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Dietary capsaicin upregulates uncoupling protein 2/3 expression in visceral adipose tissue and enhances acetylcholine-induced hypotensive effect in mice

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Titolo
La capsaicina introdotta con la dieta up-regola l'espressione di 2/3 proteine disaccoppianti nel tessuto adiposo viscerale e aumenta l'effetto ipotensivo acetilcolina-indotto nei topi

Key words
Capsaicin, transient receptor potential vanilloid type-1, uncoupling protein, hypotension

Parole chiave
Capsaicina, recettore vanilloide potenziale transiente di tipo 1, proteina disaccoppiante, ipotensione

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Summary
Obesity and hypertension are major risk factors for cardiovascular events. Some plant-based diets lower the prevalence of hypertension and prevent weight gain. Capsaicin, a major pungent ingredient in chili pepper, can specifically activate transient receptor potential vanilloid type-1 (TRPV1) channel. Our previous studies demonstrated that activation of TRPV1 channel by dietary capsaicin prevents adipogenesis and obesity in mice. However, little is known about the mechanism of TRPV1 activation mediated the reduction of adipogenesis and prevention of obesity. Furthermore, it is unclear whether long term dietary capsaicin could modulate blood pressure. Here, we report that capsaicin significantly increased the uncoupling proteins (UCP2 and UCP3) expression in mature adipocytes which indicated that capsaicin promoted the fat oxidation. Mice on high-fat diet (HFD) for 4 months developed obesity and increased UCP2 and UCP3 expression in visceral fat. Dietary capsaicin markedly prevented the development of obesity and further increased UCP2 and UCP3 expression in visceral fat from mice on HFD. Chronic administration of capsaicin also dose–dependently increased the acetylcholine-mediated hypotensive responses in mice, however, this effect was absent in TRPV1 deficient mice. We conclude that TRPV1 activation by dietary capsaicin increased fat oxidation and promoted hypotensive effects in mice. Dietary capsaicin may become a novel therapeutic strategy for obesity and its related cardiometabolic diseases.

Riassunto
L'obesità e l'ipertensione sono fattori di rischio maggiore per gli eventi cardiovascolari. Alcune diete a base vegetale riducono la prevalenza di ipertensione e prevengono l'aumento di peso. La capsaicina, il "pungente" componente principale nel peperoncino, può attivare in modo specifico i canali del recettore vanilloide potenziale transiente di tipo 1 (TRPV1). I nostri precedenti studi hanno dimostrato che l'attivazione del canale TRPV1 da parte della capsaicina introdotta con la dieta impedisce l'adipogenesi e l'obesità nei topi. Tuttavia si sa poco del meccanismo di attivazione di questo canale che media la riduzione dell'adipogenesi e la prevenzione dell'obesità. Inoltre non è chiaro se la capsaicina in-

trodotta attraverso la dieta a lungo termine possa modulare la pressione arteriosa. In questo studio si segnala che la capsaicina ha notevolmente aumentato l'espressione di alcune proteine disaccoppianti (UCP2 e UCP3) in adipociti maturi il che indica che la capsaicina ha promosso l'ossidazione dei grassi. Topi sottoposti a dieta ad alto contenuto di grassi (HFD) per 4 mesi hanno sviluppato obesità e hanno aumentato i livelli di espressione di UCP2 e UCP3 nel grasso viscerale. Si è visto che la capsaicina introdotta con la dieta ha marcatamente impedito lo sviluppo dell'obesità e l'ulteriore aumento dell'espressione di UCP2 e UCP3 nel grasso viscerale prelevato da topi sottoposti a dieta ad alto contenuto di grassi. La somministrazione cronica di capsaicina ha anche aumentato in modo dose-dipendente nei topi la risposta ipotensiva acetilcolina-mediata, tuttavia, questo effetto è stato assente in topi che presentavano una deficienza nel TRPV1. Possiamo concludere che l'attivazione del TRPV1 da parte della capsaicina introdotta con la dieta ha aumentato l'ossidazione dei grassi e promosso effetti ipotensivi nei topi. La capsaicina introdotta con la dieta può diventare una nuova strategia terapeutica per l'obesità e per le patologie cardiometaboliche correlate.

Introduction

The prevalence of obesity and hypertension in both developed and developing countries has increased dramatically in recent years. Plant-based diets can lower the risk of cardiometabolic diseases and prevalence of hypertension (1-3). Capsium species, or hot peppers, are used worldwide as vegetables and spices. Capsaicin, 8-methyl-N-vanillyl-trans-6-nonenamide, is a major pungent ingredient in red pepper and is used as a food additive (4). Recently, the pivotal role of capsaicin in the regulation of multiple physiologic processes was highlighted (5). Our recent studies showed that transient receptor potential vanilloid type-1 (TRPV1) channel was functional expressed in 3T3-L1-preadipocytes and visceral adipose tissue from mice and humans. *In vitro*, the TRPV1 agonist capsaicin induced calcium influx and prevented the adipogenesis in stimulated 3T3-L1-preadipocytes in a concentration-dependent manner. RNA interference knockdown of TRPV1 in 3T3-L1-preadipocytes attenuated the capsaicin-induced calcium influx, and adipogenesis in stimulated 3T3-L1-preadipocytes was no longer prevented. By comparing with lean counterparts, the visceral adipose tissues from obese db/db and ob/ob mice and from obese humans showed less TRPV1 expression. The lowered TRPV1 expression in the visceral adipose tissue from obese humans was accompanied by a reduced capsaicin-induced calcium influx. Oral administration of capsaicin for 4 months prevented obesity in wild-type mice but not in TRPV1 knockout mice on high-fat diet (HFD). However, little is known about the

mechanism of TRPV1 activation mediated the reduction of adipogenesis and prevention of obesity. Furthermore, it is unclear whether chronic dietary capsaicin could regulate blood pressure. Uncoupling protein 2 (UCP2) and UCP3 play an important role in fat oxidation and associated with the regulation of blood pressure (6). Thus, we investigated whether TRPV1 activation by dietary capsaicin can impact on UCPs and blood pressure modulation.

Methods

All mice (C57BL/6 wild-type mice and TRPV1 knockout mice) were purchased from the Jackson Laboratory (Bar Harbor, Maine). The local Animal Care and Use Committee approved all animal protocols. Protein expression of UCP2 and UCP3 in adipocyte and adipose tissue were determined by Western blotting. After administration of normal diet or normal diet plus capsaicin for 4 months, mice were anesthetized with pentobarbital sodium (50 mg/kg IP), and while breathing spontaneously, the right carotid artery was cannulated for measurement of direct mean arterial pressure (MAP) with a pressure transducer (model MLT 1030, Power Lab, Australia). The left jugular vein was cannulated for intravenous infusion of nitroglycerin (NTG) or acetylcholine (Ach). After obtaining baseline MAP values during a 1-hour control period, a bolus (50 µl) of NTG or Ach was injected into the jugular vein at increasing dosages (from 0.05 to 5 nmol). MAP was allowed to return to control value before the next bolus was given, which normally occurred within 5 to 10 min.

Results and discussions

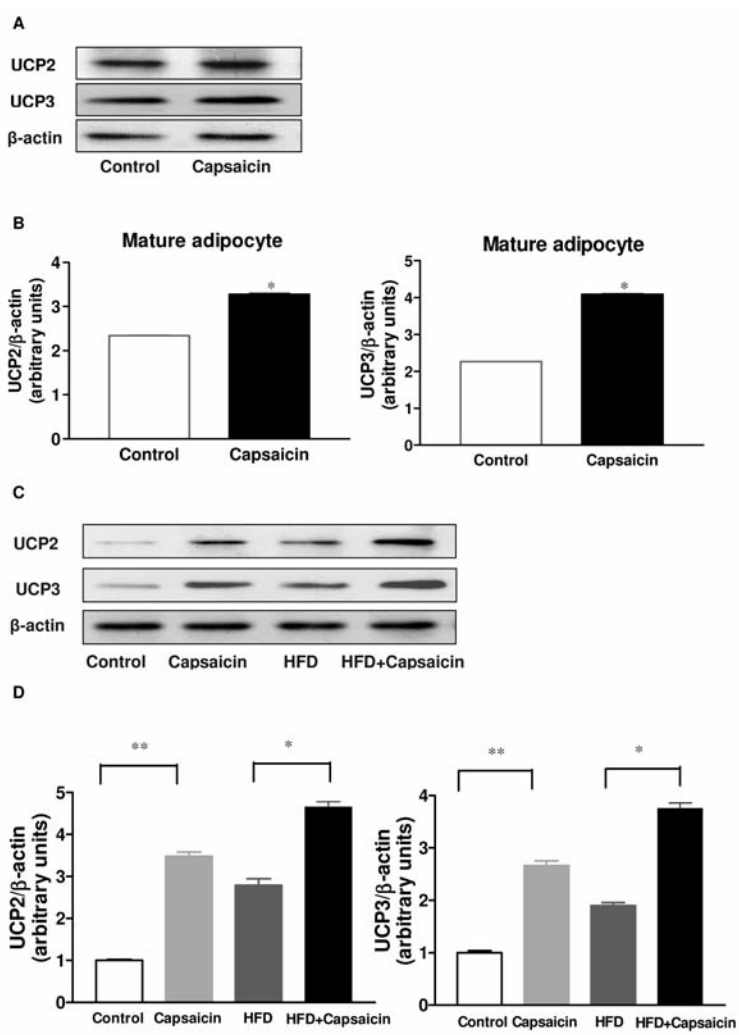
Capsaicin upregulates UCP2 and UCP3 in cultured adipocyte and adipose tissue

Our previous study has demonstrated that oral administration of capsaicin can suppress adipogenesis in stimulated 3T3-L1 preadipocytes and prevent HFD-induced obesity in C57BL/6J mice through inhibition of PPAR gamma and fatty acid sythase expression under the activation of TRPV1 channel in adipocyte and adipose tissue (7). However, it is unknown whether capsaicin also affects UCP2 and UCP3, which are involved in fat oxidation (8, 9). As the energy uncoupling plays a crucial role in the modulation of adipose tissue growth (10), we studied the effect of dietary capsaicin on UCP2 and UCP3 expression in adipocyte and adipose tissue. The results showed that expressions of UCP2 and UCP3 proteins were significantly increased in adipocyte treated with capsaicin compared with that untreated with capsaicin (Figure 1A and 1B). Similar finding was obtained from *in vivo* study; mice on HFD developed obesity and had higher UCP2 and UCP3 protein expression in visceral adipose tissue. In contrast, mice on HFD treated with dietary capsaicin for 4 months markedly further increased expressions of UCP2 and UCP3 protein in visceral adipose tissue compared with mice on HFD without capsaicin treatment (Figure 1C and 1D). Mitochondrial UCP2 and UCP3 have been implicated in energy expenditure and the development of type 2 diabetes and its related cardiovascular diseases (11). These findings suggest that the capsaicin induced upregulation of UCP2 and UCP3 may contribute to the prevention of weight gain.

Capsaicin enhances acetylcholine-induced hypotensive effect

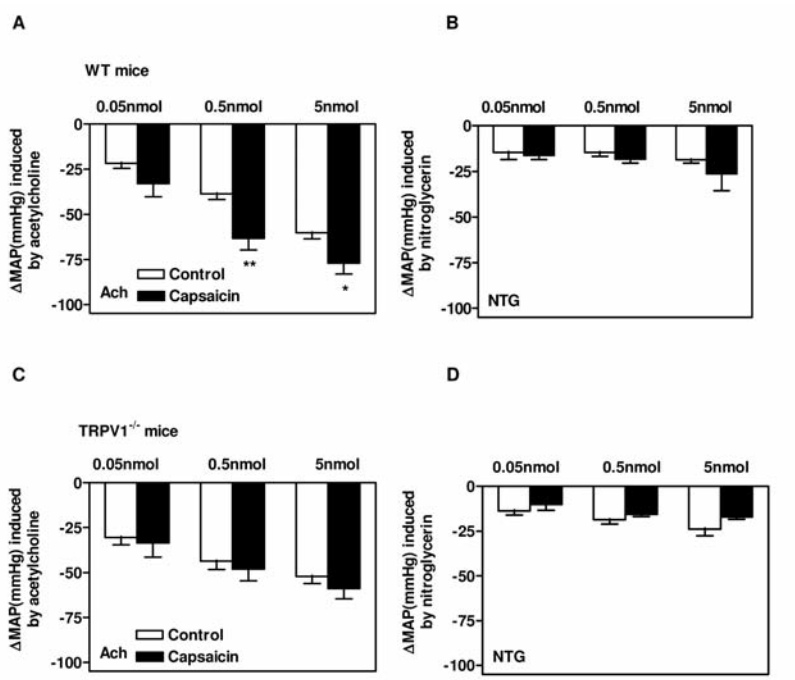
TRPV1 activation by capsaicin is implicated in the regulation of vascular tone and blood pressure (12). Acute or short-term administration of capsaicin either increases or lowers blood pressure transiently in human and rodents. It is unknown whether a long-term dietary capsaicin intervention

Figure 1 - Protein expressions of UCP2 and UCP3 in cultured adipocyte and visceral adipose tissue. Representative immunoblots of UCP2, UCP3 and loading control (β-actin) in adipocyte treated with or without capsaicin (1 μM, 24 hours) (A). Bar graphs showing the relative expression of this protein after normalization to β-actin expression (B). Values are means ± SEM; n=3-6 per each group. *P<0.05 vs. Control. Representative immunoblots of UCP2, UCP3 and loading control (β-actin) in visceral adipose tissue from C57BL/6J mice placed on standard chow (Control), 0.01% capsaicin diet, high-fat diet (HFD), and HFD plus 0.01% capsaicin. (C). Bar graphs showing the relative expression of this protein after normalization to β-actin expression (D).Values are means ± SEM; n=3-6 per each group. *P<0.05, **P<0.01



might modulate blood pressure changes. Thus, blood pressure was examined in wild-type and TRPV1 deficient mice treated with standard chow or 0.01% capsaicin diet for 6 months. Our results showed that acetylcholine dose-dependently enhanced hypotensive effects in wild-type mice treated with capsaicin compared with those without capsaicin treatment (Figure 2A). By contrary, acetylcholine-induced blood pressure lowering effects were abolished in TRPV1 deficient mice with capsaicin treatment (Figure 2C). However, nitroglycerin-induced blood pressure reductions were comparable between wild-type and TRPV1 deficient mice either in the presence or absence of capsaicin treatment (Figure 2B and Figure 2D). The results indicated that chronic dietary capsaicin promoted blood pressure lowering effects in mice. Capsaicin, as a functional ingredient, has the potential to produce significant effects on cardiometabolic system. The present study demonstrates for the first time that capsaicin activates TRPV1 channel that is necessary to prevent obesity and lower blood pressure. Thus, dietary capsaicin intervention could be considered as a novel strategy for management of obesity and hypertension.

Figure 2 - Acetylcholine and nitroglycerin-induced hypotensive effects in mice. The dose-dependent hypotensive responses to acetylcholine and nitroglycerin in 0.01% capsaicin-treated and vehicle-treated C57BL/6J wild-type mice for 6 months (A) and TRPV1-deficient one (B). Values are means ± SEM; n=3 per each group. *P<0.05, **P<0.01 vs. Control



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