Hyperbaric oxygen therapy increases insulin sensitivity in overweight men with and without type 2 diabetes

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Abstract

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Aims: The onset of insulin resistance is an important metabolic event in the development of type 2 diabetes. For patients with type 2 diabetes, we recently showed that peripheral insulin sensitivity was increased during hyperbaric oxygen treatment (HBOT). This study aims to investigate whether this occurs in a non-patient population with and without type 2 diabetes, along with the mechanism of this effect.

Methods: Overweight and obese male participants were recruited from the community, 11 without and eight with type 2 diabetes. Insulin sensitivity was measured by the glucose infusion rate (GIR) during a hyperinsulinaemic euglycaemic clamp (80 $mU \cdot m^{-2} \cdot min^{-1}$) at baseline and during the third HBOT session. Monocyte chemo-attractant protein-1 (MCP-1), tumour necrosis factor-χ(TNF-χ) and interleukin-6 (IL-6) were measured in fasting serum and adipose tissue samples taken for their gene expression at baseline and immediately following four HBOT sessions. Additional fasting serum samples were collected during the first HBOT at 0, 60 and 120 minutes, and 24-hours after the last HBOT.

Results: In response to HBOT, GIR was increased by 29 ± 32% in those without (n = 10, P = 0.01), and by 57 ± 66% in those with type 2 diabetes (n = 7, P = 0.04). This increase was maintained for 30 minutes post HBOT. Reduced MCP-1 and TNF-χwere observed after HBOT, whereas IL-6 was increased only in individuals without diabetes and this correlated with the increase in insulin sensitivity (r^2 = 0.72, P = 0.004).

Conclusions: Peripheral insulin sensitivity was increased following HBOT in overweight or obese males with and without type 2 diabetes; this increase was maintained for at least 30 minutes post HBOT. Changes in inflammatory cytokines may partly explain this effect.

Key words

Endocrinology, hyperbaric oxygen, obesity, diabetes, inflammation, metabolism, hyperbaric research

Introduction

Hyperbaric oxygen treatment (HBOT) is defined as breathing 100% oxygen at a pressure greater than 101.3 kPa and is used clinically to treat a range of conditions including non-healing wounds.[1] When patients with type 2 diabetes undergo HBOT they sometimes report symptoms of hypoglycaemia, while studies have shown that fasting glucose levels are reduced by a greater amount during HBOT as compared to room air in patients with type 2 diabetes.[2,3] In a recent pilot study of hospital patients with type 2 diabetes who were receiving a prescribed course of HBOT for a medical condition, we showed that insulin sensitivity, as measured by the hyperinsulinaemic euglycaemic clamp technique, was increased during the third and the thirtieth HBOT sessions.[4] The mechanism was not investigated and it was unknown whether the insulin-sensitising effect was influenced by their medical conditions improving over time.

Insulin resistance is defined as a relative impairment in the ability of insulin to exert its effect on glucose metabolism in target tissues (e.g., skeletal muscle, liver) and is considered one of the best predictors of the future development of type 2 diabetes.[5] Obesity is also associated with insulin resistance,[6] and both obesity and type 2 diabetes are increasing in prevalence and have become major health issues globally. Obesity-related insulin resistance is closely associated with a chronic, low-grade inflammatory response within adipose tissue, characterised by immune cell infiltration, altered cytokine production and activation of inflammatory signalling pathways.[7] Pro-inflammatory cytokines linked to insulin resistance include tumour necrosis factor (TNF)-χ[8] monocyte chemo-attractant protein (MCP)-1,[9,10] interleukin (IL)-6[11,12] and members of the IL-1 family; IL-1, IL-1 receptor antagonist (IL-1ra) and IL-18.[13–15]

This study aims to determine whether the insulin-sensitising effect of HBOT can be demonstrated in a relatively healthy urban population including those with and without type 2 diabetes, whether the effect is still measurable after exit from the hyperbaric chamber and whether HBOT-induced changes in insulin resistance are associated with changes in pro-inflammatory cytokines in serum and adipose tissue known to be associated with insulin resistance.

Methods

The study received ethics approval from the University of Adelaide and the Royal Adelaide Hospital (approval no: 100615). All investigations were conducted in accordance with the Declaration of Helsinki and all subjects provided written informed consent.

SUBJECTS AND SCREENING

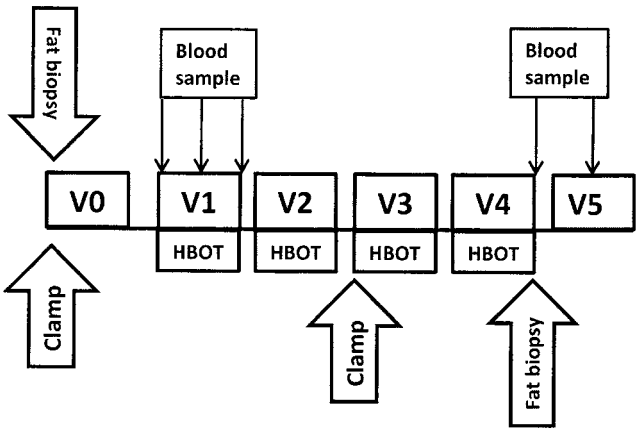
Advertisements and a web-recruitment company were

used to enlist overweight and obese male volunteers (BMI > 25kg·m^{-2}) who had no other excluded medical conditions apart from the sub-group with type 2 diabetes. As insulin sensitivity can vary throughout the menstrual cycle, only male volunteers were recruited. We undertook no specific investigation of the diabetes status of the volunteers, the diagnosis of type 2 diabetes was made from their personal medical history together with the prescription of appropriate medication. Excluded medical conditions included anything that could potentially alter insulin response or the inflammatory pathways being investigated, such as: smoking; consumption of more than three standard alcoholic drinks per day; vigorous exercise more often than twice a week; conditions that might be associated with a pathological inflammatory process or could influence inflammatory markers (such as sleep apnoea, malignancy, autoimmune and inflammatory diseases) and medication that might affect angiogenesis, lipid metabolism or have anti-inflammatory properties. Each volunteer was assessed for suitability to enter the hyperbaric chamber by a hyperbaric physician according to the standard clinical criteria used at the facility; this included history, examination and audiology assessment. Body composition was measured by dual-emission X-ray absorptiometry (DXA) to calculate fat mass and fat-free mass (FFM). Nineteen male volunteers were recruited, aged 45–70 years old, with BMI in the range of 24.3 to 45 kg·m^{-2}.

STUDY VISITS

Volunteers attended the Hyperbaric Medicine Unit at the Royal Adelaide Hospital on six occasions following a 10-hour overnight fast (Figure 1). Testing was undertaken at approximately the same time each morning and sampling was undertaken at a similar time each visit. Baseline assessments (V0) were performed one week and the following week participants attended the facility for five consecutive days (V1 to V5). Visits V1 to V4 included a routine 2-hour HBOT exposure. This involved compression to 203 kPa while breathing 100% oxygen for 90-minutes, followed by a linear decompression over 30 minutes and was administered in a rectangular twin-lock multiplace hyperbaric chamber (Fink Engineering/Cowan Engineering, Australia, 1994).

Figure 1
Timeline of study visits; V1–V5 were at same time of day on consecutive days; V0 was during the preceeding week



The 3.5-hour hyperinsulinaemic euglycaemic clamp was performed at baseline (V0) and visit V3. The baseline clamp was performed in normobaric room air, outside the hyperbaric chamber, as previously described.[16] Briefly, two intravenous cannulae were inserted into veins on opposite arms. One cannula was connected to an infusion of insulin (Actrapid®, Novo Nordisk, Baulkham Hills, Australia) at a fixed rate of 80 mU·m^{-2}·min^{-1}, together with a variable-rate infusion of 25% dextrose (Baxter Healthcare, Toongabbie, Australia). The other cannula allowed five to 10-minutely blood sampling to assess blood glucose levels by a hand-held glucometer (Accu-Chek Performa, Roche Diagnostics, Australia). The target blood glucose level was 6 mmol·L^{-1}. Insulin sensitivity was calculated from the glucose infusion rate (GIR) during two separate 30-minute steady-state (SS) periods at the end of the 3.5-hour clamp; SS1 corresponded with the period 2.5 to 3 hours and SS2 with 3 to 3.5 hours. The GIR was then standardized against FFM for each volunteer. The clamp was repeated during visit V3 with the two-hour HBOT session administered between the one- and the three-hour period of the clamp.

Therefore, when considering insulin sensitivity results, SS1 represented the last 30 minutes of the HBOT session while SS2 reflected the first 30 minutes immediately post-HBOT. Serum insulin was measured during both steady state periods. To avoid any physical effort that might influence glucose uptake, the volunteers remained sedentary in a chair which was wheeled in and out of the hyperbaric chamber. One non-diabetic subject was unable to adequately perform middle ear equalization during the first HBOT and took no further part in the study. Data from SS2 were not available for two volunteers.

Blood samples were taken at three time points during the first HBOT at visit V1: at time zero (pre-HBOT) and at 60 and 120-minutes relative to the 2-hour HBOT session. Further blood samples were taken at visit V4 (immediately after the fourth HBOT) and V5 (24 hours later). Blood samples were analysed for fasting glucose and insulin as well as cytokine markers of inflammation that are known to be associated with insulin resistance (TNF-χ IL-6, IL-18, IL-1ra and MCP-1). Abdominal subcutaneous adipose tissue was biopsied at baseline (V0) and visit V4 according to previously described techniques, snap frozen in liquid nitrogen and subsequently analysed for gene expression of inflammatory markers (IL-6, IL-1ra, TNF-χand MCP-1).[16]

LABORATORY ANALYSIS

Blood glucose samples sent to the laboratory were analysed by the hexokinase method (Olympus 4500, Beckman, USA) and insulin was measured by radioimmunoassay (Merck

Table 1
Baseline characteristics of men, stratified by diabetes status; median (95% CI), * $P \leq 0.001$

	Type 2 diabetes (n = 8)		**No diabetes** (n = 11)	
Age (years)	53	(49–60)	64	(53–66)
Height (m)	1.76	(1.69–1.79)	1.74	(1.69–1.80)
Weight (kg)	99.1	(87.9–111.5)	92.8	(80.4–108.5)
Body mass index (kg m^{-2})	30.8	(29.8–35.5)	30.5	(27.5–34.6)
Body fat (%)	35	(30–40)	32	(29–38)
Glucose (mmol·L^{-1})	9.8	(8.0–12.9) *	5.4	(5.0–5.9)
HDL (mmol·L^{-1})	1.2	(0.9–1.3)	1.2	(0.9–1.5)
LDL (mmol·L^{-1})	3.4	(2.1–4.2)	3.4	(2.1–4.2)
Triglycerides (mmol·L^{-1})	1.8	(0.8–4.9)	1.7	(1.0–2.0)
Total cholesterol (mmol·L^{-1})	5.6	(4.4–6.8)	5.3	(4.5–5.9)

Millipore, Billerica, MA, USA). Serum cytokine levels were determined using ELISA (R&D systems, Minneapolis, MN, USA). Total RNA was extracted from 100 mg adipose tissue using TRIzol reagent (Invitrogen, Carlsbad, CA). The integrity and concentration of RNA was assessed by spectrophotometry (Nanodrop, 2000, Thermoline). cDNA was synthesized using Omniscript RT kit (Qiagen, GmbH, Germany) and recombinant RNAsin ribonuclease inhibitor (Promega, Madison, WI) according to kit instructions. For RT-PCR analyses, we used gene-specific primer probes from Taqman (MCP-1, IL-6, TNF-χ IL1-ra) and Taqman universal PCR master mix (Applied Biosystems, Darmstadt, Germany). The samples were run in duplicate on an ABI Fast 7500 system (Applied Biosystems, Darmstadt, Germany) with internal negative controls and a standard curve. The cycle threshold (CT) value for each sample was normalized to the CT value of 18S ribosomal RNA to normalise for any changes in sample amplification, which was not different between V0 and V4.

STATISTICS

Statistical analysis was performed using SPSS for Windows (Version 19, SPSS Inc., Chicago, IL). Data were checked for normality by Shapiro-Wilk and log transformed prior to analysis if necessary. Differences between groups were analysed using one-way ANOVA. All other outcomes were analysed with linear mixed effects models using maximum likelihood estimation. Correlations were analysed by linear regression with coefficient of determination (r^2) and P value (Statistica v6, Statsoft, Tulsa, OK). Baseline characteristics, GIR and serum insulin were reported as median with 95% confidence intervals (CI_{95}). Significance was considered at $P < 0.05$.

Results

The baseline characteristics of groups stratified by diabetes status are shown in Table 1. Those with type 2 diabetes had higher fasting glucose ($P < 0.001$) and lower insulin sensitivity by hyperinsulinaemic clamp (Figure 2,

Figure 2
(A) Glucose infusion rate at baseline (V0) vs. HBOT (V3) during Steady State-1 (last 30 min of HBOT) in individuals with and without type 2 diabetes; (B), Glucose infusion rate at baseline vs. HBOT at Steady State-2 (first 30 min after HBOT) (mean and SEM, * $P < 0.05$, † $P < 0.01$)

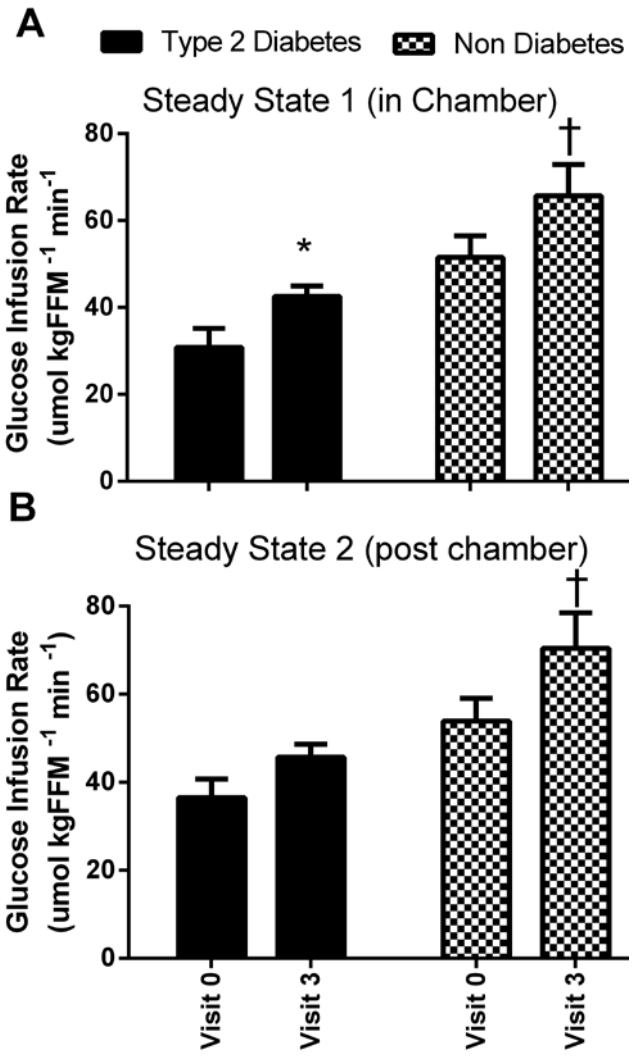


Figure 3

(A) Fasting glucose; (B) Insulin; (C); Monocyte chemotactic protein 1 – MCP-1; (D) Tumour necrosis factor – TNFχ (E) Interleukin–6 – IL-6 concentrations taken prior to and during the first HBOT exposure at 60 and 120 minutes, immediately following the 4th HBOT and 24 hours after the final HBOT (mean and SEM, * $P < 0.05$, † $P < 0.01$)

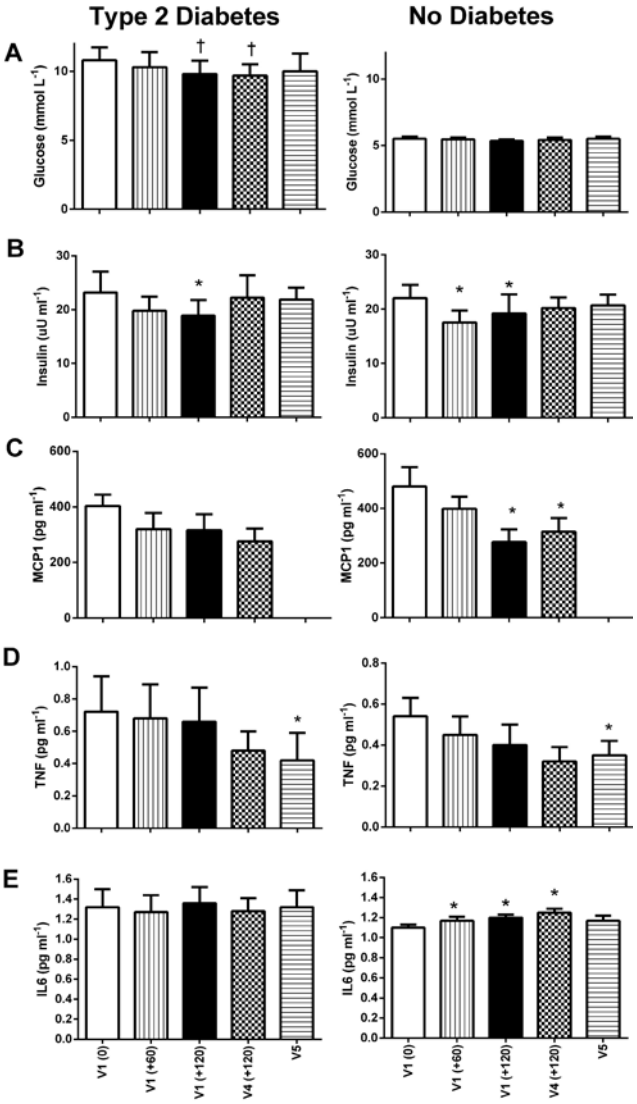
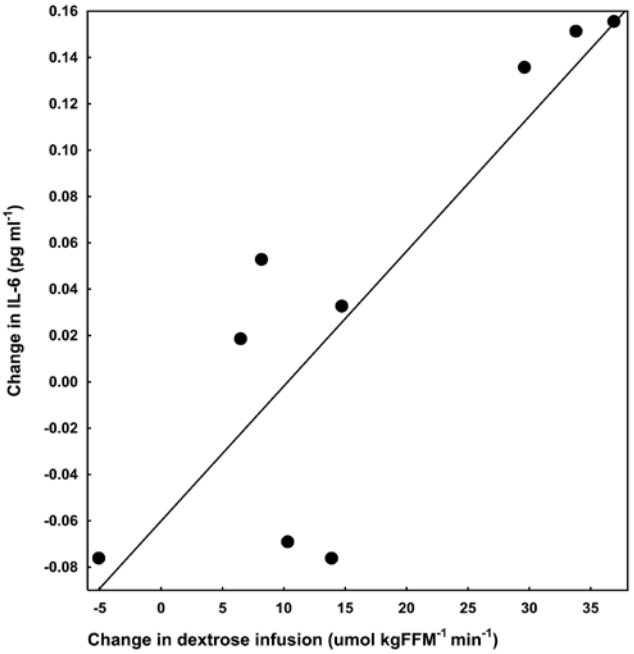


Figure 4

Relationship between the change in insulin sensitivity measured at visit V3 and change in serum interleukin–6 (IL-6) at visit V4 in non-diabetics ($r^2 = 0.72$, $P = 0.004$)



$P = 0.006$). A significant time effect was observed in the change in insulin sensitivity during the HBOT session (Figure 2A). For the group without diabetes, the median GIR at baseline in SS1 was 49.8 (39.6–62.7) μmol·kg·FFM^{-1}·min^{-1}. This increased during HBOT to 61.7 (49.4–82.1) μmol·kg·FFM^{-1}·min^{-1}. For the group with type 2 diabetes, baseline median GIR at SS1 was 32.6 (20.1–41.6) μmol·kg·FFM^{-1}·min^{-1}, increasing to 39.1 (36.6–48.5) μmol·kg·FFM^{-1}·min^{-1} during HBOT. The increase in insulin sensitivity was maintained for an additional 30 minutes after exit from the hyperbaric chamber whilst breathing normobaric air in those without diabetes ($n = 9$, $P = 0.008$, Figure 2B), but this was not significant in the group with diabetes ($n = 6$, Figure 2B). During the baseline hyperinsulinaemic euglycaemic clamp, steady state serum insulin was 204.3 (182.8–229.4) μU.ml^{-1} during SS1 and 199.2 (184.1–229.0) μU.ml^{-1} during SS2, with no significant difference during HBOT.

We observed significant time effects for the change in glucose, insulin, MCP-1, TNF-χand IL-6 with HBOT (all $P < 0.02$), with a time*group (diabetes/no diabetes) interaction observed in the change in fasting glucose only ($P = 0.03$). Further analysis by group revealed significant reductions in fasting glucose during the first and fourth HBOT sessions at 120 minutes only in those with type 2 diabetes (Figure 3A). Serum insulin was reduced during the first HBOT session in both groups (Figure 3B). MCP-1 was significantly reduced after HBOT at visits V1 and V4 in those without diabetes (Figure 3C), but this did not reach statistical significance in those with type 2 diabetes (Figure 3C). TNF-χ was significantly reduced 24-hours after the final HBOT in both groups (Figure 3D). In contrast, serum IL-6 was elevated in those without diabetes during and after HBOT at visits V1 and V4 (Figure 3E). The increase in IL-6 from baseline to visit 4 in the group without diabetes correlated with the increase in insulin sensitivity during SS2 ($n = 9$, $r^2 = 0.72$, $P = 0.004$, Figure 4). Neither group showed any significant changes for IL-1ra and IL-18 (data not shown). Adipose tissue was analysed for gene expression of IL-6, MCP-1, TNF-χand IL-1ra; however, no significant changes were detected (data not shown).

Discussion

In this study, we have demonstrated that peripheral insulin sensitivity is increased following HBOT in a relatively healthy urban population sample. Moreover, we have demonstrated that the increase in insulin sensitivity occurs in overweight and obese males without diabetes as well as those with type 2 diabetes. Importantly, the insulin sensitising effect was maintained after exit from the hyperbaric chamber for at least 30 minutes. We also observed small changes in inflammatory cytokines following HBOT that may have partly contributed to the observed increases in insulin sensitivity.

Diabetes is a common contributing or coincidental factor in patients referred for HBOT. Within hyperbaric medicine practice, it has been recognised for some time that patients with diabetes are prone to a fall in blood glucose during HBOT.[2,3] We also observed a significant fall in the blood glucose levels during the first HBOT in those with type 2 diabetes. Although greater decreases in fasting glucose inside versus outside the chamber have been reported,[2] we did not test this in our study and the changes could also be due to the prolonged length of the fast. Fasting glucose is predominantly under the control of hepatic glucose production; however, this was not specifically assessed in the current study. We also observed a fall in serum insulin during the first HBOT session in both groups; although other studies have found no effect of HBOT on insulin levels.[2,17] Our previous study tested a patient population during clinical HBOT exposure,[4] whilst the current study, which found a similar increase in insulin sensitivity, was in volunteers with no clinical indication for HBOT.

HBOT may induce an insulin-sensitizing effect by a number of possible mechanisms. Here, we studied circulating concentrations of pro-inflammatory cytokines since these have been observed in obesity and are closely associated with insulin resistance.[7] TNF-χis a pro-inflammatory cytokine which is overproduced from adipose tissue in human obesity,[8,18] and infusion of TNF-χinduces insulin resistance in humans.[19] The pro-inflammatory cytokine MCP-1 is also overproduced from adipose tissue in obesity[20] and impairs the insulin signalling cascade in a murine adipose tissue model independent of the associated macrophage infiltration.[9,10] Reductions in both TNF-χand MCP-1 were observed following HBOT and may partly explain the insulin-sensitizing effect, although the reduction in these cytokines did not correlate with the increase in insulin sensitivity.

IL-6 is a pleiotropic cytokine displaying both pro- and anti-inflammatory actions. Increased IL-6 is associated with human obesity and insulin resistance.[11,21] Conversely, exercise, a known insulin sensitiser, is associated with a transient release of IL-6 from muscle,[22] and acute infusion of IL-6 in humans leads to an increase in insulin sensitivity as measured by clamp studies.[12] IL-6 was not changed in those with type 2 diabetes, but was acutely increased by HBOT in those without diabetes. Interestingly, this was positively associated with increased insulin sensitivity. However, the changes in IL-6 are clinically small and may be a chance finding. We did not observe changes in IL-6 expression in adipose tissue, but no other tissues were investigated in this study.

The literature is mixed regarding the effect of HBOT on circulating cytokines, although most studies support an anti-inflammatory action of HBOT. Animal models suggest HBOT has, in part, an anti-inflammatory action in positive outcomes to abdominal sepsis,[23] multi-organ dysfunction[24] and development of atherosclerosis.[25] Human clinical data suggest HBOT-induced immunomodulation may be behind reduced restenosis following coronary angioplasty and stenting,[26] better outcome following cardio-pulmonary bypass,[27] and following ischaemia-reperfusion-related soft-tissue crush injury.[28] Even HBOT in the treatment of decompression illness is recognised to include an anti-inflammatory modulation of neutrophil activity as part of the therapeutic mechanism.[29] However, isolated cytokine changes should be interpreted with caution since the final effect on insulin sensitivity may depend on "*a subtle balance of their relative concentrations (high or low), kinetics (acute or chronic) and targets*".[15]

Alternatively, it has been proposed that insulin resistance may be induced by adipose tissue dysfunction secondary to hypoxia.[30] The growth of adipocytes in obesity is not matched by the blood supply, which may result in reduced oxygen delivery and regions of relative hypoxia.[30] Certainly, lower oxygen partial pressures have been measured in the adipose tissue of obese humans compared to lean controls.[31] However, another study concluded that adipose tissue had low oxygen consumption and the measurement of lactate/pyruvate ratios in blood draining this tissue revealed no evidence of metabolic stress.[32] The effects of hyperbaric oxygen on adipose tissue physiology have not been reported previously. However, studies investigating the reverse, using a hypoxic breathing gas mixture, have produced conflicting results. In two human studies using hyperinsulinaemic euglycaemic clamps, insulin resistance increased during acute exposure to hypoxia,[33] but decreased after a more chronic hypoxia protocol.[34] The substantial rise in tissue oxygen tensions associated with HBOT will also be accompanied by a transient increase in reactive oxygen species (ROS). This warrants further investigation since ROS, whilst having the potential to cause cell damage, also act as vital messengers in cell signalling,[35] including a positive effect on insulin signalling.[36]

This study employed the hyperinsulinaemic euglycaemic clamp which is considered to be the gold standard technique to assess peripheral insulin sensitivity.[37] Performing the clamp in a hyperbaric chamber was novel and required consideration of some technical issues and physiological

responses. Our glucometer used glucose dehydrogenase as the strip reagent, found to be more accurate than glucose oxidase when exposed to increased ambient oxygen.[38] Microvascular alterations in blood flow can influence measurement of insulin sensitivity as a consequence of varying the glucose delivery to the tissues.[39] Therefore, it is relevant to consider that vasoconstriction is an expected physiological response to hyperbaric oxygenation.[40] While the effects of HBOT on the microvasculature have not been tested, the sustained increase in insulin sensitivity observed upon exit from the hyperbaric chamber suggests our results were not influenced by changes in tissue blood flow.

Insulin resistance is a pivotal early change in obesity-related type 2 diabetes. The identification of pathways that influence insulin responsiveness may potentially lead to clinical therapies that prevent the development or progression of this disease. This study introduces a pathway that has not previously been exploited. The new findings, that HBOT can also increase insulin sensitivity in those without diabetes and also that the effect is sustained for a period after HBOT, have implications beyond diabetes involving obesity and glucose metabolism broadly. Further studies are now required to describe the precise mechanisms involved and to define the time course of the insulin sensitising effect – how much HBOT is required to initiate the effect and how long it persists after leaving the hyperbaric chamber.

Conclusions

This study has demonstrated that hyperbaric oxygen leads to an increase in insulin sensitivity in an overweight and obese male population with and without type 2 diabetes mellitus. Furthermore, the increase in insulin sensitivity was still evident 30 minutes after exiting the hyperbaric chamber. We have also demonstrated a favourable modulation of inflammatory markers in response to HBOT that may partly explain this effect on insulin sensitivity.

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The database of randomised controlled trials in hyperbaric medicine maintained by Michael Bennett and his colleagues at the Prince of Wales Hospital Diving and Hyperbaric Medicine Unit, Sydney is at:
<http://hboevidence.unsw.wikispaces.net/>

Assistance from interested physicians in preparing critical appraisals is welcomed, indeed needed, as there is a considerable backlog. Guidance on completing a CAT is provided. Contact Associate Professor Michael Bennett: <m.bennett@unsw.edu.au>