

## Ambient temperature: An important but neglected parameter in metabolic measurement systems

By John Lighton, Ph.D., president of Sable Systems International and author of “Measuring Metabolic Rates: A Manual for Scientists”, published in 2008 by Oxford University Press.

**E**specially if you’re using a “black box” metabolic measurement or metabolic screening system, your metabolic measurements may be fatally compromised if your study organisms – such as mice – are measured at a temperature outside their **thermal neutral zone** or TNZ.

**The vast majority of metabolic measurements of model organisms such as mice are misleading.**

If you’re unfamiliar with the thermal neutral zone, you’re not alone. Most researchers in the biomedical field are unaware of it, or may recall it from a course taken long ago but consider it irrelevant to their research. Nothing could be further from the truth.

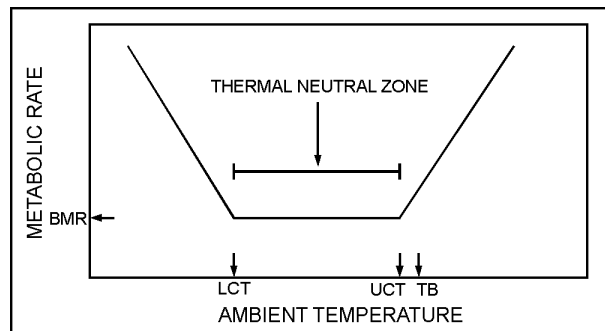
**TNZ basics.** The thermal neutral zone is easy to understand. Mammals and birds are *homeothermic endotherms*, meaning that they maintain a set, high body temperature (which we’ll call  $T_b$ ), usually in the range of 37-39°C. Because their biochemical pathways are evolutionarily optimized to their normal  $T_b$ , they will defend that temperature at all costs<sup>1</sup>.

If the ambient temperature (which we’ll call  $T_a$ ) is well below  $T_b$ , the animal must produce heat in order to defend its  $T_b$ . In some mammals such as mice this is achieved by activating mitochondria in a specialized tissue called brown adipose tissue or BAT. Alternatively, other mechanisms of heat generation can be used, such as shivering. Collectively, the various mechanisms for generating the heat required to defend  $T_b$  are referred to as thermogenesis, and they have one thing in common: *They increase metabolic rate (MR).*

<sup>1</sup> As comparative biologists know, some small animals can drop  $T_b$  to low levels for variable periods of time. However the common model animals of biomedical research, the mouse and rat, maintain and defend a relatively fixed  $T_b$ .

Conversely, if  $T_a$  is at or above  $T_b$ , then active processes such as sweating are recruited to defend  $T_b$ . These also increase MR.

At this point the alert reader, realizing that both low *and* high  $T_a$ s elevate MR in experimental animals such as mice and rats, is wondering whether a  $T_a$  exists that yields the lowest MR. In fact there is a *range* of  $T_a$ s that yield the lowest MR, and this range is called the thermoneutral zone or TNZ. Here’s a graph to clarify the concept.



As you can see, the TNZ extends from the lower critical temperature LCT to the upper critical temperature UCT, which is typically a few degrees below  $T_b$ . Between LCT and UCT, the animal changes the rate of heat transfer between it and its surroundings to maintain a constant  $T_b$  without incurring any additional metabolic cost. For example, it may change its posture, the depth of its fur layer, or its peripheral vasomotor tone to increase or decrease heat transfer without affecting its MR. But it can only go so far, and when  $T_a$  falls outside its thermoneutral zone, the animal’s ability to modulate heat transfer using cheap and easy methods has come to an end, and its MR must increase.

**MR Gold standard.** Plainly, the best measure of an animal’s MR is obtained within its TNZ. This “gold standard” MR is the basal MR or BMR, with is the MR measured within the TNZ of an animal that is neither active nor digesting food. When comparing the MRs of animals, such as different experimental groups of mice, the BMR is the best measure to use.

But, the TNZ of a typical mouse is between 30 and 34°C, though this varies with the experimental setup - in some cases the LCT may be as low as 28°C. These temperatures may feel uncomfortably warm to a clothed human. This explains why most biomedical researchers are unaware that a mouse exposed to a “comfortable” room temperature of, say, 20 or 25°C is, in fact, cold stressed and elevating its MR to defend its  $T_b$ ! This arises as a result of the difference between mice and humans in their surface areas (which exchange heat with their surroundings) and their volumes (which produce heat). A mouse produces heat at a sevenfold greater rate, per gram of body mass, than a human, but its surface area to volume ratio is close to 200-fold greater. This makes the human subjective experience of ambient temperature quite different from that of a mouse.

**Disturbingly, in the biomedical field the vast majority of metabolic measurements of model organisms such as mice are measured outside their TNZ. This has a host of negative implications.**

**Error: First example.** Consider comparing two experimental groups of mice with the same body masses. The ambient temperature is 25°C, so they are below their LCT, thus raising their MR. The extent to which the MR of a mouse is elevated depends on its conductance, which is the slope of the MR vs. temperature graph outside the TNZ. The effective conductance of an animal depends on a host of factors, such as its ability to change its peripheral vasomotor tone, its preferred  $T_b$  setpoint, the density and erectility of its fur, its BAT content, its behavioral response to hypothermia, its hormonal status, and the thickness of its subcutaneous fat deposits.

It's entirely feasible that one experimental group of mice differs from another not in its BMR, but in its conductance below its TNZ, and thus in its *measured* MR. Blissfully unaware of the TNZ, the researcher publishes this result in terms of differences in BMR – a result that is bogus and, worse, misleading to other workers because it may conceal a real and more relevant difference, such as a difference in vasomotor control or

BAT activation outside the TNZ, that alters MR as a secondary effect.

**Error: Second example.** Consider comparing two groups of mice, such as normal and ob/ob, that differ in body mass (and thus surface area to volume ratio) *plus* the extent of their subcutaneous fat deposits, which are excellent thermal insulators. Both effects will alter an ob/ob's LCT *and* its UCT relative to normal mice. If, per usual practice, the MRs of both groups are measured outside either or both of their TNZs, then the results may be entirely misleading. This doesn't even consider the equally misleading methods of analyzing the effects of body mass in much biomedical metabolic research, which rely on outdated and incorrect assumptions about the mass scaling of MR. I will deal with this subject in a future white paper.

**Why the errors?** Most makers of metabolic screening equipment have no desire to mislead their customers. But companies exist that aggressively market metabolic screening systems without an in-depth knowledge of biological science. Most lack any relevant scientific credentials. Their customers, in turn, are highly trained in their specialized fields but generally lack training in the systems biology of intact, functional non-human animals interacting with their physical environment. This “perfect storm” may, over the years, have been responsible for creating data that future scientists will find difficult or impossible to interpret.

**What to do?** My advice to scientists contemplating a metabolic screening system is first and foremost to reconsider “following the herd.” The best-known, most aggressively marketed systems are made by companies that lack Ph.D.-level personnel with documented qualifications in animal systems biology and, specifically, the theory and practice of animal respirometry. But systems do exist that will produce the accurate data you need. They are also accompanied by expert advice you can trust. If you'd like specific advice, you may wish to contact me personally at [mail@respirometry.org](mailto:mail@respirometry.org).