Enhanced mitochondrial biogenesis driven by nitric oxide (•NO), synthesized by the endothelial nitric oxide synthase (eNOS), is proposed to be a central effect in calorie restriction (CR). The goal of this work was to uncover the mechanisms associated with enhanced •NO signaling during CR, both in vivo and in vitro. Female Swiss mice were submitted to 40% CR. A tissue-specific increment in basal Akt and eNOS phosphorylation, related to enhanced mitochondrial biogenesis, was observed. To unveil the mechanism behind insulin signaling effects on •NO levels, serum from Sprague-Dawley CR rats was used in VSMC cell cultures, an in vitro CR protocol. CR sera enhanced insulin receptor (IR) and Akt phosphorylation, as well as the expression of eNOS and nNOS (neural NOS) and eNOS phosphorylation. The effects of CR sera were reversed by Akt inhibition or serum adiponectin immunoprecipitation. Cerebellar neurons were also cultured with CR or ad libitum sera and also presented striking increments in •NO signaling, associated with mitochondrial biogenesis and decreased senescence. Mitochondrial effects promoted by CR were also observed in insulin-secreting cells (INS1). However, under CR, insulin secretion stimulated by glucose was impaired. The likely explanations are reduced mitochondrial reactive oxygen species generation, or alterations in mitochondrial morphology. Overall, CR enhances •NO signaling due to increased insulin sensitivity, through adiponectin-stimulated Akt activity, and results in augmented mitochondrial biogenesis. Increments in mitochondrial mass enhance the cellular reserve respiratory capacity and survival. Mitochondrial morphology alterations are associated with impaired insulin release, possibly maintaining the low levels of plasmatic insulin secretion under CR.

Keywords: adiponectin; mitochondrial biogenesis; mitochondrial morphology; nitric oxide; calorie restriction; insulin signaling.

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