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**Ghrelin Inhibits Autonomic Function in Healthy Controls, but
has No Effect on Obese and Vagotomized Subjects**

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3 **Title: Ghrelin Inhibits Autonomic Function in Healthy Controls, but has No**
4 **Effect on Obese and Vagotomized Subjects**
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45

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47
48 None.
49

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ABSTRACT

Objective: Ghrelin inhibits sympathetic nervous system (SNS) activity in rodents.

We studied the effect of ghrelin on healthy humans, in obesity or in vagotomized subjects.

Design: Randomized, double blind, placebo controlled crossover

Subjects: 7 lean (mean body mass index (BMI) $23.6 \pm 0.9 \text{ kg/m}^2$), 7 morbidly obese (mean BMI $50.9 \pm 4.4 \text{ kg/m}^2$) and 7 post-gastrectomy subjects (mean BMI $22.0 \pm 1.1 \text{ kg/m}^2$).

Measurements: Subjects were randomized to intravenous ghrelin (5 pmol/kg/min) or saline over 270 minutes. Subjects had a fixed calorie meal and a free choice buffet during the infusion. Heart rate variability (HRV) was measured. Total power (TP) represents overall autonomic function, low frequency (LF) power represents sympathetic and parasympathetic activity and high frequency (HF) power represents parasympathetic activity. Very low frequency (VLO) represents the frequency band associated with thermogenesis.

Results: Preliminary ANOVA analysis, looking at all three subject groups together showed that ghrelin had an overall highly significant inhibitory effect on TP ($p=0.001$), HF power ($p=0.04$), VLO power ($p=0.03$) and no effect on LF ($p=0.07$). Further subset analysis revealed that ghrelin had a significant effect on TP ($p=0.03$), borderline effect on LF power ($p=0.06$) and no effect on HF power ($p=0.1$) in healthy controls. By contrast in obese subjects, ghrelin had no effect on TP ($p=0.3$), LF ($p=0.5$) and HF ($p=0.06$) and also no effect in the vagotomized subjects on TP ($p=0.7$), LF ($p=0.7$) and HF ($p=0.9$). Ghrelin had no effect on the LF/HF ratio.

Conclusions: Ghrelin inhibits SNS activity in healthy controls with a moderate effect on PNS activity but had no effect on obese subjects. Vagotomized subjects also did not respond to ghrelin suggesting the vagus nerve is important for the effects of peripheral ghrelin on the SNS.

INTRODUCTION

Ghrelin was discovered as the natural ligand for the growth hormone secretagogue receptor GHS-R 1a,¹ and appears to have vasoactive properties; administration of ghrelin in humans causes a decrease in mean arterial pressure, but does not change heart rate or norepinephrine levels.² This has led to speculation as to whether ghrelin inhibits activation of the sympathetic nervous system (SNS) during hypotension.

In support of this, two rodent studies have shown that peripheral ghrelin can inhibit the expected increase in SNS activity seen in sepsis and post myocardial infarction.^{3;4} Another study on conscious rabbits has shown that central administration of ghrelin can modulate the SNS by decreasing arterial pressure and suppressing renal sympathetic nervous activity.⁵ Furthermore, the parasympathetic nervous system (PNS) may be involved in initiating ghrelin secretion. Cholinergic agonists and antagonists respectively have been found to increase and decrease circulating ghrelin levels in humans.⁶ The vagus nerve has been shown to have an important role in signalling between peripheral ghrelin and central appetite centres. The detailed effects of ghrelin on SNS and PNS activity in humans have not previously been well described.

Ghrelin also regulates long-term bodyweight and promotes fat deposition and weight gain,⁷ perhaps in part through suppression of energy expenditure and thermogenesis in brown adipose tissue via inhibition of the SNS, which has been shown recently by administering central ghrelin in rats.⁸ Obese subjects have low circulating ghrelin and increased SNS activity, but whether there is a causal relationship between these observations is unknown.

We therefore studied the effects of peripheral ghrelin on heart rate variability (HRV) during a ghrelin/ placebo infusion in three groups of subjects; healthy controls, morbidly obese subjects and to examine the role of the vagus nerve, surgically vagotomized subjects.

METHODS

Volunteers were recruited by advertisement, from a local weight management clinic and from a surgical database. The study was approved by the South Sefton Research Ethics Committee (project registration number EC.05.03) and was performed in accordance with the principles of the Declaration of Helsinki. Volunteers gave written informed consent and had a normal physical examination and electrocardiogram. Urea and electrolytes and fasting glucose were normal in all subjects.

Subjects: We studied 7 healthy volunteers aged 31-62 years (mean \pm standard error of the mean (SEM), 44.7 \pm 4.6), body mass index (BMI) 20.4-27.2 (23.6 \pm 0.9) kg/m², 7 obese volunteers aged 31-45 years (mean 39.3 \pm 1.9), BMI 38.4-73.0 (50.9 \pm 4.4) kg/m² and 7 post gastrectomy volunteers aged 39-75 (mean 59.9 \pm 5.2), BMI 17.0-26.0 (22.0 \pm 1.1) kg/m².

Subject numbers were based on recruitment and previous published work showing a minimum of seven subjects were needed to show clear differences in appetite and energy intake.⁹ Obesity was defined as BMI>30 kg/m² according to the criteria of the World Health Organisation and the International Obesity Task Force. All gastrectomy subjects underwent total gastrectomy for gastric carcinoma at least 12 months previously and were in remission (none had any evidence of recurrence by gastroscopy within the last 12 months). All subjects had a truncal vagotomy as part of the procedure. The mean time between surgery and the study was 6.6 \pm 1.6 yrs (mean \pm SEM). None of the subjects had chemotherapy/ radiotherapy within the last three years. Subjects in all groups with ischaemic heart disease, type 2 diabetes and those aged over 75 years were excluded. None of the subjects had a diagnosis of obstructive sleep apnoea, cardiac failure or hypertension and no subjects were on any medications that could impact on the autonomic nervous system.

Protocol: Subjects were asked to fast and drink only water from 2100 on the night prior to the study, and to refrain from alcohol or strenuous exercise in the 24 hours preceding the study

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3 day. Each subject was studied on two occasions and received two infusions: 0.9% saline and
4
5 ghrelin (5 pmol/kg/min), in a randomised, double-blind, crossover design. The dose of 5
6
7 pmol/kg/min was chosen, as previous studies have shown an intravenous infusion of ghrelin
8
9 at this dose leads to a significant increase in food intake in lean and moderately obese
10
11 subjects.^{10;11} Human ghrelin was supplied in the acylated (octanoylated) form and produced
12
13 by Clinalfa Products, Merck Biosciences AG, Weidenmattweg 4, CH-4448 Laufelfingen,
14
15 Switzerland.
16
17

18
19 Subjects attended at 0730 on each study day. On arrival, external time cues were removed
20
21 until the end of the study. After venous cannulation the subjects relaxed for 30 minutes. The
22
23 infusion was started at 0830 (t=0) and lasted for 270 minutes. Previous studies have shown
24
25 that plasma ghrelin levels during an infusion reach a steady state within 60 minutes,¹¹ and
26
27 hence a fixed energy breakfast was given to all subjects at 60 minutes from the start of the
28
29 infusion. This consisted of 40g cornflakes, 250ml whole milk, 3.5g sugar (1 sachet) and
30
31 100ml fresh orange juice (1550 kJ, 62% carbohydrate, 13% protein and 25% fat). This meal
32
33 was chosen, as it represents a typical European style breakfast, which was palatable to most
34
35 tastes and was a suitable meal for the timing of the study. The subjects remained relaxed on a
36
37 couch tilted at 30 degrees for the remainder of the study.
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41 They were subsequently offered a free buffet lunch at 1230 (240 minutes after starting the
42
43 infusion). The items offered at the buffet lunch were consistent between subjects, and were
44
45 designed to be acceptable to a wide range of palates. The infusion was stopped at 1300 after
46
47 the buffet lunch was completed. The food was weighed pre- and post-prandially by a blinded
48
49 investigator. The purpose of providing a fixed meal and a buffet meal was to be able to
50
51 compare responses to a fixed energy meal, and then to subsequently assess food intake during
52
53 the infusion.
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55
56 Blood samples were taken at baseline, 30, 60, 75, 90, 105, 120 minutes and then hourly until
57
58 the end of the study. Samples for plasma ghrelin estimation were collected into plastic EDTA
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60 tubes containing 0.07 mg aprotinin (500 Kallikrein Inactivator Units (KIU)). Serum samples
were collected in serum separator tubes (SST) and allowed to stand for 15 minutes at -4°C

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3 before centrifugation. All samples were then immediately stored at -80°C until assayed. Heart
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5 rate and blood pressure were measured in the supine position at hourly intervals.
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8 9 10 **Assays**

11 Plasma total (acylated and desacylated) ghrelin was determined in duplicate by a
12
13 commercially available radioimmunoassay (Phoenix Pharmaceuticals, Belmont, CA). Intra-
14
15 assay and inter-assay coefficients were 7.3 % and 9.5 % respectively.
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17

18 Insulin and growth hormone (GH) was measured using a solid phase, two site,
19
20 chemiluminescent enzyme-labelled immunometric assay run on an Immulite 2000 automated
21
22 analyser (Diagnostic Products Corporation-UK, Llanberis, Gwynedd UK). Glucose was
23
24 measured by the glucose hexokinase method using the ADVIA 1650 system (Bayer UK Ltd).
25
26 The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the
27
28 formula:
$$\text{Insulin resistance} = \frac{\text{Fasting insulin} \times \text{Fasting glucose}}{22.5}^{12}$$

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33 **Heart Rate Variability (HRV)**

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35 HRV (the measure of the beat-to-beat variation in heart rate) is a useful and safe tool to
36
37 measure autonomic nervous system activity.¹³ HRV was measured for 20 minute intervals at
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39 0805, 0905, 1005, 1105, 1205, 1305 and 1405 ie. pre and post prandially at hourly intervals.
40
41 All tests were performed in a quiet temperature controlled (21±1°C) room. All HRV
42
43 measurements were taken with subjects lying supine, with a 30° head up tilt. Fifteen minutes
44
45 of continuous ECG data were collected using the BIOPAC (Biopac Systems Inc,
46
47 SantaBarbara, CA) data acquisition system via 3 ECG limb leads. This system consists of an
48
49 electrocardiogram amplifier module (ECG100B), signal conditioning module (UIM100) and
50
51 an analogue to digital signal converter (MP100). The ECG signals were sampled at 200Hz
52
53 and were transmitted to MP100 and stored on AcqKnowledge software (BIOPAC Systems
54
55 Inc, Santa Barbara).
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3 Data stored in AcqKnowledge were analysed using a commercial HRV analysis software
4 package (PowerMedic, OkiiMura, Taiwan). After manually excluding all ectopic beats, the
5 data were resampled at 2Hz and the Hamming spectral window was employed to reduce
6 spectral leakage.¹⁴ The fast Fourier transformation (FFT) method was used to calculate the
7 power spectral density. Total power (TP) is defined as the sum of absolute, very low
8 frequency (VLF), low frequency (LF) and high frequency (HF), and is considered to be an
9 indirect measure of cardiac autonomic tone. LF and HF were defined as the power in the
10 spectral bands 0.04 and 0.15 and 0.15 and 0.4Hz, representing sympathetic and
11 parasympathetic activity, and VLF as 0 to 0.04 Hz. LF and HF can also be expressed as
12 normalised units (n.u.) ie nLF and nHF, which is an expression of the percentage of total
13 power, with the VLF component subtracted.¹⁴ The LF/HF ratio is considered an indirect
14 measure of sympathovagal balance. **Therefore, an increased LF/HF ratio indicates a**
15 **sympathetic predominance. This interpretation of power spectral analysis has been validated**
16 **by international consensus and by several independent investigators.**¹⁴⁻¹⁶ Matsumoto and
17 colleagues also identified specific power spectral peaks associated with thermogenesis which
18 they called the VLO frequency component (0.007 to 0.035 Hz).¹⁷ An alternative method of
19 representing sympathetic and parasympathetic nervous system activity is by calculating a
20 SNS index (VLO + LF/HF) and a PNS index (HF/TP).¹⁷⁻¹⁹

41 42 **Statistical analysis**

43
44 The Statistical Package for the Social Sciences version 15.0 (SPSS, Chicago, IL, USA) was
45 used for data analysis. Data that was not normally distributed were log transformed before
46 analysis. Mean values are expressed as arithmetic means +/- SEM; data that has been log
47 transformed is presented as a geometric mean with a 95% confidence interval. Within-group
48 and group interactions with time were analysed using ANOVA for repeated measures. If an
49 overall interaction was found using ANOVA then post-hoc *t*-tests were done to identify
50 significant differences between individual groups. Total area under the curve (AUC) was
51 calculated using the trapezoid rule. Two tailed p values were considered significant when
52 p<0.05.

RESULTS

Baseline characteristics

Baseline characteristics are shown in table 1. The lean and obese groups were well matched for age and gender, but the gastrectomy group tended to be older (lean 44.7 ± 4.6 v gastrectomy 59.8 ± 5.2 $p=0.051$). The mean BMI in the obese group (50.9 ± 4.4 kg/m^2) was significantly higher than in the lean group (23.6 ± 0.9 kg/m^2). Weight, waist circumference and body fat percentage were also higher in the obese group. There was no significant difference in BMI, weight, waist circumference and body fat percentage in the gastrectomy group compared to the lean group.

Plasma ghrelin

Fasting ghrelin levels were significantly lower in the obese group compared to the lean group (lean 792.4 ± 40.6 pg/ml v obese 509.7 ± 101.2 pg/ml $p=0.02$) and lower still in the gastrectomy group (lean 792.4 ± 40.6 pg/ml v gastrectomy 266.3 ± 70.4 pg/ml $p<0.0001$). Plasma ghrelin levels during the ghrelin infusion reached steady state after 60 minutes. There was no significant difference between the mean steady state plasma ghrelin or area under the curve (AUC) in any of the three groups (table 1).

Plasma insulin and glucose

Ghrelin increased serum insulin levels in the healthy controls, but not significantly in obese and gastrectomy subjects (table 2). All three groups had a significant increase in serum glucose levels during the ghrelin infusion compared to during the saline infusion (table 2). As expected obese subjects were more insulin resistant than lean controls (HOMA IR lean 0.77 ± 0.12 v obese 8.38 ± 2.38 $p=0.02$), but there was no significant difference in insulin resistance between lean controls and gastrectomy subjects (HOMA IR lean 0.77 ± 0.12 v gastrectomy 0.69 ± 0.13 $p=0.63$).

Plasma growth hormone

GH levels were low during the saline infusion but showed a marked GH response during the ghrelin infusion in all three groups. The obese group had a significantly impaired GH

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3 response compared to the lean group (AUC lean 30095.8 ± 4701.8 mIU/l/min v obese
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5 7998.2 ± 1308.5 mIU/l/min $p=0.001$) but there was no difference in GH response between the
6
7 lean and gastrectomy subjects (AUC lean 30095.8 ± 4701.8 mIU/l/min v gastrectomy
8
9 30972.6 ± 4556.4 mIU/l/min $p=0.89$).

12 Energy intake

13
14 Energy intake during the buffet lunch was increased in lean subjects by $41 \pm 14\%$ on the
15
16 ghrelin infusion day compared with the saline infusion visit (3707 ± 414 kJ v 2626 ± 238 kJ
17
18 $p=0.008$). Obese subjects also increased their energy intake significantly by $35 \pm 14\%$ during
19
20 the ghrelin infusion (3873 ± 575 kJ v saline 2867 ± 501 kJ $p=0.04$). Gastrectomy subjects
21
22 however did not increase energy intake during the ghrelin infusion (1923 ± 226 kJ v saline
23
24 1764 ± 205 kJ $p=0.4$). This data has been published and discussed previously.²⁰

27 HRV

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29 Using ANOVA for repeated measures, ghrelin had an overall highly significant inhibitory
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31 effect on total power ($p=0.001$), HF ($p=0.04$), VLO ($p=0.03$) and a borderline effect on LF
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33 ($p=0.07$). Ghrelin also had an effect on nLF ($p=0.03$) but not on nHF ($p=0.20$). This ANOVA
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35 was used only as a preliminary analysis, looking for an overall effect as the three groups were
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37 different. We then followed this by detailed analysis for each group of subjects.

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42 Hence, to ascertain the effect of ghrelin on HRV in individual subject groups the AUC was
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44 compared for each treatment. These are shown in detail in table 3. In lean subjects ghrelin had
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46 a significant effect on total power (geometric mean (95% confidence interval) (6.4×10^5 (4.2 to
47
48 9.7×10^5) ms^2/hr v 9.2×10^5 (7.0 to 11.3×10^5) ms^2/hr $p=0.03$) (figure 1a), a borderline effect on
49
50 LF (1.3×10^5 (0.9 to 2.3×10^5) ms^2/hr v 1.9×10^5 (1.5 to 2.6×10^5) ms^2/hr $p=0.06$) but no effect
51
52 on HF (1.7×10^5 (0.7 to 3.6×10^5) ms^2/hr v 2.7×10^5 (1.7 to 4.2×10^5) ms^2/hr $p=0.1$). By
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54 contrast ghrelin appeared to have no effect on obese subjects on total power ($p=0.3$) (figure
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56 1b), LF ($p=0.5$) and HF ($p=0.06$), and had no significant effect in gastrectomy subjects (total
57
58 power $p=0.7$ (figure 1c), LF $p=0.7$ and HF $p=0.9$). As shown in table 3, ghrelin had no
59
60 significant effect on LF/HF ratio in any of the three groups. There was however a significant

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3 difference in LF/HF ratio between the groups in the saline condition; obese subjects had a
4 higher LF/HF ratio than both lean subjects ($p=0.007$) and gastrectomy subjects ($p=0.003$),
5 but lean and gastrectomy LF/HF ratio were similar ($p=0.06$).
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9 In addition, the SNS and PNS index were calculated for each of the groups during the saline
10 condition and are shown in figures 2a and 2b. Obese subjects had a higher SNS index to both
11 lean and gastrectomy subjects ($p=0.006$) and interestingly gastrectomy subjects had a higher
12 PNS index than the other two groups ($p=0.0002$).
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18 **Heart rate and Blood pressure**

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20 Ghrelin had an overall significant inhibitory effect on systolic blood pressure (ghrelin
21 $116.7\pm 1.5\text{mmHg}$ v saline $120.0\pm 1.7\text{mmHg}$ ANOVA $p=0.02$) in lean subjects but no effect on
22 diastolic blood pressure ($p=0.78$) or heart rate (ghrelin 70.2 ± 0.9 beats/minute vs 74.0 ± 1.1
23 beats/minute $p=0.07$). Ghrelin had no significant effects on systolic/ diastolic blood pressure
24 or heart rate in obese or post-gastrectomy subjects.
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33 **DISCUSSION**

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35 This is a novel study that attempts to ascertain the effects of ghrelin on the autonomic nervous
36 system (ANS) in humans. Our study suggests that ghrelin does indeed have an inhibitory
37 effect on the ANS in humans, and that this response is diminished in obese subjects. We also
38 report for the first time that this response is absent in vagotomized subjects, suggesting an
39 important role for the vagus nerve in ghrelin's effects on the ANS.
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49 Our study shows for the first time that peripheral ghrelin acts directly on the ANS in healthy
50 controls by altering various parameters of heart rate variability (HRV). Heart rate variability
51 is considered a sensitive indirect measure of cardiac autonomic tone, and although has some
52 limitations - autonomic tone can vary from organ to organ and the assumed generalisation of
53 autonomic tone is not always appropriate - the method is non-invasive and has been
54 extensively validated.¹⁴ Ghrelin appeared to have the greatest effect on total power (TP) and
55 low frequency (LF) power but did not reach significance with high frequency (HF) power.
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3 This suggests that ghrelin had a more pronounced effect on sympathetic activity rather than
4 parasympathetic activity but this distinction is likely to be minimal as the LF/HF ratio was
5 unchanged during the ghrelin infusion.
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11 Previous data have suggested a post-prandial increase in cardiac autonomic sympathetic
12 activity, but this was not seen in our cohort perhaps due to small numbers. Nevertheless, there
13 was a significant reduction in total power during the ghrelin infusion in lean individuals,
14 which was not apparent in the obese and gastrectomized subjects.
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22 Ghrelin decreases arterial pressure without altering heart rate, but the mechanism has not been
23 examined previously. We report for the first time that human subjects who have had a
24 previous vagotomy do not demonstrate any significant SNS response to the peripheral
25 administration of ghrelin. This is demonstrated in our study by the clear lack of a change in
26 HRV parameters between saline and ghrelin infusion arms. This is further supported by the
27 observation that ghrelin did also not affect blood pressure or heart rate in these subjects. This
28 suggests that peripheral ghrelin communicates with the NTS and DMV via vagal afferents.
29
30 Our group and others, have shown previously the lack of orexigenic effect of ghrelin in
31 vagotomized subjects and this further supports the new findings.^{20,21} It was interesting to note
32 that despite vagotomy our gastrectomy subjects had significantly higher PNS index than the
33 other two groups, but a similar SNS index, suggesting increased parasympathetic activity. Our
34 gastrectomy group were older but previous studies have suggested that all HRV indices are
35 thought to decrease with increasing age, so is unlikely to be a major factor.²²
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53 We have also confirmed previous studies in showing that ghrelin decreases arterial pressure
54 but does not alter heart rate in healthy controls.² This suggests that the normal compensatory
55 response to lowering of blood pressure ie SNS mediated increase in heart rate, does not occur
56 and further confirms that ghrelin is likely to inhibit SNS activity in healthy controls. It is
57 known that the growth hormone secretagogue receptor (GHS-R) is abundant in human
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3 vasculature²³ and recent data suggest that ghrelin inhibits vascular superoxide production in
4
5 hypertensive rats and hence may act directly on vascular endothelium.²⁴ Previous
6
7 investigators have demonstrated that central ghrelin induces *c-fos* expression in the nucleus of
8
9 the solitary tract (NTS) and the dorso-motor nucleus of the vagus (DMV) in the brainstem.²⁵
10
11 It is also known that the NTS is an important regulator of blood pressure and the SNS. It
12
13 follows therefore that central ghrelin may act via the NTS and the DMV to modulate the SNS
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15 and blood pressure ; an elegant study by Lin and colleagues supports this hypothesis, showing
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17 that ghrelin acted directly on the NTS in rats to suppress the SNS and decrease arterial
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19 pressure.²⁶
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25 As mentioned above, previous animal studies have suggested that central and peripheral
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27 ghrelin administration suppresses SNS activity.^{3-5;8} Interestingly, centrally administered
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29 leptin has the opposite effect by promoting increased SNS activity and increasing arterial
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31 pressure,²⁷ and as there is good evidence that ghrelin and leptin antagonize each other in the
32
33 regulation of appetite and feeding behaviour,²⁸ it is possible that they have similar roles in
34
35 SNS regulation. Ghrelin is known to increase neuropeptide Y (NPY) and agouti-related
36
37 protein (AGRP) mRNA levels in the arcuate nucleus.²⁹ Studies have shown that NPY
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39 decreases arterial pressure and renal sympathetic nerve activity in rabbits³⁰ and AGRP
40
41 antagonizes leptin activation of the melanocortin-3 and -4 receptors (MC3-R and MC4-R)³¹;
42
43 therefore the mechanism behind ghrelin's influence on the central control of SNS activity can
44
45 be at least partially explained via increasing NPY and AGRP expression,⁵ in addition to
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47 actions on the NTS and DMV as mentioned above.
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53 From our data and the above published data we can therefore postulate that an intact vagus
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55 nerve is needed for peripheral ghrelin to signal to the NTS/DMV in the brainstem, and that
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57 central ghrelin may decrease SNS activity via NPY and possibly antagonises leptin's effects
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59 on the SNS.
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3 Although ghrelin is a potent stimulator of growth hormone (GH) release, intravenous GH
4 injection in healthy volunteers failed to change arterial pressure or heart rate in a recent
5 study.³² However it is well known that growth hormone deficiency (GHD) is associated with
6 abnormalities in cardiac autonomic tone, and these are partially corrected with treatment.³³ It
7 is possible that the acute cardiovascular and sympathetic effects are secondary to ghrelin
8 alone but it is likely that enhanced GH release will play an important confounder here.
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18 Another important finding was a diminished response on HRV parameters to ghrelin by the
19 obese subjects. The obese group was typically a more heterogeneous group and this is
20 reflected by the wide confidence intervals which are apparent in table 3. However the number
21 of subjects was the same as the lean control group so should have been adequately powered to
22 show a difference. Obese subjects are typically thought to have an impairment of vagal tone,
23 but this was not demonstrated in our study.³⁴⁻³⁶
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33 The reason for a reduced effect of ghrelin on obese subjects may be related to the marked
34 hyperinsulinaemia and insulin resistance demonstrated by these subjects. Obese subjects are
35 well known to have hyperinsulinaemia and insulin resistance, and insulin is thought to have a
36 physiologically relevant effect on increasing SNS activity.³⁵ More recent data has shown that
37 insulin alters sympathovagal balance measured by HRV in insulin sensitive but not in insulin
38 resistant individuals.³⁷ The precise mechanism of insulin's action on heart rate variability is
39 complex, and furthermore the interactions between ghrelin and insulin both peripherally and
40 centrally, are controversial. As shown by previous groups obese groups have a diminished
41 GH response to ghrelin, but the GH response is preserved in the gastrectomy group.^{10;38} The
42 mechanism behind the diminished GH response is not known although may involve a
43 downregulation in pituitary GHS receptors.³⁹ As discussed above, GH is likely to have an
44 effect on autonomic function and clearly this could be involved in the diminished response on
45 the SNS in the obese. Nogueiras and colleagues have recently shown that central glucagon-
46 like peptide (GLP-1) modulates adipocyte metabolism at least partially via the SNS, and that
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3 this effect is blunted in diet-induced obese mice.⁴⁰ It is possible that there is similar obesity-
4 induced adipocyte resistance to the effects of peripheral ghrelin on the SNS; this is an area for
5 future research.
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11 The main limitation of this study was that the ghrelin infusion achieved supra-physiological
12 plasma levels of total ghrelin. We, in common with other groups who have used a synthetic
13 acylated ghrelin infusion, did not specifically measure acylated ghrelin however and this may
14 have added valuable information. In addition, it is known that acylated ghrelin makes up only
15 around 10% of circulating total ghrelin, and the administration of supra-physiological doses
16 of acylated ghrelin may have overtaken the influence of non-acylated ghrelin.^{41:42} Similarly
17 we would accept that due to small numbers, conclusions from detailed sub-set analysis may
18 have limitations but all the data and analysis has been presented and made available to the
19 reader.
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32 In conclusion we have shown that ghrelin inhibits autonomic nervous system activity in
33 healthy controls, particularly the SNS. There appears to be a diminished effect of ghrelin on
34 obese subjects and an absent effect in vagotomized gastrectomy subjects. The precise
35 mechanism behind the lack of response in obese subjects may involve hyperinsulinaemia and
36 insulin resistance or a diminished GH response. The absence of response in vagotomized
37 subjects confirms the importance of the vagus nerve in communicating the peripheral gut
38 signals to the brainstem.
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50 ACKNOWLEDGEMENTS

51 The authors would like to thank Jackie Cleator, Janet Cashen, Barbara Martin and David
52 Kerrigan for their contributions to the study, as well as all of the volunteers who took part.
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Table 1 Baseline characteristics of lean, obese and gastrectomy subjects †

	Lean	Obese	Gastrectomy
n	7	7	7
Sex (male)	3(43%)	2(29%)	2(29%)
Age (yrs)	44.7±4.6	39.3±1.9	59.8±5.2*
BMI (kg/m ²)	23.6±0.9	50.9±4.4*	22.0±1.1
Weight(kg)	64.4±4.8	141.0±19.2*	61.4±3.1
Waist (cm)	80.6±5.8	14.3±13.2*	82.2±3.5
% Body Fat	26.6±0.9	49.1±1.3*	28.3±3.0
Fasting Ghrelin			
(pg/ml)	792.4±40.6	509.7±101.2*	266.3±70.4*
Mean Steady state			
ghrelin (pg/ml)	5444.6±50.5	6112.6±447.5	5559.6±209.1
Mean ghrelin AUC**			
(pg/ml.min)	1.4x10 ⁶ ±0.2x10 ⁶	1.7x10 ⁶ ±0.2x10 ⁶	1.5x10 ⁶ ±0.2x10 ⁶

† Plus-minus values are means ± SEM

* p<0.05 when compared to lean values

** AUC area under the curve

Table 2

		Ghrelin†	Saline†	p value
AUC Insulin (μ U/ml.min)	Lean	8608 \pm 507	6604 \pm 696	0.04
	Obese	23177 \pm 4165	20214 \pm 3125	0.37
	Gastrectomy	6292 \pm 1262	4940 \pm 543	0.17
AUC Glucose (mmol/l.min)	Lean	2123 \pm 132	1725 \pm 87	0.009
	Obese	2486 \pm 168	2071 \pm 148	0.02
	Gastrectomy	2384 \pm 160	1924 \pm 95	0.006

† mean \pm standard error of the mean (SEM)

Table 3

HRV Parameters	Ghrelin infusion*	Saline infusion*	p value
AUC TP (ms ² /hr)			
Lean	6.4x10 ⁵ (4.2 to 9.7x10 ⁵)	9.2x10 ⁵ (7.0 to 11.3x10 ⁵)	0.03
Obese	14.1 x10 ⁵ (7.5 to 28.9 x10 ⁵)	17.6 x10 ⁵ (10.9 to 31.6 x10 ⁵)	0.3
Gastrectomy	6.1 x10 ⁵ (3.2 to 10.6 x10 ⁵)	6.1 x10 ⁵ (3.6 to 10.1 x10 ⁵)	0.7
AUC LF power (ms ² /hr)			
Lean	1.3 x10 ⁵ (0.9 to 2.3 x10 ⁵)	1.9 x10 ⁵ (1.5 to 2.6 x10 ⁵)	0.06
Obese	4.4 x10 ⁵ (1.9 to 10.6 x10 ⁵)	5.8 x10 ⁵ (3.2 to 11.1 x10 ⁵)	0.5
Gastrectomy	0.9 x10 ⁵ (0.6 to 1.3 x10 ⁵)	1.0 x10 ⁵ (0.7 to 1.3 x10 ⁵)	0.7
AUC HF power (ms ² /hr)			
Lean	1.7 x10 ⁵ (0.7 to 3.6 x10 ⁵)	2.7 x10 ⁵ (1.7 to 4.2 x10 ⁵)	0.1
Obese	3.1 x10 ⁵ (1.0 to 9.0 x10 ⁵)	5.7 x10 ⁵ (2.9 to 11.3 x10 ⁵)	0.06
Gastrectomy	1.7 x10 ⁵ (0.4 to 4.3 x10 ⁵)	1.7 x10 ⁵ (0.6 to 3.8 x10 ⁵)	0.9
AUC nLF power (n.u./hr)			
Lean	8386(6796 to 10347)	9021(7862 to 10352)	0.4
Obese	10134(8347 to 12440)	11439(10077 to 12979)	0.3
Gastrectomy	4788(3489 to 6508)	6738(6239 to 7275)	0.03
AUC nHF power (n.u./hr)			
Lean	9972(7932 to 12650)	12322(10855 to 13980)	0.2
Obese	8298(6732 to 10267)	10356(8036 to 13258)	0.02
Gastrectomy	11246(6776 to 18641)	10572(7438 to 15348)	0.6
AUC VLO power (ms ² /hr)			
Lean	1.6 x10 ⁵ (1.0 to 2.6 x10 ⁵)	2.1 x10 ⁵ (1.6 to 2.7 x10 ⁵)	0.08
Obese	3.4 x10 ⁵ (1.9 to 7.1 x10 ⁵)	4.8 x10 ⁵ (3.1 to 8.2 x10 ⁵)	0.1
Gastrectomy	1.3 x10 ⁵ (0.7 to 2.3 x10 ⁵)	1.5 x10 ⁵ (0.7 to 2.8 x10 ⁵)	0.2
AUC LF/HF ratio (hrs)			
Lean	364.5(247.7 to 481.2)	348.2(275.9 to 420.5)	0.7
Obese	579.2(417.9 to 740.6)	513.2(490.0 to 363.3)	0.4
Gastrectomy	184.8(108.8 to 260.7)	236.7(129.1 to 344.3)	0.1

* Geometric mean (95% confidence interval)

LEGENDS

Figure 1 Total power in a.)lean, b.)obese and c)gastrectomy subjects during the ghrelin and saline infusions.

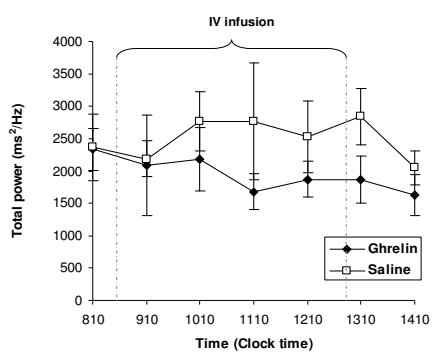
Figure 2 a) SNS index and b) PNS index in healthy controls, obese and gastrectomy subjects.

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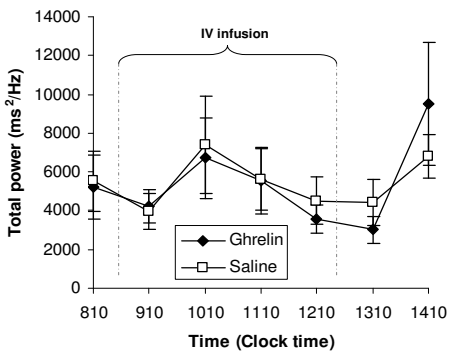
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Figure 1
a



b



c

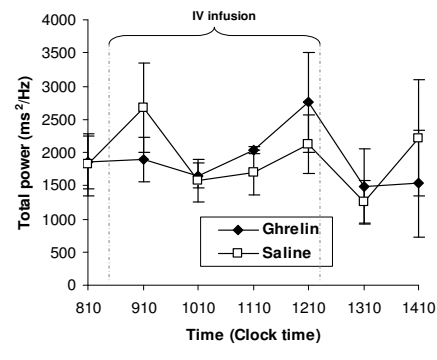
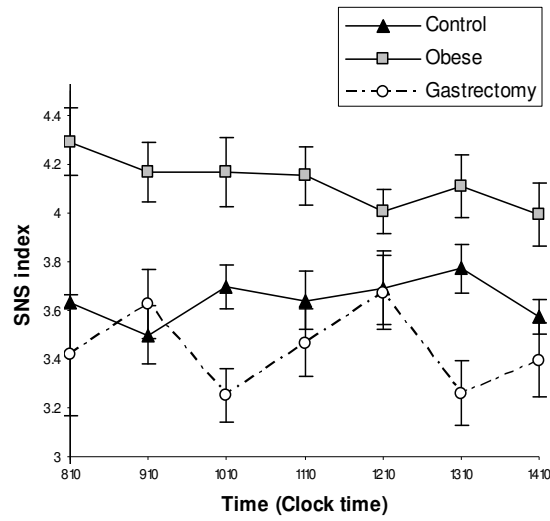


Figure 2

a



b

