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SHORT COMMUNICATION

Farnesoid X receptor - a molecular predictor of weight loss after vertical sleeve gastrectomy?

F. Scott[1], S. Elahi[1], M. Adebibe[2], U. Parampalli[2,3], K. Mannur[2], A. Góralczyk[2] and G. J. Sanger[1]

[1]Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London; [2]Bariatric Surgery Department, Homerton University Hospital, London; [3]Royal Sussex County Hospital, Brighton;

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Correspondence: Gareth J. Sanger, National Bowel Research Centre, 1st Floor, Abernethy Building, 2 Newark Street, London, E1 2AT.
E-mail: g.sanger@qmul.ac.uk

Summary

Objective

To determine the expression of the bile acid receptor, farnesoid X (FXR), in human gastric mucosa and investigate correlations between expression and body-mass index (BMI) and in patients with obesity, with changes in weight and BMI following vertical sleeve gastrectomy (VSG).

Methods

Human gastric mucosa was obtained from normal/overweight individuals (macroscopically-normal tissue following surgery for malignancy) or from patients with obesity (VSG). The expression of FXR and its isoforms (FXRα, FXRβ) were examined by quantitative PCR and compared with the G protein-coupled bile acid receptor, GPBA. In patients with obesity, changes in BMI and weight loss were determined following VSG.

Results

FXRα was the predominant isoform in normal/overweight individuals. FXR expression was higher in patients with obesity but GPBA receptor expression was unchanged. For those with obesity (n = 19), no correlation was found between FXR expression and change in Body-Mass Index (BMI)/month or weight loss/month, taken 3 ± 1 months after surgery, or in BMI or weight at surgery.

Conclusions

Obesity is associated with increased FXR expression in the gastric mucosa. The findings are preliminary but suggest that this increase in FXR expression is a consequence of obesity, rather than its cause.

Keywords: FXR, human, obesity, stomach.

Introduction

Bile acids, released into the small intestine from the liver where they are synthesized, regulate lipid absorption from the intestine. They also act at the farnesoid X receptor (FXR; a nuclear hormone receptor) and at the G protein-coupled bile acid receptor, GPBA (cell membrane bound and also known as TGR5 (1)). The latter is widely expressed, regulating for example, insulin sensitivity and glucose homeostasis through secretion of glucagon-like peptide-1, energy expenditure in brown adipose tissue and skeletal muscle, and production of pro-inflammatory cytokines by macrophages (2). FXR is expressed predominantly within the liver and gastrointestinal (GI) tract, where it is thought to regulate target gene activity in response to a ligand, modulating damage induced by inflammation (eg. gastric epithelial cells (3,4)) or ischaemia (5). In addition, FXR activation may repress cytochrome P450 7A (6) and induce the intestinal hormone, fibroblast growth factor 19, to activate hepatic FGF receptor 4 signalling and inhibit bile acid synthesis (7).

Of particular interest, observations using the FXR knock-out (FXR KO) mouse and mice with site-specific inhibition of FXR, provide evidence for a causative role of FXR in obesity acquisition, obesity related metabolic dysfunction and in the response to bariatric surgery (e.g.

the use of vertical sleeve gastrectomy (VSG) to reduce stomach volume and control the amount of food consumed) (8–11). For example, the weight loss caused by VSG in obese FXR KO and wild-type (WT) mice (fed with a high-fat diet) was not sustained in the KO mice; further, the FXR KO mice ate more following recovery from the surgery and exhibited impaired glucose tolerance. Together, these data suggested that FXR is involved in suppression of rebound hyperphagia which would follow VSG and the resultant calorie restriction (10). Subsequently bile acids and FXR have been proposed as key mediators of diabetes resolution and weight loss following bariatric surgery (12).

Little is known about the role of FXR in the response to VSG or in the development and maintenance of human obesity. Haeusler et al[13] found no correlation between Body-Mass Index (BMI) and total FXR expression (all isoforms) in human ileum and liver, but positive correlation with expression of the α isoform of FXR in the human liver. Here it is important to note that utilization of two alternative promoters of the FXR gene gives rise to FXRα and FXRβ isoforms with divergent N terminals (14) later characterized, respectively, as FXR 1/2 isoforms and as FXR 3/4 isoforms (13). Each isoform has two further splice variants, differing in a 12 BP insert in the hinge region between DNA and ligand binding. FXR isoforms are differentially expressed in tissues and differ in their activation of FXR gene target promoters, therefore providing the potential for a range of different cell-specific transcriptional activities mediated via FXR (14).

In humans, surgical intervention for obesity (VSG or Roux-en-Y gastric bypass) has been reported to increase circulating plasma concentrations of bile acids (15–17), argued to contribute to weight loss and restoration of normal glucose sensitivity (18,19). Similarly, in patients with obesity, bile acids within the plasma may be high during fasting, with further increases blunted post-prandially and in response to insulin (13). However, the expression and activity of FXR during and following bariatric surgery in humans is not known. In obese rats, VSG is associated with higher bile acids and also increased FXR mRNA expression in the liver (20).

This study has looked for an association between body-mass index (BMI) and levels of FXR, FXRα and FXRβ isoform expression in human proximal stomach mucosa obtained from normal/overweight individuals (macroscopically-normal tissue from patients with cancer) and during VSG from patients with obesity, comparing the findings with expression of the GPBA receptor in each of the patient groups. The focus on the stomach was justified by the exposure of this organ to bile acids circulating in the blood in general, increased during obesity and also after VSG or Roux-en-Y gastric bypass (see references above). In preliminary studies, these data were also used to determine if levels of gene expression could predict changes in BMI and if in patients with obesity, predict weight loss during the three month follow-up after the VSG procedure.

Methods

After approval by the local ethics committee (REC 10/H0703/71), written informed consent was obtained for use of gastrointestinal tissue from patients undergoing elective surgery (vertical sleeve gastrectomy) or the resection of gastric tumour.

Proximal stomach tissue was obtained from the excised vertical sleeve after surgery (n = 19) or macroscopically normal region of tissue excised in malignancy surgeries prior to chemotherapy (n = 7, 2 of whom were classified as obese). This region consists of gastric fundus and corpus, being defined as up to and including 5 cm from the most proximal point of resected tissue (vertical sleeve resections for control of obesity) or, when obtained from stomach removed because of malignancy, by a pathologist (sample taken from macroscopically normal areas, at least 10 cm from the tumour). Patients undergoing vertical sleeve resection (n = 19) were aged 25–54 years and had a BMI range of 43–68 kg/m^2 (class III obesity). These patients were followed up for measurement of weight loss and changes in BMI 3 ± 1 month after surgery. Patients undergoing resection for gastric malignancy were subdivided into normal-overweight (n = 5); aged 28–88 years, BMI range of 20–29 kg/m^2, and class I obese (n = 2); aged 48–61, BMI range of 32–37 (see https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight for WHO classification of overweight and obesity).

Human colon was obtained from patients undergoing surgery for excision of non-obstructing bowel cancer; again, macroscopically-normal tissue, at least 10 cm from the tumour was provided by a pathologist.

Tissues were transferred from theatre to the research laboratory within 1 hour of resection in Krebs solution (mmol·L^{-1}: NaCl 121.5, $CaCl_2$ 2.5, KH_2PO_4 1.2, KCl 4.7, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 5.6) at room temperature, equilibrated with 5% CO_2 and 95% O_2. Mucosa was removed by blunt dissection and stored in RNAlater® (Sigma) at −80°C.

Tissue stored in RNAlater® was thawed, immersed in TriReagent® (Sigma) and homogenized using a TissueRuptor (Qiagen). Tissue debris was sedimented by centrifugation and RNA was extracted and purified from the supernatant using Direct-zol™ RNA Miniprep (Zymo Research). cDNA was synthesized from 1.9 μg

RNA using SuperScript™ VILO™ cDNA Synthesis Kit (Invitrogen).

Real Time Polymerase Chain Reaction (PCR) amplification of FXR (5'-ACAACAAAGTCATGCAGGGAGA, 5′-CCTGAGGCATCCTCTGTTTG), FXRα (5'-GCTGGGATC TGGAGAGGAAGA, 5′- TTGGGTCAGAGATGGACTT TCA) and FXRβ (5'-GCTGTACGCCGTCAGGATTT, 5-GGACCTGCCACTTGTTCTGTTAA) of the proximal stomach mucosa and ascending colon mucosa was followed by agarose gel electrophoresis.

FXR, GPBA (TGR5) receptor, FXRα, FXRβ and GAPDH expression was subsequently quantified using TAQMAN™ (Applied Biosystem) PCR measurement and assay IDs Hs01026596_m1 (FXR), Hs00544894_m1 (GPBA receptor), Hs01026591_m1 (FXRα) and Hs02758991_g1 (GAPDH). An additional assay for FXRβ was custom designed utilizing an intron spanning sequence specific to FXRβ.

cDNA was diluted (1:4) and reactions were conducted in triplicate using StepOnePlus™ real time PCR System (Applied Biosystem).

Expression of each gene was normalized to reference GAPDH and relative expression calculated (ΔΔCt method). Data are expressed as median and interquartile ranges (IQR) and outliers are defined as <1.5 IQR from the nearest quartile. Statistical significance ($P \leq 0.05$) was determined using Mann–Whitney t-test or by Spearman's rank-order correlation.

Results

Preliminary experiments found FXRα and FXRβ, respectively, to be the dominant FXR isoforms in human proximal gastric and ascending colon mucosa (Figure 1a). a). Subsequently, using the TAQMAN™ system, FXRα and FXRβ transcripts were detected in human gastric mucosa from normal/overweight individuals; consistently, FXRα was the predominant isoform (Figure 1b).

FXR expression was higher in proximal gastric mucosa of patients with obesity compared to normal/overweight subjects (respectively 1.81 [1.21–2.70] and 0.83 [0.76–1.33]; $n = 21$ and 5; $P = 0.032$). However, no difference was observed in GPBA receptor expression in tissues from these two groups of patients (Figure 2a), suggesting the obesity-related increase in expression is specific to FXR and not a generalized effect on bile acid receptors.

The obesity related increase in total FXR expression could not be attributed to specific changes in expression of FXRα or FXRβ isoforms (Figure 2b).

Changes in BMI status after vertical sleeve gastrectomy were monitored for any evidence of a correlation

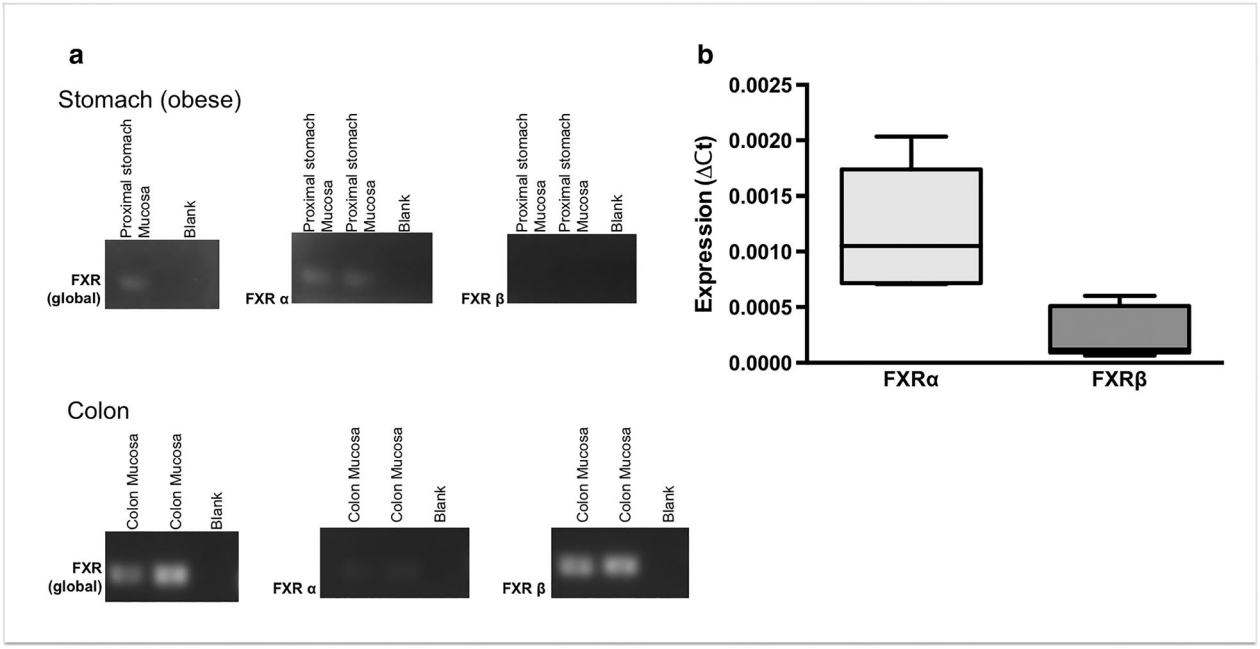

Figure 1 Expression of FXRα and FXRβ isoforms in the mucosa of human proximal stomach and colon. Tissue was obtained from patients undergoing vertical sleeve gastrectomy (stomach) and bowel cancer (macroscopically-normal colon removed at least 10 cm from the tumour) and gene expression was investigated and quantified using TAQMAN™ assays. (a) Stomach and colon FXR expression was validated in PCR and agarose gel analysis. (b) In the stomach, expression was quantified and normalized to reference GAPDH and relative expression calculated (ΔΔCt method). Data are expressed as median and interquartile ranges. Statistical significance between expression of the two isoforms was determined using the Mann–Whitney U-test; $P = 0.008$, $n = 5$ patients.

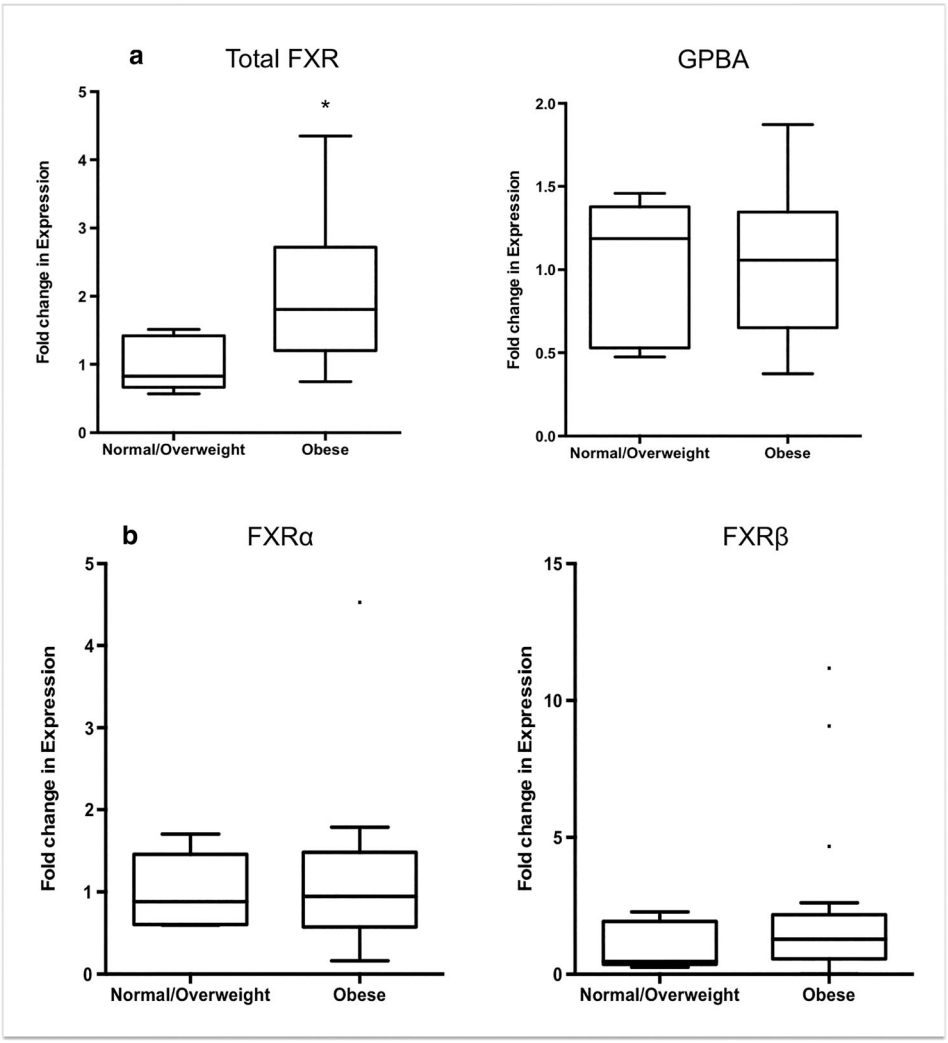

Figure 2 Relative expression of (a) total FXR, GPBA receptor and (b) FXRα and FXRβ in normal/overweight and in patients with obesity, in proximal stomach mucosa. Tissue was obtained from patients undergoing resection of gastric tumour (normal/overweight patients; macroscopically-normal tissue removed at least 10 cm from the tumour; n = 5) and vertical sleeve gastrectomy (class I/III obese patients; *n* = 21). Gene expression was quantified using TAQMAN™ assays. Expression was normalized to reference GAPDH and relative expression calculated (ΔΔCt method). Data are expressed as median and interquartile ranges; note the different scales used for the vertical axes. Statistical significance between expression in the two groups was determined using the Mann–Whitney U-test; * $P > 0.05$.

between FXR expression and the response to surgery. Clinical information collected at the first follow up appointment (3 ± 1 month) from the cohort of patients who underwent vertical sleeve gastrectomy (*n* = 19) was used to investigate any influence of FXR expression on an individual's response to the bariatric surgery, measured by percentage change in BMI per month.

Figure 3 and Table 1 demonstrates the absence of correlation between total FXR expression (FXRα and β isoforms), nor specifically FXRα or FXRβ expression, and the response to the bariatric surgery (measured by ΔBMI/month and as ΔWeight-loss/month, calculated from BMI/Weight measurements taken 3 ± 1 months following surgery). There was also no association between the total and α- and β- isoform expression and the BMI (kg/m^2) or weight (kg) at time of surgery (Table 1).

Fifteen of the 19 patients undergoing vertical sleeve gastrectomy were previously diagnosed as diabetic. Of those patients, there was no apparent association between HbA1c levels or random glucose levels, with gastric expression of FXR, FXRα or FXRβ; further there was no apparent association between the diagnosis of diabetes or the absence of this diagnosis and the expression of FXR or its isomers ($P > 0.1$ each; data not shown).

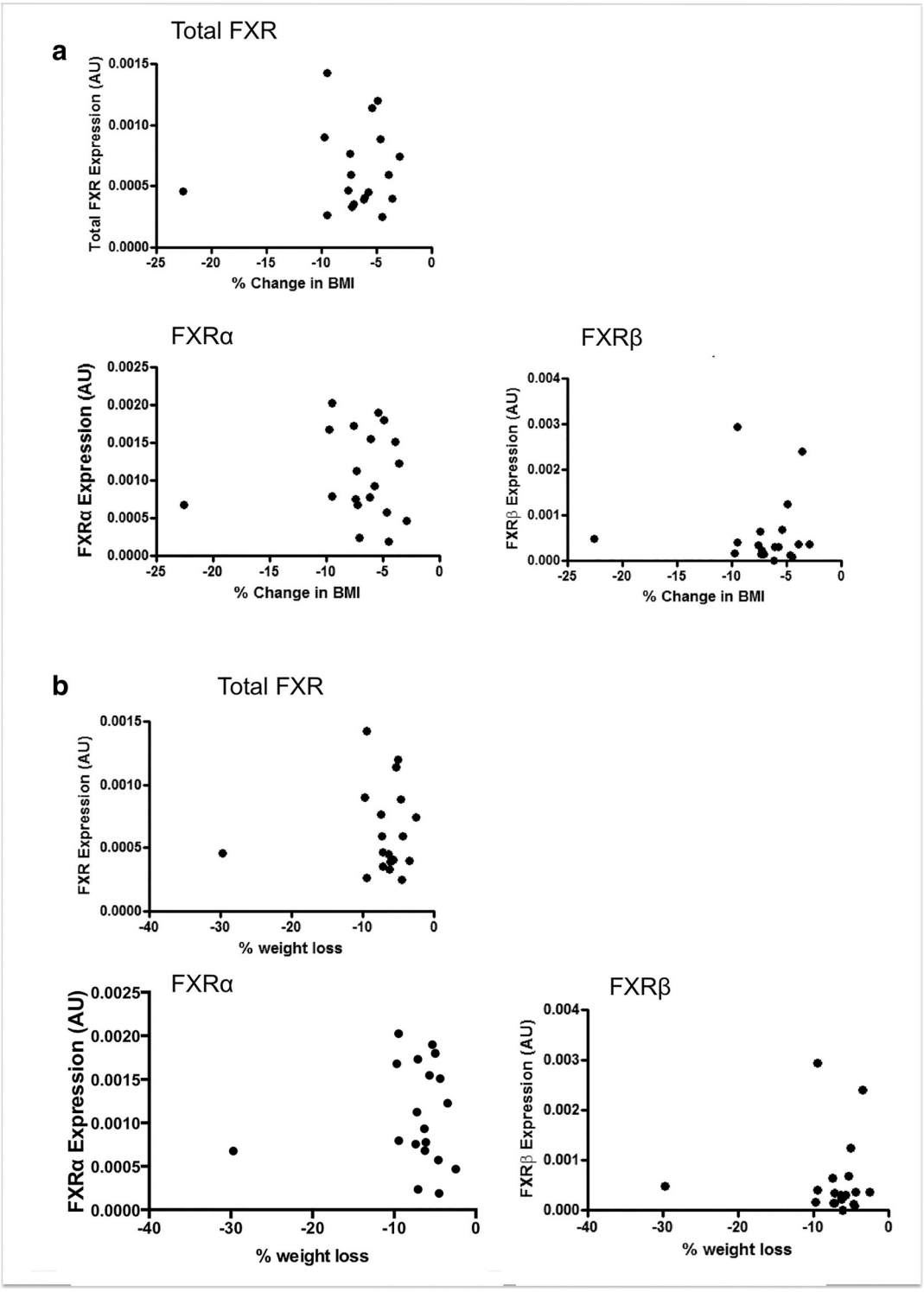

Figure 3 Changes in (a) BMI and (b) weight following vertical sleeve gastrectomy for obesity (class I/III obese patients) do not correlate with FXR (respectively $P = 0.85$ and 0.76; Spearman's rank-order correlation; $n = 19$), FXRα ($P = 0.42$ and 0.50) or FXRβ ($P = 0.81$ and 0.79) expression in the stomach at the time of surgery. BMI and weight loss measurements were taken 3 ± 1 month's following surgery and changes are expressed per month. The expression of FXR and its isomers were quantified in proximal stomach mucosa using TAQMAN™ assays. Expression was normalized to reference GAPDH and relative expression calculated (ΔΔCt method).

Table 1 Responses to vertical sleeve gastrectomy in obese patients, determined as changes in BMI and weight per month, compared with the expression of FXR in the excised stomach at time of surgery. The data are given in descending order according to the patients with the greater BMI at time of surgery. Weight and BMI was measured on the day of surgery. FXRα, FXRβ and total FXR expression was measured in the proximal stomach mucosa of the excised sleeve and is expressed relative to GAPDH control ($2^{-\Delta Ct}$). Response to bariatric surgery at the first follow up (3 ± 1 month) is tabulated as percentage change in weight or BMI per month.

Patient	BMI at Surgery (kg/m^2)	Weight at Surgery (kg)	FXRα Expression ($2^{-\Delta Ct}$, AU $\times 10^{-4}$)	FXRβ Expression ($2^{-\Delta Ct}$, AU $\times 10^{-4}$)	Total FXR Expression ($2^{-\Delta Ct}$, AU $\times 10^{-4}$)	Δ BMI/month	Δ Weight/month
1	63.7	178	15.47	3.13	4.09	−6.02%	−5.68%
2	63.3	168	11.23	1.52	5.92	−7.27%	−7.20%
3	62.4	198	6.76	4.79	4.61	−22.52%	−29.69%
4	61.7	147	16.81	1.73	8.98	−9.72%	−9.68%
5	61.6	157	5.72	1.33	8.85	−4.59%	−4.59%
6	60.1	150	1.88	0.76	2.46	−4.49%	−4.50%
7	58.2	162	20.24	29.53	14.25	−9.45%	−9.46%
8	57.0	156	2.34	1.40	3.51	−7.02%	−7.07%
9	55.5	198	18.98	6.88	11.38	−5.41%	−5.32%
10	51.4	201	7.94	3.98	2.66	−9.44%	−9.43%
11	51.2	132	4.69	3.54	7.43	−2.86%	−2.46%
12	50.8	108	9.33	3.08	4.55	−5.71%	−6.29%
13	49.6	150	17.99	12.34	12.04	−4.91%	−4.96%
14	49.4	136	17.32	3.35	4.63	−7.52%	−7.09%
15	49.2	146	7.76	0.00	3.92	−6.10%	−6.13%
16	46.6	140	6.81	2.25	3.29	−7.19%	−6.23%
17	46.6	117	12.26	23.96	3.96	−3.51%	−3.45%
18	46.1	121	7.56	6.53	7.66	−7.38%	−7.37%
19	43.7	120	15.10	3.55	5.97	−3.89%	−4.36%

Discussion

FXR expression within the human proximal gastric mucosa was measured in normal/overweight patients and compared with similar measurements in patients with established and predominantly severe (Class III) obesity. Although FXRß was detected, in each group, FXRα was the predominant isoform in the stomach. In separate experiments, FXRß was found to be the predominant isoform in the ascending colon. Expression of FXRß in the colon is consistent with the findings of Huber et al[14] but the present data obtained using human gastric mucosa contrast with the low levels or lack of detection of both FXRα and FXRß by Huber et al[14] in this area of the human gastrointestinal tract. Perhaps the difference is explained by the use of proximal gastric mucosa in the present study – Huber et al[14] did not specify the use of any particular region of stomach (or colon) and did not indicate that the mucosa and muscle had been separated.

In the gastric mucosa, the expression of FXR was higher in patients with obesity compared to normal/overweight subjects, but no difference was observed in GPBA receptor expression in tissues from these two groups of patients. These data suggest that the obesity-related increase in expression is specific to FXR and not a generalized effect on bile acid receptors. Among the 19 patients with obesity, 15 had been diagnosed with diabetes. Studies in rats have suggested that FXR expression within the liver may be reduced as a result of diabetes (21). In the present study, there was no apparent association between FXR expression, diabetes diagnosis, HbA1c levels or random glucose levels, but such data are difficult to interpret in the absence of data pertaining to the full history of the diagnosis and its treatment.

The obesity-related increase in total FXR expression could not be attributed to specific changes in expression of FXRα or FXRβ isoforms. This is difficult to explain. Unlike the median fold-changes for FXRα, those for FXRß were greater than those for the normal/overweight patients, but the variation in data made it impossible to draw conclusions; perhaps a more extensive study, examining a greater numbers of patients, is required. Notably, Haeusler et al[13], using human ileum (mucosa and muscle together) and liver, found no correlation between BMI and total FXR expression (all isoforms) but recorded a positive correlation between BMI and expression of the α isoform of FXR in the liver. The data obtained in this study and in the present work may therefore be regarded as broadly consistent with the suggestion of an association between obesity and the level of FXR expression, but with the exact relationship depending on the tissue being studied.

Nevertheless, such an association does not distinguish between a cause or effect relationship between FXR and obesity. To gain some insight into the answer to this question, changes in BMI status and weight loss after vertical sleeve gastrectomy were monitored for any evidence of a correlation with FXR expression (at time of surgery). The data are preliminary but suggest no clear association between FXR expression (FXR, FXRα and β isoforms or ratio of FXRα to β) and response to the bariatric surgery (measured as change in BMI/month or as weight-loss/month, calculated from measurements taken 3 ± 1 month following surgery). Different possibilities exist to explain these data. For example, FXR receptors are expressed in different regions of the gastrointestinal tract, potentially affecting multiple metabolic pathways and influencing obesity in ways not measured during the present study. Alternately, our findings can be argued to suggest that the observed changes in FXR expression during human obesity are an effect of the obesogenic state/sustained intake of excess energy, rather than a cause.

In conclusion, the present study is preliminary and short-comings need to be addressed in future studies. These include the need to measure bile concentrations circulating in the blood and the expression of FXR protein before and after VSG, in both the stomach and the intestine (exposed to higher concentrations of bile). In addition, it remains a possibility that the data may be somehow confounded by different dietary behaviours and weight trajectories between obese and cancer patients and by aberrant gene expression within the 'macroscopically-normal' mucosa adjacent to the tumour (22). Careful studies of patient behaviours, in conjunction with measurements of bile concentrations and FXR expression are therefore required to confirm or modify the present conclusions. Future studies should also monitor changes in BMI over longer periods of time following the gastrectomy (and in larger groups of patients), so as to maximize the opportunity to detect any trends towards weight reduction. Nevertheless, the correlation between FXR expression in the gastric mucosa and obesity (in the absence of any correlation between obesity and expression of the G protein-coupled bile acid receptor), together with the increased expression of FXRα in the liver of the obese (13), begin to raise significant questions about the precise tissue specific role of FXR in the mechanisms of obesity and in methods of regulating FXR function to control obesity (e.g. when considering FXR agonism as a therapeutic strategy). Furthermore, it is important to note that these data, obtained in human tissues, appear to contrast with a reduction in FXR expression in the liver of rats made obese by exposure to a high-fat diet (23) and with no change in FXR expression in the liver of obese mice exposed to a high fat diet (24); in the latter experiments FXR expression was reduced during weight loss when switching to a low fat diet. Several reasons could explain these differences between the human and rodent data (e.g. model of obesity and tissue being studied). Nevertheless, the difference raises additional questions about the degree of translation from rodent models to humans, especially in terms of gastrointestinal functions (e.g. relating to those changes in gastric anatomy, innervation, functions and gene expression related to the inability of these animals to vomit and experience true nausea (25,26)) and the functions of bile acids, which themselves differ markedly between the species (27).

Finally since in the present study, measurements were made using human stomach removed during VSG it is important to note that this organ is exposed to bile acids circulating in the blood and as a result of gastro-duodenal reflux, exposure to bile acids is increased during obesity and also after Roux-en-Y gastric bypass (see Introduction for references). In this organ, FXR is thought to regulate genes involved in gastric epithelial protection (28,29). Elsewhere in the GI tract FXR may help regulate functions of mucosal endocrine cells (30) and has been implicated in intestinal barrier integrity, controlling the response to and growth it's of commensal bacteria (31). The extent to which these and other actions of FXR, determined in animal models, translate to humans now needs to be studied in greater detail.

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Conflict of interest statement

GJS is currently in receipt of funding from Takeda Pharmaceuticals and currently consults for Takeda and Zealand Pharma.

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Author contributions

F.S. conducted the experiments and designed primers for this study. S.E. helped to identify patients, collate their clinical data and assisted in stomach collections. U.P.

M.A. and A.G. identified the patients with obesity and obtained informed consent. U.P. and A.D. initiated the interest in FXR and its potential role in obesity. F.S., G.J.S., wrote the manuscript.

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