Plasma apelin levels in obstructive sleep apnea and the effect of continuous positive airway pressure therapy

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Abstract

Apelin is a peptide hormone with cardiovascular and glucose homeostasis properties, and obstructive sleep apnea (OSA) is complicated by cardiovascular and metabolic comorbidities. Plasma apelin has not been previously assessed in OSA. We investigated the response of plasma apelin to a 2-h 75 g oral glucose tolerance test (OGTT) and the effect of 3 months compliant continuous positive airway pressure (CPAP) therapy in 15 obese males with newly diagnosed OSA. Plasma apelin and serum cortisol were recorded 10 minutely, while serum insulin and glucose were measured 30 minutely. Ten subjects had plasma apelin measured at intervals across a 24-h period to investigate for circadian variation in apelin levels, and this was repeated following 3 months compliant CPAP therapy. Fasting (0.342 ± 0.038 vs 0.288 ± 0.024 ng/ml, $P=0.04$), 30 min (0.399 ± 0.035 vs 0.312 ± 0.036 ng/ml, $P=0.007$) and 120 min (0.402 ± 0.030 vs

0.259 ± 0.024 ng/ml, $P<0.001$) apelin levels were reduced following CPAP. The area under curve for apelin OGTT response was lower post-CPAP (44.1 ± 3.3 vs 35.8 ± 2.3 ng/ml per min, $P<0.001$). Mean OGTT apelin levels showed a significant treatment effect ($P=0.006$) and a time effect ($P<0.001$), and the effect of time was different pre- versus post-CPAP ($P=0.005$). No significant variability in apelin levels existed across the 24-h period at diagnosis. Lower levels were evident overnight following treatment ($P=0.004$). Improvements in insulin and glucose parameters and reduced cortisol levels were found post-CPAP. In summary, untreated OSA was associated with elevated plasma apelin levels, altered apelin secretory dynamics in response to oral glucose and lack of an apparent circadian variability, which was restored following CPAP.

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Introduction

Obstructive sleep apnea (OSA) is a common condition, with an estimated prevalence of 3–7% for adult men and 2–5% for adult women in the general population (Punjabi 2008). It is associated with intermittent upper airway obstruction and subsequent hypoxia and autonomic arousal during sleep, resulting in fragmented sleep with daytime somnolence (Buckley & Schatzberg 2005). Morbidity and mortality from OSA are primarily due to cardiovascular disease (Peter *et al.* 1995, Collop 2007), but it is also independently associated with an increased prevalence of the metabolic syndrome (Coughlin *et al.* 2004) and impaired glucose metabolism (Seicean *et al.* 2008). These may be ameliorated with continuous positive airway pressure (CPAP) therapy (Harsch *et al.* 2004, Bradley & Floras 2009).

Apelin is a novel peptide hormone discovered in 1998 using reverse pharmacology and was first isolated from bovine

stomach extract (Tatemoto *et al.* 1998). It is produced as a 77-amino acid prepropeptide that is cleaved to C-terminal fragments of varying size including apelin-13, apelin-17 and apelin-36 (Tatemoto *et al.* 2001, Kleinz & Davenport 2005). All apelin peptides activate APJ (listed as APLNR in Hugo Database), an orphan G-protein-coupled receptor, which shares about 30% homology with the angiotensin (AT-1a) receptor (O'dowd *et al.* 1993). However, ATII does not bind to APJ.

Apelin may have an important role in respiratory physiology. There are relatively high amounts of APJ receptor mRNA located in the rat medulla oblongata (Hosoya *et al.* 2000), and microinjections of apelin-13 into the nucleus tractus solitarius result in apnea (Seyedabadi *et al.* 2002). Apelin and *Apj* mRNA are highly expressed in the rat lung suggesting a paracrine effect in this tissue (Hosoya *et al.* 2000, Kawamata *et al.* 2001). Plasma apelin levels are reduced in patients with chronic, severe parenchymal lung disease and

preserved cardiac function (Goetze *et al.* 2006). In a rat model of chronic hypoxic pulmonary hypertension, apelin protein concentration in lung tissue decreased, although total pulmonary apelin content remained stable as a result of a corresponding increase in pulmonary tissue mass, with evidence of altered downstream signalling from the APJ receptor (Andersen *et al.* 2009).

Apelin is found in the myocardium (Foldes *et al.* 2003) and endothelial cells (Kleinz & Davenport 2004) and has several cardiovascular properties including regulation of blood pressure (Tatemoto *et al.* 2001, Lee *et al.* 2005), cardiac contractility (Szokodi *et al.* 2002, Ernst *et al.* 2006) and possibly fluid balance (Chandrasekaran *et al.* 2008). It has also recently been shown to be an adipokine secreted from adipocytes (Boucher *et al.* 2005) and is thought to be involved in glucose homeostasis and obesity (Boucher *et al.* 2005, Dray *et al.* 2008). Circulating levels are altered in subjects with impaired glucose metabolism (Li *et al.* 2006, Erdem *et al.* 2008) and dyslipidaemia (Tasci *et al.* 2007). Given these observations, we conducted a study to investigate apelin levels in obese patients with newly diagnosed OSA and examined the effect of compliant CPAP therapy.

Materials and Methods

Subjects

Fifteen male patients were recruited from the Sleep Disorders Clinic and participated in the study after provision of written informed consent approved by the Bath Research Ethics Committee. Each was newly diagnosed with OSA following overnight polysomnography (Alice 5, Respiromics (UK) Ltd, Chichester, UK) with continuous recording of electroencephalogram, electromyogram, electrooculogram, nasal airflow, body position, thoracic and abdominal respiratory efforts and arterial oxyhaemoglobin saturation recorded by pulse oximeter and were analysed according to established criteria (Rechtschaffen *et al.* 1968). An apnea was defined as a 10 s cessation of breathing, and a hypopnea was defined as a 10 s decrease of 50% or more in airflow measured by nasal–oral cannulae. Arousals and desaturations were not included in the scoring of hypopneas. According to the apnea–hypopnea index (AHI), patients were included with either moderately severe (AHI 15–30) or severe (AHI > 30, $n = 13$) OSA.

Other inclusion criteria included: normal physical examination; body mass index (BMI) ≥ 30 ; normal renal, liver and thyroid function. Specific exclusion criteria were: any other respiratory disorder; acute illness; symptomatic chronic medical disorder; diabetes mellitus; depression; uncontrolled hypertension; medications known to alter cortisol levels (specifically, any glucocorticoid therapy within the preceding 3 months); AT converting enzyme inhibitor medication; ATII receptor blocker medication; current smoker; alcohol intake of > 21 units per week.

Anthropomorphic measurements

Body weight and height were measured by the same observer using a scale and stadiometer to 0.5 kg and 0.5 cm respectively. Waist (WC) and hip circumferences were measured to 0.5 cm with a non-elastic tape using standard techniques (World Health Organisation 2000). The waist-to-hip ratio (WHR) was calculated from the ratio of waist and hip circumferences. Neck circumference was measured at the level of the superior border of the cricothyroid cartilage to 0.5 cm. The means of two consecutive recordings were used. Systolic and diastolic blood pressures were measured twice at 5-min intervals in the sitting position.

Oral glucose tolerance test

Participants attended in the morning after at least 12-h fasting and were connected to an automated sampling system as previously described (Henley *et al.* 2009). Briefly, participants attended 1 h prior to commencement of sampling for cannulation of a suitable peripheral arm vein. This was attached to the sampling system in an adjacent room and a 30-min acclimatisation sequence performed. Blood for plasma apelin and serum cortisol was collected at baseline (fasting) and then 10 min for 2 h following a 75 g oral glucose load. Three baseline samples for serum insulin and glucose were drawn at 5-min intervals (Wallace *et al.* 2004) for homeostatic model assessment of insulin resistance (HOMA-IR) estimates and then at 30-min intervals after the oral glucose. A fasting lipid profile was also assessed. Following the initial study, participants commenced nasal CPAP therapy (REMstar Auto, Respiromics (UK) Ltd), and the exact protocol was repeated 3 months later while using CPAP.

Ten of the participants had serial plasma apelin measurements over a 24-h period to assess for diurnal variation. Samples were drawn at 2200, 0200, 0800, 1200 and 1800 h. This 24-h study, designed to assess ultradian hypothalamic–pituitary–adrenal (HPA) axis activity, commenced at 1900 h, and lights were switched off from 2300 h until 0700 h. Standardised meals were served at 2000, 0800 (after sample drawn) and 1230 h.

Hormone assay

Samples for apelin were collected in chilled 1 ml EDTA tubes, centrifuged immediately at 4 °C and then stored at –80 °C until assayed. Plasma apelin-12 level was determined by ELISA (Phoenix Pharmaceuticals, Belmont, CA, USA), with a sensitivity of 0.07 ng/ml, intra-assay coefficient of variation (CV) 3.8% and inter-assay CV 9.9%. There is 100% cross-reactivity with human apelin-12, apelin-13 and apelin-36. Cortisol concentrations were measured by solid phase, competitive chemiluminescent enzyme immunoassay (Immulate 2000, Siemens Medical Solutions, Camberley, UK). Intra- and inter-assay CV were 5.9 and 11% respectively. Serum insulin was measured by

electrochemiluminescence immunoassay (Roche Modular, Roche Diagnostics Ltd) with intra- and inter-assay CV of 1.5 and 4.9% respectively. Glucose was assayed using the enzymatic hexokinase method (Roche Modular, Roche Diagnostics Ltd) with intra- and inter-assay CV of 1.0 and 1.7% respectively. Plasma total cholesterol and triglyceride concentrations were determined enzymatically by cholesterol esterase and lipase hydrolysis respectively using an Olympus AU2700 platform (Watford, UK).

Statistical analysis

The data are presented as mean $\pm$ S.E.M. Kolmogorov–Smirnov test was used to determine distribution characteristics. Comparisons of mean values were made with paired *t*-test (or Wilcoxon signed-rank test for non-parametric data). Two-way repeated-measures ANOVA was used to assess serial mean apelin and cortisol data with pairwise multiple comparison via the Holm–Sidak method. One-way repeated-measures ANOVA was used to compare circadian apelin data with pairwise multiple comparison using the Tukey’s test. The relationship between variables was analysed by Pearson’s correlation (or Spearman’s correlation for non-parametric data). All reported *P* values are two sided, and the significance level was set to 0.05.

Results

The clinical characteristics of the patients are shown in Table 1. The mean age was 51.2 ± 2.7 years (range 34–65). There were no significant differences in anthropomorphic measurements or lipid profile at diagnosis compared with post-CPAP therapy. Systolic and diastolic blood pressures were significantly reduced after CPAP treatment. There was a significant reduction in the AHI and Epworth Sleepiness Scale consistent with successful CPAP therapy. CPAP usage was determined objectively with an internal compliance meter.

Oral glucose tolerance test

Table 2 summarises the plasma apelin and serum insulin, glucose and cortisol levels in response to the oral glucose tolerance test (OGTT). Basal (fasting), 30 and 120 min apelin levels were reduced following CPAP therapy (Fig. 1A). There was also a significant reduction in the area under curve (AUC) for apelin after CPAP (Fig. 1B). The mean plasma apelin at 30 min was significantly higher than the baseline level pre-CPAP (0.399 ± 0.035 vs 0.342 ± 0.028 ng/ml, $P=0.02$), but not after treatment (0.312 ± 0.026 vs 0.288 ± 0.024 ng/ml, $P=0.45$). Two-way repeated-measures ANOVA of mean plasma apelin levels during the OGTT found a significant treatment effect ($P=0.006$) and time effect ($P<0.001$), as well as a different effect of time pre- versus post-treatment

Table 1 Demographic and clinical data

Variable	Baseline	3 Months post-CPAP
Age (years)	51.2 ± 2.7	–
BMI (kg/m ²)	36.1 ± 1.3	36.1 ± 1.3
WC (cm)	118.7 ± 2.8	117.7 ± 2.9
WHR	1.0 ± 0.0	1.0 ± 0.0
SBP (mmHg)	134.7 ± 3.4	$127.6 \pm 3.3^*$
DBP (mmHg)	86.8 ± 2.1	$83.7 \pm 1.3^†$
Total cholesterol (mmol/l)	4.4 ± 0.3	4.4 ± 0.2
HDL-cholesterol (mmol/l)	1.0 ± 0.1	1.0 ± 0.0
LDL-cholesterol (mmol/l)	2.6 ± 0.3	2.6 ± 0.2
Triglyceride (mmol/l)	1.8 ± 0.2	1.8 ± 0.3
ESS	11.7 ± 1.2	$3.5 \pm 0.6^*$
AHI	55.0 ± 5.8	$3.8 \pm 0.5^*$
Desaturation index (number/h) ^a	45.3 ± 9.4	–
Average daily CPAP use (h)	–	5.3 ± 0.3
CPAP duration (days)	–	93.8 ± 2.2

BMI, body mass index; WC, waist circumference; WHR, waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ESS, Epworth Sleepiness Scale; AHI, apnea-hypopnea index. * $P<0.001$ for comparison with baseline value, by paired *t*-test. [†] $P=0.03$ for comparison with baseline value, by paired *t*-test.

^aDesaturation defined as 4% drop below baseline.

($P=0.005$; Fig. 2), suggesting a change in the secretory dynamics in response to glucose.

There was a significant reduction in the mean fasting insulin level and the AUC during OGTT with CPAP therapy. There was also a significant decrease in HOMA-IR together with a concomitant increase in insulin sensitivity after therapy, but no change in β -cell function. The mean fasting, 30 and 120 min glucose levels were significantly lower following CPAP, as was the glucose AUC.

The mean fasting cortisol levels were reduced with CPAP; however, given the pulsatile nature of cortisol release, the significance of this is uncertain. More importantly, the mean AUC for cortisol was reduced, and two-way repeated-measures ANOVA of mean cortisol levels during the OGTT revealed a significant treatment effect ($P=0.002$; Fig. 3).

Prior to treatment, AHI correlated positively with WC ($r=0.539$, $P=0.04$), BMI ($r=0.618$, $P=0.01$), fasting insulin ($r=0.529$, $P=0.04$), HOMA-IR ($r=0.778$, $P<0.001$) and β -cell function (%B) ($r=0.662$, $P=0.01$), and negatively with insulin sensitivity (%S) ($r=-0.780$, $P<0.001$). Both BMI and WC correlated positively with fasting insulin ($r=0.562$, $P=0.03$; $r=0.623$, $P=0.01$ respectively), HOMA-IR ($r=0.658$, $P=0.01$; $r=0.712$, $P=0.004$ respectively), %B ($r=0.548$, $P=0.04$; $r=0.606$, $P=0.02$ respectively) and insulin AUC ($r=0.529$, $P=0.04$; $r=0.609$, $P=0.01$ respectively), and negatively with %S ($r=-0.660$, $P=0.01$; $r=-0.726$, $P=0.002$ respectively). Similarly, WHR had a positive correlation with fasting insulin ($r=0.522$, $P=0.04$), %B ($r=0.573$, $P=0.03$) and insulin AUC ($r=0.600$, $P=0.02$). The desaturation

Table 2 Comparison of oral glucose tolerance test (OGTT) hormone levels pre- and post-continuous positive airway pressure (CPAP)

	Pre-CPAP	Post-CPAP	P value
Fasting apelin (ng/ml)	0.342 ± 0.028	0.288 ± 0.024	0.04 ^a
30 min apelin (ng/ml)	0.399 ± 0.035	0.312 ± 0.026	0.01 ^a
120 min apelin (ng/ml)	0.402 ± 0.030	0.259 ± 0.024	<0.001
AUC ₁₂₀ apelin ((ng/ml) × min)	44.1 ± 3.3	35.8 ± 2.3	<0.001
Fasting insulin (pmol/l)	181.9 ± 37.4	135.8 ± 30.8	0.002
30 min insulin (pmol/l)	735.4 ± 121.7	637.4 ± 102.7	0.113
120 min insulin (pmol/l)	935.3 ± 184.6	573.9 ± 126.7	<0.001
AUC ₁₂₀ insulin ((pmol/l) × min)	108 586 ± 16 130	78 343 ± 13 266	<0.001
HOMA-IR	2.9 ± 0.4	2.1 ± 0.3	<0.001 ^a
HOMA-%B	153.4 ± 19.8	143.4 ± 24.5	0.423
HOMA-%S	48.7 ± 8.1	61.8 ± 8.3	0.02
Fasting glucose (mmol/l)	5.7 ± 0.2	5.2 ± 0.2	<0.001 ^a
30 min glucose (mmol/l)	8.7 ± 0.5	7.9 ± 0.3	<0.001 ^a
120 min glucose (mmol/l)	6.9 ± 0.6	6.2 ± 0.5	<0.001 ^a
AUC ₁₂₀ glucose ((mmol/l) × min)	1003 ± 55	920 ± 52	<0.001 ^a
Fasting cortisol (nmol/l)	310.9 ± 26.1	237.5 ± 22.5	0.01
AUC ₁₂₀ cortisol ((nmol/l) × min)	31 445 ± 2110	26 027 ± 1985	0.001

AUC₁₂₀, area under curve from baseline to 120 min; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-%B, homeostasis model assessment of β-cell function; HOMA-%S, homeostasis model assessment of insulin sensitivity.
^aWilcoxon signed rank test.

index (Table 1), which relates to the severity of oxygen desaturation, correlated positively with HOMA-IR ($r=0.696$, $P=0.02$), %B ($r=0.658$, $P=0.03$) and insulin AUC ($r=0.607$, $P=0.03$).

Following CPAP therapy, WC had a negative correlation with fasting apelin ($r=-0.561$, $P=0.03$), although the negative correlation for BMI just failed to reach significance ($r=-0.509$, $P=0.05$). However, both WC ($r=-0.570$, $P=0.02$) and BMI ($r=-0.588$, $P=0.02$) correlated

negatively with apelin AUC. None of these correlations were present at diagnosis. Both WC and BMI retained positive correlations with fasting insulin ($r=0.722$, $P=0.002$; $r=0.717$, $P=0.002$ respectively), HOMA-IR ($r=0.689$, $P=0.006$; $r=0.694$, $P=0.005$ respectively) and insulin AUC ($r=0.565$, $P=0.03$; $r=0.592$, $P=0.02$ respectively), as well as the negative correlation with %S ($r=-0.672$, $P=0.008$; $r=-0.667$, $P=0.008$ respectively).

Circadian apelin measurements

Plasma apelin levels measured at five time points throughout the 24-h period revealed no significant variation across this time domain at diagnosis ($P=0.838$; Fig. 4). However, following 3 months compliant CPAP therapy, one-way repeated-measures ANOVA of mean plasma apelin levels found that the 1800 h level was significantly higher than both the 2200 and 0200 h levels ($P<0.05$). Therefore, CPAP may have restored some form of circadian pattern in apelin secretion with the lowest levels overnight.

Discussion

Patients with OSA have significant cardiovascular and metabolic co-morbidities, and apelin itself is known to have cardiovascular and glucose homeostatic effects, as well as being produced in myocardial, endothelial and adipocyte cells. We found that untreated OSA was associated with significantly elevated apelin levels with evidence of altered secretory dynamics in response to an oral glucose load. Furthermore, for the first time, we provide evidence for a circadian pattern of apelin secretion.

Figure 1 Summary of plasma apelin data. (A) Time-specific plasma apelin levels during OGTT at diagnosis (■) and following 3 months compliant CPAP therapy (□). (B) Plasma apelin area under curve during OGTT similarly before and after treatment. Values are the means ± s.e.m. * $P=0.04$, ** $P=0.01$, *** $P<0.001$.

Figure 2 Plasma apelin levels during OGTT at diagnosis ($\diamond$) and following 3 months compliant CPAP therapy ($\blacksquare$). Values are the means $\pm$ S.E.M. of levels at each time point.

Apelin is an adipokine that has been linked to obesity and insulin secretion (Boucher *et al.* 2005). In this group of obese patients with OSA, we found elevated plasma apelin levels in the fasting state and during a standard 2-h OGTT at diagnosis as compared with 3 months compliant CPAP therapy. There was a significant treatment effect; however, the different effect of time across the OGTT with multiple sampling suggests a difference in secretory dynamics before and after treatment. This patient group is unique compared with other clinical studies on plasma apelin previously reported in other pathophysiologic groups (Boucher *et al.* 2005, Li *et al.* 2006, Tasci *et al.* 2007, Erdem *et al.* 2008) and supports the view that apelin synthesis/secretion is complex with multiple regulatory factors.

Fasting plasma apelin levels are reported to be increased in obese subjects (Boucher *et al.* 2005, Heinonen *et al.* 2005) and to correlate positively with BMI (Heinonen *et al.* 2005). However, the prevalence of OSA in these subjects was not documented. Other studies in patient groups with newly diagnosed type 2 diabetes (Erdem *et al.* 2008), isolated hypercholesterolaemia (Tasci *et al.* 2007) and heart failure (Chong *et al.* 2006) did not find any correlation of apelin with BMI. Similarly, we found no correlation between fasting plasma apelin and BMI in untreated OSA. However, there was a negative correlation of WC with fasting plasma apelin and both WC and BMI with apelin OGTT AUC after

Figure 3 Serum cortisol levels during OGTT at diagnosis ($\diamond$) and following 3 months compliant CPAP therapy ($\blacksquare$). Values are the means $\pm$ S.E.M. of levels at each time point.

Figure 4 Mean ($\pm$ S.E.M.) plasma apelin levels recorded at five time points across the 24-h period in ten patients with OSA at diagnosis ($\blacksquare$) and following 3 months compliant CPAP therapy ($\square$).

* $P < 0.05$.

3 months compliant CPAP therapy. Thus, adiposity *per se* is unlikely to be a major determinant in circulating apelin levels in different pathophysiological settings. In OSA, other factors such as insulin resistance, oxidative/hypoxic stress, inflammatory cytokines, glucocorticoids and particularly endothelial dysfunction may have a multifactorial role.

Insulin exerts a direct positive action on adipocyte apelin production, and hyperinsulinaemia rather than obesity appears to be the main determinant of adipocyte apelin expression and secretion (Boucher *et al.* 2005). Apelin has a glucose-lowering effect associated with enhanced glucose utilisation in normal and insulin-resistant murine models (Dray *et al.* 2008). Thus, it may be postulated that in this group of obese, insulin-resistant patients' apelin is up-regulated to improve glucose utilisation and post-CPAP, with the amelioration of hyperinsulinaemia and improvement in insulin sensitivity, apelin is down-regulated.

Dyslipidaemia modifies plasma apelin levels in non-obese patients with isolated hypercholesterolaemia (Tasci *et al.* 2007, 2009). In our study, the dyslipidaemia remained unchanged with CPAP therapy and therefore contributed insignificantly to the changes reported in apelin levels. However, the APJ/apelin system appears to have a role in the development of hypercholesterolaemia-associated atherosclerosis (Hashimoto *et al.* 2007). Therefore, although elevated plasma apelin levels in untreated OSA may be beneficial in terms of glucose homeostasis (and hypertension – see below), there are putative deleterious consequences for oxidative stress-linked vascular disease, which warrant further investigation.

A tight positive correlation between apelin and tumour necrosis factor α (TNF α) expression in human adipose tissue has been reported (Daviaud *et al.* 2006). This link between apelin expression and inflammatory mediators may be particularly relevant in OSA where circulating levels of TNF α are known to be elevated (Vgontzas *et al.* 2000, Ciftci *et al.* 2004). CPAP therapy lowers circulating TNF α (Dorkova *et al.* 2008) and soluble TNF α receptor-1 levels (Arias *et al.* 2008). Reduced activation of the TNF α system may be another mechanism by which CPAP affects plasma apelin levels.

Glucocorticoids may have a role in regulating apelin gene expression in adipocytes with suppression of apelin production in a dose-dependent manner (Wei *et al.* 2005). We have found that significant disruption to the HPA axis signalling occurs in untreated OSA with abnormalities in cortisol secretory dynamics (D E Henley, G M Russell, J A Douthwaite, S A Wood, F Buchanan, R Gibson, W W Woltersdorf, J R Catterall & S L Lightman, unpublished data). This may in turn affect cortisol signalling and so disrupt the normal glucocorticoid inhibitory effect on apelin expression.

In the heart, apelin and APJ are highly expressed in myocardial and endothelial cells, and in the periphery, apelin is widely expressed in endothelial cells of blood vessels (Foldes *et al.* 2003, Kleinz & Davenport 2004). Apelin has a role in modulating cardiac function and blood pressure. It is a potent positive inotropic agent (Szokodi *et al.* 2002), exerts hypotensive effects mediated through nitric oxide-dependent mechanisms (Tatemoto *et al.* 2001) and exhibits direct cardioprotective activity against ischaemia/reperfusion injury (Carpene *et al.* 2007). Endothelial dysfunction is well established in OSA (Atkeson & Jelic 2008) and is ameliorated by CPAP therapy (Jelic *et al.* 2008). Hypoxia-induced myocardial/endothelial apelin expression has been demonstrated via hypoxia-inducible factor (HIF) pathways (Glassford *et al.* 2007, Ronkainen *et al.* 2007), and the HIF-1 pathway is activated in patients with OSA (Atkeson & Jelic 2008). The pulmonary apelin-producing endothelium is particularly sensitive to hypoxia (Sheikh *et al.* 2008). Thus, upregulation of apelin in OSA may be a protective mechanism against OSA-associated hypertension and recurrent hypoxia/hypoxaemia, and endothelial dysfunction may represent the dominant mechanism for circulating plasma apelin levels in this patient group. Reversal of these factors by effective CPAP therapy is another means by which apelin may be down-regulated post-treatment.

We found no variation in plasma apelin levels throughout the 24-h period in untreated OSA, but a reduction was noted overnight following CPAP therapy. Nocturnal hypoxia/hypoxaemia may be a significant factor, but does not explain the lower level found at 2200 h, before the lights-out period. Interestingly, we did not find significant associations between oximetry parameters and nocturnal apelin levels (data not shown). Other factors such as inflammatory cytokines may also have a role. In addition, sympathetic nervous system (SNS) hyperactivity is well documented in OSA with high sympathetic drive present across the 24-h period, even during daytime wakefulness and there is a reduction following CPAP (Narkiewicz & Somers 2003). Chronic SNS activation may indirectly affect apelin expression via its effects on endothelial dysfunction, hypertension and hyperinsulinaemia (Narkiewicz & Somers 2003). Direct effects of SNS activity on the apelin/APJ system have not been investigated. Why apelin should exhibit circadian variability is unclear. While apelin/APJ is expressed in the hypothalamus and pituitary gland, apelin localises with ACTH in corticotrophs

(Reaux-Le *et al.* 2007) and hypothalamic *Apj* mRNA expression is up-regulated by acute and chronic stress (O'Carroll *et al.* 2003); understanding of the exact role in HPA functioning is only now emerging (Newson *et al.* 2009) and the 24-h variability in plasma apelin levels in this study did not match the typical cortisol circadian rhythm.

We found a reduction in insulin levels and HOMA-IR with a concomitant increase in insulin sensitivity following CPAP therapy. However, there was no change in HOMA-calculated β -cell function, which may reflect relative changes in parasympathetic and sympathetic autonomic activity at both a pancreatic and peripheral tissue level (Gilon & Henquin 2001). Markers of OSA severity together with anthropomorphic measurements correlated with OGTT insulin parameters. The OGTT, a dynamic test of the HPA axis (Reynolds *et al.* 2003), provided further evidence for HPA axis activation in untreated OSA (Henley *et al.* unpublished data) with a significant reduction in cortisol post-CPAP.

This study is the first to examine the effect of CPAP on plasma apelin levels in OSA patients. It is limited by its relatively small sample size and inability to recruit an appropriate control group. We are also unable to determine cardiovascular contributions. However, the dynamic changes in plasma apelin in response to OGTT suggest altered apelin regulation and secretory dynamics in untreated OSA. Whether the upregulation of apelin has predominantly beneficial or potentially negative effects should be the focus of future investigations, which should also include concurrent measures of cardiac status and endothelial dysfunction. This study highlights the importance of considering coexisting medical conditions in the interpretation of clinical data on apelin physiology/pathophysiology.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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