changes in plasma levels of gut hormones following a 400 kcal test meal in 12 subjects. Expressed as mean (SEM).

a) PYY, b) GLP-1 and c) ghrelin

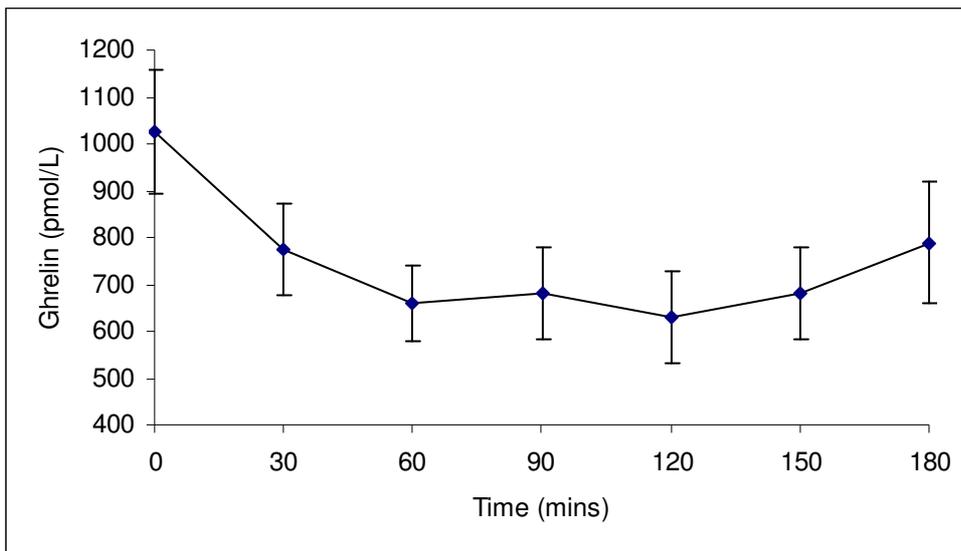
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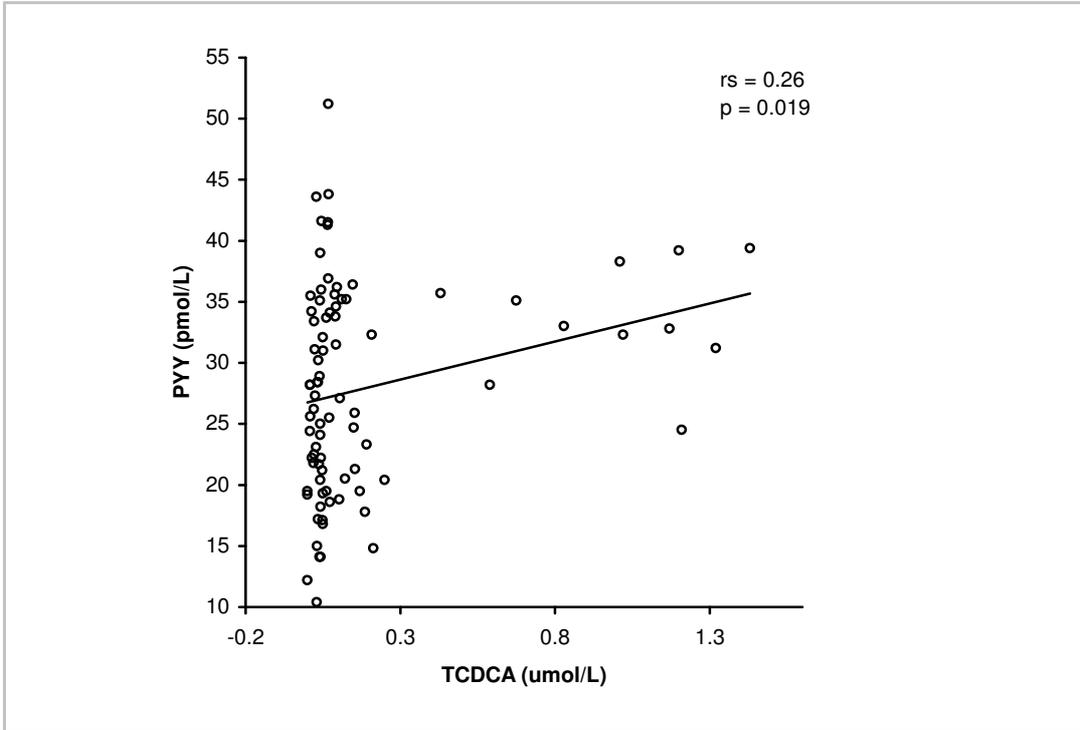
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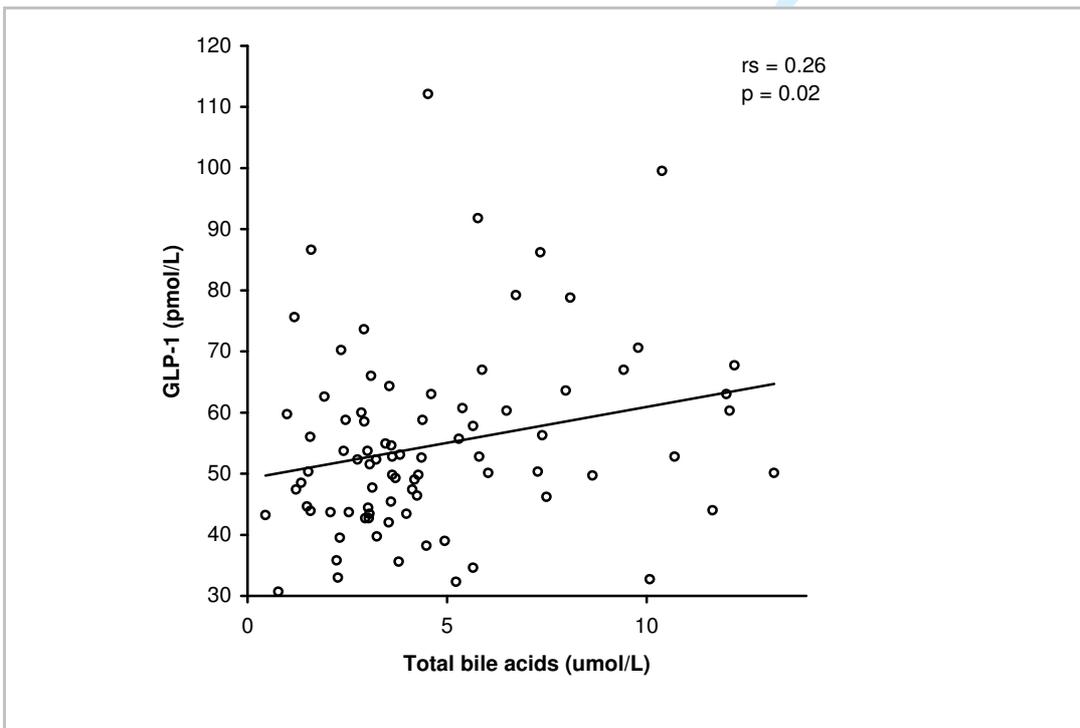
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Figure. 3. Scatter graph demonstrating the correlation between a) taurochenodeoxycholic acid (TCDCA) and PYY; b) total bile acids and GLP-1 and c) glycochenodeoxycholic acid (GDCA) and ghrelin.

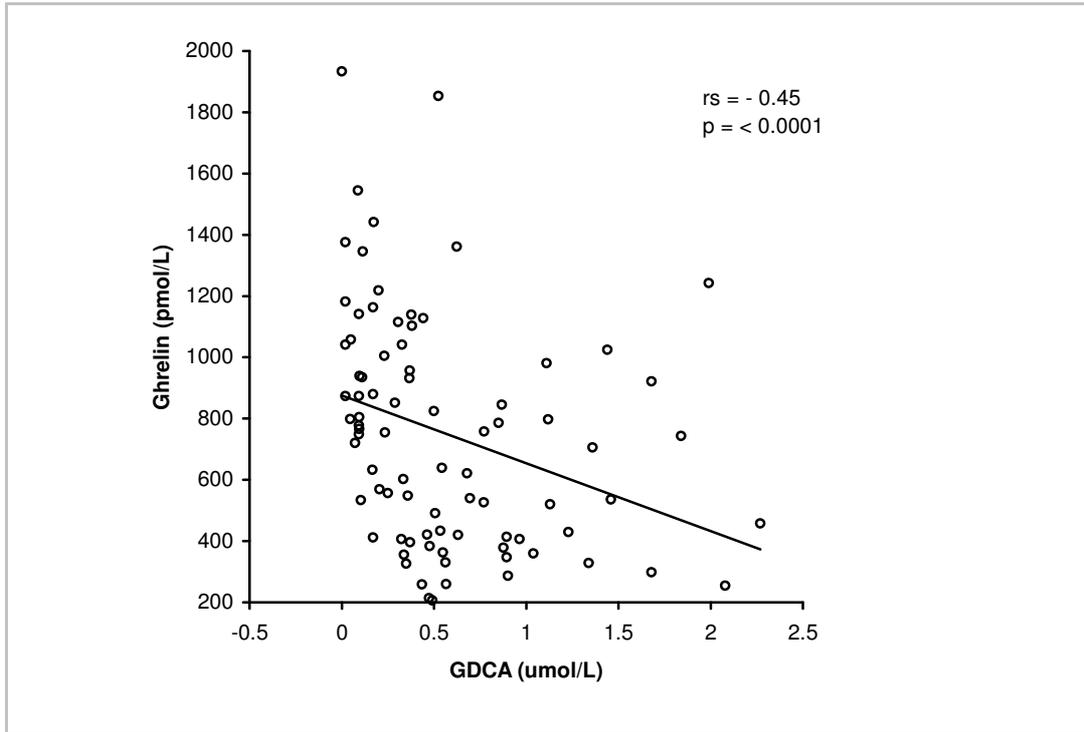
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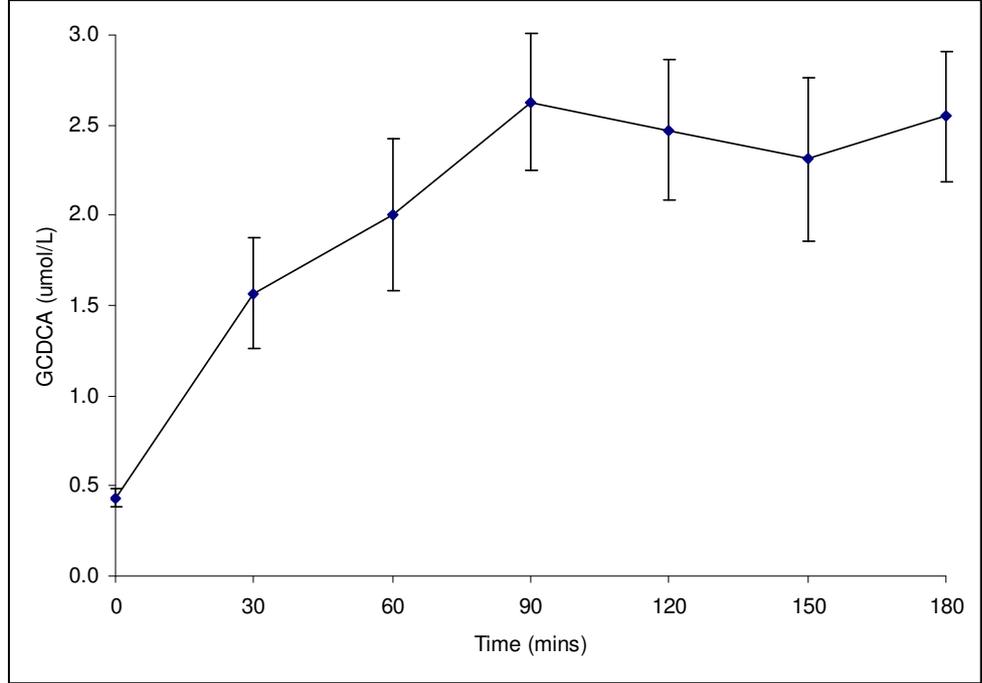


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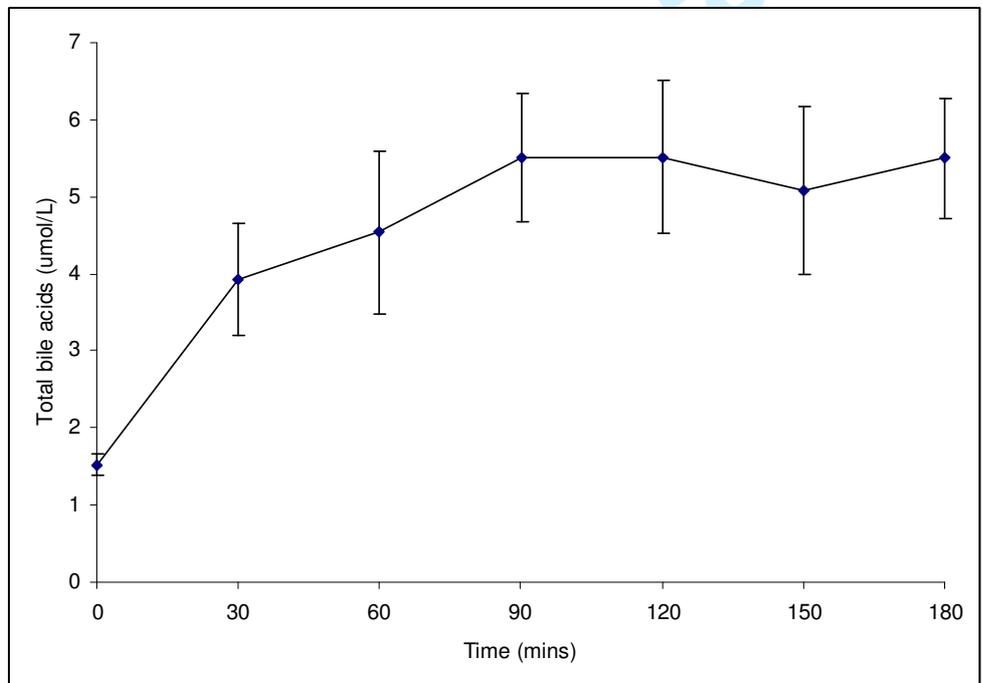
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Figure. 4. Line graphs demonstrating the response of a) glycochenodeoxycholic acid (GCDCA) and b) total bile acids following a 400 kcal test meal in 9 subjects. Expressed as mean (SEM).

a)



b)



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