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In pursuit of the Unicorn.

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This short review was prompted by The Physiological Society's recent online symposium on variability (https://www.physoc.org/events/variability-how-to-deal-with-it-interpret-it-and-learn-from-it/). It does not deal with a specific methodology, but rather with the myth that certain environmentally-induced clinical conditions be identified, quantified, simplified and monitored with a single methodology. Whilst this may be possible with some clinical conditions, others resist the prevailing reductionist approach of minimising rather than exploring variation in pathogenesis and pathology, and will not be fully understood until the variation in cause and effect are embraced. This is likely to require comprehensive methodologies and collaboration.

When exposed to extreme environments, the human response lies on a continuum from the physiological to pathophysiological. At the extreme end lie conditions like drowning, hypothermia, heat illness, cold injury and high altitude related illness (acute mountain sickness, high altitude cerebral or pulmonary oedema). For some of these, such as drowning and hypothermia, the pathogenesis, pathophysiology and therefore treatment are relatively well understood. For many more, however, this is not the position, and there are lessons to be learnt from asking why this is the case.

Take the enigma that is non-freezing cold injury (NFCI). Despite its prevalence, impact and long history (Golden et al, 2013) little is known about its pathogenesis and pathology. We have little idea of the dose of cold or cold/wet condition that might result in the injury or of what causes that injury, nor why one person may be gravely affected and another not when exposed to a similar challenge. Whilst some of the factors that appear to predispose to NFCI have been identified (Burgess and MacFarlane 2009; Golden et al, 2013; Kuht et al, 2019),

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none apply in all conditions, and even their combined use has limited sensitivity in predicting individual risk.

Attempts to elucidate the pathophysiology of NFCI have focussed on two separate elements: neural and vascular. This is largely to do with the expertise of the groups involved rather than the NFCI itself, which could very well have a neurovascular basis: nerve injury affecting vascular function; vascular injury causing nerve damage; both occurring independently; or purely neural in some cases and vascular in others. Individual variation might exist in the susceptibility to either or both elements. Most human phenotypes result from the interaction of genetic elements with the environmental challenge, and such variation might thus result from innate genetic variation, past environmental exposure (with possible past injury or epigenetic impacts), differences in 'intrinsic' environmental milieu (sex hormones, stress responses, hydration status, age), and the duration and anatomical nature of exposure. Some variation may be accounted for by unmeasured factors ("latent" variables), measurement error or unreliability, all limits of our methodological capabilities. Some may result from subtly different conditions between days. Repeated experimentation in the same individuals can help elucidate such factors but, in general, inter-individual differences in experimental responses remain, indicating that a mixed and varying pathology may be compounded by variation between and within individuals in the susceptibility to the different mechanisms of injury. For NFCI, this may relate to factors such as age, fitness and underlying genetic inheritance (including sex and race).

One way to explore the variation in the pathophysiological responses to extreme environments is to apply a standardised environmental challenge to animals in whom genetic variation is minimised: the study of purebred animals housed in standardised conditions. Figure 1 shows previously unpublished data relating to the vascular response of the tail of Wistar rats exposed to a standard cold challenge before and after a longer, colder exposure of just the tail. Such studies have limitations: rats are not clones, and thus genetic variation does exist amongst them. Thus, even before prolonged cold exposure the variation in the time to vasodilate following a brief cold exposure is large, even in littermates. This time increased post-prolonged cold exposure for five of the six animals, and the variation remained.

INSERT FIG 1 HERE

Similar previously unpublished data for humans with no cold injury or previous unusual exposure to cold are presented in Figure 2. Again, the variation in the response to a highly controlled, standardised brief cold exposure is remarkable.

INSERT FIG 2 HERE

In both examples (Figs 1 & 2), those with the most intense vasoconstriction on exposure to the cold, and those slowest to rewarm are the individuals who will receive the largest "dose" of cold making them, from this perspective, more likely to acquire a NFCI.

The picture is similar for heat illness (HI), which is often described as the progression along a continuum from heat cramps to heat stroke, but might better be thought of as a number of different conditions that often occur in the presence of heat. Although the pathology of some of the conditions that constitute HI are better understood than NFCI, they can still vary in terms of the organs affected in different individuals, and the sensitivity of different individual to the same conditions and responses. As with NFCI, lists of factors that predispose to HI have been produced (Westwood et al, 2020) but none of these apply in all conditions.

Figure 3 outlines the complex physiological responses to heat exposure. The putative mechanisms responsible for impaired aerobic performance in the heat and heat exhaustion (a form of HI) remain a matter for debate. This is most likely the case because individuals fail for different reasons and combinations of reasons: there are 120 ways of combining the five regulated variables in Figure 3 (bottom row). Measuring just one of these variables is unlikely to provide sufficient information to predict or inform heat exhaustion or its causation. Even if all five variables were measured, due to individual variability in susceptibility to conditions such a dehydration or raised body temperature, there is unlikely to be a fixed profile for these variables that predicts HI across individuals. Furthermore, it would not be unreasonable, following an integrative, holistic theme, to add links to other systems influencing function such as acid-base balance, thereby increasing complexity and potential variability further.

Thus, a mixed and varying pathology is compounded by variation between and within individuals in the susceptibility to the different mechanisms of injury. As for NFCI, this may relate to factors such as age, fitness and underlying genetic inheritance (including sex), the heat and exertional challenge faced, and factors such as clothing worn.

All of this means that the methodologies employed to investigate conditions like NFCI and HI must consider and seek to identify all sources of variation; this is likely to require a greater number of more comprehensive measurements and assessments, as well as statistics that look at different clusters of relevant factors across individuals (e.g. the Dominance-based rough set approach, Chakhar et al. 2015).

INSERT FIG 3 HERE

The concept of clinical conditions being variants of a larger syndrome, or an end- state that can be reached by differing routes, is increasingly understood to apply to other 'more conventional' disease states. Whilst Amyotrophic Lateral Sclerosis appears to be one readily identified form of Motor Neurone Disease, it is now understood to be 'one end phenotype' which can be reached through multiple paths: 70 % are familial, 5-10 % directly inherited from parents, and 15 % sporadic. Twenty-five different genes, in varying combination, are now known to be associated with its development, and many others may modulate its course (Mathis et al. 2019).

Whilst 'variation in the causes of variation' may seem to impede progress in understanding the fundamental (patho) physiological drivers of the response to extreme environments, the detailed study of such variation may actually offer some advantages. This has been demonstrated by the use of controlled gene-environment interaction studies. Thus, the left ventricular growth response in humans is hard to explore, but standardising the hypertrophic stimulus (identical exercise training) in identical conditions (military training facilities) in those of single sex and race means that the role of variation in specific systems (and the genetic elements which influence them) is enriched. In this way, the systems which control human cardiac growth (Brull et al, 2001), and also skeletal muscle performance (Montgomery et al, 1998; Williams et al, 2000), can be identified. A similar approach has been used to the study of human hypoxic adaptive responses (Horscroft et al, 2017).

However, barriers to such methodological approaches exist. Investment in 'physiology' as a discipline has been lacking in recent decades. Physiological expertise is often compartmentalised (e.g. the study of 'vascular' or 'neural' responses in NFCI noted above). Interdisciplinary collaboration with groups experienced in dealing with latent variables (e.g. psychology and social sciences, Bollen, 2002) or other relevant skills (e.g. computational biologists, geneticists and more) has been limited, and funding for such studies is hard to obtain. How do we enable these methodological approaches? Firstly, we need to expand training in physiology. Without the relevant expertise across broad disciplines such studies cannot be done. Secondly, we need to break down silos and facilitate collaboration; breakthroughs will come when precise data relating to inter-individual variation in responses are married to physiological mechanistic, genetic, proteomic and metabolomic data, often analysed using cutting edge computational biological approaches. Thirdly, new funding models that enable this collaboration need to be created.

Conclusion

Dr Ian Hampton, a human physiologist and friend from Leeds University, was once asked how he managed "all those variables in whole body integrative physiology". His retort was, "at least I don't choose to ignore them". In science, we often constrain experimental design to actively reduce variation and thereby increase statistical power: we try to increase internal validity and reduce between participant variance. However, this reduces

the generalizability of the research. Rather, we would argue that the time has come to embrace variation in experimentation, to seek latent variables of influence and to utilise appropriate statistical methods to deal with these experimental approaches. A thorough understanding of some pathophysiological conditions may demand such an approach.

Conditions like NFCI and heat illness are more "syndrome" than "disease". They can be acquired through a variety of routes and, whilst the presentation may appear similar, the underlying mechanistic aetiology of these conditions may be subtly (or substantially) different. As with other medical conditions, a variety of responses to a battery of tests, together with an assessment of each patient's specific sensitivity to the aspect each test assesses, will be needed. The only way to report the combined outcome of such tests will be as a range, group or index constructed from a composite score. Broad, rather constrained, phenotyping is necessary, cross-disciplinary collaboration essential, and routes to funding likewise.

Thus, the idea that each of the clinical conditions resulting from exposure to extreme environments can be reduced to one simple, small and discrete set of causative conditions and a single pathology that can be assessed by a single test is a myth: one might as well be pursuing a unicorn.

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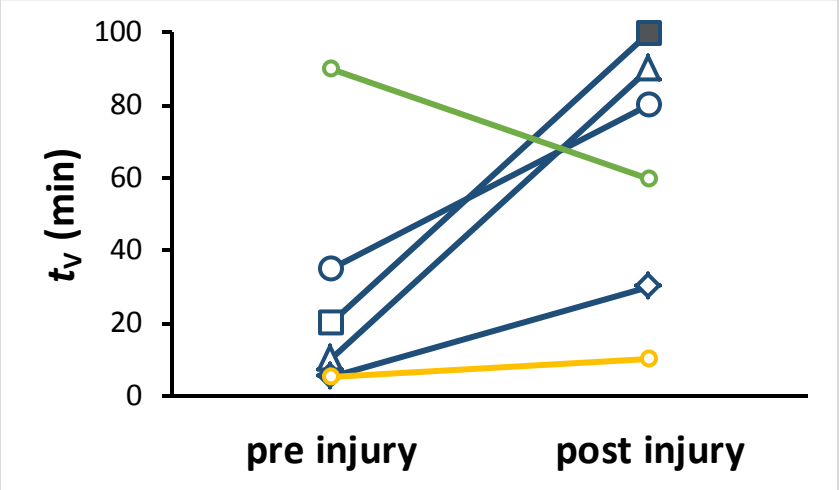

Figure 1. Individual time to vasodilation in the rat tail following a brief cold exposure (tail immersed in 15°C water for 2 minutes) before and after a prolonged cold exposure (tail immersed in 1°C water for 3 hours). Blue lines = littermates, solid symbol indicates vasodilation did not occur within maximum duration of test (100 min).

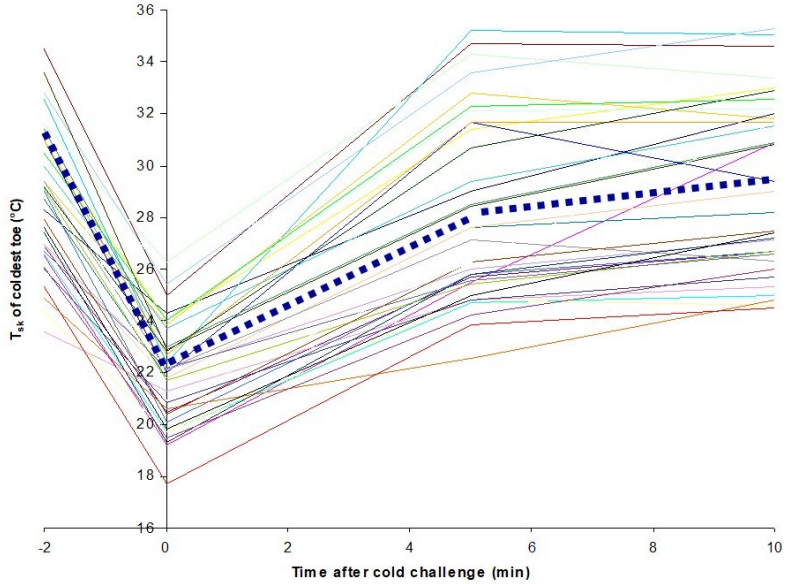

Figure 2. Skin temperature responses of "Control" participants to a cold sensitivity test involving preconditioning for 30 minutes in a room at 30 °C and then immersion of a foot (placed in a plastic bag to keep it dry) in 15 °C water for 2 minutes followed by spontaneous rewarming in 30 °C air. Each line represents the temperature profile of the coldest toe from each participant (n=35) with skin temperature measured prior to immersion (-2 min), immediately following immersion (0 min) and 5 and 10 minutes of rewarming using a thermal imaging camera. The dotted line represents the data obtained from an unperfused finger model (sausage) following the same protocol (Drawn as a line graph for clarity).

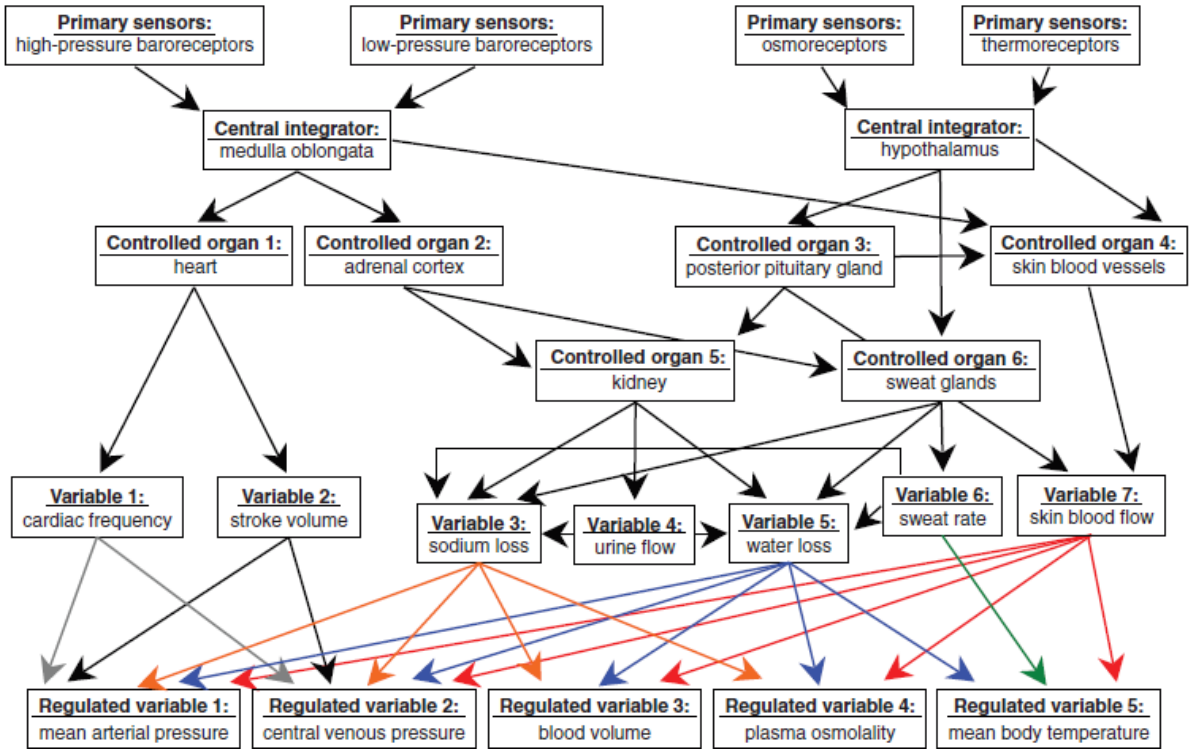

Figure 3. The homeostatic mechanisms and physiological responses accompanying hot thermal challenges. Five critical variables (bottom row) must be kept within ranges conducive to optimal physiological function while simultaneously avoiding states that are either hazardous or life threatening (from Taylor 2014 with permission).