

## **IMPAIRED COLD TOLERANCE IN EP4 KNOCKOUT MICE IS NOT DUE TO COMPROMISED RECRUITMENT OF UCP1**

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**OBJECTIVE:** Uncoupling protein 1 (UCP1) is the key component of beta-adrenergically controlled non-shivering thermogenesis in brown adipose tissue (BAT). Certain white adipose tissue (WAT), upon exposition to cold, can undergo a process known as browning where it takes on characteristics of BAT, notably induction of UCP1 and the presence of multilocular lipid droplets and multiple mitochondria. Our preliminary data indicated that mice deficient in EP4 (one subtype of PGE2 receptor) are cold-intolerant, suggesting that EP4 influence thermogenesis capacity. The aim of this project was to investigate whether or not the impaired thermogenesis in EP4 knockout mice is due to reduced recruitment of UCP1 in fat.

**METHODS:** Male EP4 wild-type and knockout mice (12-15 weeks old) were treated with CL316243 (a highly selective beta 3-adrenergic agonist, 1 mg/kg/day *i.p.*) or saline for 10 days. Expression of UCP1, as well as other thermogenesis-related genes in subcutaneous white adipose tissues (sWAT), epididymal white adipose tissue (eWAT) and BAT of experimental mice were compared. In addition, isolated fat from the experimental mice were stained with hematoxylin and eosin and their morphology were compared.

**RESULTS:** After chronic CL316243 treatment, eWAT of EP4 knockout were morphologically indistinguishable from that of wild-type mice. However, sWAT and BAT of EP4 knockout have smaller multilocular lipid droplets than wild-type mice. The expression of UCP1 and other thermogenesis-related genes (including epithelial V like antigen 1, type II thyroxine deiodinase, cell death inducing DFFA like effector A) in eWAT, sWAT and BAT did not differ between EP4 knockout and wild-type mice. Interestingly, after CL316243 treatment, there is a substantial increase in UCP3 mRNA in sWAT of EP4 knockout as compared to wild-type mice. An enhanced level of UCP3 was also observed in BAT of EP4 knockout as compared to wild-type mice under saline treatment.

**CONCLUSIONS:** Impaired cold tolerance in EP4 knockout mice is not due to compromised recruitment of UCP1 in WAT and BAT. The enhanced level of UCP3 in sWAT (after CL316243 treatment) and BAT (under basal conditions) in EP4 knockout mice may possibly serve as a compensatory mechanism to counteract the impaired thermogenesis observed in these mice.