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5-hydroxytryptamine signaling in irritable bowel syndrome with diarrhoea: effects of gender and menstrual status

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ABSTRACT

Background and Aims: Symptomatology and physiology differ between males and females, and across the menstrual cycle in IBS. Ovarian hormones influence 5-HT, an amine known to play a role in gut motor-sensory function. Our aim was to assess the effects of gender and menstrual status on platelet depleted plasma (PDP) 5-HT concentration in IBS-D patients compared with healthy volunteers (HV).

Methods: PDP 5-HT concentrations were assessed under fasting and fed conditions in 73 IBS-D patients (aged 18-58 yr;18 male) and 64 HV (aged 18-50 years;24 male). Females were divided into those with low or high progesterone/oestrogen (P/O) levels.

Results: IBS-D patients had higher PDP 5-HT concentrations than HV under fasting ($p=0.002$) and fed ($p=0.049$) conditions. This was particularly related to IBS-D males having higher PDP 5-HT concentrations than healthy controls ($p=0.002$). Moreover PDP 5-HT concentrations in IBS-D females with low P/O levels were similar to healthy controls.

Conclusions: Similarly to IBS-D females with high P/O levels,[13,14] IBS-D males also have raised PDP 5-HT concentrations. 5-HT concentration normalizes at menses in IBS-D females, suggesting a shift in the mechanisms responsible for abnormal 5-HT signaling in these patients.

INTRODUCTION

Various observations point to a possible role for sex hormones in the clinical presentation and pathophysiology of irritable bowel syndrome (IBS). For example, IBS is twice as common in women than men,[1] and is diagnosed more often in women with than without dysmenorrhea.[2] Women with IBS also report a worsening of symptoms and a loosening of stools immediately prior to and during menses,[3] the latter being more exaggerated than that reported by healthy volunteers.[4] Physiologically, female IBS patients exhibit increased rectal sensitivity compared with male patients[5] which is further enhanced at menses compared with the other phases of the menstrual cycle.[3] This contrasts with observations in healthy volunteers, where generally there are no differences in rectal sensitivity between men and women[6] or across the menstrual cycle.[7] Despite a lack of information on the effect of gender and menstrual status on gastrointestinal motility in IBS, studies in healthy volunteers have shown women to generally have slower gastric emptying[8] and colonic transit,[9] and reduced colonic motility[6] to men. In addition, gastrointestinal transit tends to be slower in the luteal compared with the follicular phase of the menstrual cycle in healthy women.[10] Although these observations are thought to be related to the inhibitory effects of oestrogen, and particularly progestrone, [10,11] the precise mechanisms of action remain unclear.

5-Hydroxytryptamine (5-HT) is known to play a key role in the motor-sensory function of the gastrointestinal tract.[12] Moreover, we and others have shown that platelet depleted plasma 5-HT concentration increases with meal ingestion,[13,14,15,16] and that IBS patients with diarrhea (IBS-D) exhibit abnormally elevated[13,14,16] but IBS patients with constipation (IBS-C) reduced[14,15] postprandial 5-HT concentrations compared with healthy volunteers. These observations may be associated with the increased and decreased motility seen in IBS-D and –C patients, respectively.[1] Indeed we have recently shown that platelet depleted plasma 5-HT concentration directly correlates with colonic motility during the luteal phase of the menstrual cycle of both IBS and healthy subjects.[17] Whether endogenous plasma 5-HT concentrations relate to visceral sensation is unknown but studies which have pharmacologically raised plasma 5-HT concentration with selective serotonin re-uptake inhibitors ,[18] have shown no effects on visceral sensation[18,19,20] or compliance[18,19, 20] under fasting conditions, although they have been

shown to enhance the amplitude of the meal-induced fundic relaxation.[19] However, in none of the latter visceral sensitivity studies was the time of the menstrual cycle controlled.

As oestrogen and/or progesterone have been shown to be capable of influencing the 5-HT system within the brain,[21] by reducing serotonin reuptake transporter (SERT)[22] and monoamine oxidase (MAO) mRNA[23,24] expression, and increasing the availability of the 5-HT precursor, tryptophan,[23,25] all of which could potentially increase the availability of 5-HT, it was the aim of this study was to assess the effect of gender, and progesterone and oestrogen status on both fasting and fed concentrations of platelet depleted plasma 5-hydroxytryptamine (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in patients with IBS-D and to compare them with healthy volunteers.

MATERIALS AND METHODS

Subjects

This study was carried out on 73 IBS patients with diarrhea (aged 18-58 yrs; mean age 32 yrs; 18 male) and 64 healthy volunteers (aged 18-50 yrs: 28 yrs; 24 male). IBS patients with either constipation or an alternating bowel habit were excluded from the study. IBS patients were recruited from the Out Patients Departments of the University Hospital of South Manchester NHS Foundation Trust (tertiary patients excluded), local general practices, advertisement in regional news papers and an existing departmental volunteer pool of patients, and all satisfied the Rome II criteria for IBS with diarrhea.[26] No subject had co-existent disease and all had normal haematology, biochemistry, urinalysis and sigmoidoscopy, together with a normal colonoscopy or barium enema if aged over 50 years. Age and sex matched healthy volunteers were recruited by advertisement, and all had normal laboratory investigations (as above) and negative toxicology for substances of abuse. Subjects were excluded if they: had a history of gastrointestinal surgery (other than appendectomy and hiatus hernia repair); had gastrointestinal symptoms related to or exacerbated by consumption of milk or milk products; or were taking drugs that might modify either gastrointestinal function or the 5-HT system, such as analgesic medication, tranquillizers or antidepressants. Female subjects were excluded if they were pregnant, breast feeding or hysterectomised, and all were post-pubertal, pre-menopausal with a

regular menstrual cycle (mean 28 days). None of the females described chronic gynaecological symptoms. All medications and cigarette smoking were stopped for 48 hours prior to the study. All subjects drank below the recommended safe alcohol limit (<21 units/week), smoked < 5 cigarettes per day, and had not participated in a clinical trial of any drug within the previous 30 days. Written consent was obtained from all subjects and the study was approved by the South Manchester Medical Research Ethics Committee.

Study protocol

Female IBS-D and healthy subjects were studied either during the luteal phase of the menstrual cycle (days 18-20), when progesterone and oestrogen levels are high, or whilst taking combined non-phased oestrogen/progesterone contraceptive medication (ie high P/O) (39 IBS-D females, aged 19-52 yrs; mean 32 yrs; 19 healthy females, aged 19-50 yrs, 30 yrs) or at menses (days 2-3) when hormone levels are low (ie low P/O) (16 IBS-D females, aged 18-45 yrs, 28 yrs and 21 healthy females, aged 19-48 yrs, 25 yrs). In addition, 18 male IBS-D patients, aged between 20 – 58 yrs (mean age 37 yrs) and 24 healthy male volunteers aged 18 – 46 yrs (28 yrs) were studied.

All subjects presented to the Neurogastroenterology Unit after fasting for at least 10 hours, and an arm vein was cannulated. Nine milliliters of blood was taken via EDTA vacutainer at 0 hours for platelet count and 5-HT/5-HIAA analysis. Additional 5 ml blood samples were taken at hourly intervals for 2 hours under fasting conditions and at half hour intervals for a further four hours following ingestion of a standard carbohydrate-rich meal, consisting of 200g spaghetti in tomato sauce (Heinz, Stockley Park, Uxbridge, UK), 2 medium slices of toast, a jam and fresh cream scone (Marks and Spencer, London, UK) and 200 ml water (totaling 65.5g carbohydrate, 12g protein and 16g fat, calorie content of 457 kcal), which was consumed within 10 minutes.[13,14]

Symptomatology was assessed on attendance at the laboratory with the question “Is your IBS active at the moment?” In addition, at hourly intervals (0, 1, 2, 3 hours….) throughout the study, questions targeting the presence and severity of abdominal pain/discomfort, bloating, and bowel urgency were asked, eg. “In the past hour, have you experienced abdominal pain/discomfort?” If the subjects reported “yes” they were then

asked to grade the severity of that symptom using the scale 1=mild, 2=moderate, 3=intense, and 4=severe.

Measurement of platelet depleted plasma 5-HT and 5-HIAA concentrations

The collected blood (t = -2 to 4 hours postmeal) was transferred to tubes containing 0.5 ml of 3.12% trisodium citrate and centrifuged (room temperature) twice to ensure no platelet contamination of supernatant; initially at 2500 rpm for 10 minutes and then at 4000 rpm for a further 10 minutes. Platelet depleted plasma was aspirated, and duplicate samples stored at -70^{o}C for later batch analysis which was carried out blind to subject status. 5-HT and 5-HIAA concentrations were measured in duplicate using reverse-phase, high-performance liquid chromatography (HPLC) with fluorimetric detection and the sensitivity of the HPLC system was as previously described.[13,14, 27] Previous studies[13,14,17] in which we employed the exact same method of blood collection and preparation showed that there was little or no platelet activation, as indicated by undetectable levels of β-thromboglobulin, a marker for platelet activation. The β-thromboglobulin detection limit in these studies was 0.1fg per well, which equates to less that 0.1% of platelet activation.

Measurement of platelet 5-HT concentration

The blood collected at t = -2 hours was transferred to a tube containing 0.9 ml 3.12% trisodium citrate and initially spun only once at 900 rpm for 5 minutes. One aliquot of aspirated, platelet-rich plasma was then used to assess platelet count (Advia Centaur Analyser; Bayer Ltd, Berkshire, UK), whereas additional duplicate samples were used for 5-HT analysis of platelet-rich plasma, and following further centrifugation (as above) for analysis of platelet-depleted plasma 5-HT/5-HIAA concentrations.[13,14]

Data and statistical analysis

The following end-points were analysed for platelet depleted plasma 5-HT and 5-HIAA concentrations: (i) fasting concentrations, calculated as the average of the preprandial measurements (nmol/L); (ii) fed concentration, calculated as the average of the postprandial measurements (nmol/L); and (iii) ratio of average fed to fasting concentrations.

Factorial analysis of variance and analysis of covariance models were fitted to the log transformation of the above variables to assess changes and differences between groups.

Additionally, analyses of covariance were carried out on subgroups of the data. All models were checked for the validity of model assumptions and included terms for subject group and age. The increase in 5-HT concentration following meal ingestion within each group were also analysed using Student's paired *t*-test (two tailed), adjusting for multiple comparisons using Bonferroni's correction. Data are expressed as adjusted geometric mean and 95% confidence interval unless otherwise stated.

The sum of the individual symptom scores for pain, urgency and bloating at each time point were used to calculate an overall hourly symptom score (max score 12), from which the mean pre-meal (2 hours) and post-meal (4 hours) symptom scores were then calculated for each subject. Differences in fasting and fed symptom scores among the subject groups were assessed using the Kruskal Wallis test, and between fasting and fed scores using the Wilcoxon test. Data are expressed as median and IQR.

In addition, Spearman rho correlations between platelet and plasma 5-HT concentrations were calculated.

P-values less than 0.05 were considered as showing formal statistical significance.

RESULTS

Symptomatology:

Table 1 shows both demographic and baseline symptom data in all subject groups. Assessment of symptoms on the study day showed that, as expected all IBS-D patients had significantly worse symptoms under both fasting and fed conditions compared with healthy volunteers ($p < 0.001$) (Figure 1). Moreover, meal ingestion was associated with worsening of symptoms in all IBS-D sub-groups ($p<0.05$) but not in healthy volunteers who continued to report minimal or no symptoms. There was no difference in symptomatology between the three IBS-D subgroups either under fasting or fed conditions (Figure 1).

Platelet-depleted plasma 5-HT concentration:

Under fasting conditions, there was no difference in platelet depleted plasma 5-HT concentration between healthy male, and healthy female volunteers with either high or low P/O levels (Figure 2). Overall, IBS-D subjects had higher fasting platelet depleted plasma 5-HT concentrations compared with healthy volunteers ($p=0.002$). Furthermore,

there were differences between the gender/hormonal status subgroups in the comparisons between the IBS-D and healthy volunteers subgroups (interaction, borderline significance, p=0.055) stratifying by gender/menstrual status group. IBS-D males (p=0.002) but not IBS-D females with high (boarderline significance, p=0.07) or low P/O levels exhibited higher platelet depleted plasma 5-HT concentrations compared with healthy volunteers (Figure 2).

Meal ingestion increased platelet depleted plasma 5-HT concentrations in all IBS-D and healthy volunteer sub-groups ($p<0.05$). There was no overall difference in postprandial platelet depleted plasma 5-HT concentrations between the IBS-D patients and healthy volunteers after adjusting for fasting concentrations (ie 5-HT response to ingestion of the meal) (p=0.46). In addition, there were no significant differences in the 5-HT response to meal ingestion between the various healthy volunteer (p=0.24) or IBS-D patient (p=0.25) sub-groups (Figure 2).

Comparison of the actual postprandial platelet depleted plasma 5-HT concentrations (ie not correcting for fasting concentrations) however, showed that IBS-D patients have higher concentrations than healthy volunteers (p=0.049) and that there were differences between the gender/hormonal status sub-groups (p=0.046), with females with high P/O levels (both IBS-D and healthy subjects) having significantly higher platelet depleted plasma 5-HT concentrations compared with females with low P/O levels ($p<0.05$). There were no significant differences between the IBS-D patients sub-groups and corresponding healthy volunteers, or between the gender/hormonal status subgroups with subject group (borderline significance male IBS-D v healthy volunteers, p=0.07).

Platelet-depleted plasma 5-HIAA concentration:

Under fasting conditions, there was no difference in platelet depleted plasma 5-HIAA concentration between the IBS-D and healthy subjects (p=0.33), and no evidence of a difference between the gender/hormonal status subgroups (p=0.16) (Figure 3).

Meal ingestion increased platelet depleted plasma 5-HIAA concentrations in all IBS-D and healthy volunteer sub-groups, but significantly only in the healthy and IBS-D females with high P/O levels and IBS-D males ($p<0.05$). There were no significant

differences in the 5-HIAA response to meal ingestion (postprandial concentrations adjusted for fasting concentrations) between IBS-D patients and healthy volunteers, or between the gender/hormonal status subgroups (Figure 3).

Comparison of the actual postprandial platelet depleted plasma 5-HIAA concentrations revealed that IBS-D patients have higher concentrations than healthy volunteers ($p=0.04$), with both IBS-D females with high P/O levels ($p=0.032$) and IBS-D males ($p=0.052$) having higher concentrations than healthy volunteers. Moreover there were differences between the three IBS-D patient ($p=0.038$) but not healthy volunteer ($p=0.76$) subgroups; such that IBS-D males ($p=0.056$) but not IBS-D females with high P/O levels ($p=0.07$) tended to have higher platelet depleted plasma 5-HIAA concentrations than IBS-D females with low P/O levels (Figure 3).

5-HIAA:5-HT Ratio:

Under fasting conditions, the ratio of 5-HIAA:5-HT was significantly lower in IBS-D than healthy subjects ($p=0.001$), such that male IBS-D patients ($p=0.043$) and female IBS-D patients with high P/O levels ($p=0.038$) had lower 5-HIAA:5-HT ratios than their healthy volunteer counterparts. However, there was no difference between the gender/hormonal status subgroups ($p=0.96$) (Figure 4).

Meal ingestion decreased the 5-HIAA:5-HT ratio in IBS-D and healthy volunteer females with high P/O levels, and male healthy volunteers ($p< 0.05$). The magnitude of the decrease in turnover was similar between the IBS sub-groups and their healthy volunteer counterparts ($p=0.79$) and similar between the gender/hormonal status subgroups ($p=0.69$) (Figure 4). Moreover comparison of the actual postprandial 5-HIAA:5-HT ratios showed there was no difference between the IBS-D patients and healthy volunteers ($p=0.21$) and no differences between the gender/hormonal status subgroups ($p=0.74$).

Platelet 5-HT concentrations:

There was no difference in platelet 5-HT concentrations between healthy volunteer and IBS-D patients overall ($p=0.71$) or between the various gender/hormonal status subgroups (Figure 5).

***Correlation between platelet depleted plasma 5-HT concentration and platelet 5-HT*:**

Fasting platelet depleted plasma 5-HT concentrations were inversely correlated with platelet 5-HT concentrations in female IBS-D patients with high P/O levels (ρ = - 0.559; $p<0.001$). There were no significant correlations between these two parameters for either male IBS or female IBS with low P/O patients or for healthy males, and healthy females with low and high P/O levels.

DISCUSSION

These data extend those of our previous studies in female IBS-D patients who were assessed either in the luteal phase of their menstrual cycle or whilst taking the combined oral contraceptive (ie high progesterone/oestrogen levels),[13,14] by showing that male IBS-D patients also have raised fasting platelet depleted plasma 5-HT concentrations compared with healthy volunteers. Furthermore, they show for the first time that at menses (ie low progesterone and oestrogen levels) platelet depleted plasma 5-HT concentrations in female IBS-D patients are no different from healthy controls. Raised concentrations of plasma 5-HT tended to be associated with similar but less marked differences in 5-HIAA and thus lower 5-HIAA:5-HT ratios (a surrogate measure of 5-HT turnover)[14].

Our observations that male IBS-D patients have elevated fasting platelet depleted plasma 5-HT concentrations, but that the postprandial relative to fasting 5-HT concentrations was similar to healthy males, is consistent with our data obtained in female IBS-D patients with high P/O levels[13,14] and suggests that both male and female IBS-D patients may have a disorder of metabolism and/or reuptake rather than synthesis and/or release of 5-HT. This is further supported by the ratio of 5-HIAA:5-HT being significantly reduced under fasting conditions for both patient groups. The lack of statistical difference between female IBS-D patients with high progesterone and oestrogen levels compared with their healthy volunteer counterparts in this study (fasting, $p<0.07$) is probably related to a type II error, as our previous study evaluated 55 rather than only 39 patients, and showed a statistically significant difference.[14] Exactly what mechanism is responsible for this reduction in 5-HT removal is unclear as although these observations are consistent with some studies showing a reduced expression of the SERT transporter protein[28,29] and increased frequency of the serotonin reuptake transporter (SERT) deletion/deletion (ss)

genotype[30,31] in these patients, both of which are associated with less serotonin re-uptake, they are inconsistent with others showing either a reduction in ss genotype[32,33] or no difference compared with healthy controls,[34, 35, 36, 37, 38] with the latter study also reporting no difference in colonic mucosal expression of SERT. Indeed, a recent meta-analysis[39] carried out on 576 IBS-D patients from the above mentioned studies [30- 37] has suggested that no genetic polymorphism in the gene encoding for activity of SERT is associated with IBS-D. However, SERT transporter protein expression does not necessarily relate to the SERT genotype [38] and factors such as the presence of gastrointestinal inflammation and/or depression which are often associated with IBS-D[1] and linked with reduced SERT expression[40,41, 42] may be playing a role. Regrettably the presence of gastrointestinal inflammation and/or depression, the latter which is also known to be associated with increased frequency of the SERT (ss) genotype[43,44] were not assessed in this, or previous studies assessing the association between the SERT polymorphisms and IBS.

An interesting but unexpected finding was that platelet depleted plasma 5-HT and 5-HIAA concentrations, and the ratio 5-HIAA:5-HT under both fasting and fed conditions in menstruating female IBS-D patients were no different from healthy controls. Previous studies have shown an association between plasma 5-HT concentration and IBS symptom severity[13] but in the present study symptom severity in menstruating IBS-D females was not significantly different from the other patients groups. However, as previously mentioned, an interaction between female sex hormones and the 5-HT system is well documented with studies showing that oestrogen and/or progesterone can reduce SERT mRNA expression,[22] reduce monoamine oxidase (MAO) mRNA expression,[23, 24] an enzyme responsible for 5-HT metabolism and increase the availability of the 5-HT precursor, tryptophan [23, 25] in the central nervous system of non human primates. Whether similar interactions occurring peripherally are responsible for our findings in IBS patients cannot be determined from this study, but studies in healthy volunteers have shown a reduction in the B_{max} for platelet [^{3}H]paroxetine binding at the mid-luteal stage of the menstrual cycle.[45] The lack of effect of menstrual status in our healthy volunteers might just reflect the fact that we were assessing normal concentrations of endogenous plasma 5-HT whereas the latter study was investigating the binding of an exogenous substance in excess to the platelet serotonin transporter. Studies assessing whole blood 5-

HT concentration across the menstrual cycle in healthy volunteers have similarly shown no differences.[46] The fact that menstrual status did influence plasma 5-HT concentration (ie IBS-D and healthy females with high P/O levels > IBS-D and healthy females with low P/O levels) is maybe further evidence that variation in progesterone and oestrogen status can influence that 5-HT system.

The elevated plasma 5-HT concentrations seen in male IBS-D patients as well as previously in female IBS-D patients with high P/O levels[13,14] is consistent with its potential association with the increased motility and transit generally seen in IBS-D patients compared with healthy controls.[1] Indeed we have recently shown that plasma 5-HT concentration directly correlates with colonic motility.[17] Whether the elevated levels of 5-HT cause or are the consequence of these motility patterns and increased transit is unclear, but the fact that disturbances in gastrointestinal transit tend to be modest in IBS[47] despite the significantly elevated plasma 5-HT concentrations seen under both fasting and fed concentrations in IBS-D patients[14] and the fact that the time to peak 5-HT is similar to IBS-C patients who have limited or no increase in plasma 5-HT in response to meal ingestion[14] would suggest that 5-HT is causing, at least in part the motility patterns associated with IBS-D. This is further supported by the observations in our previous studies[13,14] that plasma 5-HT concentration tended to be raised during the luteal phase of the menstrual cycle when transit might be expected to be slightly slower, and the recent observations that citalopram, a selective serotonin reuptake inhibitor, which has been shown to increase plasma 5-HT concentration,[48] increases colonic motility in healthy controls.[49] However, the fact that plasma 5-HT concentration decreases at menses, a time usually associated with looser stools and worse symptoms[3,4] is supportive of these changes being related not to 5-HT but maybe to prostaglandin production,[50] which is thought to cause looser stools at this time by inhibiting transepithelial ion transport in the small intestine.[51] Furthermore, as plasma 5-HT concentrations are normal at menses in IBS-D females and modulation of plasma 5-HT concentrations using SSRIs have generally not been shown to cause changes in visceral sensation, prostaglandin production is maybe again more likely to be the cause of the increased rectal sensitivity and symptomatology seen at this time,[3] especially as they can induce afferent nerve sensitization.[52]

Finally, our observations that there were no differences between the IBS-D and healthy volunteer subgroups is consistent with our previous findings[14] and suggests that the increased plasma 5-HT concentrations seen in IBS-D patients despite possibly reduced SERT efficiency is maybe sufficient to maintain normal platelet concentrations. The fact that there was only an inverse correlation between fasting platelet depleted plasma and platelet 5-HT concentrations in IBS-D females with high P/O levels but not those with low P/O levels and IBS-D males, or healthy volunteers, might again be suggestive of compromised SERT function in IBS-D females being more likely to be influenced in the presence of high concentrations of progesterone and oestrogen.

In conclusion, these data suggest for the first time that both male and female IBS-D patients have raised platelet depleted plasma 5-HT concentrations maybe as a consequence of reduced uptake or metabolism which is attenuated in IBS-D females when their progesterone and oestrogen levels drop at menses.

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Table 1: Patient demographics and baseline symptoms.

	Healthy volunteers			IBS-D patients		
	Male	**Female High P/O**	**Female Low P/O**	**Male**	**Female High P/O**	**Female Low P/O**
Number	24	19	21	18	39	16
Age	28(18,46)	30(19,50)	25(19,48)	37(20,58)	32(19,52)	28(18,45)
Pain	0(0,0)	0(0,0)	0(0,0)	0(0,2.5)[a]	1(0,3.0)[a]	1(0,4.5)[a]
Bloating	0(0,0)	0(0,0)	0(0,0)	0(0,2.3)[b]	0(0,3.0)[a]	0(0,1.0)
Urgency	0(0,0)	0(0,0)	0(0,0)	0(0,2.0)[a]	0(0,1.0)[c]	0(0,1.5)[c]
Overall	0(0,0)	0(0,0)	0(0,0)	0.5(0,2.0)[a]	0.7(0.3,1.3)[a]	0.5(0.1,2.2)[a]

Age data expressed as mean (range) and symptom data as median (IQR). [a] *$p \leq 0.001$,* [b] *$p < 0.005$, and* [c] *$p < 0.02$ compared with healthy volunteers.*

FIGURE LEGENDS

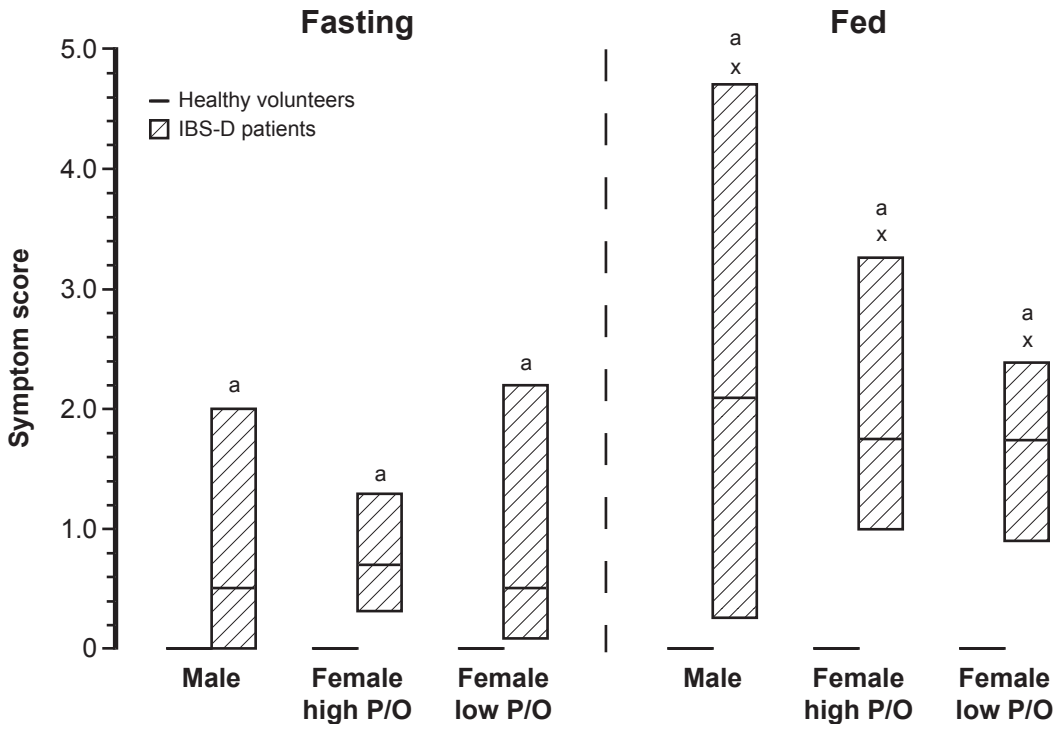
Figure 1: Overall symptom scores under fasting and fed conditions in female IBS-D and healthy subjects with high and low progesterone/oestrogen levels, and male IBS-D and healthy subjects. Data are expressed as median and IQR. [a] $p<0.001$ compared with healthy subjects; [x] $p<0.05$ compared with fasting.

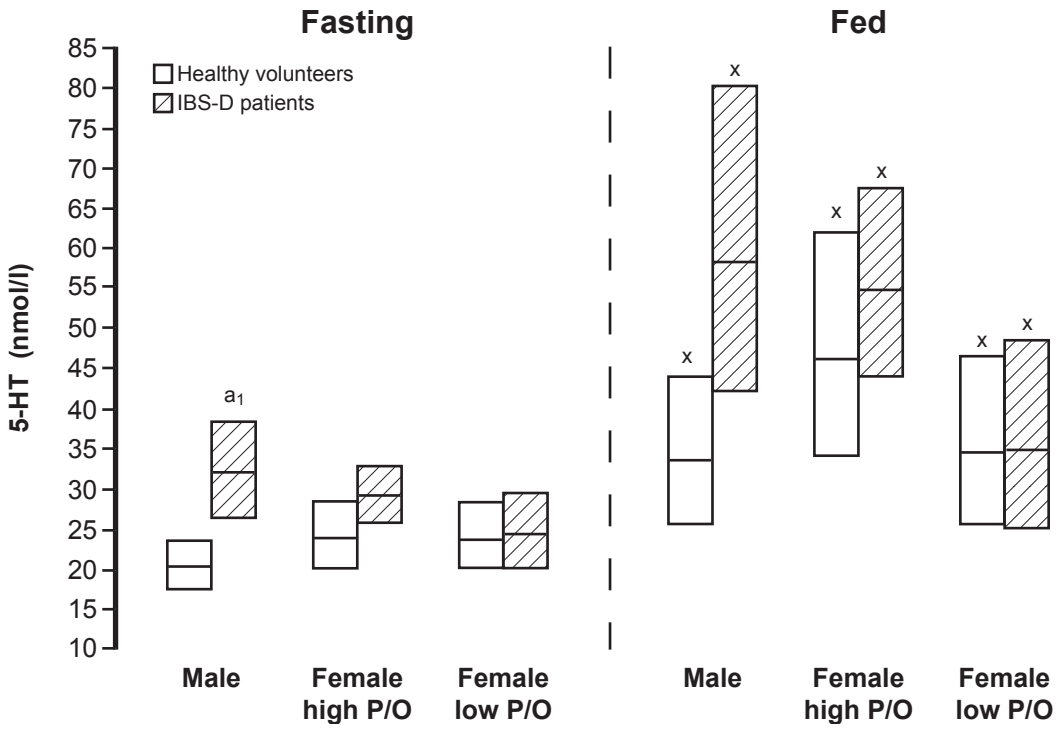
Figure 2: Comparison of platelet depleted plasma 5-HT concentrations among female IBS-D and healthy subjects with high and low progesterone/oestrogen levels, and male IBS-D and healthy subjects under fasting and fed conditions. Data are expressed as geometric mean and 95% CI. [a1] $p=0.002$, compared with healthy volunteers; [x] $p<0.05$ compared with fasting.

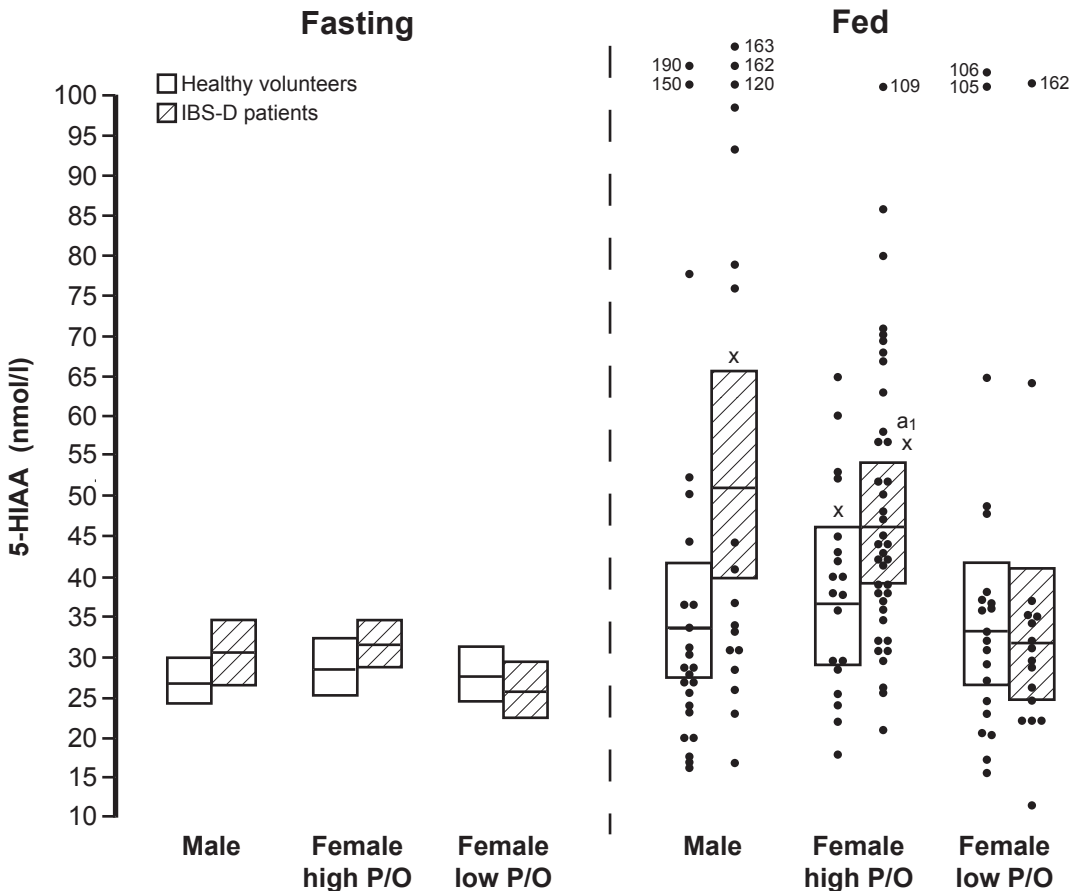
Figure 3: Comparison of platelet depleted plasma 5-HIAA concentration among female IBS-D and healthy subjects with high and low progesterone/oestrogen levels, and male IBS-D and healthy subjects under fasting and fed conditions. Data are expressed as geometric mean and 95% CI. [a1] $p=0.032$, compared with healthy volunteers; [x] $p<0.05$ compared with corresponding fasting period. Data points for each subject are shown as dots.

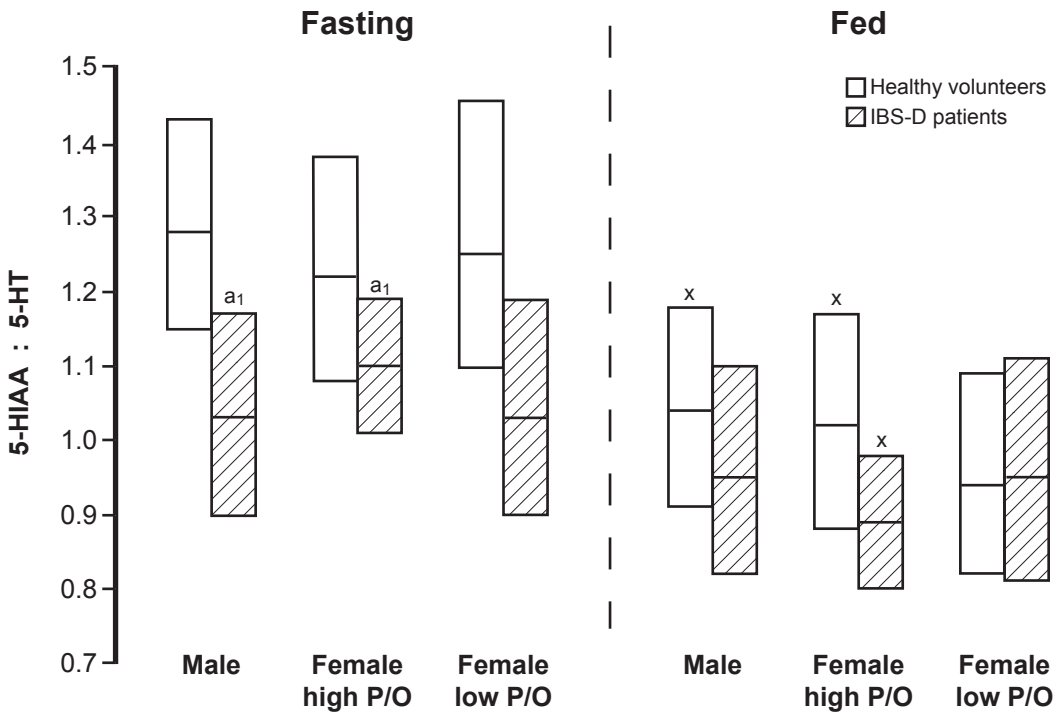
Figure 4: Comparison of the 5-HIAA:5-HT ratio among female IBS-D and healthy subjects with high and low progesterone/oestrogen levels, and male IBS-D and healthy subjects under fasting and fed conditions. Data are expressed as geometric mean and 95% CI. [a1] $p<0.05$, compared with healthy volunteers; [x] $P<0.05$ compared with fasting.

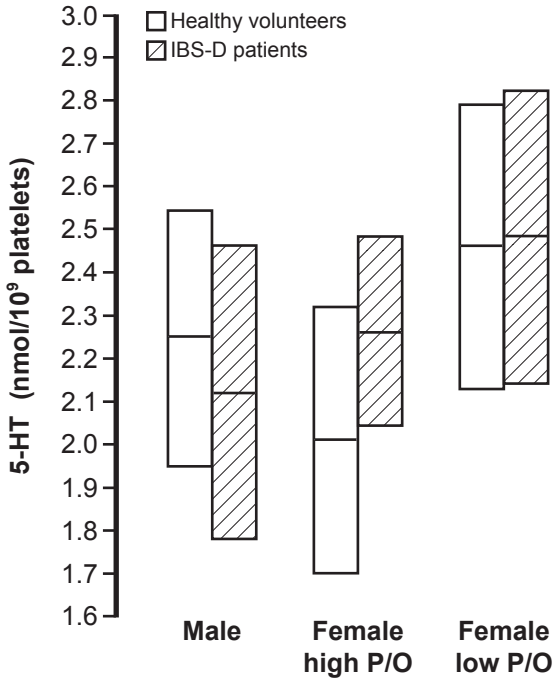
Figure 5: Comparison of platelet stores of 5-HT among female IBS-D and healthy subjects with high and low progesterone/oestrogen levels, and male IBS-D and healthy subjects under fasting and fed conditions. Data are expressed as geometric mean and 95% CI.


Fasting
Fed
Symptom score
5.0
4.0
3.0
2.0
1.0
0
Healthy volunteers
IBS-D patients
a
a
a
a
x
a
x
a
x
Male
Female high P/O
Female low P/O
Male
Female high P/O
Female low P/O


Fasting
Fed
Healthy volunteers
IBS-D patients
5-HT (nmol/l)
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
a1
x
Male
Female high P/O
Female low P/O
Male
Female high P/O
Female low P/O


Fasting
Fed
Healthy volunteers
IBS-D patients
5-HIAA (nmol/l)
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
190
150
163
162
120
109
106
105
162
x
x
x
a1
Male
Female high P/O
Female low P/O
Male
Female high P/O
Female low P/O


Fasting
Fed
Healthy volunteers
IBS-D patients
5-HIAA : 5-HT
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
a1
a1
x
x
x
Male
Female high P/O
Female low P/O
Male
Female high P/O
Female low P/O


Healthy volunteers
IBS-D patients
5-HT (nmol/10⁹ platelets)
3.0
2.9
2.8
2.7
2.6
2.5
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
Male
Female high P/O
Female low P/O