Skeletal Muscle Uncoupling Proteins in Obesity Mice Models

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Obesity and accompanying type 2 diabetes are among major and increasing worldwide problems that occur fundamentally due to excessive energy intake during its expenditure. Endotherms continuously consume a certain amount of energy to maintain core body temperature via thermogenic processes, mainly in brown adipose tissue and skeletal muscle. Skeletal muscle glucose utilization and heat production are significant and directly linked to body glucose homeostasis at rest, and especially during physical activity. However, this glucose balance is impaired in diabetic and obese states in humans and mice, and manifests as glucose resistance and altered muscle cell metabolism. Uncoupling proteins have a significant role in converting electrochemical energy into thermal energy without ATP generation. Different homologs of uncoupling proteins were identified, and their roles were linked to antioxidative activity and boosting glucose and lipid metabolism. From this perspective, uncoupling proteins were studied in correlation to the pathogenesis of diabetes and obesity and their possible treatments. Mice were extensively used as model organisms to study the physiology and pathophysiology of energy homeostasis. However, researchers should be aware of interstrain differences in mice models of obesity regarding thermogenesis and insulin resistance in skeletal muscles.

Keywords: uncoupling protein ; skeletal muscle ; obesity ; mouse models ; UCP1 ; UCP2 ; UCP3 ; Sex Differences in UCP Expression

1. UCP Homologs and Their Roles

The role of UCPs in the pathogenesis of diabetes mellitus has recently become a popular topic since five homologs have been found in mammals[1]. Their structure is similar, but their distribution in different tissues varies considerably[2]. The physiological functions of UCPs have been studied intensively in the last three decades, yet they are still not completely elucidated. They are known for their antioxidative activity[3][4] and as glucose and lipid metabolism enhancers or regulators. Several gene polymorphisms of UCP1, UCP2, and UCP3 have been found in human diabetic and obese individuals, linking them to the development of glucose metabolism and insulin signaling pathologies[2] (**Figure 1**). In contrast to UCP1, which can represent as much as 10% of proteins in the inner mitochondrial membrane[5], UCP2 and UCP3 usually comprise less than 0.1% of the membrane protein content. They need specific activation for their proton transporting function[6].

Figure 1. UCP homologs are present in different tissues and have distinct roles. Arrows up represent an increased activity, arrows down represent a decreased activity (created with BioRender.com, (accessed on 19 January 2022)).

1.1. UCP1

Studies in rodents have shown that BAT starts to develop in the interscapular region during embryonic days E15–16 and that UCP1 mRNA expression increases around days E18–19 just before birth. The BAT continues to develop postnatally until between postnatal days P15–21 and remains present throughout adult life[7][8]. Recent research revealed the existence of two subpopulations of brown adipocytes in mice. One subpopulation has high thermogenic activity and high UCP1 expression, and the other has low thermogenic activity and low UCP1 expression[9]. At birth, all adipocytes express high levels of UCP1 and have a high thermogenic activity to meet newborns' thermal requirements. Postnatally, some adipocytes begin to convert to the subpopulation with low UCP1 expression so that both subpopulations coexist in adult mice and might switch between each other during normal thermogenesis at room temperature. When exposed to cold, the transcription of genes in the subpopulation with the low UCP1 expression increases, thereby increasing the total thermogenic capacity of BAT[10]. During long-term cold exposure, de novo adipogenesis was observed in BAT[11][12]. In senescence, the capacity of adipocytes to increase UCP1 expression after cold exposure becomes impaired[10].

UCP1 mainly localizes to the inner mitochondrial membrane of BAT. Its proton conductance increases in elevated concentrations of long-chain free fatty acids (FFAs)[13] and is controlled by insulin[14][15]. Apart from BAT, recent studies also reported UCP1 expression in white adipose tissue, skeletal muscle, longitudinal smooth muscle layers, retinal cells, and Langerhans islet cells[2][16].

In skeletal muscle mitochondria, the expression of UCP1 reaches only 13% of the expression in BAT and increases the GDP-sensitive proton leak[17]. The roles of UCP1 are decreasing membrane potential, reducing reactive oxygen species (ROS) generation, increasing energy expenditure, and increasing nonshivering thermogenesis[18][19][20]. Compared to BAT, the ability of UCP1 in skeletal muscle to increase glutathione levels and reduce ROS production is far greater, suggesting different specific roles and possibly distinct mechanisms of UCP1 in both tissues[17]. Some research shows that diabetes and obesity development involve specific polymorphisms of the *Ucp1* gene[21]. Mutations in *Ucp1* affect the activity or expression of the UCP1 protein and reduce regulated or basal energy expenditure, resulting in altered pancreatic function and insulin secretion[22][23].

1.2. UCP2

UCP2 mRNA is expressed in many tissues, such as muscle, spleen, pancreas, kidney, central nervous system, and immune system. The UCP2 gene is already expressed during fetal life in murine skeletal muscle. Its expression increases immediately after birth, reaching a maximum on day 2, and steadily declines after that regardless of the lactating mother's diet[24].

UCP2 is most widely present and highly expressed among UCPs in diabetic pancreatic beta-cells[25]; therefore, its involvement in diabetes development has been proposed. Its role in the pancreas as a negative regulator of insulin secretion has been studied intensively in ob/ob mice. The activation of UCP2 by ROS causes mitochondrial membrane proton leak, which reduces ATP synthesis in pancreatic β-cells and downregulates glucose-stimulated insulin secretion[26][27][28]. The ob/ob mice lacking UCP2 have increased ATP synthesis and glucose-stimulated insulin secretion from beta-cells in Langerhans islets[29][30]. DeSouza et al. (2007) used an antisense oligonucleotide to *Ucp2* in ob/ob mice and Swiss mice with hyperlipidemic diet-induced obesity and diabetes to inhibit UCP2 expression, resulting in metabolic improvement[28]. Finally, results from a human study on ethnicity differences in UCP2 polymorphisms demonstrated that in Asians, the UCP2-866G/A polymorphism is protective against, while the UCP2 Ala55Val polymorphism is susceptible to, type 2 diabetes[31]. Similar traits might also exist in mice, but these have not been thoroughly researched yet.

One of the reported other roles of UCP2 is controlling immune cell activation by modulating MAPK pathways and mitochondrial ROS production[32][33]. Additionally, a neuroprotective role has been proposed. By regulating mitochondrial membrane potential, production of ROS, and calcium homeostasis, UCP2 modulates neuronal activity and inhibits cellular damage[34].

1.3. UCP3

UCP3 is expressed in skeletal muscle and BAT[35][36][37][38]. In BAT, UCP3 is almost one order of magnitude more abundant than in skeletal muscle or heart and is directly correlated with the abundance of UCP1[39]. The predominant isoform in skeletal muscle is UCP3, and its expression is highly skeletal-muscle-specific[40]. In mice, UCP3 mRNA levels were highest in skeletal muscle, followed by heart, white adipose tissue, and spleen, which was somewhat different than in rats, where the expression in tissues other than skeletal muscle was negligible[41].

UCP3 expression was almost undetectable in murine muscle tissue during fetal life. In contrast, its expression became noticeable soon after birth in response to suckling and lipid intake and steadily increased for 15 days. Interestingly, after 15 days of life, the UCP3 mRNA levels became dependent on dietary interventions. If lactating mice were fed regular high-carbohydrate chow, UCP3 expression levels in pups started to decrease, whereas if mothers were fed a high-fat diet, the levels of UCP3 expression in pups remained high[24]. Research shows that nutritional factors regulate UCP3 expression. Specifically, its expression is induced by elevated circulating FFAs, which is typical for fasting or starvation[24][42]. Pedraza et al. reported that the UCP3 expression in skeletal muscle is dramatically downregulated in lactating mice, and this effect is reversed with weaning. These changes come hand-in-hand with changes in circulating FFAs, which are reduced during lactation and return to normal after weaning[43].

Pancreatic beta cells also express UCP3[44], linking its role to energy expenditure, glucose metabolism, diabetes, and obesity[45][46]. Pancreatic UCP3 also affects insulin secretion but acts differently than UCP2[44]. In humans, the expression of the *Ucp3* gene in skeletal muscle and pancreas of diabetic patients is decreased[47], suggesting *Ucp3* involvement in the development of type 2 diabetes. Muscle UCP3 is also important in FFA metabolism. It protects mitochondria from oxidative stress induced by lipids and modulates insulin sensitivity[48], making it a potential player in type 2 diabetes development. UCP3 protein levels are upregulated when FFAs' supply to the mitochondria exceeds their oxidative capacity and downregulates when oxidative capacity is improved.

The degradation of both UCP2 and UCP3 is very rapid[49], making their half-lives only approximately 30 min[50]. In comparison, the half-life of UCP1 is around 30 h[51]. The short half-lives of UCP2 and UCP3 enable rapid adjustments of their protein levels, which are needed when facing the rapidly changing metabolic needs and different rates of ROS production during mitochondrial oxidative processes. Because of this rapid degradation, the UCP2 protein level can decrease before the level of its mRNA drops[52]. It is crucial to consider this when evaluating data and drawing conclusions solely on mRNA expression.

1.4. Other UCPs

UCP4 and UCP5 are mainly expressed in the central nervous system, where they play roles in brain metabolism and thermoregulatory heat production and are therefore often named neuronal UCPs[53][54]. However, their expression has also been determined in skeletal muscle, controlling energy expenditure and lipid oxidation. UCP5 is expressed in human skeletal muscle in three different isoforms, with UCP5L being the most abundant isoform, followed by UCP5S and UCP5SI[55]. UCP4 and UCP5 have a similar role in the protection against oxidative stress and mitochondrial dysfunction as other homologs[56]. High levels of UCP5 mRNA have been detected in testes and lower levels in the kidneys and liver[55].

2. Sex Differences in UCP Expression

Studies with rodents of both genders have shown significant sex-associated differences in the regulation of UCPs, which occur due to sex hormones and other distinct gender-based biological functions[57]. Sex hormone receptors are localized in the mitochondria of specific cells and can affect mitochondrial physiology[58]. In rodents, sex hormones influence different features of skeletal muscle, such as fiber diameter and myosin heavy-chain expression[59]. They also regulate UCP1 expression in brown adipocytes[60][61].

Age plays a vital role in the sex dimorphism of UCP expression. In prepubertal age in mice, UCPs are expressed at similar levels in both sexes, with significant differences, especially in UCP1 and UCP3 expression, being observed only later in adulthood. Expression of these proteins decreased with time in adult males, while in females, UCP1 and UCP3 expression decreased during young adulthood and increased later[62]. This age-dependent UCPs expression pattern correlates with weight gain. In several studies, weight gain with aging was more significant in males than in female mice, which showed a slight increase in body weight with senescence. This finding suggests that upregulation of UCP1 and UCP3 in BAT helps female mice avoid triglyceride accumulation in skeletal muscle and prevents obesity development[45][62][63].

Caloric diet feeding causes different overweight-induced expression of UCP3 in muscle and UCP1 in BAT in males than in females. Females tend to have a higher capacity to store fat when food is in excess than males, resulting in weight gain[64]. On the other hand, experiments with fasting showed interesting sex-dependent differences in UCP expression. Bazhan et al. (2019) studied sex asymmetry in the fasting effects on the transcription of the *Ucp3* gene in muscle. A

significant upregulation of muscle *Ucp3* occurred in females after fasting for 24 h, while these changes were much less evident in males[65].

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