The Reproducibility Issue within Hypoxia Chambers and a Simple Solution to Fix it

by Justin Croft

Introduction

In the last two decades, researchers have become aware that across most scientific fields there have been countless examples of high-profile studies exhibiting major reproducibility issues. As this continues to be at the front of investigators' minds, it suggests researchers themselves are better able to look at their own experimental setups and find areas where they may run into one or more of these reproducibility issues. One area in cell research that has been growing steadily and providing a reproducible and physiologically relevant micro-environment has been hypoxia/physoxia workstations and incubators. These systems modulate oxygen conditions and allow cells to grow in environments that more closely imitate the *in vivo* condition.

Given that more research is using physoxic environments to help curb the reproducibility crisis, then what is the issue here? Well, it turns out that there is a fundamental shortcoming in the majority of hypoxic/physoxic workstation in use today, which is that systems are unable to fully replicate results from day-to-day, season-to-season, or location-to-location. The simple issue being that these systems employ a relative measurement of environmental oxygen (percent), limiting the ability to accurately compare results over time or across locations. A simple switch to reading oxygen levels in absolute units of partial pressure, such as mmHg or kPa, would resolve this limitation.

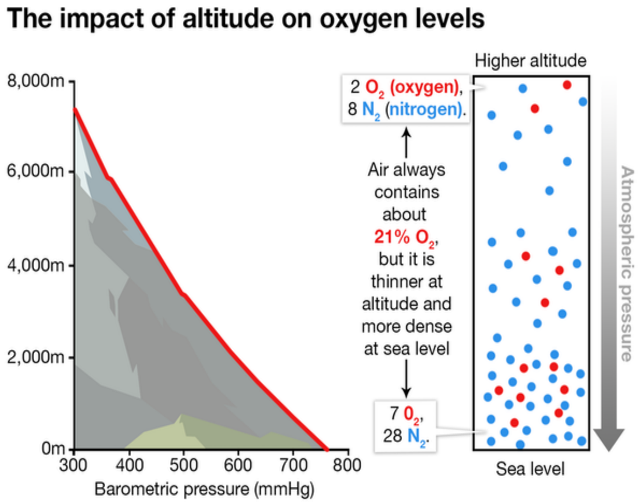
The questions you should consider as you read this are: Why are relative measurements (i.e. percentage) being used to measure and control oxygen levels and would an absolute measurement, like mmHg or kPa, be more appropriate? What are the inherent issues with using percentages in tri-gas and hypoxia workstation? And finally, will a shift occur from relative to absolute measurements and who is leading the charge on this?

A Hypoxic History

So how did percent O_2 become the standard measurement unit within hypoxia workstations? When looking at the first commercially available tri-gas incubators, by Fisher Scientific in 1979, these systems used percent O_2 for their measurement. Why Fisher chose to implement percent oxygen as the standard for this first incubator is unknown. One simple reason for this could be because nearly 40 years ago it would have been far more expensive and difficult to implement the accessories necessary for taking absolute measurements (in mmHg or kPa). Developing a technology platform using the relative percentage metric back in 1979, likely made the hypoxia incubator accessible to more researchers. This was a net positive, of course, as it allowed more scientists to access these systems and helped to grow the physoxic movement.

It may be surprising but very little has truly changed since the late 1970's. While absolute measurement technology is readily available today, nearly every hypoxia workstation and tri-gas incubator still use percent to measure and modulate oxygen levels. This only seems to be the case because "this is the way it has always been done" and is widely accepted in the literature. A paper by McKeown (2013) makes this abundantly clear when they declare how absolute measurements are more precise and relevant, but researchers should use percent, as historically it's more "physiologically meaningful than mmHg". While there may not be much of a case to disagree with the historical precedent of this, the article then downplays any major differences in absolute and relative measurements by comparing percent O_2 to an average absolute pressure. This is highly problematic, however, as percentage does not and cannot, account for variances in atmospheric pressure, to which there are many.

pO_2 vs. percent oxygen



Roughly one fifth of earths atmosphere consists of oxygen. As the density of the atmosphere decreases with reduced pressure, or inversely increases with increased pressure, so does the availability of oxygen (however, it always remains a fixed proportion). Controlling internal chamber oxygen using percentage does not account for these barometric pressure changes, which can vary by as much as +/-5% in a day in any given geographic location. For example, if a researcher sets a chamber to 8% oxygen and atmospheric pressure drops by 5%, then they are, unwittingly, exposing cells to 5% less oxygen. On the other hand, if a researcher were able to set a chamber to 38 mmHg O_2 and atmospheric pressure drops by 5%, the oxygen density in the internal chamber atmosphere would remain constant. This should be a major concern as most researchers perform studies over days or even weeks where weather and seasonal changes are almost guaranteed. It would seem to be imperative that investigators should recreate the same O_2 conditions regardless of when they use a system or at least have the realization that the conditions in the chamber will have variation from one day to the next, which could be substantial, when using percentage measurements.

Similarly, percentage does not correct for altitude. While this is far less of an issue it is still arguably relevant for science being carried out in locations such as Denver, Colorado, that has an average O_2 pressure of 633 mmHg compared to the 760 mmHg at sea level. This is a 16.7% difference, that is unaccounted for using the relative percent measurement. It may therefore become a challenge to accurately replicate experiments from one lab to another. This is not a new problem and will continue to be a concern that all hypoxia researchers need to be aware of if percent measurements do remain the norm. Some of the most long-standing researchers in the field do seem to understand this problem and are doing their best to convert their percentage results into pO_2 using local barometric pressures, to make their results more reproducible, but this is not standard practice yet.

This leads to a further point, which is when a researcher needs to replicate the results of another group; percentage measurements lead to more questions whereas absolute units provide a definitive place to start. When using absolute units there is no guessing if altitude or swings in daily barometric pressures influenced results. In studies where the difference between growth or differentiation is miniscule, investigators should insist on absolute units of oxygen being the standard. At a time when pressure is mounting to ensure studies can be replicated, and journals are becoming ever more stringent, absolute units offer a safeguard to ensuring researchers are accounting for a key factor that could influence their results.

Finally, there seems to be a discrepancy in the current literature where studies cite *in vivo* and *in vitro* studies in completely different units. Tissue oxygen studies almost unanimously express this metric in partial pressures, yet researchers doing cellular studies, in hypoxic/physoxic workstations, compare their cells to these specific tissues using the non-absolute function, percentage. This creates a seemingly avoidable disconnect, considering there is no solid scientific reason why tissue oxygen and cell oxygen should be expressed using fundamentally different units. For a standard, apples-to-apples, comparison an absolute measurement in cellular research would bring *in vivo* and *in vitro* research onto the same plane.

The way forward

With all this in mind, why are hypoxia chamber manufacturers not making the switch to partial pressure control, or at a minimum, making this an option? The technology to do so, is more widely available and easier to implement than ever before. Perhaps, it is because the percentage measurement is still widely accepted, and researchers have become content. Regardless, like all scientific advancements, once it is fully understood there is a better and more reproducible way to conduct cellular research, partial pressure measurements will almost certainly become the norm. If researchers can avoid years of research being scrutinized as results cannot be replicated from one lab to another this switch will be more than worth the small effort to change a long-standing habit.

It is also becoming clear, as major scientific journals find these areas of common reproducibility issues, more demanding controls will be placed upon submitted manuscripts to ensure the research can be replicated. As such, being able to control the exact amount of oxygen that cells 'see' regardless of any other factor should become a primary focus for research moving forward. If the central purpose of physoxic systems is to modulate O_2 levels tightly then using an absolute measure that can compensate for pressure (density) change by adapting the partial pressure of oxygen should be freely adopted. Of note, one of the most in-depth reviews on physiological normoxia by Keeley and Mann (2018) seems to fully grasp this issue when they state "Ideally, [O2] should be defined in units of partial pressure (mmHg or kPa) in compliance with Henry's Law" and even make the effort throughout this lengthy review standardise all cited research into kPa.

While nearly every hypoxia chamber and tri-gas incubator on the market today still uses percent to measure and control oxygen content, the HypoxyLab by Oxford Optronix, UK stands out as the first system to control its internal environment using absolute units of oxygen. The HypoxyLab is a fully loaded, compact, standalone hypoxia/physoxia workstation and incubator that uses partial pressure to measure and control its oxygen environment. With the science of absolute oxygen measurement behind it, all researchers needing precise oxygen control should take note of this system.

McKeown SR. *Defining Normoxia, Physoxia and Hypoxia in Tumours—Implications for Treatment Response*. Br J Radiol 2014; 87: 20130676

Keeley TP & Mann GE. *Defining Physiological Normoxia for Improved Translation of Cell Physiology to Animal Models and Humans.* Physiological Reviews 2018; Vol 99:1