Lack of Association Between Impaired Glucose Tolerance and Appetite Regulating Hormones in Patients with Obstructive Sleep Apnea

Ioannis Papaioannou, Ph.D.¹; Michael Patterson, Ph.D.²; Gillian L. Twigg, Ph.D.¹; Ali Vazir, Ph.D.¹; Mohammad Ghattei, Ph.D.²; Mary J. Morrell, Ph.D.¹; Michael I. Polkey, F.R.C.P.¹

¹NIHR Respiratory Disease Biomedical Research Unit at the Royal Brompton and Harefield NHS Foundation Trust and Imperial College London, London, UK; ²Section of Investigative Medicine, Division of Diabetes, Endocrinology and Metabolism, Imperial College London, London, UK

Study Objectives: Understanding the etiologic mechanisms underlying impaired glucose tolerance in obstructive sleep apnea (OSA) would assist development of therapies against this comorbidity. We hypothesized that in patients with OSA impaired glucose tolerance (IGT) would be associated with elevated levels of hormones associated with appetite regulation (leptin, ghrelin, neuropeptide Y [NPY] and peptide tyrosine-tyrosine [PYY]).

Method: We studied 68 OSA patients (mean AHI 22 events/h) and 37 age and weight matched healthy controls recruited by advertisement. All participants received a standardized evening meal, attended polysomnography and an oral glucose tolerance test (OGTT) on waking. Hormones were measured in blood taken before sleep (22:30) and at the start of the OGTT.

Results: Impaired glucose tolerance was present in 54% of patients and 32% of controls ($p = 0.05$). The only differences between groups was that leptin was significantly higher at 22:30 in OSA patients compared to controls (9.6 ng/L vs 7.9 ng/L, $p = 0.05$). OSA patients had marginally elevated plasma NPY

levels at 22:30 (56.6 [52, 67] pmol/L vs 51.1 [47.3, 61] pmol/L; $p = 0.04$). No differences in ghrelin, PYY or NPY were observed between patients with IGT and those without. However OSA patients with IGT had significantly higher value of leptin at both 22:30 (10.9 [7.7, 15.9] ng/mL vs 7.4 [5.6, 12.3] ng/mL, $p = 0.02$) and 07:00 (11.6 [7.6, 16.2] ng/mL vs 6.9 [5.4, 12.6] ng/mL, $p = 0.024$) than those without. In multivariate analysis the only major association of leptin was body mass index.

Conclusion: Clinically significant abnormalities of appetite regulating hormones are not present in OSA. Appetite regulating hormones did not differ in OSA patients with and without impaired glucose tolerance.

Keywords: Obstructive sleep apnea, impaired glucose tolerance, appetite regulating hormones

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BRIEF SUMMARY

Current Knowledge/Study Rationale: Impaired glucose tolerance is a common and important co-morbidity of obstructive sleep apnea yet its pathogenesis, beyond the shared etiology of obesity is unknown. Here we tested the hypothesis that OSA might induce increases of appetite regulating hormones which might suggest a cause for IGT.

Study Impact: In fact the data did not support our hypothesis, so providing an "important negative." Strengths of the study were first that we used a larger sample size than has commonly previously been the case and, more important, our control subjects were "true" controls rather than patients referred to the sleep laboratory subsequently found to have a low AHI. These design considerations could inform future studies.

Untreated obstructive sleep apnea (OSA) is associated with insulin resistance,^{1,2} and some studies suggest that treatment with CPAP improves glucose control,^{3,4} although this was not found in a randomized controlled trial of diabetics with sleep apnea.⁵ The observation that established type 2 diabetics cannot improve glucose control when their OSA is treated with CPAP, emphasizes the importance of understanding the pathophysiology of insulin resistance associated with OSA.

One mechanism linking OSA with impaired glucose tolerance could be sympathetic nerve activity which is a recognized phenomenon in OSA⁶; indeed it has previously been hypothesized that this process is responsible for the elevated leptin levels seen in newly diagnosed OSA patients.⁷ Consistent with this sibutramine, a serotonin and noradrenaline reuptake inhibitor, was able to improve insulin resistance in OSA independent of visceral fat.⁸ Moreover two studies raise the hypothesis that hormones involved in appetite regulation could contribute to impaired glucose tolerance. Broglio et al.⁹ demonstrated that ghrelin administered to healthy subjects simultaneously with

glucose, resulted in a transient decrease of insulin concentration that was coupled with significant increase in glucose levels. In addition it has been reported that IR is associated with increased plasma leptin concentration independent of the body fat.¹⁰ Consistent with this hypothesis, previous studies have reported that OSA is independently associated with neuroendocrine dysregulation; in fact OSA has been reported to be

associated with increases in plasma concentrations of 3 hormones involved in the appetite regulation. Specifically OSA patients have been reported to display elevated ghrelin,¹¹⁻¹³ leptin,¹¹ and neuropeptide Y (NPY).¹² The effect, if any, on a fourth identified neuroendocrine hormone, peptide tyrosine-tyrosine (PYY) is unknown. However glucose tolerance was not related to hormone levels in any of the studies, and in most cases sample sizes were small.

Thus the aim of the present study was to test the hypothesis that IGT in OSA patients is associated with elevated leptin and ghrelin concentration. In order to fully interpret the findings we also measured the two other known hormones involved in appetite regulation, NPY and PYY.

METHODS

Subjects

Participant recruitment is illustrated in flow chart (**Figure 1**). Thirty-three consecutive patients and eleven healthy controls were studied and, since the numbers then exceeded the numbers studied Harsch et al.,¹¹ an interim analysis was performed. This interim analysis showed that we had sufficient data to answer our hypothesis with regard to ghrelin but revealed potential trends to significance in the other 3 hormones (see supplementary **Table S2**, supplementary material is available online at www.aasmnet.org/jcsm) and we extended the study. A total of 68 OSA patients and 37 healthy controls were studied for leptin, PYY, and NPY and 33 OSA patients and 11 controls for ghrelin. The patients were recruited from our sleep service. Inclusion criteria were a diagnosis suggested by the finding of snoring, sleepiness, and in most cases a respiratory sleep study performed outside our hospital. For the purposes of the study a subsequent attended in-hospital polysomnography was performed (see below). The controls were, importantly, recruited not from the sleep service but rather from the staff of the hospital or by advertisement. The protocol was approved by the ethics committee of the Royal Brompton Hospital and Imperial College (05/Q0404/69) and all participants provided written informed consent.

Protocol

Participants in this study arrived at the sleep laboratory in time to receive a standard meal (680 kcal, 51% carbohydrates) at 18:00; no further food was permitted until the completion of the study. They then received a structured clinical evaluation comprising a physical examination (including measurement of height and weight) and measurement of arterial blood gases (this procedure was not performed in healthy subjects). Subjective sleepiness was assessed using the Epworth Sleepiness scale.¹⁴

Polysomnography (Jaeger-Toennies, Hoechburg, Germany) was performed according to standard guidelines.¹⁵ We measured the electroencephalogram (C3/A2 and C4/A1), and electrooculogram (ROC/A1 and LOC/A2). The EEG was recorded using gold-cup electrodes (Grass instruments, West Warwick, USA). Airflow was measured using nasal pressure cannulae (Embla, Broomfield, USA). Ribcage and abdominal movement were assessed non-invasively with

Figure 1—Recruitment flow diagram for OSA patients and healthy controls

P, patients; C, controls.

two effort belts. Arterial oxygen saturation (SpO₂) was measured with a built-in pulse oximeter. Tracheal sounds and snoring were recorded with a snoring-microphone (diameter 26 mm) taped in the anterior part of the neck. Anterior tibialis EMG activity was recorded from one leg. Two electrodes were placed in the long axis of the anterior tibialis muscle. Finally an integrated sensor was also used to assess the body position during sleep.

Blood tests were taken after 4 h of fasting at 22:30, just before the initiation of the full polysomnography. Immediately after awakening an intravenous cannula was sited in the antecubital fossa, and all participants participated in an oral glucose tolerance test (OGTT) using the protocol of the American Diabetes Association (ADA)¹⁶ with a glucose load of 75 g. Blood was drawn immediately before and 2 h after administration of oral glucose. All blood samples were collected into Vacutainers with EDTA and immediately spun for 10 min at 0°C. The clear plasma supernatant was then stored at -80°C until final analysis.

Glucose and insulin were measured in the morning fasting sample in order to calculate the IR using the homeostatic model assessment (HOMA) method; standard kits were supplied by Beckman Coulter Inc., Fullerton, USA. Hormones were measured by established specific and sensitive radioimmunoassays. Briefly the ghrelin radioimmunoassay was carried out as previously described.^{17,18} The assay cross-reacted fully (100 %) with both acylated and des-acylated ghrelin

and did not cross-react with any other known gastrointestinal or pancreatic hormone. The assay detected changes of 25 pmol/L with a 95% confidence limit. Our laboratory has recorded intra-assay and inter-assay coefficients of variation of 5.5% and 9.5%, respectively. Plasma leptin was measured by human leptin radioimmunoassay purchased from Linco Research (St. Charles, MO, USA). The NPY radioimmunoassay was carried out as previously described.¹⁹ The assay cross-reacts fully with 100 % human NPY and did not cross-react with pancreatic polypeptide, PYY or any other known gut hormone. The assay detected changes of 4 pmol/L with a 95% confidence limit. Our laboratory has recorded intra-assay and inter-assay coefficients of variation of 6.8% and 10.9%, respectively. The PYY radioimmunoassay was carried out as previously described.²⁰ The assay cross-reacted fully with PYY 1-36 and PYY 3-36 and did not cross-react with pancreatic polypeptide, NPY, or any other known gastrointestinal hormone. The assay detected changes of 2 pmol/L with a 95% confidence limit. Our laboratory has recorded intra-assay and inter-assay coefficients of variation of 5.8% and 9.8%, respectively.

Data Conventions and Statistical Analysis

Patient characteristics, metabolic and sleep parameters, and hormones are expressed as means $\pm$ standard deviation (SD) or median (IQR). Impaired glucose tolerance (IGT) was defined as fasting glucose > 6.1 mM/L or a 2-h glucose > 7.8 mM/L after administration of the oral glucose load. Age, body mass index (BMI), fat free mass, sleep parameters, glucose metabolism indices and hormones were compared between different groups; since variables were in general not normally distributed statistical differences were sought between OSA and non OSA patients, and patients with and without IGT, using the Mann-Whitney test. Hormone levels and sleep parameters such as apnea-hypopnea index (AHI) and oxygen desaturation index (ODI) were tested for any relationship using linear regression analysis or spearman correlation when variables were not normally distributed. All factors were first tested using simple regression and then a multiple regression model adjusted for age and BMI. The percentage of IGT in the OSA group and the control group, which could not be normally distributed (being a dichotomous variable), was compared using Fisher exact test. In all cases, $p < 0.05$ was considered statistically significant.

Table 1—Characteristics of patients and controls

	Patients	Controls	p value
Number of subjects	68	37	
Age (y)	49 (9.9)	45 (10.2)	0.07
BMI (kg/m ²)	30 (4.6)	28 (4.5)	0.06
Stage 1&2 (% TST)	73 (65, 77.8)	61 (54.2, 67)	0.001
REM sleep (% TST)	15 (5.9)	19 (6.8)	0.001
SWS sleep (% TST)	13 (7.8)	21 (7.7)	0.001
Sleep efficiency (%)	78 (69.6, 84.5)	80 (71.6, 84)	0.7
AHI (events/h)	22.4 (11.4, 37)	2.5 (1.85, 3.4)	
ODI (/h)	13 (5, 27.7)	1 (0, 2)	< 0.001
Arousal index (/h)	18.9 (13.8, 34)	11.0 (96, 18.4)	0.001
ESS (of 24)	11 (7, 13)	4 (2, 7)	0.001

Figures in parenthesis denote SD or upper and lower limit of interquartile range for non-normally distributed data.

RESULTS

Characteristics of the OSA patients and healthy controls are shown in **Table 1**. The baseline demographics and characteristics of the patients and controls at the time of the interim analysis (and therefore of the ghrelin subset) is shown in supplementary **Table S1**. There were no statistical differences between OSA patients and controls for age or BMI. Details of the interim analysis may also be found in the online supplement at www.aasmnet.org/jcsm.

The endocrine and metabolic parameters for both OSA patients and controls are given in **Table 2**. OSA patients had a greater 2-h post load blood glucose values than controls (7.1 [5.5, 8.2] mmol/L vs 5.35 [4.7, 6.3] mmol/L; $p = 0.003$). However there was only a trend for OSA to have increased fasting plasma glucose ($p = 0.07$) despite the higher IR, as judged by the HOMA method in OSA patients ($p = 0.01$).

Table 2—Endocrine and metabolic parameters

	Patients	Controls	p value
Impaired glucose tolerance (No of subjects)	37/68	12/37	0.05
Insulin Resistance (a.u.)*	1.9 (1.24, 2.54)	1.2 (0.82, 2.1)	0.01
07:30 fasting plasma glucose (mmol/L)	5.5 (5.1, 6)	5.3 (5.1, 5.5)	0.07
2-h Glucose (mmol/L)	7.1 (5.5, 8.2)	5.4 (4.7, 6.3)	0.003
Plasma leptin 22:30 (ng/mL)	9.6 (6.8, 15)	7.9 (4, 12.9)	0.05
Plasma leptin 07:00 (ng/mL)	11 (6.2, 15.4)	7.9 (3.8, 15.5)	0.08
Plasma NPY 22:30 (pmol/L)	56.6 (51.6, 67)	51.1 (47.3, 61)	0.04
Plasma NPY 07:00 (pmol/L)	53 (13.2)	53 (13.6)	0.98
Plasma PYY 22:30 (pmol/L)	20 (16.7, 25.9)	22 (18.5, 27.5)	0.3
Plasma PYY 07:00 (pmol/L)	15 (11, 20)	15 (10.6, 19.)	0.69

Figures in parenthesis denote SD or upper and lower limit of interquartile range for non-normally distributed data. Insulin resistance was measured by homeostatic model assessment (HOMA) in arbitrary units. An increase in the figure represents an increase in insulin resistance. The result demonstrates the patient group are more insulin resistant than the controls.

Neuropeptides and OSA

Leptin was significantly higher at 22:30 in OSA patients than controls ($p = 0.05$), although this did not reach significance at 07:00 in ($p = 0.08$). In OSA patients, leptin (measured at 22:30) was found to correlate weakly with IR ($r = 0.3$, $p = 0.01$) and with BMI ($r = 0.31$, $p = 0.01$). Leptin is plotted against BMI in **Figure 2**; a relationship is observed in patients with and without IGT. Multiple regression analysis was therefore used to explore any relation between IGT and leptin. Leptin was found to be significantly associated with IR adjusted for age and AHI; however, when BMI was entered into the model, only BMI was related to IR in OSA patients (**Table S4**).

OSA patients had a statistically higher level of NPY at 22:30 (56.6[52, 67] pmol/L vs 51.1[47.3, 61] pmol/L; $p = 0.04$); although the difference was small, and it did not correlate with BMI or IR. NPY at 07:00 was similar in both groups ($p = 0.98$). Finally PYY and ghrelin (see **Table 2** and supplementary **Table S2**) were similar in both OSA and control subjects at 22:30 and at 07:00 (PYY: $P = 0.3$ and 0.69 ; ghrelin: $p = 0.4$ and 0.41 , respectively).

Neuropeptides and Impaired Glucose Tolerance

54.4 % of OSA patients (37/68) were found to have IGT the demographics and characteristics of patients with and without

IGT are given in **Table 3**. Except for age, there was no statistically significant difference between OSA patients with IGT and those without; although, as expected, OSA patients with IGT had a tendency to be heavier, which marginally failed to achieve statistical significance (BMI: 31 [3.9] kg/m² vs 29 [5.1] kg/m², $p = 0.06$).

Neuropeptide values are shown in **Table 4**. OSA patients with IGT had significantly higher value of leptin at both 22:30 (10.9 [7.7, 15.9] ng/mL vs 7.4 [5.6, 12.3] ng/mL, $p = 0.02$) and 07:00 (11.6 [7.6, 16.2] ng/mL vs 6.9 [5.4, 12.6] ng/mL, $p = 0.024$). No differences in ghrelin (shown in **Table S3**), NPY, or PYY between patients with and without IGT were observed.

DISCUSSION

Our data show that, with the exception of leptin and NPY (and only modestly in the case of NPY) the hormones associated with appetite regulation do not differ between patients

Table 3—Characteristics of OSA patients with and without IGT

	OSA with IGT	OSA without IGT	p value
Number of subjects	37 (54.4 %)	31 (45.6 %)	
Age (y)	51.4 (9)	46.1 (10)	0.025
BMI (kg/m ²)	31 (3.9)	29 (5.1)	0.06
Insulin resistance (a.u.)	5.2 (4.9, 5.3)	1.3 (1.1, 2.39)	
Fasting glucose (mmol/L)	6.0 (5.6, 6.5)	5.2 (4.9, 5.3)	
2-h glucose (mmol/L)	8.1 (7.1, 10.5)	5.6 (4.7, 7.0)	
Stage 1&2 (% TST)	75 (6.4, 78.2)	73.5 (66.7, 77.4)	0.9
REM (% TST)	15 (6.2)	14 (5.6)	0.73
SWS (% TST)	12 (6.2)	14 (8.3)	0.26
Sleep efficiency (%)	74 (13.6)	77 (10.7)	0.3
AHI (events/h)	23 (14.8, 46.4)	20 (8.2, 36.5)	0.18
ODI (/h)	17 (7.5, 31)	8 (2.0, 25.7)	0.07
Arousal index (/h)	19 (14.1, 35.1)	19 (13.1, 32.2)	0.6
ESS (of 24)	10 (4.1)	10 (5.2)	0.6

Figures in parenthesis denote SD or upper and lower limit of interquartile range for non-normally distributed data.

Figure 2

Fasting leptin at 22:30 versus body mass index in OSA patients (●) with IGT (upper regression line; $r = 0.48$, $p = 0.003$). Fasting leptin at 22:30 versus body mass index in OSA patients (○) without IGT (lower regression line; $r = 0.16$, $p = 0.38$).

Table 4—Metabolic and endocrine parameters in OSA patients with and without IGT

	OSA with IGT	OSA without IGT	p value
Plasma leptin 22:30 (ng/mL)	10.9 (7.7, 15.9)	7.4 (5.6, 12.3)	0.02
Plasma leptin 07:00 (ng/mL)	11.6 (7.6, 16.2)	6.9 (5.4, 12.6)	0.024
Plasma NPY 22:30 (pmol/L)	60.5 (13)	57.0 (11)	0.22
Plasma NPY 07:00 (pmol/L)	55.0 (49, 61.7)	54.5 (46.4, 59.3)	0.41
Plasma PYY 22:30 (pmol/L)	19.3 (16.5, 22.6)	21.7 (17.5, 26.6)	0.22
Plasma PYY 07:00 (pmol/L)	14.9 (10.8, 19.55)	15.4 (11.7, 19.8)	0.8

Figures in parenthesis denote SD or upper and lower limit of interquartile range for non-normally distributed data.

and controls nor between OSA patients with and without IGT. Although the biggest driver of elevated leptin was obesity, consistent with prior reports, insulin resistance was greater in those with more elevated leptin levels. We conclude that important abnormalities of the hormones associated with appetite regulation are not present in OSA nor do they cause IGT in OSA.

Critique of the Method

Our cross-sectional data must be interpreted with caution. Care must always be exercised when drawing conclusions from studies without an intervention, although in this case our conclusions are stronger since they are negative. We did not continue ghrelin analysis after 33 patients had been studied, since no positive results were found; but because we were not confident about the other hormones we extended the study.

The strengths of our study are that all participants had their sleep evaluated using polysomnography, and that IGT was determined formally with a glucose tolerance test. Importantly, our control subjects were truly controls rather than, as is often the case, snorers referred to the sleep clinic who prove to have a low apnea hypopnea index (AHI). The diet prior to sleep was carefully controlled, and hormone concentrations were measured both before and after sleep. Appetite-regulating hormones are by nature dynamic, and conceptually it is possible that circadian rhythm differences between patients and controls could have masked a positive finding. However, we specifically investigated circadian rhythm in a separate study²¹ and showed that important differences did not exist between patients and controls.

Lastly, although the mean AHI of our patients was lower than many prior studies, we found like previous investigators, that our cohort of patients had a high prevalence of IGT; thus, if hormone abnormalities had occurred in our study we should have captured them. We were unable to distinguish between those with IGT, and those without using polysomnography. In passing we note that unlike Barcelo and coworkers,²² we also found no differences in subjectively assessed sleepiness between OSA patients with and without IGT.

Significance of the Findings

Prior to our study the most information was available for leptin. Phillips et al. studied 32 patients with newly diagnosed OSA and found leptin elevated compared with control subjects.⁷ Harsch et al. measured leptin in 30 OSA patients and found elevated leptin levels which decreased after 2 months of CPAP therapy, without a change in BMI.¹¹ Similarly Ciftci et al. after a study of 30 OSA patients and 22 controls also found leptin elevated in patients with OSA.²³ Unlike Ciftci et al., we observed no association between leptin and indices of sleep disordered breathing. Barcelo et al. studied leptin and NPY in 47 patients with OSA and 37 controls split by obesity. Again, obesity seemed the strongest predictor of leptin, although levels fell in non-obese patients after the initiation of CPAP therapy.²⁴ Our results are also in agreement with those of Vgontzas et al.,²⁵ who reported higher leptin ($p < 0.05$) at both 22:30 and 07:00 in 14 obese OSA patients with severe disease (AHI > 30) compared to lean and obese healthy controls. Ip et al.²⁶ investigated the relation between OSA and leptin, which was found to be

increased, and positively correlated with indices of sleep disturbance and body fat stores in an Asian population. Lastly, a more recent study,²⁷ reported that leptin was significantly higher in 21 OSA patients, compared to 21 healthy subjects matched for age and BMI, but this was not retained in multiple regression analysis which accounted for BMI.

Three previous studies have investigated the relation between OSA and ghrelin.^{11,13,23} Harsch et al.¹¹ found levels to be elevated in 9 patients, and that levels fell after CPAP. Takahashi et al. also found elevated levels of ghrelin, and that levels were reduced by 1 month of CPAP therapy in 21 patients with COPD.¹³ On the other hand Ciftci et al., like us, found no elevation of ghrelin in patients with OSA.²³ The likelihood that our conclusion (that ghrelin is not elevated in OSA) is correct is further supported by the lack of a relationship between ghrelin and AHI, or other indices of oxygen desaturation.

With regard to the remaining peptides, NPY was elevated in the evening sample of OSA patients compared with controls; two previous studies investigated the relation of NPY and OSA. The first included eleven obese OSA patients²⁸ who were not fully matched to the healthy control subjects for age or BMI. NPY in the OSA patients was not increased and the authors explained their negative results by the low sympathetic nerve activity (SNA) of the patients they included, as NPY is a marker of sympathetic activation.²⁹ However the SNA was assessed during wakefulness and NPY was measured in the afternoon in a non-fasting state. Given these limitations, one can draw only limited conclusions from the above study on the effect of OSA on NPY. In a larger study,¹² NPY was measured between 08:00-10:00, and NPY was elevated in both obese and non-obese OSA patients ($n = 47$), consistent with the data of the present study. The diurnal variance of NPY has not been previously reported; in contrast NPY in healthy subjects was not found different between 22:30 and 07:00 ($p = 0.7$). It is of interest that hypoxia itself can be a stimulus to elevation of NPY^{30,31} so the above observations are biologically plausible.

The lack of association between ghrelin and IGT *in vivo* is best explained as a dosage phenomenon; specifically we speculate that ghrelin probably causes only hyperglycemia if it is administered intravenously and reaches supra-physiological levels. Previous studies reported significantly increased ghrelin in healthy subjects who report short sleep duration³² and in those who have imposed sleep restriction³³; however the findings of this study suggest that sleep deprivation may have different physiologic effects on ghrelin levels than the intermittent sleep fragmentation seen in OSA patients.

This study has also showed that higher leptin levels in OSA patients are associated with IR especially in OSA patients with IGT; several studies are consistent with a causal role for leptin in IR. Previous studies have shown leptin to decrease basal insulin secretion from the pancreatic islets³⁴ and down-regulates insulin gene transcription.³⁵ Leptin receptors have been demonstrated on the β -pancreatic cell,³⁶ and in animal studies acute physiologic increases in leptin significantly inhibited glucose stimulated insulin secretion in a dose-dependent manner.³⁷ Plasma levels of leptin, adequate to significantly suppress insulin, are found in mild obesity³⁸ and patients with IGT.³⁹ Furthermore, leptin is associated with features of the metabolic syndrome such as IGT

and IR in a large prospective population-based cohort study.⁴⁰ Therefore any increase of leptin in OSA, in addition to that due to body fat, has clinical significance and may constitute another mechanism (apart from obesity) mediating IGT in OSA.

In summary, IGT was common in OSA patients referred for evaluation of sleep disordered breathing. Although leptin was elevated in patients compared with controls and in patients with IGT compared to those without, this was mainly explained by the amount of obesity of the study population. In contrast to previous studies, we found that when measured under carefully controlled conditions, that there is no elevation of ghrelin in patients with OSA compared with control subjects.

ABBREVIATIONS

AHI, apnea-hypopnea index
CPAP, continuous positive airway pressure
IGT, impaired glucose tolerance
NPY, neuropeptide Y
OGTT, oral glucose tolerance test
OSA, obstructive sleep apnea
PYY, peptide tyrosine kinase
SpO₂, fingertip oxygen saturation

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Professor Polkey, Dr. Morrell, and Dr. Gbatei conceived the study and designed it. Professor Polkey prepared the 1st draft of the manuscript based on a chapter in the Ph.D. thesis submitted by Dr. Papaioannou under the supervision of Professor Polkey and Dr. Morrell. Dr. Papaioannou performed all the OGTT and clinical assessments with Dr. Twigg and Dr. Vazir undertook the attended PSGs, under the supervision of Dr. Morrell. Dr. Patterson and Dr. Gbatei undertook the analyses of appetite regulating hormones. All authors reviewed and contributed to the revised manuscript.

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Address correspondence to: Professor Michael I. Polkey, Ph.D., FRCP, NIHR Respiratory Biomedical Research Unit, Royal Brompton Hospital, Fulham Road, London SW3 6NP, UK; E-mail: m.polkey@rbht.nhs.uk

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SUPPLEMENTAL RESULTS

Details of Interim Analysis Performed after Study of 33 Patients and 11 Controls

Demographic data are shown in **Table S1**.

Leptin, ghrelin, neuropeptide Y, and PYY, known to be involved in the appetite regulation, were measured at 22:30 and 07:00. Leptin and ghrelin was similar in OSA patients and control subjects, but OSA patients had a higher NPY at 07:00 (54.0 ± 14.3 pmol/L vs 46.3 ± 10.6 pmol/L, $p = 0.05$) and a lower level of PYY at 22:30 (21.0 ± 7.5 pmol/L vs 25.2 ± 6.9 pmol/L, $p = 0.04$) (**Table S2**).

Forty-six percent of the OSA patients (15/33) were found to have IGT. The patient group subdivided in 2 groups—those with IGT and those without; there were no demographic differences between those with IR and those without.

The analysis of neuropeptides has shown that OSA patients with IGT had significantly higher value of leptin at both 22:30 (14 ± 6.5 ng/mL vs 10 ± 9 ng/mL, $p = 0.018$) and 07:00 (14.9 ± 7.8 ng/mL vs 11 ± 10 ng/mL, $p = 0.038$) however no differences in NPY, PYY, or ghrelin between patients with IGT and patients without IGT were observed (see **Table S3**).

Taking into account the above results we continued the recruitment of the patients and controls however we did not perform further the ghrelin assays.

Table S1—Demographic data of patients and controls at the time of the interim analysis

	Patients	Controls	p value
Number of subjects	33	11	
Age (y)	48 (10.5)	43 (11.6)	0.17
BMI (kg/m ²)	30 (5)	28 (5.4)	0.25
Stage 1&2 (% TST)	66 (13)	60 (15)	0.26
REM sleep (% TST)	15.4 (7.3)	20 (10.2)	0.11
SWS sleep (% TST)	16 (9.1)	19.6 (9.2)	0.26
Sleep efficiency (%)	73 (11)	76 (15)	0.37
AHI (events/h)	30 (24.8)	2 (1)	
ODI (/h)	17 (5, 3)	0 (0, 1)	< 0.001
Arousal Index (/h)	17.2 (12.8, 31)	7.8 (6.2, 14.4)	0.006
ESS (of 24)	11 (6.25, 13)	4 (3, 6.75)	0.002

Table S2—Metabolic and endocrine parameters in patients with obstructive sleep apnea and controls

	Patients	Controls	p value
IGT (Number of Subjects)	15/33 (46%)	4/11 (36%)	
Plasma ghrelin 22:30 (pmol/L)	294 (208)	219 (136)	0.4
Plasma ghrelin 07:00 (pmol/L)	303 (237)	275 (201)	0.41
Plasma leptin 22:30 (ng/mL)	11.8 (8)	9.5 (7.5)	0.29
Plasma leptin 07:00 (ng/mL)	12.7 (9.2)	11 (9.5)	0.34
Plasma NPY 22:30 (pmol/L)	60.8 (13)	55.6 (7.9)	0.30
Plasma NPY 07:00 (pmol/L)	54 (14.3)	46.3 (10.6)	0.05
Plasma PYY 22:30 (pmol/L)	21 (7.5)	25.2 (6.9)	0.04
Plasma PYY 07:00 (pmol/L)	16.4 (6)	15.9 (7.6)	0.5

Table S3—Metabolic and endocrine parameters in obstructive sleep apnea patients with and without impaired glucose tolerance

	OSA with IGT (n = 15)	OSA without IGT (n = 18)	p value
Plasma ghrelin 22:30 (pmol/L)	247 (99)	338 (270)	0.62
Plasma ghrelin 07:00 (pmol/L)	250 (85)	355 (321)	0.36
Plasma leptin 22:30 (ng/mL)	14 (6.5)	10 (9)	0.018
Plasma leptin 07:00 (ng/mL)	14.9 (7.8)	11 (10)	0.038
Plasma NPY 22:30 (pmol/L)	62 (14.6)	60 (11.6)	0.6
Plasma NPY 07:00 (pmol/L)	57.7 (10.4)	50 (17)	0.2
Plasma PYY 22:30 (pmol/L)	20.4 (7.7)	21.7 (7.3)	0.4
Plasma PYY 07:00 (pmol/L)	16 (7)	16.5 (5.1)	0.5

Table S4—Multiple regression model for insulin resistance in OSA patients in order to separate the effect of BMI and Leptin. Elevated leptin was thus shown to be an epiphenomenon of obesity alone

Variables	Coefficient	Standard error	p value
BMI	0.18	0.04	< 0.001
Age	-0.0266	0.0176	0.141
Leptin 22:30	0.038	0.024	0.122
AHI	-0.00007	0.0077	0.99