Assessment of insulin sensitivity during hyperbaric oxygen treatment

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Abstract
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Introduction: Previous studies using a hyperinsulinaemic, euglycaemic glucose clamp have demonstrated an increase in peripheral insulin sensitivity in men with and without Type-2 diabetes mellitus on the third and thirtieth hyperbaric oxygen treatment (HBOT) session. In two studies using different techniques for assessment of insulin sensitivity, we investigated the onset and duration of this insulin-sensitising effect of HBOT.
Methods: Men who were obese or overweight but without diabetes were recruited. One study performed a hyperinsulinaemic euglycaemic glucose clamp (80 $mU.m^{-2}.min^{-1}$) at baseline and during the first HBOT exposure (n = 9) at a pressure of 203 kPa. Data were analysed by paired t-test. The other study assessed insulin sensitivity by a frequently sampled intravenous glucose tolerance test (FSIGT) at three time points: baseline, during the third HBOT and 24-hours post-HBOT (n = 9). Results were analysed by repeated-measures ANOVA.
Results: There was a significant 23% increase in insulin sensitivity by clamp measured during the first HBOT exposure. The FSIGT showed no significant changes in insulin sensitivity.
Conclusions: The hyperinsulinaemic, euglycaemic glucose clamp demonstrated a significant increase in peripheral insulin sensitivity during a single, 2-hour HBOT session in a group of men who were obese or overweight but without diabetes. As an alternate technique for assessing insulin sensitivity during HBOT, the FSIGT failed to show any changes during the third HBOT and 24-hours later, however modification of the study protocol should be considered.

Introduction

While hyperbaric oxygen treatment (HBOT) is not used to treat diabetes mellitus *per se*, it has been observed that when people with diabetes undergo HBOT they may experience a decrease in blood glucose levels (BGL), potentially inducing clinical hypoglycaemia.[1,2] One study showed a substantial average BGL decrease of 3.5 $mmol.l^{-1}$ during a 2-hour HBOT session, with no change in serum insulin concentrations, suggesting an increase in insulin sensitivity as an underlying mechanism.[3]

Insulin resistance is defined as a relative impairment of the action of insulin on target tissues, particularly muscle and liver. The development of insulin resistance is the best predictor of those likely to develop type-2 diabetes mellitus (T2DM) in the future.[4] The inverse of insulin resistance is termed insulin sensitivity. In addition, obesity is strongly associated with the development of insulin resistance and T2DM via activation of a chronic inflammatory state.[5] The insulin resistance has effects on peripheral tissue glucose uptake as well as hepatic glucose production although an important effect is found in the peripheral tissues, specifically muscle.[6]

Of the many techniques available to assess insulin sensitivity, the hyperinsulinaemic, euglycaemic glucose clamp is the gold standard.[7,8] In a preliminary study of men (with and without T2DM) who were receiving a course of 30 HBOT sessions for medical indications, the glucose clamp technique revealed a substantial and significant increase in insulin sensitivity from baseline during their third (37% increase) and thirtieth (41% increase) HBOT sessions.[9] On subgroup analysis, this increase was significant only in those with T2DM, however numbers were small. A subsequent study, again using the glucose clamp technique, enrolled men who were obese or overweight (body mass index (BMI) > 25 $kg.m^{2}$), with and without T2DM.[10] This study demonstrated significant increases in insulin sensitivity during the third daily HBOT session in those with T2DM

(57% increase) and without (29% increase). The increased insulin sensitivity was still measurable 30-minutes after exit from the hyperbaric chamber.

Unanswered questions include how quickly the insulin-sensitising effect of HBOT occurs, how long it persists and its underlying mechanisms. To investigate this, we planned to assess insulin sensitivity during the first HBOT using the hyperinsulinaemic euglycaemic glucose clamp. However, while the glucose clamp technique is accurate, it is labour intensive and made more complicated by being performed within a hyperbaric chamber under pressure. We therefore designed a further study to assess an alternative, technically easier method of assessing insulin sensitivity in the chamber, which, if sufficiently accurate, could be more easily used for repeated studies on the same participant. Having previously shown that the insulin-sensitising effect could be demonstrated in men without T2DM, we designed these studies using men who were obese or overweight (BMI > 25 $kg.m^{-2}$) but without diabetes. This paper reports two studies: the use of the hyperinsulinaemic, euglycaemic glucose clamp to test the effect on insulin sensitivity during the first HBOT session and secondly, the use of a frequently sampled intravenous glucose tolerance test (FSIGT) to assess insulin sensitivity during HBOT and after 24-hours.

Methods

Both studies were approved by the Human Research Ethics Committee of the Royal Adelaide Hospital (RAH121212a, RAH140321) and the University of Adelaide and entered on a trial registry site (NCT02009813; NCT02136615). Both studies were carried out in accordance with the Declaration of Helsinki. All participants provided written, informed consent.

PARTICIPANT SELECTION

Both studies enrolled participants via local advertisement and a web-based recruitment company. Only men were studied as insulin sensitivity in women can vary throughout the menstrual cycle. Other inclusion criteria included age over 18 years with no history of diabetes; participants were obese or overweight (BMI > 25 $kg.m^{-2}$). Exclusion criteria included: prescribed or non-prescribed medication that may affect glucose homeostasis (e.g., corticosteroids); smoking; alcohol intake > 140 $g{\cdot}week^{-1}$; regular, high-intensity exercise (> twice weekly); blood donation or involvement in any other study within the last three months. All participants were assessed for fitness to undertake HBOT by a hyperbaric physician.

HYPERINSULINAEMIC EUGLYCAEMIC GLUCOSE CLAMP STUDY DESIGN

The hyperinsulinaemic, euglycaemic glucose clamp was first described by DeFronzo in 1979.[11] Insulin is infused at a constant rate that is above fasting levels, to stimulate glucose disposal in peripheral tissues but suppress hepatic glucose output. A variable dose glucose infusion is guided by regular blood sampling to measure BGL and 'clamp' the BGL at a pre-determined level (in this case, 6 $mmol.l^{-1}$). After running the infusions for a period of time, a steady-state can be reached where BGL and glucose infusion are stable. At this point, the glucose infusion rate (GIR) is equal to the glucose disposal rate. The GIR is a direct measure of whole body glucose disposal for a given level of hyperinsulinaemia.[8]

Ten participants were enrolled. A dual-emission X-ray absorptiometry scan (DXA) was performed at baseline for all participants to determine fat free mass (FFM). All participants attended the hyperbaric medicine unit after a 10 h overnight fast. Two intravenous cannulae were inserted into contralateral arms, one for the insulin and glucose infusions and the other for blood sampling. A primed insulin (Actrapid, Novo Nordisk, Baulkham Hills, Australia) solution (80 $mU{\cdot}m^{-2}{\cdot}min^{-1}$) was infused for 3.5 h as previously described.[10] Blood samples were taken at 5–10 min intervals and BGL measured by glucometer (Accu-Chek Performa, Roche Diagnostics, Sydney, Australia). BGL was maintained at 6 $mmol{\cdot}l^{-1}$ with a variable infusion of 25% dextrose (Baxter Healthcare, Old Toongabbie, Australia). Insulin sensitivity was determined by 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 h and SS2 with 3–3.5 h. The GIR was standardised for FFM from the DXA scan.

The following day, all participants returned after overnight fasting and the 3.5 h glucose clamp was repeated using the same protocol, this time overlayed with a 2 h HBOT session. The twin-lock, multiplace hyperbaric chamber (Fink Engineering/Cowan Engineering, Australia, 1994) was compressed to 203 kPa followed by breathing 100% oxygen by mask or hood during 90 min at 203 kPa and a 30 min linear decompression to 101.3 kPa. Insulin sensitivity was determined by the GIR during the same two SS periods, so SS1 coincided with the last 30 min of the 2 h HBOT session and SS2 with the first 30 min after exit from the chamber. Statistical analyses were performed using Statistica (version 12, Statsoft, Tulsa, OK, USA). A paired *t*-test was used to compare GIR. Statistical significance was considered at $P < 0.05$.

FREQUENTLY SAMPLED INTRAVENOUS GLUCOSE TOLERANCE TEST STUDY DESIGN

An indirect measure of insulin sensitivity was developed by Bergman in 1979 using mathematical modelling of glucose and insulin data from an intravenous glucose tolerance test.[12] Following the glucose bolus, frequent measurement of blood glucose and insulin are made. The complex relationship between glucose and insulin in the disposal of glucose from the blood is built into pharmacokinetic models that are fit to the data. Parameters that provide best fit are derived.

This includes insulin sensitivity (S_I), defined as fractional glucose disappearance per insulin concentration unit.[8] Other parameters include: glucose effectiveness (S_G), the ability of glucose to promote its own disposal; the acute insulin response to glucose (AIR_G) or first-phase insulin response; the disposition index (DI), a product of insulin sensitivity and insulin secretion, which is a constant. The mathematics to calculate these parameters has been packaged into a commercially available software program (MINMOD Millennium, Pasadena, CA, USA). The FSIGT has shown reasonable correlation with the glucose clamp (r = 0.54).[7]

Twelve participants were enrolled. On the first study day (Day 1) all participants attended the hyperbaric medicine unit at the Royal Adelaide Hospital after a 10-hour overnight fast. A baseline FSIGT was performed in room air with the participant resting in a chair outside of the hyperbaric chamber according to the following protocol. Two intravenous cannulae were inserted into contralateral forearms and blood taken for time zero. A glucose bolus was given into one of the cannulae at time zero over one minute. The weight-dependant bolus used 25% dextrose (Baxter Healthcare, Old Toongabbie, Australia) at 300 mg·kg^{-1} to a maximum dose of 120 ml (30 g dextrose). Blood sampling from the other cannula was performed at 2, 4, 6, 8, 10, 12, 14, 16, 19, 22, 25, 30, 40, 50, 60, 70, 90, 120, 150 and 180 minutes.

Each participant then underwent three HBOT sessions on consecutive days (Days 2–4), with compression to 203 kPa breathing oxygen for 90 min and a 30 min decompression. During the third HBOT session on Day 4, another FSIGT was performed using the same protocol as on Day 1. Compression of the chamber to 203kPa takes 7 minutes and time zero for the dextrose bolus aligned with the start of oxygen breathing during the 90 min period at 203 kPa. A further FSIGT was performed 24 h later on Day 5, in air outside the hyperbaric chamber. The three FSIGTs were performed at a similar time of the day.

Blood samples taken at each of the time points were analysed for glucose and insulin. Insulin was measured by radioimmunoassay (Millipore, St. Charles, MO, USA). Glucose was measured using commercial enzymatic kits on a Beckman AU480 clinical analyser (Beckman Coulter, Brea, CA, USA). All samples for each subject were analysed within the same analytic run to minimise instrument variation. The glucose and insulin data were entered into the minimal model software to derive insulin sensitivity and the other parameters. These measures were statistically analysed by repeated measures ANOVA using SPSS for Windows (Version 22, SPSS, Chicago, IL, USA). Statistical significance was considered at $P < 0.05$.

Table 1
Demographics of participants in the glucose clamp study, $n = 9$; DXA – dual-emission X-ray absorptiometry scan

Parameter	Mean (SD)
Age	47 (5.7)
Height (cm)	176.4 (10.3)
Weight (kg)	97 (15.1)
Body mass index (kg.m^{-2})	31.1 (3.0)
DXA fat free proportion (%)	64.3 (0.1)
Baseline insulin sensitivity (mg.kgFFM^{-1}.min^{-1})	8.57 (3.02)

Results

HYPERINSULINAEMIC EUGLYCAEMIC GLUCOSE CLAMP

One participant sustained a minor middle ear barotrauma during compression at the start of the HBOT. He was removed from the hyperbaric chamber and excluded from the study. Characteristics of the remaining nine participants are shown in Table 1. The GIR data were normally distributed by Shapiro-Wilk and Kolmogorov-Smirnov tests. Figure 1A shows the GIR during SS1 (the last 30 min of the HBOT session). There was a significant increase in insulin sensitivity from Day 1 to Day 2, as measured by the GIR ($t = -2.89$, df = 8, $P = 0.02$). Figure 1B shows the GIR during SS2 (the first 30 min after leaving the chamber), the rise was not statistically significant ($t = -1.87$, df = 8, $P = 0.10$).

FREQUENTLY SAMPLED INTRAVENOUS GLUCOSE TOLERANCE TEST

One participant sustained a minor middle ear barotrauma at the start of compression and was removed from the hyperbaric chamber; another withdrew for personal reasons. On laboratory analysis, another participant had glucose and insulin levels on arrival for the FSIGT on the third HBOT and again 24 h later which suggested a failure to follow the fasting protocol, and these data were excluded. Characteristics of the remaining nine participants are shown in Table 2. The results of the minimal model analysis of the FSIGT are shown in Table 3. Data sets for all parameters showed large variances and there were no significant changes in any of the measured parameters.

Table 2
Demographics of participants in the FSIGT study, $n = 9$

Parameter	Mean (SD)
Age	37.1 (13)
Weight (kg)	99.3 (15.2)
Height (cm)	172.6 (3.8)
Body mass index (kg.m^{-2})	33.2 (4.1)

Figure 1
(**A**) Glucose infusion rate (GIR) at baseline vs. HBOT during SS1 (last 30 min in chamber); (**B**) GIR at baseline vs. HBOT during SS2 (first 30 min after HBOT); * $P = 0.02$

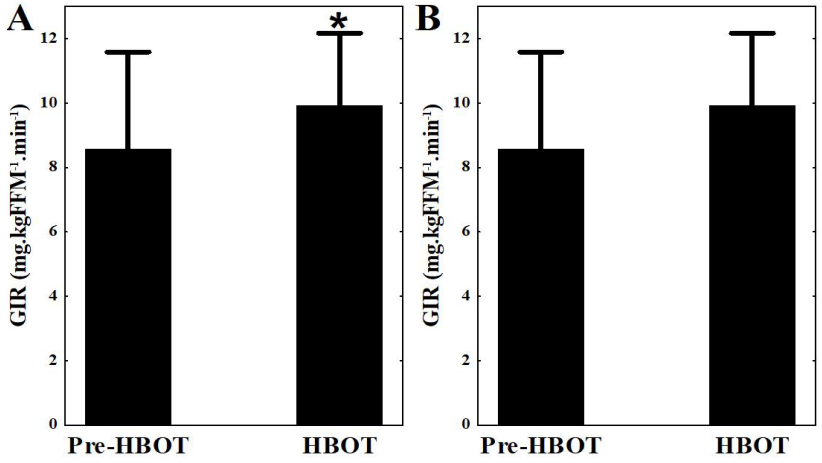

Table 3
Insulin sensitivity and other parameters derived from minimal model analysis; data are mean (SD); S_I = Insulin Sensitivity, S_G = Glucose effectiveness, AIR_G = Acute insulin response to glucose

Parameter	Day 1	Day 4	Day 5
S_I ($mU \cdot l^{-1} \cdot min^{-1}$)	3.35 (1.27)	3.82 (2.09)	4.23 (3.38)
S_G (min^{-1} x100)	1.55 (0.79)	1.58 (0.92)	1.48 (0.82)
AIR_G ($mU \cdot l^{-1} \cdot min^{-1}$)	720 (462)	573 (275)	706 (364)
Disposition index	2304 (2004)	1862 (1115)	2165 (1089)

Discussion

Using an in-chamber hyperinsulinaemic euglycaemic glucose clamp technique, we have previously shown that routine HBOT typically used for clinical indications is associated with significant increases from baseline in peripheral insulin sensitivity on the third day of daily HBOT sessions.[9,10] Utilising the same clamp technique, we have now found that the HBOT-induced increase in insulin sensitivity occurs during the very first HBOT session. This study also confirms the previous findings that the insulin-sensitising effect of HBOT can be identified in overweight/obese men without diabetes and is not specific to those with diabetes. The findings that the effect can be identified during the first HBOT exposure and in men without diabetes should make future studies examining the effects of HBOT on insulin sensitivity and the effects underlying them easier to undertake.

In our previous study using the glucose clamp technique, HBOT significantly increased insulin sensitivity not only during the final 30 min of the 2 h spent under HBOT conditions, but also during the first 30 min after exit from the hyperbaric chamber, when performed on the third HBOT exposure.[10] The current study used the glucose clamp technique on the first HBOT and found significantly increased insulin sensitivity under hyperbaric conditions (during SS1). In contrast, there was not a significant increase over baseline insulin sensitivity during the first 30 min after leaving the chamber (SS2). There is a trend towards an increase in insulin sensitivity, however small sample size and large variance in the data make statistical significance more difficult to achieve. Another consideration as to why SS2 did not achieve significance in the current study could be that one HBOT has less impact than three; there was a 23% increase in insulin sensitivity during the first HBOT compared to a 29% increase in men without diabetes during the third HBOT.[10] There may be some accumulation of the HBOT effect with repeated exposures, however its duration of effect is not known. It is clear however, that one 2 h HBOT session is sufficient to see a change in insulin sensitivity. This finding is also consistent with clinical practice in hyperbaric medicine where anecdotally, people with diabetes have experienced a fall in their BGL during their first HBOT session.

Our previous studies performed the clamp on the third HBOT session for two reasons: to improve the chances of identifying an effect if some accumulated exposure was important, and also to give the participant the opportunity to practice middle ear equalisation manoeuvres that are required during pressurisation of the hyperbaric chamber,

prior to undergoing the glucose clamp procedure. While potential difficulty with ear equalisation was assessed during their initial medical review, middle ear barotrauma continues to be the most frequent complication associated with clinical HBOT (approximately 2%).[13] Indeed, one of our participants in this study had been established on his second glucose clamp with infusions of glucose and insulin when he was wheeled into the chamber only to find he could not satisfactorily equalise his ears on compression, resulting in his removal from the chamber and from the study. Despite the small sample size in this study, a significant increase in insulin sensitivity was identified, consistent with the two previously published studies.

Our attempts to replace the glucose clamp technique with the simpler FSIGT have not been successful. While the FSIGT requires frequent blood sampling over several hours, it avoids the necessity of passing samples through the medical lock for immediate glucometer analysis and the rapid decisions required to maintain blood glucose concentrations during a glucose clamp. However, under the same HBOT conditions as in our three glucose clamp studies, all of which showed increased insulin sensitivity during the first or third HBOT session, we found no significant effect of HBOT on insulin sensitivity when assessed by the FSIGT during the third HBOT and at 24 h later.

There are a number of reasons the FSIGT may have failed to pick up such an effect. First, the sample size was small and there was substantial variation in the data. Second, the FSIGT is known to be less reliable in people with insulin resistance. Several modifications to this technique have been suggested, such as giving tolbutamide or an insulin infusion early in the FSIGT, which has improved the correlation with glucose clamp studies.[7] However, in pursuit of a simpler technique and with a group of men without diabetes, we did not modify the FSIGT.

Third, and perhaps more likely, we performed the FSIGT too soon after the participants started their HBOT session. While we have demonstrated an increase in insulin sensitivity during steady state periods 2.5 to 3.5 h into the clamp (at the end of an HBOT exposure), we have not specifically tested insulin sensitivity earlier in the HBOT session using a glucose clamp technique. If the insulin-sensitising effect of HBOT requires some duration of exposure to activate, then giving the glucose bolus of the FSIGT at the beginning of the HBOT session may not be the best time. The bulk of the glucose disposal would have taken place in the early part of the HBOT session and missed a later-onset effect identified in the clamp studies. Future studies using the FSIGT should perform the procedure towards the end of the HBOT session. On a cautionary note, such a study design may create the potential for the fasting participant with diabetes to develop hypoglycaemia during their HBOT session prior to the FSIGT, and they would need regular monitoring of their in-chamber BGL. If hypoglycaemia occurred during the HBOT, intervention would be required and the FSIGT would not be able to proceed.

The third FSIGT performed 24-hours post HBOT also did not demonstrate an effect of HBOT on insulin sensitivity, but we cannot say whether this is because such an effect was not present (i.e., a stimulatory effect of the previous day's HBOT had worn off), or whether such an effect was present but could not be detected due to limitations with the FSIGT technique.

The FSIGT was chosen because it was anticipated to be easier to perform and more easily tolerated by the participant than the glucose clamp. In the end, both techniques were found to be labour-intensive in a hyperbaric chamber. Importantly for undertaking assessment of insulin sensitivity in the novel environment of a hyperbaric chamber, every endeavour was made to perform these techniques according to established protocols. The fasting participants were tested at the same time of the day. They were kept sedentary in comfortable chairs for the duration of the study and wheeled into and out of the hyperbaric chamber. The glucometer utilised a glucose dehydrogenase reagent as opposed to glucose oxidase, making it less sensitive to ambient oxygen pressures.[14]

Our hyperbaric facility, along with many others, manages potential hypoglycaemia in patients with diabetes by monitoring their BGL before they enter the hyperbaric chamber and by repeating it if clinically indicated. Continued investigation is warranted in this field, both for the safety of hyperbaric patients with diabetes but also for the potential to identify novel pathways of glucose control.

Conclusion

The glucose clamp performed during the first HBOT session demonstrated a significant increase in insulin sensitivity, earlier than in our previously published studies which showed an increase in insulin sensitivity in men with and without diabetes on the third and thirtieth HBOT.[9,10] The hyperinsulinaemic, euglycaemic glucose clamp appears to be a useful tool to undertake these investigations. The FSIGT in its current design is probably not a good tool to assess insulin sensitivity in a hyperbaric chamber.

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