Supplementary Historical perspective

The functions of isolated brown adipocyte mitochondria have been intensely studied for decades. It is clear from these early studies that freshly isolated brown fat mitochondria contains significant amounts of free fatty acids which are partially responsible for the uncoupled state[1, 2]. The high sensitivity of brown fat mitochondria to uncoupling by fatty acids suggested a physiological regulator of uncoupling, from which the concept of 'fatty acid uncoupling' was developed and later confirmed and widely accepted[3]. In fact, albumin which acts as an acceptor of fatty acids must be used in isolation and respiration medium for coupled respiration to be observed. Thus, bovine serum albumin (BSA) was generally used in the respiration buffer of isolated brown adipocyte mitochondria. Perhaps due to this valuable knowledge, in early publications on oxygen consumption measurements in isolated brown adipocytes following norepinephrine stimulation, 4 % bovine serum albumin (0.6mM, equate to the concentration found in blood) was also used in the respiration medium [4-7]. In 1979, based on the rationales that (1) brown fat when activated release fatty acids and (2) in vivo those released fatty acids are bound by albumin in blood, Nedergaard and Lindberg investigated the effect of albumin on the metabolism of isolated brown fat cells and found that the addition of albumin increases norepinephrine-induced fatty acid release and induces a more stable norepinephrine-stimulated respiration rate[8]. This study provides direct evidence that addition of albumin has beneficial effects on brown adipocytes metabolism. By using this setup, in 2000, Matthias et al. reported marked difference in oxygen consumption between isolated brown adipocytes from wild-type and UCP1 knock-out mice, both following NE stimulation and stimulation with oleate[9]. This study also showed that there was no difference in basal respiration between the two preparations demonstrating UCP1 was not active without stimulation. These observations were further confirmed by Shabalina et al. when studying the bioenergetics of wild-type and UCP1 knock-out brown-fat mitochondria[10]. In addition, investigations with trypsinized primary cultures of brown and brite cells by Petrovic et al. in 2008 and 2010, respectively, showed a correlation between UCP1 expression (as induced by rosiglitazone) and NE-stimulated oxygen consumption[11, 12]. In all these studies, UCP1-mediated leak respiration was observed when albumin was present in respiration medium using Warburg apparatus or Clark-type oxygen electrode systems. Nowadays, microplate-based respirometry has become a mainstream method for measuring UCP1-mediated leak respiration in cultured brown and brite cells. However, in the respiration buffer defined by the respirometry manufacturer albumin is absence. Notably, no studies have been performed so far to validate this setup with cultured UCP1 knock-out (KO) cells as the ultimate model to test the causality between uncoupled respiration and presence of UCP1. This relationship seems to have been taken for granted.

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