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Original article

Adipose tissue is influenced by hypoxia of obstructive sleep apnea syndrome independent of obesity

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ABSTRACT

Aims. – Obstructive sleep apnea syndrome (OSAS) is associated with increased cardiovascular risk and diabetes independent of obesity. We investigated whether adipose tissue dysfunction is exacerbated due to increased tissue hypoxia.

Methods. – Adipose tissue (AT) oxygenation was measured with a Clarke-type electrode ($p_{AT}O_2$) in 16 men with OSAS before and after 4 months of continuous positive airway pressure therapy (CPAP) and in BMI-matched controls. Oxygenation was simultaneously monitored in arterial blood by pulse oximetry (SaO_2); mixed blood in AT microcirculation by reflectance spectroscopy ($S_{AT}O_2$) along with blood flow. Markers of hypoxia, adipo- and angiogenesis, inflammation and fibrosis were analysed in AT and serum.

Results. – OSAS subjects were more insulin resistant. Despite lower arterial SaO_2 (95.4 ± 1.3% vs. 97.1 ± 1.6%, $P = 0.013$) in subjects with OSAS, there was no difference in the oxygen content of AT microcirculation (61.6 ± 18.4 vs. 72.2 ± 7.0%, $P = 0.07$) or $p_{AT}O_2$ (49.2 ± 7.5 vs. 50.4 ± 14.7 mmHg, $P = 0.83$) between groups. Resting AT blood flow was higher in OSAS compared to controls (108.5 ± 22.7 vs. 78.9 ± 24.9 au, $P < 0.005$) and strongly associated with inflammation markers IL-6 and MCP-1. AT of OSAS subjects showed increased inflammation (TNFA $P = 0.049$) and fibrosis (COL3A1 $P = 0.02$), a trend of higher HIF1A expression ($P = 0.06$) and reduced adipogenesis (PPARG $P = 0.006$). After CPAP, only expression of the lipid deposition marker LPL increased (30%, $P = 0.047$).

Conclusions. – Adipose tissue of awake OSAS subjects appears no more hypoxic than adipose tissue of BMI-matched controls despite daytime hypoxaemia. Increased adipose tissue blood flow may be explained by an increased inflammatory response. We observe features of adipose dysfunction in subjects with OSAS, which attribute to increased cardiometabolic risk associated with this condition.

Introduction

Impaired adipose tissue (AT) function plays a crucial role in the pathophysiology of cardiovascular disease (CVD) and type 2 diabetes. One of the main drivers of AT dysfunction is considered to be hypoxia [1,2]; however, there is limited, contradictory literature on in vivo measurements of AT oxygenation in humans [3–5] with none, to our knowledge, addressing the effects of obstructive sleep apnea syndrome (OSAS) on oxygenation and/or AT function. With increasing weight gain, AT becomes inflamed as characterised by macrophage infiltration and secretion of inflammatory cytokines resulting in subclinical systemic inflammation [2,6]. Hypoxia is considered to play a major part in the process of inflammatory cytokine induction and secretion [7]. Hypoxia induces gene expression of the transcription factor hypoxia-

Abbreviations: ADIPOQ, adiponectin; AHI, apnea hypopnea index; ATBF, adipose tissue blood flow; CD 14, panmacrophage marker; CD 86, M1 macrophage marker; CDH5, cadherin 5 (CD144); COL, collagen; CPAP, continuous positive airway pressure; DSI, oxygen desaturation index; FASN, fatty acid synthase; Hb, deoxyhaemoglobin; HbO_2, oxyhaemoglobin; HIF1A, hypoxia inducible factor 1 alpha; HOMA-IR, homeostasis model assessment-estimated insulin resistance index; IL-6, interleukin-6; LEP, leptin; LPL, lipoprotein lipase; MCP-1 (CCL2), monocyte chemoattractant protein-1; OSAS, obstructive sleep apnea syndrome; $p_{AT}O_2$, partial pressure of oxygen in adipose tissue; PECAM, platelet endothelial cell adhesion molecule (CD31); PPARG, peroxisome proliferator activating receptor γ; S_aO_2, arterial oxygen saturation; $S_{AT}O_2$(deep), oxygen saturation of blood in AT microcirculation at 20 mm depth; $S_{AT}O_2$(superficial), oxygen saturation of blood in AT microcirculation at 4 mm depth; TGFB1, transforming growth factor β1; TNFA, tumour necrosis factor α; VEGFA, vascular endothelial growth factor A.

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induced factor 1 alpha (HIF1A), a key signal of low oxygenation, which mediates adaptive responses to hypoxia. Increased HIF1A have been found in AT with human obesity [8]. However, it is less clear whether intermittent airway obstruction, which is a feature of obstructive sleep apnea syndrome (OSAS), and which results in intermittent arterial desaturation, has a chronic effect on AT oxygenation and/or contributes to pre-existing hypoxia. Although there are acute effects of hypoxia on tissue during periods of apnea, the chronic long-term consequences are less well understood [5]. Therefore, the aim of this study was to investigate whether in vivo AT partial oxygen pressure ($p_{AT}O_2$), AT blood flow (ATBF), and mixed blood oxygenation ($S_{AT}O_2$) in the AT microcirculation were reduced not during periods of sleep apnea but in awake subjects with OSAS and excessive day time sleepiness compared to BMI-matched controls. In addition, expression of selected genes relevant to AT dysfunction were established to study potential chronic effects of night-time oxygen desaturation and the effect of the use of continuous positive airway pressure (CPAP) devices on AT. In this study, we have used non-invasive optical techniques using visible and near infrared light to measure ATBF and AT oxygenation as a novel application in this tissue, in conjunction with a measurement of the partial pressure of oxygen directly in the subcutaneous AT through the insertion of a Clarke-type electrode.

Materials and methods

Subjects

Sixteen men (mean BMI 32.7 ± 4.0 kg/m^2 [mean $\pm$ SD]) were recruited at the time of diagnosis of OSAS from routine clinical practice prior to starting overnight CPAP treatment (Aria LX; Respironics). They were consented through the NIHR Exeter Clinical Research Facility. The study was approved by the North West Research Ethics Committee (08/H1005/91) and conducted in accordance with the Declaration of Helsinki. Fourteen age and BMI-matched control males with no history of OSAS or daytime sleepiness (Epworth Score < 5) were recruited from the Exeter 10,000 study (09/H0106/75) research database. Exclusion criteria included active or passive smoking within the last 3 years; active treatment for other respiratory disorders; recent use of steroids (< 3 months); active endocrine dysfunction; previous weight reduction including use of weight loss drugs, prior bariatric surgery and a history of CVD.

OSAS diagnosis and CPAP

OSAS was diagnosed using NICE/BTS criteria to include apnea (10 seconds breathing pauses) and hypopnea episodes (ventilation reduction by 50% from baseline) identified by the presence of recurrent arterial oxygen desaturation of $> 4\%$ (oxygen desaturation index: ODI; Minolta 3iA pulse oximeter) during sleep screening. We graded the severity of OSAS according to the American Academy of Sleep Medicine based on the frequency of apneas and hypopneas/hour of sleep (Apnea/Hypopnea Index: AHI), as having mild (AHI 5–14), moderate (AHI 15–30) or severe (AHI > 30) OSAS [9]. CPAP treatment was optimised following CPAP titration (AutosetTM self-adjusting CPAP device, ResMed Autoscan). Compliance with therapy was verified using the incorporated CPAP-recording devices (Smartcard Encore; Respironics).

Study protocol

Fat distribution was characterised by: BMI, waist/hip ratio and % fat mass by body impedance measurement (Tanita BF350, Tokyo, Japan). All volunteers were screened for abnormal ECG. Subjects were studied fasted, in the morning, having refrained from caffeine and alcohol from 22:00 hours the previous evening. Blood was taken during the 20 minutes acclimatization to a room temperature of 24.0 ± 1.5 °C. Oxygenation levels were assessed in:

- arterial blood by pulse oximetry (S_aO_2, Nellcor^{TM} N560) on the right index finger;
- mixed blood in abdominal AT's microcirculation by reflectance spectroscopy to assess $S_{AT}O_2$ using O2C (Lea Medizintechnik GmbH, Giessen, Germany) and NIRO200 (Hamamatsu Photonics KK, Japan);
- partial oxygen tension in AT ($p_{AT}O_2$) with a Clarke-type electrode (LICOX RECON, Germany) inserted through an 18 gauge cannula using the Seldinger technique (Fig. S1; see supplementary material associated with this article online).

The O2C and NIRO200 probes were secured with adhesive tape to the abdominal wall approximately 10 cm lateral to the left of the umbilicus. Abdominal $p_{AT}O_2$ was continuously recorded 10 cm lateral to the right of the umbilicus at a depth of $\sim$8–10 mm reaching a steady state $\sim$5 minutes after insertion. Baseline data from all instrumentation were recorded for 20 minutes. Consent to record the invasive measure of $p_{AT}O_2$ was given by 15 subjects with OSAS and 8 controls. All other non-invasive parameters were obtained from all subjects, except when not possible for occasional technical reasons. The postprandial response was monitored for a further hour following a standardized 75 g oral glucose challenge in 14 controls and 8 OSAS subjects.

Following the administration of a local anaesthetic, a subcutaneous abdominal AT biopsy was removed by scalpel incision at 10 cm laterally to the right of the umbilicus, processed and analysed for candidate genes as described previously [10]. Biopsies were flash frozen in liquid nitrogen and stored at –80 °C.

Blood samples were analysed for routine chemistry for serum lipids and HbA_{1c} at the Royal Devon and Exeter Hospital (Exeter, UK). Plasma glucose concentrations were measured using a glucose oxidase technique on the YSI2300 Glucose Analyser (Yellow Springs Instruments). Serum leptin was assessed by ELISA (Invitrogen, UK) according to manufacturer's instruction.

Assessment of superficial and deep AT blood oxygenation and blood flow

Reflectance spectroscopy is able to derive a measure of the concentration of oxyhaemoglobin [HbO_2] and deoxyhaemoglobin [Hb] at tissue depths dependent upon the wavelength of light and the separation between the light source and detector at the skin surface. The sampled volume of tissue is considered to be to a depth of approximately half the probe spacing [11] and from which a measure of the mean blood oxygen saturation = $[HbO_2] \times 100/([HbO_2] + [Hb])$ can be derived. In this study, the O2C uses visible light (500–600 nm) and a source detector spacing of 8 mm to interrogate subcutaneous AT blood oxygenation [$S_{AT}O_2$(superficial)] at a depth of $\sim$4 mm. NIRO200 uses 4 pulsed laser diodes at wavelengths of 775, 825, 850 and 905 nm and a source detector spacing of 4 cm to determine deep AT blood oxygenation [$S_{AT}O_2$(deep)] at a depth of $\sim$20 mm. AT blood flux (ATBF) was assessed by a 830 nm (30 mW) laser diode adjacent to the ORS white light source incorporated within the O2C probe at a depth of $\sim$4 mm.

Adipose tissue analysis

Tissue was washed thoroughly in $1 \times$ PBS (1%pen/strep), homogenised in Tri reagent (Life Technologies) using a Retsch Mixer Mill MM400 and the guanidinium-thiocyanate-phenol-chloroform RNA extraction method used. RNA quantity and purity were determined spectrophotometrically using Nanodrop^{TM} technology. RNA was processed using DNase I to remove any residual