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**EFFECT OF RIMONABANT ON ESOPHAGEAL MOTOR  
FUNCTION IN MAN**

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## EFFECT OF RIMONABANT ON ESOPHAGEAL MOTOR FUNCTION IN MAN

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30 relaxations, gastroesophageal reflux disease.  
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## SUMMARY

**Introduction:** Cannabinoid type 1 (CB<sub>1</sub>) receptors are implicated in the control of transient lower esophageal sphincter relaxations (TLESRs) in animals. In man, it is unclear whether CB<sub>1</sub> receptors are involved in the control of esophageal function. **Aim:** To study the effects of the CB<sub>1</sub> receptor antagonist rimonabant on fasting and postprandial LES function in healthy subjects. **Methods:** Twelve healthy volunteers underwent two esophageal manometry studies with administration of wet swallows and a meal after 3 days premedication with placebo or rimonabant 20 mg. **Results:** Rimonabant did not significantly alter preprandial LES pressure (21.1±4.0 vs. 17.3±3.0 mmHg, NS), but postprandial LES pressures were significantly enhanced (9.9±1.9 vs. 17.1±2.7 mmHg in the first and 10.0±1.4 vs. 19.3±3.6 mmHg in the second postprandial hour, both p<0.05). Swallow-induced relaxations and amplitude of peristaltic contractions were not altered, but rimonabant significantly increased the duration of peristaltic contractions at all time points (e.g. 5.0±0.3 vs. 8.0±0.3 sec preprandially and 5.0±0.2 vs. 8.2±0.3 sec at 60 min postprandially, both p<0.01). The number of postprandial TLESRs (3.1±0.5 vs. 1.2±0.5, p<0.05) and acid reflux episodes (1.4±0.2 vs. 0.3±0.1, p<0.05) were significantly lower after rimonabant. **Conclusion:** The CB<sub>1</sub> receptor antagonist rimonabant enhances postprandial LES pressure and decreases TLESRs in healthy subjects.

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## INTRODUCTION

Gastroesophageal reflux disease (GERD) is defined as the presence of symptoms or lesions that can be attributed to the reflux of gastric contents into the esophagus. (1,2) The pathophysiology of GERD is multifactorial and involves several mechanisms such as failure of the anti-reflux barrier, impaired esophageal clearance, the presence of offensive factors in the refluxate and defective esophageal tissue resistance (3). Among the dysfunctions of the antireflux barrier, transient lower oesophageal sphincter relaxations (TLESRs) are the major mechanism underlying gastro-oesophageal reflux events in normal subjects, and in most GERD patients (4-6). TLESRs are a vago-vagal reflex, triggered by activation of stretch receptors of the proximal stomach, and organized in the brain stem. (5-7)

Pharmacological restoration of the anti-reflux barrier is considered a relevant target for the control of GERD (8). Several gastroprokinetic agents which also enhance lower esophageal sphincter (LES) pressure and esophageal motility have been developed and used in the treatment of GERD (2,8) Because of side-effects or lack of efficacy, none of these drugs has led to a currently established treatment for GERD. Drugs inhibiting TLESRs are considered a novel and attractive target for GERD therapy. (9-11) Baclofen, an agonist at the gamma-aminobutyric acid receptor type B (GABA-B), has been demonstrated to inhibit considerably the occurrence of TLESRs and gastro-oesophageal reflux, with an acceptable side-effect profile (11-14). It is currently thought that the anti-reflux effect of baclofen depends on peripheral inhibition of mechanosensitive gastric vagal afferents, thereby raising the threshold for action potential firing, and partly on central inhibition of vagal afferents leading to reduced transmitter release (15).

Cannabinoid receptors (CBRs) have many similarities with GABA-B receptors. For example agonism of both GABA-B receptors and CBRs inhibits transmitter release presynaptically and postsynaptically hyperpolarizes neurons in the central nervous system (5CNS) (16,17). Common CBR-mediated actions in vivo include skeletal muscle relaxation, hypothermia, and antinociception (18). CBRs have an important role in inhibition of emesis triggered by stimuli in the gastrointestinal tract or CNS (19-21). They have been localized to specific points along the emetic pathway in the central nervous system that receive input from gastrointestinal vagal afferents. There is considerable anatomical overlap of

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3 pathways involved in emesis and triggering of TLESRs (22,23). CBRs, like GABA-B  
4 receptors, belong to the G protein-coupled receptor superfamily and are subdivided into 2  
5 groups, CBR1 and CBR2 (24). Delta-9- tetrahydrocannabinol (delta(9)-THC) is the  
6 principal psychoactive compound of marijuana and is well established as an agonist at  
7 CBRs. More recently, endogenous ligands (endocannabinoids) have been isolated, the  
8 most important of which are arachidonylethanolamide (anandamide) and 2-arachidonyl  
9 glycerol (24-26). The endocannabinoids appear to act as retrograde messengers, i.e. they  
10 can be released by postsynaptic neurons and diffuse to nerve terminals where they reduce  
11 transmitter release (25,26). A number of synthetic ligands for CBRs have been made, and  
12 whereas CBR agonists are moderately subtype selective, antagonists show definitive  
13 selectivity (26). The CB1 antagonist rimonabant was commercially available as a drug in  
14 the treatment of obesity, until being expelled from the market because of an increased  
15 prevalence of depression. (27,28).

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17 In dogs and in humans, delta(9)-THC significantly reduced resting LES pressure and the  
18 occurrence of TLESRs after a standard meal (29). A study in dogs established that CB1  
19 agonists inhibit the occurrence of TLESRs while rimonabant reversed this effect (30). The  
20 effects of rimonabant on LES function in man has not been addressed so far. Thus the aim  
21 of the present study was to investigate the effect of rimonabant on esophageal motility and  
22 LES function in man.  
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## MATERIALS AND METHODS

### SUBJECTS

Studies were performed in 12 healthy volunteers (five men and seven women; mean age, 30.2±1.8 years; range, 23–41 years) with a mean body weight of 66±3 kg. None of the subjects had symptoms or a history of gastrointestinal disease or upper gastrointestinal surgery, nor were they taking any medication. In addition, volunteers with a history of depression were excluded. Written informed consent was obtained from each subject and the study protocol had been approved previously by the Ethics Committee of the University Hospital.

### STUDY DESIGN

All subjects underwent two studies after 3 days premedication with rimonabant 20 mg or matching placebo in a double-blind randomised cross-over design, at least one week apart. The 3 days administration was chosen based on the SmPC information. Upon oral ingestion, maximum concentration is reached after approximately two hours. Rimonabant's half-life is longer in obese patients (approximately 16 days) than in non-obese subjects (nine days). Steady state is reached after 3 days (31). On each day of measurements, subjects were studied after an overnight fast of at least 12 h and the dose of placebo or rimonabant for that day was administered in a double blind fashion in the motility research unit. Together with a stationary manometry probe, a pH probe was passed through the mouth under topical anesthesia and positioned with the pH electrode at 5 cm above the LES. A summary of the protocol is shown in Figure 1. After placement of the assembly, the subjects remained in a sitting position for a habituation period of 20 minutes. This period allowed baseline assessment of esophageal peristalsis and LES function. Ten wet swallows of 5 ml of water were administered and followed by oral ingestion of 20 mg of rimonabant or placebo in a double-blind, randomized cross-over order. During the 30 min after administration of the drug oesophageal and lower oesophageal sphincter pressure and oesophageal pH were recorded concomitantly. Sixty minutes after drug administration the subjects ingested a mixed liquid meal (200 mL, 300 kcal, 13% proteins, 48%

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3 carbohydrates, 39% lipids; Nutridrink, Nutricia, Bornem, Belgium) and recordings  
4 continued for 2 h after. Throughout the study, 10 wet swallows of 5 ml of water were  
5 administered at 30-minute intervals. The sensations of fullness, nausea, heartburn,  
6 belching, satiety, hunger, anxiety, dizziness, sleepiness and fatigue were measured every  
7 15 min using validated 100-mm visual analogue scales (32).  
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## 13 RECORDING METHODS

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17 Following an overnight fast, an oesophageal manometric catheter fitted with a 6-cm Dent  
18 Sleeve was introduced through the mouth. Subsequently, the oesophageal catheter was  
19 positioned so that pressures could be recorded from the fundus (side hole 2 cm below the  
20 sleeve), the lower oesophageal sphincter (sleeve), oesophageal body (side holes 4, 7 and  
21 10 cm proximal to the sleeve) and pharynx (side hole 28 cm proximal to the sleeve, to  
22 detect swallows). The oesophageal catheter was infused at a flow rate of 0.5 mL/min with  
23 distilled water using a low-compliance pneumo-hydraulic capillary infusion system  
24 (Arndorfer Medical Specialties, Milwaukee, WI, USA). The infusion system was connected  
25 to external pressure transducers, and signals were recorded on a polygraph (Synectics  
26 Medical, Stockholm, Sweden).  
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35 The oesophageal pH was measured with an antimony pH electrode (Synectics Medical,  
36 Stockholm, Sweden) positioned 5 cm above the proximal margin of the sleeve. The pH  
37 electrode was calibrated in buffers of pH 1 and pH 7 before and after each study. During  
38 the study period, the oesophageal pH was recorded continuously using an ambulatory  
39 data-logger (MicroDigitrapper, Synectics Medical, Stockholm, Sweden).  
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## 54 DATA ANALYSIS

### 55 Lower oesophageal motility

56 The tracings were analysed in a blinded fashion by one of the authors (ES) and checked  
57 by a second readere (JT). The basal lower oesophageal sphincter pressure was measured  
58 at end expiration relative to the end-expiratory intragastric pressure. The basal lower  
59 oesophageal sphincter pressure was visually determined every 3 min and averaged over  
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3 15-min intervals. The influence of drug administration on the basal lower oesophageal  
4 sphincter pressure was assessed by comparing the value of the first with the value of the  
5 third pre-prandial 30-min interval.  
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8 Transient lower oesophageal sphincter relaxations were defined according to criteria  
9 previously published (33) as follows: (i) absence of a swallowing signal for 4 s before to 2 s  
10 after the onset of lower oesophageal sphincter relaxation; (ii) relaxation rate of  $\geq 1$   
11 mmHg/s; (iii) time from onset to complete relaxation of  $\leq 10$  s; and (iv) nadir pressure of  $\leq$   
12 2 mmHg. Excluding multiple swallows, lower oesophageal sphincter pressure falls that  
13 fulfil the last three criteria, but have a duration of  $> 10$  s, can also be classified as transient  
14 lower oesophageal sphincter relaxations irrespective of the timing of lower oesophageal  
15 sphincter relaxation relative to swallowing.  
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## 24 Oesophageal pH

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28 The percentage of time with an oesophageal pH  $< 4$  and the number of acid reflux  
29 episodes were calculated. Acid reflux episodes were defined as a decrease in  
30 oesophageal pH to a value below pH 4 for at least 4 s or, if the basal oesophageal pH was  
31 already below pH 4, as a rapid further drop in pH of at least 1 pH unit.  
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## 37 Statistical analysis

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40 Data are presented as the mean  $\pm$  standard error of the mean (S.E.M.). The paired t-test  
41 was used for the comparison of the mean values between the periods or between the  
42 rimonabant and placebo studies. The changes in the basal lower oesophageal sphincter  
43 pressure, was evaluated using analysis of variance for repeated measures. The frequency  
44 and duration of transient lower oesophageal sphincter relaxations, the number of acid  
45 reflux episodes and the symptom scores were compared using the Wilcoxon signed rank  
46 test. A p-value  $< 0.05$  was considered to be statistically significant. Based on previous  
47 studies, the study had a 85% power to detect 30% difference in TLESR rate at 5%  
48 significance level (14,32).  
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## RESULTS

### Conduct of the study

The positioning of oesophageal manometric catheter and pH probe were well tolerated, and all subjects completed both the sessions of studies. No adverse events were reported

### Lower esophageal sphincter pressure

Prior to drug administration, and in the preprandial period prior to the meal, LES resting pressure and swallow-induced relaxations were similar for both conditions (Table 1,2). In the placebo studies, ingestion of the meal was associated with a significant decrease in the LES pressure during both the first and the second postprandial hour (Figure 2). In contrast, in the rimonabant studies, no significant decrease in LES pressure occurred after the meal (Table 1).

### Esophageal motility

The amplitude of peristaltic contractions and swallow-induced relaxations were not significantly altered by rimonabant in the pre-prandial and post-prandial periods. However, rimonabant significantly increased the duration of peristaltic contractions in the pre-prandial and post-prandial periods (Table 1). Rimonabant did not alter the amplitude of swallow-induced relaxations, but the duration of contractions was significantly increased by rimonabant (Table 1).

### Transient lower esophageal sphincter relaxations.

The numbers of transient lower oesophageal sphincter relaxations after the administration of placebo and rimonabant are summarized in Figure 2 and Table 3. After placebo, ingestion of the meal was associated with a significant increase in the rate of TLESRs during the first and the second post-prandial hour. In contrast, no significant increase in the rate of TLESRs relaxations occurred postprandially in the rimonabant studies (Figure

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3, Table 3). Compared to placebo, the number of TLESRs in the rimonabant studies was  
4 significantly lower for the whole postprandial period and in the first postprandial hour.  
5 After placebo, postprandial TLESRs lasted significantly longer than preprandial TLESRs  
6 (26.2±3.4 vs. 31.5±2.1 sec,  $p < 0.05$ ) while no difference was seen between pre- and  
7 postprandial TLESRs with rimonabant (19.9±3.4 vs. 19.7±3.3 sec, NS). In the postprandial  
8 period, TLESR duration was significantly shorter after rimonabant compared to placebo  
9 (19.7±3.3, vs. 31.5±2.1 s;  $p < 0.05$ ).  
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## 19 Esophageal pH

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23 The percentage of time  $\text{pH} < 4$  in the oesophagus did not differ between the rimonabant  
24 and placebo studies in the pre-prandial (0.1± 0.05 % vs. 0.3±0.1%, NS) but tended to be  
25 lower after rimonabant in the postprandial period (0.1± 0.05 vs. 0.4±0.1%;  $p = 0.06$ ). The  
26 number of acid reflux episodes increased significantly after the meal in the placebo studies  
27 but not in the rimonabant studies (Figure 4). The number of acid reflux episodes during the  
28 post-prandial period after rimonabant was significantly lower than after placebo (Figure 4).  
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## 34 Symptoms

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38 No significant differences in symptom scores (calculated as area under the curve, AUC)  
39 during both the pre-prandial and post-prandial periods were found between rimonabant  
40 and placebo studies (details not shown).  
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## DISCUSSION

The aim of the present study was to investigate how suppression of endocannabinoid action by rimonabant would influence esophageal motility, the occurrence of TLESRs elicitation and gastro-esophageal reflux events in healthy volunteers. We found that rimonabant inhibited the decrease in LES pressure and the increase in the rate of TLESRS and reflux events after a meal, leading to a significant lower number of acid reflux events. In addition, the duration of peristaltic contractions in the distal esophagus was prolonged after rimonabant.

The observation that rimonabant increased LES pressure after a meal is in line with previous studies which demonstrated that the cannabinoid agonist delta(9)-THC reduced resting LES pressure after a standard meal in man (29). The inhibition of TLESRs and reflux events we observed is more surprising, as delta(9)-THC administration inhibited the occurrence of TLESRs after a standard meal in healthy volunteers (29), and in the dog rimonabant enhanced the rate of TLESRs and reflux events induced by an acidified meal and intra-gastric air insufflation (30). These observations which indicate ongoing suppression of TLESR by a CB1 receptor in the dog (30), were not confirmed in the present human study.

The reason for the apparent species difference between dog and man, and the mechanism underlying the inhibition of TLESRs and reflux events by rimonabant remains to be established. The dosing regimen differed strongly between the dog studies and the present studies: dogs received a unique dose of 0.22  $\mu\text{mol/kg}$  while the volunteers were treated for 3 days of medication with 20 mg/day. Furthermore, binding studies show that rimonabant has an affinity for the CB1 receptor in the low nanomolar range, but in functional experiments, rimonabant is not a neutral antagonist but, has rather been found to be an inverse agonist at the CB1 receptor (34,35). The effects of rimonabant in the present study therefore can reflect antagonist properties, inverse agonist effects, or both.

TLESRs are controlled by a vago-vagal reflex pathway which is triggered by gastric distention, integrated in the brainstem and which induces release of nitric oxide from intrinsic nerves at the LES (36-38). Endocannabinoids can potentially control this vago-vagal reflex pathway at many points since CB1 receptors are expressed on vagal

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3 afferents, in the brain stem, on interneurons in the wall of the gastrointestinal tract and on  
4 postganglionic fibers innervating smooth muscle in the gastrointestinal tract (39-42). In the  
5 dog, rimonabant only increased the number of TLESRs while their characteristics and  
6 esophageal motility were unchanged, and this was considered an argument in favor of a  
7 central site of action (30). The observation that fasting LES pressure was not affected by  
8 rimonabant in the present study, while the postprandial drop in LES pressure and the  
9 occurrence of TLESRs were inhibited could also argue in favor for a central action, for  
10 instance on vago-vagal reflex pathways. Rimonabant also has the potential to inhibit the  
11 occurrence of TLESRs at the level of triggering through distention of the proximal stomach.  
12 In a gastric barostat study, we demonstrated a significant decrease in postprandial volume  
13 of the proximal stomach in healthy volunteers after rimonabant pretreatment (43). In the  
14 present study in man, the duration of distal esophageal contractions was prolonged and  
15 the duration of TLESRs was shortened by rimonabant. Another important site of action for  
16 rimonabant, therefore, to consider is the enteric nervous system. Indeed, we and others  
17 previously provided evidence for a continuous endocannabinoid tone acting on CB1 to  
18 submit neurotransmitter release in the enteric nervous system (39-42). Rimonabant may  
19 thus enhance the release of for instance acetylcholine from intrinsic motor neurons, which  
20 could contribute to the observed longer duration of peristaltic contractions and higher  
21 postprandial LES pressure. The absence of any change in symptoms from the  
22 gastrointestinal tract could also be viewed as an argument against a primarily central site  
23 of action. Further clarifying the site of action where rimonabant inhibits TLESRs and reflux  
24 would require studies with a peripherally acting CB1 antagonist, and no such drug is  
25 presently available for human studies.

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46 Inhibition of TLESRs is a now well-established therapeutic target in GERD, and several  
47 drugs are being investigated for their ability to inhibit TLESRs, including GABA-B agonists,  
48 metabotropic glutamate-5 receptor antagonists and the mixed dopamine-2  
49 antagonist/cholinesterase inhibitor itopride (9-15, 32, 44-46). The magnitude of inhibition of  
50 TLESRs and reflux events observed with rimonabant is at least in the same order of  
51 magnitude as observed with these agents. On the other hand, long-term administration of  
52 rimonabant has been associated with an increased occurrence of depression (47), and this  
53 adverse event does not seem compatible with the use of rimonabant or similar CB1  
54 antagonists for GERD therapy. Development of peripherally acting CB1 antagonists could  
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3 be considered for this and other indications, provided that the site of action in TLESR  
4 inhibition is outside the blood-brain barrier (48,49).  
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10 The present study has a number of limitations. First, only a single dose of rimonabant was  
11 studied, as this was the only one available for the treatment of obesity in man. Second, the  
12 number of TLESRs and reflux events was low, which may be due to the fact that we  
13 studied asymptomatic healthy volunteers, and due to the relatively small size of the test  
14 meal. Finally, reflux events were only assessed by pH monitoring. It is conceivable that the  
15 use of esophageal impedance monitoring might have increased the number of detectable  
16 and quantifiable reflux events, especially after the meal.  
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24 In summary, in this placebo-controlled, double blind, randomized, crossover study we  
25 demonstrated for the first time that the CB1 receptor antagonist, rimonabant, inhibits the  
26 meal-induced increase in TLESRs, increases postprandial LES pressure leading to a lower  
27 number of acid reflux events. In addition, rimonabant increased the duration of distal  
28 esophageal peristaltic waves. Further studies will need to address whether this effect of  
29 rimonabant has a central or a peripheral site of action.  
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For Peer Review

## TABLES

**Table 1.** Esophageal motility parameters, before and after intake of placebo or rimonabant, and before and after the meal. \* $p < 0.05$  compared to placebo; \*\* $p < 0.001$  compared to placebo; † $p < 0.05$  compared to basal.

	LES pressure (mm Hg)		Distal contraction amplitude (mm Hg)		Distal contraction duration (sec)	
	Placebo	Rimonabant 20 mg	Placebo	Rimonabant 20 mg	Placebo	Rimonabant 20 mg
<b>Basal</b>	19.5±2.7	24.0±2.6	76.6±3.7	78.6±5.8	4.8±0.2	7.9±0.3**
<b>Post-drug</b>	17.7±3.2	21.2±3.7	83.9±5.2	80.8±5.1	5.2±0.3	8.0±0.3**
<b>Postprandial 1<sup>st</sup> hour</b>	9.9±1.9 <sup>†</sup>	17.1±2.7*	78.0±3.9	80.8±4.3	5.2±0.2	8.2±0.3**
<b>Postprandial 2<sup>nd</sup> hour</b>	10.0±1.4 <sup>†</sup>	19.3±3.6*	77.3±5.5	75.6±4.3	5.2±0.3	8.2±0.4**

**Table 2.** Characteristics of swallow-induced relaxations before and after intake of placebo or rimonabant, and before and after the meal. No significant differences occurred between groups or over time.

	Relaxation (%)		Duration (sec)	
	Placebo	Rimonabant 20 mg	Placebo	Rimonabant 20 mg
<b>Basal</b>	98.0±0.9	95.6±2.9	11.8±0.5	12.2±0.4
<b>Post-drug</b>	97.0±1.3	97.1±1.3	12.5±0.7	12.2±0.4
<b>Postprandial 1<sup>st</sup> hour</b>	94.6±1.8	96.5±1.7	11.5±0.7	12.1±0.3
<b>Postprandial 2<sup>nd</sup> hour</b>	95.8±1.8	96.7±1.8	11.8±0.6	12.7±0.4

**Table 3.** Number of transient lower oesophageal sphincter relaxation. †P < 0.05 compared to preprandial. \*p< 0.05 compared to placebo.

Pretreatment	Pre-prandial	First post-prandial hour	Second post-prandial hour	Total post-prandial
Placebo	0.3±0.1	1.4±0.2 <sup>†</sup>	1.7±0.4 <sup>†</sup>	3.1±0.5 <sup>†</sup>
Rimonabant 20 mg	0.4±0.2	0.4±0.2*	0.8±0.4	1.2±0.5*

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## FIGURES

**Figure 1. Study outline.** Healthy volunteers underwent esophageal sleeve manometry and pH measurement studies after 3 days premedication with rimonabant 20 mg or placebo. After placement of the assembly ten wet swallows of 5 ml of water will be administered, followed by ingestion of the medication. After 60 minutes a standardized meal will be administered and measurements will continue for another 120 minutes. At 30-minute intervals, 10 wet swallows will be administered. Throughout the study, at 15-minute intervals the intensity of 8 epigastric symptoms will be scored on visual analogue scales.

**Figure 2. LES pressure.** (A) Basal LES pressure was comparable for both conditions. Rimonabant pretreatment prevented the meal-induced decrease in LES pressure. \*  $P < 0.05$  compared to placebo. (B) Individual results for the LES pressure during the first postprandial hours.

**Figure 3. TLESRs.** (A) Basal LES pressure was comparable for both conditions. Rimonabant pretreatment prevented the meal-induced decrease in LES pressure. \*  $P < 0.05$  compared to placebo. (B) Individual results for the total number of postprandial TLESRs.

**Figure 4. Acid reflux episodes.** The number of acid reflux episodes during both the preprandial and post-prandial period was significantly lower after rimonabant. The pretreatment with rimonabant was not associated with a post-prandial increase of acid reflux episodes as after placebo. \*  $p < 0.05$  compared to placebo. †  $p < 0.05$  compared to preprandial.

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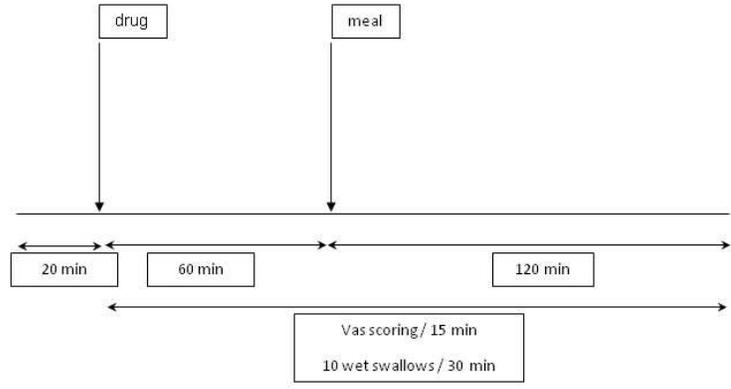
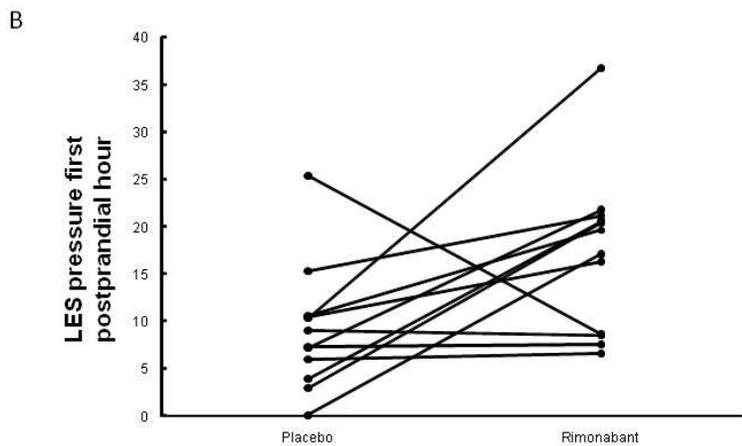
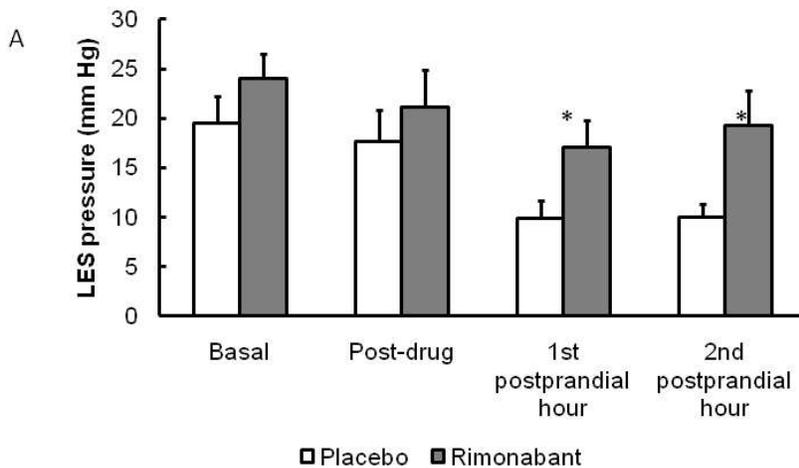
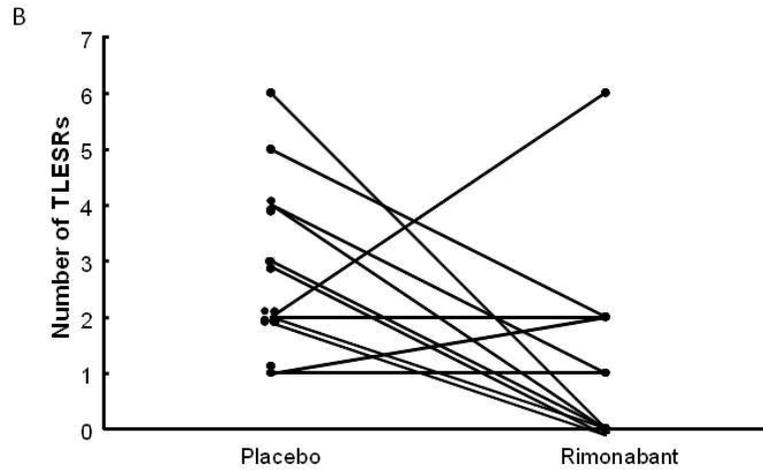
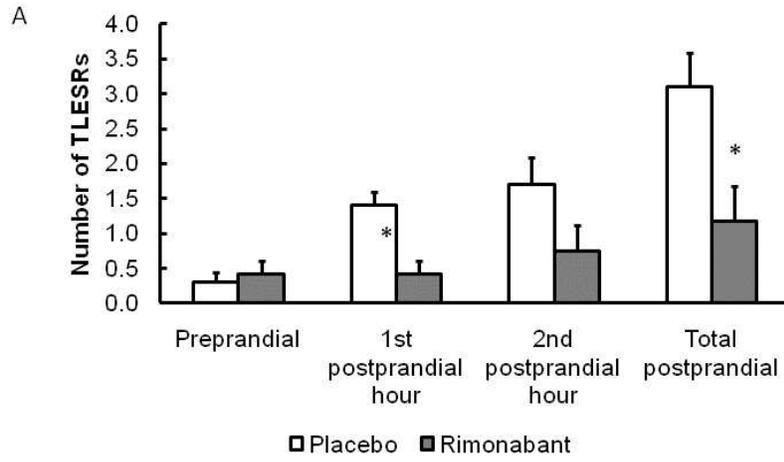


Figure 1  
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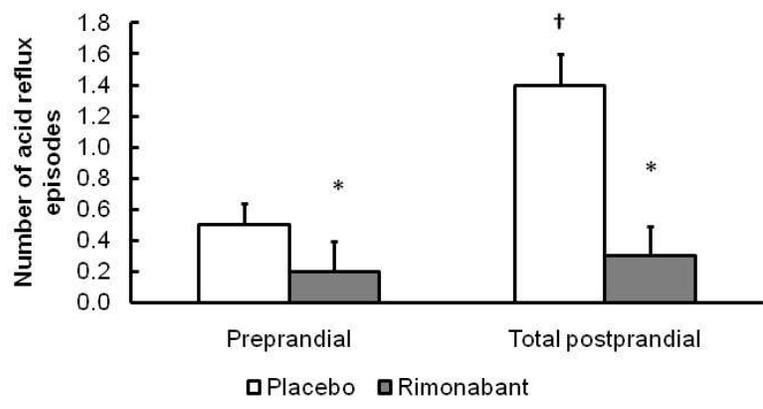


Figure 4  
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## REVIEWERS' COMMENTS TO AUTHOR:

Reviewer: 1

Comments for Transmission to the Authors

The histograms obscure what happens to individual patients – and this is even more wasteful when there are paired experiments. The individual data-points should be shown for each subject, and in paired experiments the data-points can be joined. Thus the reader can see much more about the spread of the results, and the consistency of trends by each subject. This should be done for all the figures.

*We included a total of 4 figures, some of which are composite figures. We added individual graphs for the most relevant of these, see figures 2B and 3B.*

Some missing references:

*The references (41 and 45, now 32 and 43) have been updated.*

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Reviewer: 2

Comments for Transmission to the Authors

The authors have performed a small study in healthy volunteers demonstrating that rimonabant does have some potential effects on esophageal physiology.

It is unclear how the sample size was arrived at as the statistical analysis describes no power calculation. It is also unclear whether the tracings were read in blinded fashion, though I suspect that they were.

*We made these additions to the text:*

- 1) *Based on previous studies, the study had 85% power to detect 30% difference in TLESR rate at 5% significance level. We apologise for an error in the manuscript: there were 12 subjects, not 11. This has been corrected.*
- 2) *The tracings were read in blinded fashion by two of the authors.*

The authors do well to point out the study limitations and the issues with the compound in general as well as pointing out that the magnitude of inhibition of TLESRs and reflux events similar to other agents being investigated for antireflux therapy. Early data from the reflux inhibitors cited have been underwhelming. Is there anything about rimonabant that makes it more attractive?

*Together with the safety profile of the drug, the findings of the present study would not justify therapeutic development for GERD at this point, in our opinion. We have clarified this in the manuscript.*

Table 2 could be eliminated from the manuscript if space were an issue.

*We would prefer to keep Table 2 in the manuscript: inhibition of TLESRs without interference with swallow-induced relaxation is important as it points to involvement of CBI receptors in neural pathways controlling TLESRs, but not in swallow-induced peristalsis. Inhibition of swallow-induced relaxation is undesirable as this might lead to dysphagia.*

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7 Comments for Transmission to the Authors

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9 Nice study exploring the role of CB receptor influence on GERD physiology. The  
10 methods are sound (although I would have preferred to see pH-impedance be used)  
11 and the results support the conclusion. This is an interesting target for therapy in  
12 GERD and more human data regarding this area is needed.  
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17 *Thank you for the comments. We acknowledged the potential benefit of using*  
18 *impedance for future studies. No other responses.*  
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3 Reviewer: 4

5 Comments for Transmission to the Authors

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7 The paper is very interesting and well written. The authors investigated the effect of  
8 CB1 receptors antagonist rimonabant on esophageal motility. The study is well  
9 designed and use combined manometric and pH monitoring of the physiologic effects  
10 of the drug, concluding that rimonabant enhances postprandial LES pressure and  
11 decreases TLESRs in healthy subjects.  
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17 I have several comments:

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21 Methods:

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25 1. The rationale for pretreatment with rimonabant for 3 days should be explained,  
26 including bioviability of the drug,  $t_{1/2}$  half, etc.  
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30 *The rationale for the 3 days pretreatment with rimonabant is based on the SmPC*  
31 *information. The bioavailability of rimonabant is not well established. The time to*  
32 *maximum concentration ( $T_{max}$ ) is approximately two hours, and the peak*  
33 *concentration ( $C_{max}$ ) is  $196 \pm 28.1$  ng/ml. The drug's half-life is longer in obese*  
34 *patients (approximately 16 days) than in non-obese patients (nine days). Steady state*  
35 *is reached after 3 days).*  
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42 2. It is not clear from the text (page 5), whether only the patients who were pretreated  
43 with this drug received additional dose of rimonabant during the manometry session,  
44 or it was blind?  
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47 *On the day of experiment, witnessed intake occurred in the motility unit. This was*  
48 *added to the text.*  
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53 3. Methods- please include reference for VAS for specific symptoms mentioned in  
54 paper.  
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58 *The reference for the VAS questionnaire was added (Scarpellini et al., now ref. 31).*  
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5 Discussion:  
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9 1. Despite the pretreatment with rimonabant the baseline manometric measurements  
10 did not differ between drug and placebo group. Does it mean that the drug has only  
11 short term effect or another explanation exists- needs a comment in discussion. The  
12 authors should comment practical clinical applicability based on results of the study.  
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17 *Based on the pharmacokinetics of the drug, a steady state plasma level is expected a*  
18 *the time of the measurements. The lack of an effect on LES pressure before the meal*  
19 *suggests that the effect of rimonabant on LES pressure is only present in the*  
20 *postprandial state, while preprandial LES pressure is not affected. In contrast to the*  
21 *effect on LES pressure, there is already a preprandial effect of rimonabant on the*  
22 *duration of esophageal body peristalsis, which is in line with the presence of effective*  
23 *plasma levels before drug administration on the day of the experiments.*  
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33 2. Although rimonabant has some interesting physiological effects on LES which  
34 might be helpful for GERD, low safety profile of rimonabant will most probably  
35 preclude the clinical use of this medication. This should be mentioned in discussion.  
36 Thus, the future research should be directed to find safer alternative for this  
37 medication.  
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44 *We agree with the reviewer. The poor safety profile for the drugs does not allow new*  
45 *studies in patients to verify the real impact of the present findings and requires the*  
46 *development of new drugs selectively acting on the peripheral CB1 receptor, or*  
47 *centrally acting CB1 receptor antagonists that do not induce depression; although the*  
48 *latter is unlikely to occur. This has been added to the discussion section.*  
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56 3. Please comment on the difference of results of 3-days pretreatment and acute dose  
57 of drug during manometry.  
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See above: in terms of duration of peristaltic contractions, there is already an effect of rimonabant prior to drug administration on the day of the experiments. The effect on LES pressure is only present postprandially, suggesting a selective effect on postprandial LES motor control.

Tables&figures.

I think Table 2 is unnecessary. Table 1 is difficult to read. I would suggest to make three columns with physiologic parameters next to each other (LES pressure, Amplitude, and Duration) divided into Placebo/ Rimonabant , while the timing mentioned in first left column only once, instead of three times.

*We would prefer to keep Table 2 in the manuscript: inhibition of TLESRs without interference with swallow-induced relaxation is important as it points to involvement of CB1 receptors in neural pathways controlling TLESRs, but not in swallow-induced peristalsis. Inhibition of swallow-induced relaxation is undesirable as this might lead to dysphagia. We have adapted Tables 1 and 2 as suggested.*