# Effects of exposure to mild hyperbaric oxygen on metabolism-related diseases in animal models

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## Summary

Hyperbaric oxygen therapy is an established medical treatment and is usually conducted under conditions of 2026 to 3039 hPa with 100% oxygen in a pressurized chamber, i.e., a device used for medical treatments which is effective against a variety of medical conditions related to arterial gas embolism, acute carbon monoxide poisoning, decompression sickness suffered by divers, routine wound care, and thermal burns. Hyperbaric oxygen has been shown to increase the number of invasive inflammatory cells and cause excessive production of reactive oxygen species in several tissues and organs. In addition, middle-ear barotrauma, sinus pain, and painful joints, which are often induced by high pressure. These reactions do not occur under mild hyperbaric oxygen conditions at 1266 to 1317 hPa and 35-40% oxygen because of the relatively low pressure and oxygen concentration in the chamber. Exposure to mild hyperbaric oxygen enhances peripheral blood flow and dissolved oxygen in blood plasma, and facilitates oxidative metabolism in cells and tissues. Metabolism-related diseases, such as metabolic syndrome, type 2 diabetes, and hypertension, exhibit a decreased oxidative metabolism in cells and tissues. Previous studies observed that exposure to mild hyperbaric oxygen increases a peripheral blood flow and dissolved

oxygen in blood plasma, thereby inhibited and/or improved type 2 diabetes, diabetes-induced cataract, and hypertension. Enhancement of oxidative capacity in cells and tissues might be linked to inhibit and/or improve these metabolism-related diseases. Therefore, this study examined the effects of exposure to mild hyperbaric oxygen on metabolism-related diseases and skeletal muscle atrophy.

In the first study, the effects of exposure to mild hyperbaric oxygen on the properties of the soleus muscle in rats with metabolic syndrome were examined. Metabolic syndrome is linked to physical inactivity and consumption of a high-fat and high-calorie diet and is characterized by obesity, high blood pressure, increased blood glucose levels, and hyperlipidemia. Five-week-old SHR/NDmcr-cp (*cp*/*cp*) rats were used as an animal model for metabolic syndrome in this study. Metabolic syndrome rats exposed to mild hyperbaric oxygen had a lower percentage of low-oxidative type I fibers and observed high-oxidative type IIA fibers, that were not find in metabolic syndrome rats. In addition, those rats had higher succinate dehydrogenase (SDH) staining intensity of type I and type IIC fibers in the muscle compared with the rats without exposure to mild hyperbaric oxygen. Exposure to mild hyperbaric oxygen inhibited a growth-related increase in fasting and non-fasting blood glucose, glycated hemoglobin, total cholesterol, triglyceride, insulin, and systolic blood pressure levels, and enhanced adiponectin levels, oxidative capacity (muscle SDH activity and fiber SDH staining intensity), and mRNA levels of peroxisome proliferator-activated receptor γ coactivator-1α (*Pgc-1α*) in the muscle. The increased blood flow and availability of oxygen, especially dissolved oxygen in blood plasma, which were induced by exposure to mild hyperbaric oxygen, facilitated oxidative metabolism in skeletal muscle, which is a major target of insulin-stimulated glucose uptake. High oxidative metabolism in

skeletal muscle linked to improve the impaired glucose tolerance and insulin sensitivity induced by metabolic syndrome. Exposure to mild hyperbaric oxygen inhibited a growth-related increase in blood glucose levels and decrease in muscle oxidative capacity of rats with metabolic syndrome.

In the second study, the effects of exposure to mild hyperbaric oxygen on the oxidative capacity of spinal motoneurons innervating the soleus muscle in rats with type 2 diabetes were examined. It is well known that non-obese and obese rats with type 2 diabetes exhibit decreased oxidative capacity, such as reduced oxidative enzyme activity, fiber low-intensity staining for oxidative enzymes in skeletal muscle, especially in the soleus muscle. In contrast, there are no data available concerning the oxidative capacity of spinal motoneurons innervating skeletal muscle of rats with type 2 diabetes. In this study, spinal motoneurons innervating the soleus muscle were identified using nuclear yellow, a retrograde fluorescent neuronal tracer. Eight-week-old Goto-Kakizaki (GK) rats with type 2 diabetes had a decreased oxidative capacity in the motoneurons innervating the soleus muscle. Decreased oxidative capacity of the motoneurons innervating the soleus muscle of type 2 diabetes was improved by exposure to mild hyperbaric oxygen. The increased peripheral blood flow and dissolved oxygen induced by exposure to mild hyperbaric oxygen had a beneficial impact on the type shift of fibers in skeletal muscle of GK rats. The improved oxidative capacity in motoneurons corresponded well with those observed in fibers in the soleus muscle that the motoneurons innervate, indicating that the properties and responses of motoneurons and their innervating fibers are related closely even in diabetic rats. Rats with type 2 diabetes have decreased oxidative capacity in motoneurons innervating the soleus

muscle and the decreased oxidative capacity is improved by exposure to mild hyperbaric oxygen.

In the third study, the effects of exposure to mild hyperbaric oxygen on diarrhea and colonic inflammation were examined. Morphological and pathophysiological features, e.g., mucosal damage, weakened epithelial barrier function, and ulceration were observed in human and animals in inflammatory bowel disease. Dextran sulfate sodium (DSS) is the most common agent to develop animal models for inflammatory bowel disease. In this study, five-week-old Kyoto Apc Delta (KAD) rats were given 2% DSS in drinking water for 7 days, and used as a colonic inflammation model. In the colonic inflammation model used in this study, 67% of rats exhibited bloody diarrhea immediately after DSS treatment for 1 week. Furthermore, mucosal damage was observed after the DSS treatment. Consistent with histological results, Interleukin 1β (*Il1β*) mRNA levels were higher in the inflamed distal colon. Exposure to mild hyperbaric oxygen at 1317 hPa with 40% oxygen for 3 h daily for 2 weeks improved diarrhea, but did not improve colonic inflammation. Colonic inflammation in rodent is linked to a reduction of mitochondrial biogenesis in the intestinal epithelium. Exposure to mild hyperbaric oxygen for 2 weeks might be too short to enhance the oxidative metabolism of colonic mucosa, and to improve colonic inflammation.

In the last study, whether exposure to mild hyperbaric oxygen prior to and/or after hindlimb suspension-induced unloading has pre- and/or post-conditioning effects on the recovery of the atrophy and decreased oxidative capacity in skeletal muscle associated with hindlimb unloading was examined. The hindlimb suspension method in this study allows for free movement of the forelimbs but prevents the hindlimbs from contacting the sides or the floor of the cage. This method has been successful and none

of the animals slipped out of the rigging and underwent weight bearing. Muscle weights, cross-sectional areas of all fiber types, and oxidative capacity decreased after hindlimb unloading. In addition, a type shift of fibers from type I to type IIA and type IIC was observed after hindlimb unloading. mRNA levels of *Pgc-1α* decreased, whereas those of forkhead box-containing protein O1 (*FoxO1*) increased after unloading. Muscle atrophy and decreased oxidative capacity were unaffected by either pre- or post-conditioning with mild hyperbaric oxygen at 1266 hPa with 36% oxygen. In contrast, combination of pre- and post-conditioning with exposure to mild hyperbaric oxygen can be effective against the atrophy and decreased oxidative capacity of skeletal muscle associated with unloading. Since neither preconditioning nor post-conditioning alone were effective countermeasures, it is likely that pre- and post-conditioning with exposure to mild hyperbaric oxygen triggered different signaling pathways and that only a combination of these treatments activated the signaling cascades required for the recovery of the soleus muscle mass and oxidative capacity.

In summary, this study provides that exposure to mild hyperbaric oxygen at 1266-1317 hPa with 35-40% oxygen is a valuable countermeasure for preventing and improving metabolism-related diseases including metabolic syndrome, type 2 diabetes, and colonic inflammation. It is well known that mitochondrial biogenesis decreases in metabolism-related diseases with growth. Exposure to mild hyperbaric oxygen enhances peripheral blood flow and dissolved oxygen in blood plasma, and facilitates oxidative metabolism in cells and tissues.