# **POINT-COUNTERPOINT**

# Role of creatine and creatine kinase in UCP1-independent adipocyte thermogenesis

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Targeting thermogenesis in brown and beige adipocytes for treating obesity and metabolic disease has sparked enormous interest even beyond the scientific community (4). Several more recent publications have added a new player in this field: the well-studied enzyme creatine kinase (CK) together with its substrates phosphocreatine (PCr) and creatine (Cr). These are best known for their eminent bioenergetic role in energy buffering and energy channeling via the so-called CK/PCr shuttle (3) or circuit (31) that link sites of ATP generation (e.g., mitochondria and glycolysis) with sites of ATP utilization (e.g., cytosolic and membrane bound ATPases) in tissues with high energy turnover, such as skeletal, cardiac and smooth muscle, brain, neural tissue, spermatozoa, inner ear hair cells, etc. (25, 27, 30, 31). As highly energy-dependent cells, adipocytes also express CK isoenzymes and contain the CK substrates PCr and Cr (for review, see Ref. 28). In fact, a series of elegant and very comprehensive studies by Spiegelman, Kazak, and colleagues (2, 15, 16, 18), well summarized in a recent review (17), provides ample genetic and functional evidence for a direct involvement of the CK system in adipocyte thermogenesis with an increased Cr/ PCr turnover being thermogenic. In beige or brown adipocytes, such Cr-dependent heat generation would work in parallel to uncoupling of mitochondrial respiration by uncoupling protein 1 (UCP1), a futile proton cycle that represents the canonical pathway of nonshivering thermogenesis. Since noncanonical UPC1-independent, but ATP- and Cr-dependent, thermogenesis is not only operational during short-term cold response and long-term cold acclimation, but also in diet-induced thermogenesis (16, 18), these recent advances open exciting new avenues for targeting obesity and metabolic disease.

What then is the exact molecular mechanism underlying Crdependent thermogenesis? For heat generation, increased Cr/ PCr turnover has to be linked to a futile cycle, i.e., a metabolic pathway that is not generating useful work, but dissipates free energy as heat. The mechanism put forward so far has been futile Cr-cycling (15). Here, PCr generated by mitochondrial CK in the mitochondrial intermembrane space (IMS) (31) would be immediately hydrolyzed by a postulated PCr phosphatase, colocalizing in the IMS or eventually outside mitochondria, e. g., at the endoplasmic reticulum (ER) (17). However, CK is the only confirmed enzyme that can use PCr and Cr as substrates, while the existence and subcellular localization of the proposed PCr-phosphatase are still uncertain and ill-defined, respectively. Importantly, the presence of such a phosphatase would potentially disrupt PCr-based cell energetics by depleting cellular PCr and thus lowering local PCr/ATP and ATP/ADP ratios. This would be detrimental for proper Ca<sup>2+</sup> homeostasis that depends on highly energy-demanding Ca<sup>2+</sup> pumps (10, 12, 23, 29–31).

Based on our extensive research on the molecular structure, function, and localization of CK isoforms at specific subcellular microcompartments (6, 7, 24, 25, 27, 29-31), we propose here an alternative model for CK/PCr/Cr-mediated thermogenesis in adipocytes (Fig. 1). It combines the classical CK/PCr shuttle (3, 25, 31) with futile  $Ca^{2+}$  cycling at the ER. The latter has been described in detail in recent years in beige and brown adipocytes (10, 12). In this model, PCr fulfills its classical function by shuttling energy from mitochondria to the ER (1, 5, 8, 26, 29), where it is converted by membrane-associated cytosolic CK into ATP. Since the CK/PCr shuttle can also use glycolytic ATP for PCr generation, this pathway would also work in case the mitochondrial ATP would become limiting, which seems the case in adipocytes (11, 19). In fact, there is evidence for additional, noncanonical UCP1-independent nonshivering thermogenesis, fueled by enhanced glycolytic ATP generation (9). ER-localized, cytosolic CK then locally fuels the SR/ER Ca<sup>2+</sup>-ATPase2b (SERCA2b) (23), which is also expressed in adipocytes (12), for efficient  $Ca^{2+}$  uptake into the ER lumen. Requiring a very high ATP/ADP ratio for its function, this Ca<sup>2+</sup> pump has a particular requirement for PCr-dependent fueling via CK (23, 29–31). Although such local ATP supply has not been specifically analyzed in adipocytes, it is well known and documented for skeletal and cardiac muscle, as well as for heater organs of fish (20, 31).

Futile Ca<sup>2+</sup> cycling in brown/beige adipocytes is then induced by coupling Ca<sup>2+</sup> uptake via SERCA2b to immediate Ca<sup>2+</sup> release via different routes that may operate in parallel: adipocyte ryanodine receptor (RyR2), inositol 1,4,5-triphosphate receptor type 1 (IP3-R1), or more so type 3 (IP3-R3). All these constituents have been identified in beige/brown adipocytes, and they all rely on the stimulation of adrenergic receptors ( $\alpha$ 1,  $\beta$ 3) (10, 12, 13). Adipocyte Ca<sup>2+</sup> cycling between SERCA2b and RyR2 occurs under cold exposure and/or adrenergic receptor stimulation (13), is accompanied by a significant increase of oxygen consumption rate (OCR) and heat generation, and improves cold tolerance and metabolic status (12, 13). Furthermore, evidence for an involvement of IP3-R in futile Ca<sup>2+</sup> cycling is given by the fact that RyR2 blockage by high dose ryanodine or ruthenium red only partially but not completely disrupts norepinephrine-induced thermogenesis

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Fig. 1. Proposed mechanism of creatine-driven thermogenesis in brown/beige adipocytes. The creatine kinase (CK)/phosphocreatine (PCr) shuttle (bold red and black arrows) improves energy transfer between mitochondrial and/or glycolytic ATP supply (big white circles) and ATP consumption at the endoplasmic reticulum (ER) by SR/ER Ca<sup>2+</sup>-ATPase2b (SERCA2b). This shuttle is supported by specifically associated mitochondrial CK (black square) and cytosolic CK (gray squares) isoforms. At the mitochondrial site, CK functionally interacts with adenine nucleotide translocase and voltage-gated anion channel (small white circles) to exchange matrix ATP against ADP and cytosolic creatine (Cr) against PCr, respectively (14, 27). At the ER site, cytosolic CK fuels SERCA2b for Ca<sup>2+</sup> pumping (30), which can switch to futile Ca<sup>2+</sup> cycling (blue arrows). This involves Ca<sup>2+</sup>-release by either ryanodine receptor (RyR2), potentially regulated by calstabin2 (Cal2) (12), or the inositol-3-phosphate receptors ( $\alpha$ 1,  $\beta$ 3) (10, 12, 13). This classical CK/PCr shuttle links ATP supply with ER-based ATP consumption by SERCA2b, thus supporting futile Ca<sup>2+</sup> cycling for noncanonical, UCP1-independent, but ATP-dependent, nonshivering thermogenesis. [Figure modified with permission from (27)].

in UCP1-knockout adipocytes (12). This is supported by earlier data (10) showing that elevated cytosolic Ca<sup>2+</sup>, caused by mitochondrial Ca<sup>2+</sup> release, plus elevated IP3 levels, after  $\alpha$ 1-adrenergic stimulation of adipocytes together result in a Ca<sup>2+</sup> release from the ER/SR via IP2 receptor (22). This event is likely to happen in parallel with Ca<sup>2+</sup> release via RyR2 (12). There is also evidence that the CK system and Ca<sup>2+</sup> cycling via SERCA2b operate in the same pathway for UCP1-independent thermogenesis. When using UCP1-knockout beige adipocytes, both SERCA2b depletion and CK inhibition by  $\beta$ -guanidinopropionic acid ( $\beta$ -GPA) reduced OCR, but  $\beta$ -GPA treatment had no additional effect on OCR in already SERCA2b-depleted adipocytes (12).

In conclusion, the role of Cr and CK in thermogenesis of beige and brown adipocyte has attracted much attention. Their role in UCP1-independent, but ATP-dependent nonshivering thermogenesis is clearly emerging (15–18, 21), but the detailed molecular nature of the thermal energy generator is less evident. It is our contention, however, that it does not necessarily require futile Cr cycling and the presence of a hypothetical, novel PCr phosphatase. It is proposed here that Cr and PCr may just operate as part of the classical energy shuttle, providing ATP to other thermogenic pathways, in particular for futile  $Ca^{2+}$  cycling at the ER. For the latter, ample evidence for its functioning in adipocytes is available (10, 12, 13). Much diligent experimentation, however, is still needed to clarify these issues before they can be harnessed for mechanism-based therapeutic approaches to combat obesity, type 2 diabetes, and metabolic syndrome (4).

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### DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

#### AUTHOR CONTRIBUTIONS

T.W., M.T., L.K., and U.S. drafted manuscript; T.W., M.T., L.K., and U.S. edited and revised manuscript; T.W., M.T., L.K., and U.S. approved final version of manuscript.

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