Hyperbaric oxygen but not hyperbaric air increases insulin sensitivity in men with type 2 diabetes mellitus

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Abstract
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Introduction: We have previously shown that hyperbaric oxygen treatment (HBOT) increased insulin sensitivity in men who were obese or overweight, both with and without type 2 diabetes. The aim of this study was to test whether this insulin-sensitising effect is seen in hyperbaric air (HA).
Methods: Men with type 2 diabetes who were obese or overweight were randomised to two groups: HBOT (n = 13) or HA (n = 11). A hyperinsulinaemic euglycaemic glucose clamp (80 mU·m^{-2}·min^{-1}) was performed at baseline and during hyperbaric intervention. Both groups were compressed to 203 kPa (two atmospheres absolute) for 90 minutes followed by a linear 30-minute decompression. The HBOT group breathed oxygen via a hood while the HA group breathed chamber air. Insulin sensitivity was assessed from the glucose infusion rate (GIR) during the last 30 minutes in the hyperbaric chamber (SS1) and the first 30 minutes after exit (SS2). Data were analysed for within-group effect by paired student t-test and between-group effect by one-way ANOVA.
Results: HBOT increased GIR by a mean 26% at SS1 (P = 0.04) and 23% at SS2 (P = 0.018). There was no significant change in GIR during or after HA. A between-group effect was evident for the change in GIR at SS1 in HBOT vs HA (P = 0.036).
Conclusions: The pathway by which insulin sensitivity is increased in men with type 2 diabetes requires the high oxygen partial pressures of HBOT and should be further investigated. Insulin sensitivity was not changed in hyperbaric air.

Introduction

Hyperbaric oxygen treatment (HBOT) is defined as breathing near 100% oxygen while in a hyperbaric chamber pressurised to more than 101 kPa or 1 atmosphere absolute (atm abs).[1] HBOT administered by clinical facilities typically uses pressure between 203-284 kPa (2–2.8 atm abs), with a duration of treatment 90–120 minutes. HBOT is an evidence-based treatment for conditions including decompression illness, cerebral arterial gas embolism, necrotising fasciitis, non-healing ulcers and wounds and delayed radiation injuries.[1]

Although HBOT is not used to treat diabetes mellitus *per se*, the increasing prevalence of this disease means that diabetes, particularly type 2 diabetes, is a frequent co-morbidity in patients treated with HBOT. For some years, it has been apparent that people with diabetes who undergo HBOT may experience a decrease in their plasma glucose level (PGL) during their treatment. Using a hand-held glucometer to measure PGL before and after 237 HBOT sessions in 27 patients with a mixture of type 1 and type 2 diabetes,[2] a mean fall in PGL of 2.04 mmol·L^{-1} was found. Another study measured laboratory glucose in a group of five patients with type 2 diabetes over the 2-hour duration of their HBOT session and found a mean fall of 3.5 mmol·L^{-1} at the end of HBOT.[3] There was no change in serum insulin levels.

In the present study, the effect of HBOT on insulin resistance and its reciprocal term, insulin sensitivity was investigated. Insulin resistance is defined as a relative impairment in the ability of insulin to exert its effect on glucose in target tissues (particularly muscle and liver). The development of insulin resistance is the best predictor for those likely to develop type 2 diabetes in the future.[4] Of the many investigative techniques used to assess insulin sensitivity, the hyperinsulinaemic euglycaemic glucose clamp is considered the gold standard.[5,6] In recent studies we have described an acute effect of HBOT to increase insulin sensitivity, as measured with the glucose clamp technique. A pilot study initially revealed that insulin sensitivity was increased in a cohort of men with and without diabetes receiving a clinical course of HBOT.[7] Progressively it was demonstrated that insulin sensitivity was increased during the third HBOT

session in a cohort of men with and without diabetes,[8] and most recently that the increase can be measured during the first HBOT session.[9]

The aim of this study was to determine whether the insulin-sensitising effect seen during HBOT (while breathing oxygen at a very high partial pressure) is also present during an equivalent pressure excursion but using air as the breathing gas rather than oxygen.

Methods

The study was approved by the Human Research Ethics Committee of the Royal Adelaide Hospital (R20160801) and the University of Adelaide and entered on a trial registry site (NCT03138746, clinicaltrials.gov). The study was carried out in accordance with the Declaration of Helsinki. All participants provided written, informed consent. The study was performed in the Hyperbaric Medicine Unit at the Royal Adelaide Hospital. Participant recruitment commenced in August 2018 and was closed due to reasons external to the study in December 2019.

PARTICIPANTS

Twenty-five participants were enrolled via a web-based recruitment company. Inclusion criteria were men aged 40 years or older who were obese or overweight (Body Mass Index (BMI) > 25 $kg \cdot m^{-2}$) with type 2 diabetes. Exclusion criteria included the presence of significant other medical issues, other non-prescribed medication that could affect glucose homeostasis, smoking, individuals who regularly perform high intensity exercise (> twice per week) and current intake of > 140 g alcohol per week. All participants were assessed for fitness to enter the hyperbaric chamber by a hyperbaric physician (DCW). Participants were randomised into two groups, HBOT and hyperbaric air (HA), stratified for BMI (BMI < 33 or BMI ≥ 33) by computer-generated, randomised block design in groups of 4.

STUDY DESIGN

Participants attended the Hyperbaric Medicine Unit on two occasions after overnight fasting (10 hours) and modification of their diabetic medication. On the first visit, participants sat in comfortable reclining chairs, breathing room air while the baseline glucose clamp was performed over 3.5 hours. Intravenous cannulae were inserted, one in each forearm with one for the insulin and glucose infusions and the other for blood sampling. A primed insulin (Actrapid, Novo Nordisk, Baulkham Hills, Australia) solution was infused (80 $mU \cdot m^{-2} \cdot min^{-1}$) with blood samples taken at 5–10 minute intervals and PGL measured by a hand-held glucometer (Accu-Chek Performa, Roche Diagnostics, Sydney, Australia). PGL was clamped at 5.5 $mmol \cdot L^{-1}$ with a variable infusion of 25% dextrose (Baxter Healthcare, Old Toongabbie, Australia). Insulin sensitivity can be assessed at a pre-determined point in the glucose clamp during a steady state (SS) period when glucose infusion rate (GIR) and PGL readings are stable. Insulin sensitivity was assessed using the GIR during two separate but consecutive 30-minute steady state (SS) periods in the last hour of the infusion: SS1 corresponded with 2.5–3 hours; and SS2 with 3–3.5 hours. The raw GIR data for each participant were adjusted for body surface area.

Two days later, participants returned after overnight fasting for a second glucose clamp using the same protocol, this time overlaid with a 2-hour session in the hyperbaric chamber. The insulin infusion was established one hour prior to entering the chamber. The large, triple-lock, multiplace hyperbaric chamber (Fink Engineering Pty Ltd, Warana, Australia) was compressed using air to 203 kPa (2 atm abs) and held at this pressure for 90 minutes followed by a 30-minute linear decompression back to ambient pressure. In the hyperbaric chamber, oxygen was delivered to the HBOT group via a hood system the same as used in clinical HBOT treatments (Amron International Inc, Vista, CA) which was connected on reaching 203 kPa pressure and continued for the 2-hour session (apart from a routine 5-minute 'air break' taken half-way through by temporarily detaching the hood). The HA group, who underwent exposure to the same pressure profile, breathed chamber air throughout the hyperbaric session. The participants remained in their reclining chairs once the clamp procedure had commenced and were wheeled into and out of the hyperbaric chamber. Blood samples were sent out of the chamber via the medical lock for PGL estimation. Insulin sensitivity was determined

Table 1
Participant characteristics for HBOT (n = 13) and hyperbaric air (n = 11) groups. (BMI, body mass index; BSA, body surface area)

Parameter	HBOT mean (SD)	HA mean (SD)
Age	62.3 (8.7)	56.3 (7.1)
Weight (kg)	108.2 (21.5)	102.4 (13.1)
Height (cm)	176.4 (6.7)	179.8 (10.3)
BMI ($kg \cdot m^{-2}$)	34.7 (6.8)	31.8 (4.7)
BSA (m^2)	2.23 (0.21)	2.21 (0.17)

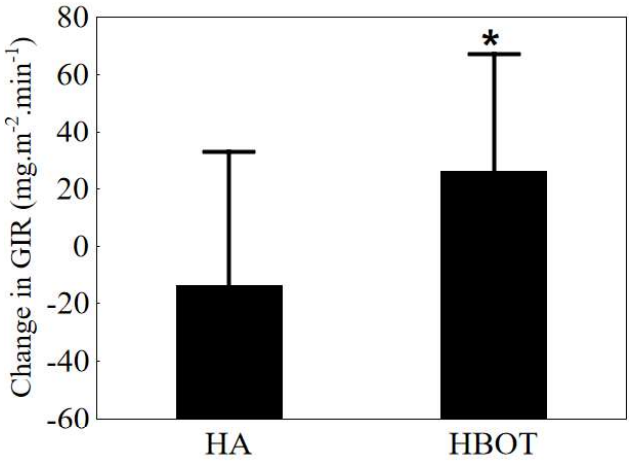
Table 2
Diabetes medication used by participants. DPP = dipeptidyl peptidase; GLP = glucose-like peptide; SGLT = sodium-glucose co-transporter

Medication	Number (n = 24)
Metformin	21
Insulin	5
SGLT-2 inhibitors	8
DPP-4 inhibitors	7
GLP-1 receptor agonists	4
Sulphonylureas	2

Table 3
Glucose infusion rates (mg·m^{-2}·min^{-1}) for HBOT and HA groups at baseline and during the hyperbaric intervention

Period	HBOT		HA	
	Baseline mean (SD)	Hyperbaric mean (SD)	Baseline mean (SD)	Hyperbaric mean (SD)
Steady state 1	151 (71)	177 (86)	180 (73)	166 (83)
Steady state 2	173 (87)	198 (85)	189 (91)	189 (81)

Figure 1
Change in glucose infusion rate (mg·m^{-2}·min^{-1}), expressed as mean change and SD, for hyperbaric air (HA) and hyperbaric oxygen treatment (HBOT) groups at steady state 1. *P = 0.036



by the GIR during the same two SS periods, so SS1 coincided with the last 30 minutes of the 2-hour hyperbaric session and SS2 with the first 30 minutes after exit from the chamber. At each visit, blood was taken for serum insulin concentration before commencing the clamp infusions for fasting levels and during SS1 and SS2 to demonstrate hyperinsulinemia. Steady-state insulin concentrations were not different between groups or between SS1 and SS2.

STATISTICAL CONSIDERATIONS

Statistical analyses were performed using Statistica (version 12, Statsoft, Tulsa, OK). Power analysis of earlier data suggested sample size of 20 in each group for power of 80% and α of 0.05 would be sufficient to detect a 25% difference in GIR between groups. GIR data were normally distributed by Shapiro-Wilk and Kolmogorov-Smirnov tests. HBOT and HA groups were analysed by paired student t-test for within-group effects and ANOVA for between-group effect. Significance was considered at $P < 0.05$.

Results

Of the 25 men enrolled, one participant experienced technical issues during his hyperbaric session and loss of data required exclusion. The other 24 participants completed the study without complication and their characteristics are shown in Table 1. There were no significant differences between the two groups. The participants were prescribed between one and four medications in the management of their diabetes (median two), summarised in Table 2.

The GIR data for both the HBOT and HA groups at baseline and during the hyperbaric exposure, for SS1 and SS2 can be seen in Table 3. Within the HBOT group, there was a mean 26% increase in GIR (median 17%) when compared to baseline, during SS1 (P = 0.04). There was a mean 23% increase in GIR (median 19%) during SS2 (P = 0.018). The HA group revealed no significant changes in GIR at SS1 or SS2.

One-way ANOVA for the change in GIR revealed a difference between groups at SS1 for HBOT vs. HA (Figure 1, P = 0.036). A trend towards a between-group difference was evident at SS2 (P = 0.088).

Discussion

This study has demonstrated that one session of HBOT significantly increased peripheral insulin sensitivity in men with type 2 diabetes, but exposure to an equivalent pressure profile without breathing supplemental oxygen (the hyperbaric air group) had no effect. The effect of HBOT persisted for at least the first 30 minutes after exit from the hyperbaric chamber.

The insulin-sensitising effect of HBOT observed in this study is consistent with that observed in earlier studies. In a group of patients referred for clinical HBOT (five men who were not obese and without diabetes and five men who were obese with type 2 diabetes), the glucose clamp revealed a significant increase in insulin sensitivity in the whole group during the third HBOT (37% increase) and the thirtieth HBOT (41% increase) although subgroup analysis revealed the change was statistically significant only in the group with diabetes.[7] A subsequent study recruited a cohort of men who were obese or overweight, both with (n = 8) and without (n = 11) type 2 diabetes.[8] A hyperinsulinemic euglycemic glucose clamp performed during the third HBOT demonstrated an increase in insulin sensitivity of 57% in those with type 2 diabetes and 29% in those without. This increase was still apparent during the first 30 minutes after exit from the hyperbaric chamber. A further study performed the glucose clamp technique during the first HBOT session on men who were obese or overweight but

without diabetes (n = 9).[9] This demonstrated a significant 23% increase in insulin sensitivity during the first HBOT session. Encouragingly, the magnitude of the insulin-sensitising effect in the current study is comparable with the effect sizes previously published and is large enough to be clinically significant. The effect has an onset of action within one HBOT session but its duration is not known. However, this study again found that the insulin-sensitising effect of HBOT was still active for at least the first 30 minutes after exit from the hyperbaric chamber.

The mechanism of action for the insulin-sensitising effect of HBOT is also unknown. However, an important new contribution from this study is the finding that the hyperbaric air group showed no change in insulin sensitivity. One can say for the first time that the hyperbaric environment itself – where the increase in absolute pressure is transmitted throughout the human body and generates a number of recognised physiological responses – has no independent effect on insulin sensitivity, in men with diabetes at least; it also requires the very high oxygen partial pressures that are only delivered during clinical HBOT to increase insulin sensitivity. There have been no reports or studies that the authors are aware of to suggest that breathing high concentrations of oxygen in the absence of hyperbaric conditions affects insulin sensitivity, and it seems likely that both high oxygen concentrations and high pressures are needed to produce this effect.

Previous findings that this effect can be detected in men with and without diabetes suggest that HBOT initiates a common metabolic response which is not confined to people with diabetes mellitus.[8] If the underlying mechanism for this insulin-sensitising effect can be identified, it may offer a new therapeutic target. In earlier work we found that the insulin-sensitising effect of HBOT was associated with some reductions in serum inflammatory cytokines;[8] however, this may only be part of the story. A number of the therapeutic benefits of clinical HBOT have now been shown to require the deliberate generation of oxidative stress as a consequence of breathing hyperbaric oxygen.[10] Reactive oxygen species can be damaging to biological tissue; however, they have other vital roles where they act as signalling molecules in a number of cellular pathways for a range of growth factors, cytokines and hormones.[11] Independently, other research has pointed out that reactive oxygen species can have both an inhibitory as well as a stimulatory effect on the intracellular glucose transport pathway.[12]

The finding that there was no change to insulin sensitivity in hyperbaric air is an important outcome in its own right. Whilst this study was not specifically designed to answer scuba diving questions, it is interesting to consider that the hyperbaric air group undertook a simulated (dry) scuba dive, albeit perhaps not a typical dive profile. Their intervention was the equivalent of diving, on air, to 10 metres' seawater (msw) for a 90-minute bottom time followed by a very slow ascent to the surface over 30 minutes (so results for any 'deeper' intervention cannot be assumed). This is relevant because people with diabetes do present to dive physicians with a desire to undertake scuba diving as recreation, with medical approval. For the dive physician, the medical assessment is complex and must consider the potentially disastrous consequences that could result from hypoglycaemia occurring underwater. Prospective observational studies have followed recreational divers with diabetes using detailed protocols for PGL management, suggesting that they can safely monitor and manage their PGL to allow diving.[13–15] However, it has never been determined if the potentially hazardous event encountered in hyperbaric medicine – the precipitous fall in PGL in a person with diabetes during HBOT – could also occur in response to the hyperbaric stimulus of the underwater environment. While other medical concerns will certainly exist for the potential diver with diabetes, this study provides the first evidence that exposure to a hyperbaric profile breathing air similar to that encountered in the recreational diving environment has no effect on insulin sensitivity. This encouraging finding may also be relevant to people in other hyperbaric environments.

One limitation to these studies is that we have only investigated men. Insulin sensitivity can change physiologically in adolescence and during pregnancy and different parts of the menstrual cycle in women. However, the studies have demonstrated an insulin-sensitising effect of HBOT that is not limited to those with diabetes and is likely to be a metabolic response to HBOT. As such, one would expect to see the same effect in women, although this has never been tested. Other limitations include the relatively small sample size. Despite this, the magnitude of the effect is large enough to achieve statistical significance and is comparable to previous studies. The already labour-intensive glucose clamp was made more complicated by performing it within a hyperbaric chamber. Previous studies have allowed the development of experience in the use of this technique in the hyperbaric environment. Strategies include keeping participants sedentary in reclining chairs and wheeling them, plus the infusions, into and out of the hyperbaric chamber to minimise exertion. The regular blood samples were passed out of the hyperbaric chamber through the medical lock for PGL analysis while the glucometer itself utilised a glucose dehydrogenase reagent which is less affected by high oxygen environments.[16]

Conclusions

This study has further strengthened the evidence that acute exposure to hyperbaric oxygen leads to a clinically significant increase in insulin sensitivity in men with type 2 diabetes. This effect is still evident during the first 30 minutes after exit from the hyperbaric chamber although its duration beyond that time is not known. Importantly, it has been shown for the first time that this insulin-sensitising

effect does not occur when breathing hyperbaric air at 203 kPa (2 atm abs, 10 msw equivalent). This may be relevant to other hyperbaric environments such as recreational diving but further work would be required to definitively establish the absence of an effect when breathing air at greater depths. The insulin-sensitising effect requires the very high partial pressures of oxygen only encountered during clinical HBOT. Further research should be encouraged to discover the mechanism for this novel effect on metabolism, as it could translate to new clinical therapies to improve glucose regulation.

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Conflicts of interest and funding

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