DISCOVERY BIOLOGY

Griffith UNIVERSITY

In vitro oxygen concentration alters *PfK*13 mutant *Plasmodium falciparum* sensitivity to DHA

Sandra Duffy & Vicky. M. Avery
Discovery Biology, Griffith Institute for Drug Discovery, Griffith University, Nathan, Qld 4111, Australia

CONCLUSION

DHA resistant K13 mutant *Plasmodium falciparum* acquires enhanced susceptibility to DHA under hyperoxic *in vitro* culturing conditions. This phenotype is due to adaptation of the parasite during a single development and proliferation cycle from early ring stage to newly invaded RBC.

INTRODUCTION

- *Pf*13 is a biological marker of artemisinin resistance associated with ACT clinical treatment failure
- *In vitro*, the *Pf*K13 mutant F32-ART5 was generated under 21% O_2 conditions
- Other attempts at *in vitro* generation of *Pf*K13 mutations by escalating drug dosing in 5% O_2 protocols have not identified K13 mutations.
- Raising the question "does oxygen influence *Pf* susceptibility to artemisinins before during and after drug exposure?" **Figure 1**

METHODS

- PFK13 containing *Pf* (MRA-1241) was cultured in 5% CO_2 and either 5% or 21% O_2.
- Cultures incubated in alternative conditions at different asexual stages of growth were then evaluated for parasite survival using the ring stage survival assay (RSA).

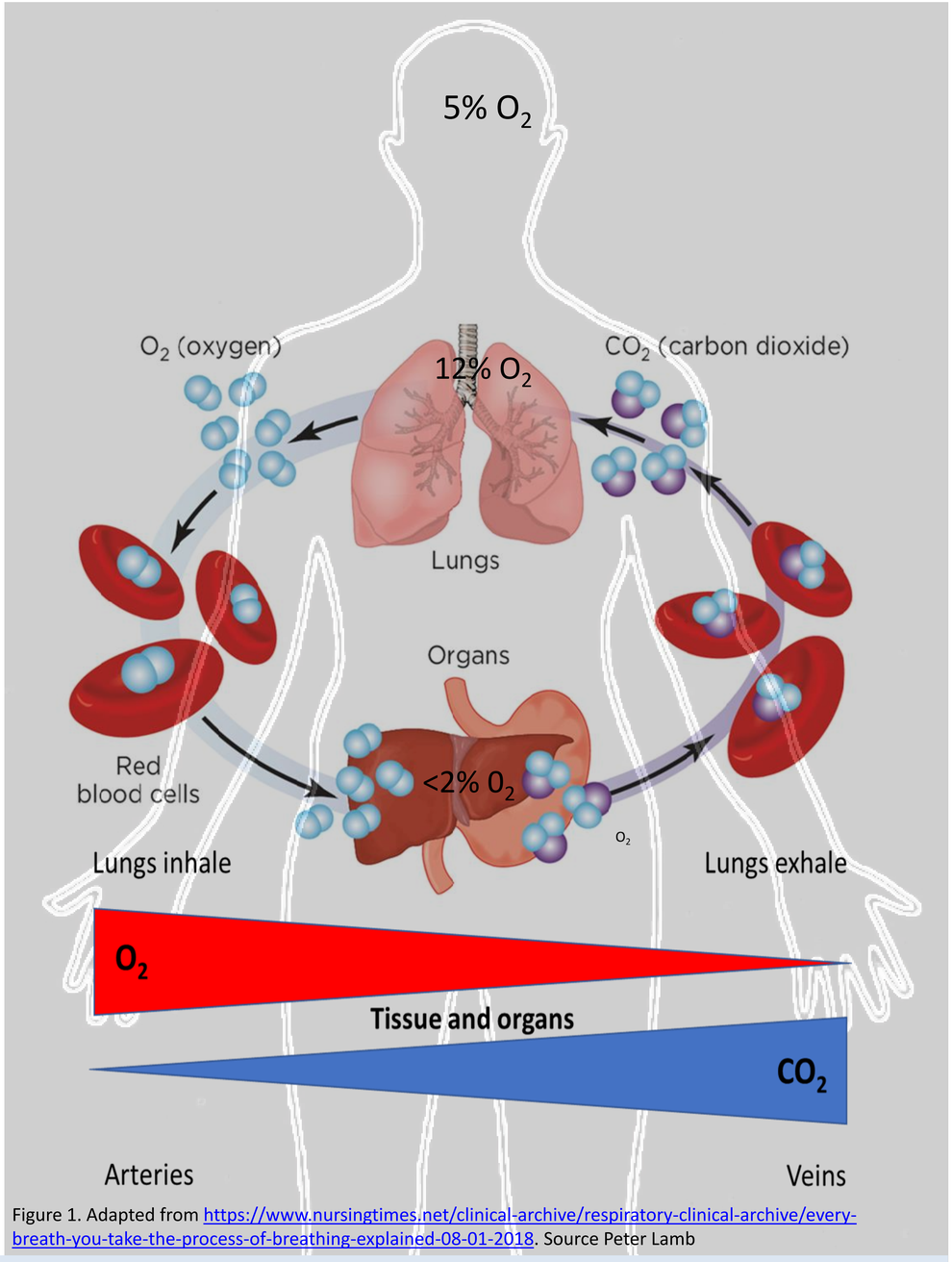


Figure 1. Adapted from https://www.nursingtimes.net/clinical-archive/respiratory-clinical-archive/every-breath-you-take-the-process-of-breathing-explained-08-01-2018. Source Peter Lamb

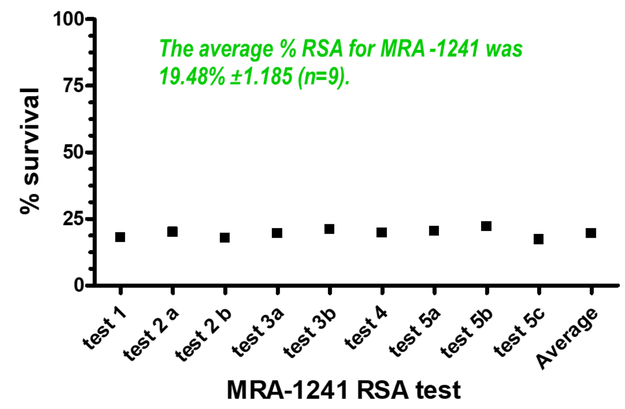
Figure 1. Illustrates the human respiratory system and variances in both O_2 and CO_2 throughout the human body. When blood passes through the lungs from the veins, CO_2 is expelled through an exhaled breath and O_2 inhaled.

RBCs transport oxygen from the lung capillaries to all areas of the body exchanging O_2 for energy production, with CO_2 as metabolic waste. The levels of O_2 and CO_2, unlike a standard laboratory incubator, are highly variable in both concentration and ratio to one another. The intra-erythrocytic form of the *Pf* parasite therefore encounters a vast array of gaseous conditions throughout an infection.

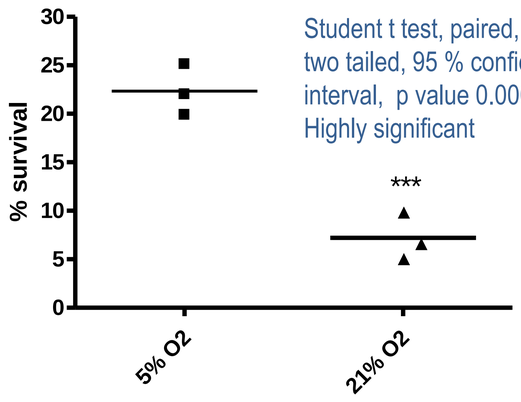
ACKNOWLEDGMENTS
ASTMH travel award. NHMRC Postgraduate Scholarships - Sandra Duffy - APP1150359, supported in part by a Griffith University Postgraduate Scholarship and a Discovery Biology Post Graduate Scholarship. Australian Red Cross Blood services for the provision of blood.

RESULTS

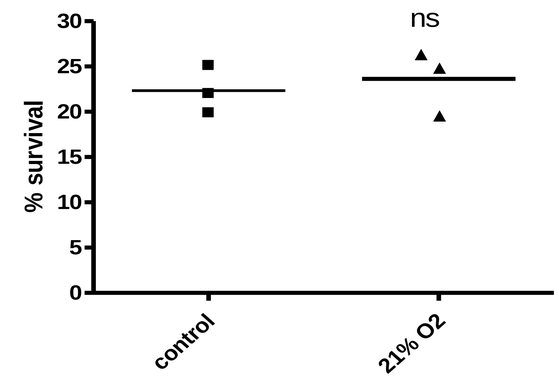
1) Reproducibility of RSA in standard conditions



2) Standard 5% O_2 vs high O_2 incubation for long term culture of MRA-1241 effect on RSA.



3) Acute change from 5% O_2 to 21% O_2 for RSA



Parasite susceptibility at high O_2 conditions is **not** a feature of DHA having greater activity in higher O_2 conditions.

4) Time in high O_2 conditions for enhanced parasite susceptibility to DHA