Alteration of blood glucose levels in the rat following exposure to hyperbaric oxygen

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Submitted 24 February 2015; accepted in final form 15 July 2015

Alteration of blood glucose levels in the rat following exposure to hyperbaric oxygen. J Appl Physiol 119: 463–467, 2015. First published July 16, 2015; doi:10.1152/japplphysiol.00154.2015.—Findings regarding blood glucose level (BGL) on exposure to hyperbaric oxygen (HBO) are contradictory. We investigated the influence of HBO on BGL, and of HBO on latency to central nervous system oxygen toxicity (CNS-OT). The study was conducted on five groups of rats: Group 1, exposure to oxygen at 2.5 atmospheres absolute (ATA), 90 min/day for 7 days; Group 2, exposure to oxygen once a week from 2 to 6 ATA in increments of 1 ATA/wk, for a period of time calculated as 60% of the latency to CNS-OT (no convulsions); Group 3, exposure to 6 ATA breathing a gas mixture with a pO2 of 0.21; Group 4, received 10 U/kg insulin to induce hypoglycemia before exposure to HBO; Group 5, received 33% glucose to induce hyperglycemia before exposure to HBO. Blood samples were drawn before and after exposures for measurement of BGL. No change was observed in BGL after exposure to oxygen at 2.5 ATA, 90 min/day for 7 days. BGL was significantly elevated after exposure to oxygen at 6 ATA until the appearance of convulsions, and following exposure to 4, 5, and 6 ATA without convulsions (P < 0.01). No change was observed in BGL after exposure to 6 ATA breathing a gas mixture with a pO2 of 0.21. Hypoglycemia shortened latency to CNS oxygen toxicity, whereas hyperglycemia had no effect. Our results demonstrate an influence of HBO exposure on elevation of BGL, starting at 4 ATA. This implies that BGL may serve as a marker for the generation of CNS-OT.

Central nervous system oxygen toxicity (CNS-OT) represents a major risk when diving with 100% oxygen (closed-circuit diving apparatus) or an oxygen-enriched gas mixture, or during hyperbaric oxygen (HBO) treatment (29, 31). It is characterized by convulsions similar to epileptic seizures and sudden loss of consciousness, sometimes without any warning symptoms. CNS-OT may thus be fatal during underwater activity, and it would be of great benefit if we could find a marker that predicts the beginning of convulsions. Apart from this, our knowledge of the mechanism responsible for CNS-OT, despite extensive investigation, is still incomplete. A better understanding of its underlying mechanism would help us to find ways of reducing the risk of its occurrence.

Glucose is a major source of cellular energy in mammals. In the brain, glucose is an obligate source of energy. The CNS requires a consistent supply of glucose for all its energy demands and to maintain metabolic homeostasis (26). Findings regarding blood glucose level (BGL) during exposure to HBO in humans are contradictory. Patients undergoing HBO therapy frequently suffer from a reduction in BGL. This was found to be the case in both diabetics and nondiabetic healthy subjects (2, 27). However, Wilkinson et al. (36) found no change in BGL following HBO treatment in nondiabetic patients.

In a rat model, animals were exposed to 100% oxygen at 2–3 atmospheres absolute (ATA), the pressure employed in clinical HBO therapy. Exposure to 2.8 ATA oxygen, 2 h daily for 7 days, did not induce any change in BGL in nondiabetic rats at 5, 8, and 12 days (20–22). In the study by Yasuda et al. (38), daily exposure for 6 h to 36% oxygen at 1.25 ATA over a period of 4 wk induced reduction of BGL in nondiabetic rats. In the study of Torbati and Reilly (33), exposure to normobaric hyperoxia for 24 h did not change BGL. This was correlated with the absence of any change in cerebral metabolic rate for glucose in the brain. However, exposure to 100% oxygen at 5–6 ATA, a pressure that produces CNS-OT in rats and mice, resulted in an increase in BGL (7, 8, 23). In the study of Torbati and Torbati (34), conscious rats were exposed to HBO at 3 and 5 ATA; the authors found a correlation between the elevation of BGL and changes in the electrocorticogram. The discrepancy between BGL obtained following exposure to lower HBO pressures (2–3 ATA), and to higher pressures that may produce convulsions (5–6 ATA), raised the question of whether elevation of the BGL could be linked to the convulsions, or to the HBO itself at these pressures. If the latter were true, elevation of BGL might be used as a predictive marker for CNS-OT.

The purpose of the study was to elucidate the influence of HBO at various pressures on BGL, and to examine whether these changes might be associated with the development of CNS-OT, thus enabling them to be used as an indicator for this process.

METHODS

Animals and maintenance. Thirty-five male Sprague-Dawley rats weighing 250–300 g were housed in plastic cages under standard conditions, with free access to drinking water and standard chow. They were kept in an 8-h light/16-h dark cycle, and the ambient temperature was maintained at 24°C. The Animal Care Committee of the Israel Ministry of Defense approved the experimental procedures, and the rats were handled and surgical procedures performed in accordance with internationally accepted humane standards.

Protocol. The study was conducted on five groups of rats. Animals in Group 1 were exposed to oxygen at 2.5 ATA, 90 min/day (a protocol employed in clinical HBO therapy) for 7 days (n = 6). Rats in Group 2 were exposed to oxygen at 6 ATA until the appearance of convulsions, to determine their baseline latency to CNS-OT (n = 7). These animals were exposed to HBO for a second time a week later (to avoid any residual effects of the previous exposure) and once each week thereafter, to pressures from 2 to 6 ATA in increments of 1 ATA/wk for 60% of their latency to CNS-OT (no convulsions). We used the latency obtained at 6 ATA to calculate
the exposure time to HBO at each pressure from 2 to 5 ATA according to the power equation: $t = (PO_2/1)^{5.62}$, where $t =$ time and $PO_2 =$ oxygen pressure in ATA, as obtained in the study of Arieli et al. (6). This equation is shown in Fig. 4 of the cited study, and represents latency to CNS-OT as a function of $PO_2$. We used the power equation derived from the curve to calculate the estimated time to convulsions at 2–5 ATA. By calculating 60% of the time obtained from the equation’s solution for each pressure, we reached the estimated 60% of latency. The calculated mean 60% latency for each pressure is presented in Table 1. The 60% latency for 2 ATA was established as 120 min, and not the calculated value, to avoid pulmonary oxygen toxicity.

Rats in Group 3 were exposed to 6 ATA breathing a gas mixture containing oxygen at a partial pressure of 0.21 (equivalent to normobaric air), with nitrogen as the diluent gas ($n = 6$). The exposure period for these animals was equivalent to that for the rats in Group 2, which were exposed for 60% of their latency to CNS-OT at 6 ATA (16.5 min). Rats in Group 4 were exposed to pure oxygen at 6 ATA to determine their baseline latency to CNS-OT ($n = 8$). A week later, these animals received 10 U/kg insulin intraperitoneal before exposure to HBO. When their BGL dropped to 50 mg/dl and lower (hypoglycemia), they were exposed to HBO at 6 ATA until the appearance of convulsions. Rats in Group 5 were exposed to 6 ATA oxygen to determine their baseline latency to CNS-OT ($n = 8$). A week later, these animals received 33% glucose in 3 ml saline intraperitoneal before exposure to HBO. When their blood glucose reached 150 mg/dl and greater (hyperglycemia), they were exposed to HBO at 6 ATA until the appearance of convulsions. Previous studies have demonstrated that there will be no effect of one exposure to HBO on latency to CNS-OT in the following exposure, as long as there are 2 days intervening (3, 4).

Blood samples a few microliters in volume were drawn from the tail vein before and after each exposure for measurement of BGL by using a FreeStyle Freedom glucometer (Abbott Diabetics Care, Alameda, CA) calibrated with the manufacturer’s solvents of known concentration.

Exposure to HBO. The rat was put in the experimental cage, which was placed in a 150-liter hyperbaric chamber (Roberto Galeazzi, La Spezia, Italy). The flow of gas through the cage was controlled by two needle valves. A small portion of the outgoing gas was directed out of the pressure chamber (controlled by another needle valve), passed through a flow meter, and was sampled by an oxygen analyzer (Servomex Model 571, Crowborough, East Sussex, UK), which monitored the concentration of $O_2$ in the experimental cage. The temperature in the cage was monitored continuously throughout the HBO exposure and was maintained within a thermoneutral range ($27 \pm 1°C$) to avoid any effect of temperature on CNS-OT. Temperature regulation was by means of water flowing from a recirculation bath via ports in the pressure chamber.

When the pressure of 2.5 ATA was reached, the flow of air was replaced by pure oxygen at a fast flow rate of 15 liters/min for 1 min to replace the cage’s atmosphere. When the oxygen level reached 95%, oxygen flow into the experimental cage was reduced to 5 liters/min. Exposure continued for 7 days, 90 min/day.

To induce convulsions, rats were exposed to HBO at 6 ATA. When the desired pressure was reached, a period of 20 min was allowed for acclimation to the experimental conditions, during which air flowed through the cage at ~5 liters/min. At the end of this period, the flow of air was immediately replaced by pure oxygen at a fast flow rate of 15 liters/min for 1 min to replace the cage’s atmosphere. When the oxygen level reached 95%, oxygen flow into the experimental cage was reduced to 5 liters/min. The exposure was terminated when the first convulsions appeared. We then changed the inflowing gas back to air, and reduced the pressure at a rate of 1 ATA/min. Latency to CNS-OT was measured starting from the time at which $O_2$ reached a level of 95% until the appearance of the first convulsions.

Statistical analysis. The Student t-test was used to evaluate differences in BGL before and after HBO exposure. One-way ANOVA was used to evaluate differences in BGL before and after HBO exposures from 2 to 6 ATA. One-way ANOVA was used to evaluate differences in latency to CNS-OT following the induction of hypoglycemia and hyperglycemia. For a posteriori analysis we used Dunnett’s test. All data are expressed as means ± SD. The level of significance was $P < 0.05$.

RESULTS

Blood glucose level following exposure to HBO at 2.5 ATA. No change was observed in BGL after this exposure protocol, neither after each individual exposure nor at the conclusion of the series of seven daily exposures (Fig. 1).

Blood glucose level following exposure to 2–6 ATA. BGL was significantly elevated ($P < 0.01$) after convulsions on exposure to oxygen at 6 ATA. The latency to convulsions was 27.5 ± 5.8 min. A similar pattern of BGL was observed after exposure to 6 ATA for 60% of the latency to CNS-OT (no convulsions) (Fig. 2). However, no change was observed in BGL after exposure to 6 ATA while breathing a gas mixture containing oxygen at a partial pressure of 0.21 (equivalent to normobaric air), with nitrogen as the diluent gas.

Figure 3 presents the BGL for weekly exposure to HBO at pressures from 2 to 6 ATA, in increments of 1 ATA/wk, for 60% of the estimated latency to CNS-OT. HBO pressure had a dose-response effect on BGL. The rise in BGL began following exposure to 4 ATA, and continued increasing significantly until the exposure to 6 ATA.

Latency to CNS-OT and blood glucose level following administration of glucose and insulin. Administration of glucose to induce hyperglycemia resulted in elevation of the BGL to 301.1 ± 74.7 mg/dl. Following exposure to 6 ATA, BGL dropped to 144 ± 60 mg/dl. Hyperglycemia did not prolong the latency to CNS-OT. Administration of insulin to induce hypoglycemia resulted in a reduction of BGL to 48 ± 10.5 mg/dl. The BGL following exposure to 6 ATA oxygen was 49.7 ± 13.9 mg/dl. Hypoglycemia significantly shortened the latency to CNS-OT ($P < 0.01$, Fig. 4).

DISCUSSION

In the present study, we have demonstrated an association between BGL and elevated levels of oxygen, starting at 4 ATA. There was a constant increase in BGL up to the pressure of 6 ATA. If there are no additional risk factors present, CNS-OT ending in convulsions will not occur in the rat at less than 4 ATA oxygen (5). Our results might therefore indicate that in the rat, BGL may serve as a marker for the development of CNS-OT before seizures are observed. In support of this hypothesis, we may note that BGL did not change following exposure to 6 ATA with a gas mixture containing oxygen at a partial pressure of 0.21 (equivalent to normobaric air). CNS-OT does not develop under these conditions, indicating

Table 1. The calculating mean time of 60% latency for each pressure

<table>
<thead>
<tr>
<th>Pressure (ATA)</th>
<th>6</th>
<th>5</th>
<th>4</th>
<th>3</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (min) ± SD</td>
<td>16.5 ± 5.8</td>
<td>32.3 ± 4.3</td>
<td>47.8 ± 12.6</td>
<td>93.8 ± 36.7</td>
<td>120.0</td>
</tr>
</tbody>
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ATA, atmospheres absolute.
that exposure to high pressure on its own is not enough to bring about an increase in BGL. This finding is further supported by Nolan et al. (23) and Torbati and Torbati (34), who showed that there was no change in BGL following exposure to 5 and 6 ATA with a gas mixture containing 50% oxygen/50% nitrogen, and that pressure per se was not sufficient to cause elevation of BGL. On the other hand, exposure to 100% oxygen did not cause elevation of BGL unless the pressure reached at least 4 ATA, such that both high pressure and oxygen are necessary to bring about an increase in BGL. Elevation of BGL starting at 4 ATA is probably due to an increase in reactive oxygen species (ROS). The combination of hyperoxia and high pressure results in the production of more ROS. Numerous studies have linked ROS to disease states such as cancer, diabetes mellitus, cardiovascular diseases, and atherosclerosis, as well as to aging, and of more relevance in the present study, to insulin resistance (1). One might argue that the elevation of BGL with increasing levels of HBO is probably due to HBO’s inducing ROS production, with the subsequent development of insulin resistance.

Exposure to 100% oxygen at 6 ATA, a protocol that induces CNS-OT in rats and mice, resulted in elevation of BGL both in the present study and in previous investigations (7, 23, 34). However, in the present study BGL increased whether or not the animals convulsed. No rat convulsed following exposure to only 60% of its latency to CNS-OT. This was not the case in the study by Nolan et al. (23), in which a 10.5-min exposure to HBO at 6 ATA had no effect on BGL, whereas after 14 min BGL increased and convulsions were observed. A correlation was also found in the study of Torbati and Torbati (34) between elevation of BGL and changes in the electrocorticogram. The authors noted that there was no change in BGL in the absence of changes in the electrocorticogram. Nolan et al. (23) explained these results as the appearance of stress due to restlessness and convulsions, resulting in elevation of BGL. In the present study, however, the BGL was elevated by the same amount whether or not the animal reached convulsions. Therefore, the stress the animal suffered just before and during convulsions is probably not the factor that led to elevation of BGL as a result of the HBO exposure. This could be explained by a gradual decrease in glycogen detected before the onset of symptoms of hyperbaric oxygen toxicity (30), and by insulin resistance resulting from the oxidative stress (1).

To evaluate the influence of glucose on the appearance of convulsions, we manipulated BGL by injection of glucose to induce a hyperglycemic state and insulin to induce hypoglycemia. It might be assumed that hyperglycemia would shorten the latency to CNS-OT, on the basis of our finding that BGL increased because of higher levels of HBO. However, hypoglycemia failed to affect latency to CNS-OT in the present study. This finding may imply that HBO affects BGL and not the reverse, that BGL affects resistance to CNS-OT. However, Beckman et al. (7) did find that hyperglycemia shortens the latency to CNS-OT, except that the level of BGL obtained following HBO exposure in their study was more than twice as high as it was in the present investigation.

Hypoglycemia shortened the latency to CNS-OT. Hypoglycemia results in the release of adrenaline, which is mediated by the sympathetic nervous system. Adrenaline inhibits the release of insulin by the pancreas and stimulates the release of glucagon, among other actions which serve to maintain BGL in a euglycemic state (35). Seizures are associated with increased sympathetic activity, as demonstrated by measurement of renal sympathetic nerve activity and plasma norepinephrine levels, and have also been associated with an increase in hemodynamic parameters. These changes were diminished when NO inhibitors were administered prior to HBO exposure at 6 ATA (13). Ozkul et al. (25) demonstrated that after acute stroke, insulin-resistant patients had a higher level of NO compared with nonresistant patients. Their findings suggested that oxidative stress is more evident following cerebral ischemia with insulin resistance.

This indicates that the gradual elevation of BGL with increasing levels of HBO, as found in the present study, may be due to changes in sympathetic nervous activity, cerebral blood flow, and insulin resistance. The resultant elevation of NO makes a significant contribution to the development of CNS-OT. We therefore speculate that hypoglycemia and HBO exposure (both seizure-promoting stimuli) have a synergistic effect on the development of CNS-OT via the autonomic nervous system. Further studies will be required to examine this hypothesis.
Previous studies investigated factors other than glucose during continuous elevation of oxygen pressure. Cerebral blood flow (CBF) decreased during HBO exposure at 2 and 3 ATA, whereas at 5 and 6 ATA it increased dramatically as exposure time continued; elevation of CBF is therefore considered a predictor of O₂ convulsions (12). During exposure to 3 ATA, CBF decreased in superoxide dismutase 3 (SOD3) knockout and WT mice but was not changed in overexpressed SOD3 mice, whereas on exposure to 5 ATA CBF immediately increased in overexpressed SOD3 mice (14). Elevation of the oxygen pressure will result in increased production of superoxide. The superoxide molecule combines with NO to generate peroxynitrite, which, against the background of this depletion of NO, causes cerebral vasoconstriction. However, in the later stages of an HBO exposure which can potentially lead to seizures, sympathetic activation brings about the release of NO from neuronal sources (nNOS), thus inducing vasodilatation. This further increases the delivery of oxygen to the brain, providing the final impetus for the development of seizures (13).

A different picture was obtained for hypoglycemia that stems from a chronic situation, rather than the acute administration of insulin. Chronic low glucose may be due to starvation (8) or may occur following a continuous ketogenic diet (37). Starvation or administration of a single dose of R,S-1,3 butanediol acetoacetate diester, which mimics fasting (although it did not lower BGL), delayed CNS-OT seizures (8, 10). Thus these situations gave quite the opposite results. Whereas acute administration of insulin resulted in higher susceptibility to CNS-OT, as demonstrated in the present study and that of Beckman et al. (7), chronic low BGL enhanced resistance to CNS-OT. Bitterman et al. (8) demonstrated that starvation and starvation plus dehydration for 24–36 h prolonged latency to CNS-OT. This was associated with a lower level of blood glucose and an elevated level of ketone bodies compared with control rats. Starvation, which lowers the BGL, or therapeutic ketosis, provides the brain with an alternative source of energy that comes from ketones (9). The metabolic alterations associated with fasting that reduced BGL improve mitochondrial function, decrease ROS production, and reduce inflammation (19). It is well known that elevation of ROS is part of the mechanism that leads to the generation of CNS-OT (11, 17, 24, 32). We suggest that in acute hypoglycemia, there is not sufficient time to produce metabolites other than glucose that might enhance resistance to CNS-OT.

In the present study, exposure to a protocol employed in clinical HBO therapy (2.5 ATA, 90 min) did not affect BGL in the rat, neither after each individual exposure nor at the conclusion of the series of seven daily exposures. This was also demonstrated in previous studies using a rat model and exposure over a period of 7 days (20–22). Data in the literature regarding the effect of exposure to HBO on BGL in humans are contradictory. Human patients showed a reduction or no change in BGL following HBO treatment. This was found in both diabetics and nondiabetics (2, 27, 36). The reduction in BGL reported in certain studies is probably due to food deprivation during HBO treatment, as claimed in the study by Peleg et al. (27). It must be emphasized that there is a clear difference between the rat model and human subjects with regard to CNS-OT, the rat being much more resistant. HBO produces convulsions at a much lower pressure in humans than it does in rats. CNS-OT will occur in humans at pressures above 2 ATA (15, 16, 18, 39), but only above 4 ATA in the rat (5, 28). For this reason, careful consideration should be given to using an animal model in studies where the same conditions will have vastly different results in the animal and the human subject.

In conclusion, our results demonstrated an association between BGL and elevated levels of oxygen in HBO starting at 4 ATA. This elevation of BGL following HBO exposure may be due to glycogen depletion and insulin resistance mediated by the sympathetic nervous system. The correlation between elevated levels of HBO and BGL may imply that BGL could serve as a predictive marker for the generation of CNS-OT.

ACKNOWLEDGMENTS

The authors thank Mr. R. Lincoln for skillful editing. The opinions and assertions contained herein are the private ones of the authors, and are not to be construed as official or as reflecting the views of the Israel Naval Medical Institute.

GRANTS

Support for this study was provided by a research grant from the IDF Medical Corps and Israeli MOD.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).
AUTHOR CONTRIBUTIONS

M.E. and Y.A. conception and design of research; M.E., M.M., N.K., and A.B. performed experiments; M.E., M.M., N.K., and A.B. analyzed data; M.E., A.B., and Y.A. interpreted results of experiments; M.E. prepared figures; M.E. drafted manuscript; M.E. and Y.A. approved final version of manuscript; A.B. and Y.A. edited and revised manuscript.

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