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Regulation of UCP2 and UCP3 by muscle disuse and physical activity in tetraplegic subjects

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Abstract

Aims/hypothesis. The regulation of uncoupling protein 2 and uncoupling protein 3 gene expression in skeletal muscle has recently been the focus of intense interest. Our aim was to determine expression of uncoupling protein 2 and 3 in skeletal muscle from tetraplegic subjects, a condition representing profound muscle inactivity. Thereafter we determined whether exercise training would modify expression of these genes in skeletal muscle.
Methods. mRNA expression of uncoupling protein 2 and 3 was determined using quantitative reverse transcription-polymerase chain-reaction.
Results. Expression of uncoupling protein 2 and 3 mRNA was increased in skeletal muscle from tetraplegic compared with able-bodied subjects (3.7-fold $p < 0.01$ and 4.1-fold, $p < 0.05$, respectively). A subgroup of four tetraplegic subjects underwent an 8-week exercise programme consisting of electrically-stimulated leg cycling (ESLC, 7 ESLC sessions/week). This training protocol leads to increases in whole body insulin-stimulated glucose uptake and expression of genes involved in glucose metabolism in skeletal muscle from tetraplegic subjects. After ESLC training, uncoupling protein 2 expression was reduced by 62 % and was similar to that in able-bodied people. Similarly, ESLC training was associated with a reduction of uncoupling protein 3 expression in skeletal muscle from three of four tetraplegic subjects, however, post-exercise levels remained increased compared with able-bodied subjects.
Conclusion/interpretation. Tetraplegia is associated with increased mRNA expression of uncoupling protein 2 and 3 in skeletal muscle. Exercise training leads to normalisation of uncoupling protein 2 expression in tetraplegic subjects. Muscle disuse and physical activity appear to be powerful regulators of uncoupling protein 2 and 3 expression in human skeletal muscle. [Diabetologia (1999) 42: 826–830]

Keywords Uncoupling proteins, exercise, tetraplegia, skeletal muscle, mRNA, gene expression, polymerase chain reaction.

Uncoupling proteins (UCP) are mitochondrial proteins that function to uncouple respiration from oxidative phosphorylation and ATP synthesis, converting fuel into heat [1]. Three uncoupling proteins have to date been described and named UCP1, 2 and 3. Uncoupling protein 1 is the best characterised and it is almost exclusively expressed in brown adipose tissue [2, 3]. In rodents, UCP1 plays an important part in regulating energy balance and protecting against hypothermia [4–6]. UCP1 is located on the inner mitochondrial membrane, where it facilitates the transport of fatty acids across the membrane. Fatty acids are then protonated and diffuse back across the membrane, thus uncoupling respiration from the generation of ATP, leading to a net heat gain [1]. Uncoupling proteins 2 and 3 show 59 and 57 % amino acid identity to UCP1, respectively [7–10]. The gene for UCP3 under-

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Abbreviations: UCP, Uncoupling protein; ESLC, electrically stimulated leg cycling.

goes alternative splicing, leading to a long form, $UCP3_L$ and a short from, $UCP3_S$ [11]. In adult humans there is little or no detectable UCP1 expression, but UCP2 is expressed in several tissues, including white adipose tissue and skeletal muscle [7, 9] and UCP3 is expressed primarily in skeletal muscle [8, 10].

Expression of UCP2 and UCP3 is regulated by diet and nutritional status [12–16]. Several lines of evidence suggest a direct role of fatty acids in regulating UCP3 expression. Infusion of lipids leads to increased UCP3 expression in rat skeletal muscle [17]. Furthermore, a strong correlation between skeletal muscle UCP3 expression and non-esterified fatty acids has been noted in obese human subjects [18]. In addition, the level of physical activity may also regulate UCP2 and UCP3 expression. Recently, muscle disuse through denervation was associated with increased UCP3 expression in rat gastrocnemius muscle after denervation [19], whereas, in mouse skeletal muscle, denervation led to a reduction in UCP3 expression [19]. Interestingly, short-term exercise increased expression of both genes in rat skeletal muscle, whereas UCP2 but not UCP3 was increased by exercise training in mouse muscle [19]. This species difference between rat and mouse is somewhat perplexing and highlights the need to investigate the regulation of these genes in human skeletal muscle.

The aim of this study was to investigate the regulation of UCP2 and UCP3 expression in human skeletal muscle under conditions of extreme inactivity and after long-term exercise training. In humans, tetraplegia following spinal cord lesions represents a state of chronic muscle inactivity, without peripheral denervation. To assess the effect of exercise training on UCP2 and 3 expression, tetraplegic subjects underwent an 8-week electrically-stimulated leg cycling (ESLC) training programme. We have shown previously that this training protocol leads to increased whole body insulin-stimulated glucose uptake, as well as increased expression of a number of genes involved in glucose metabolism in tetraplegic people [20]. In this paper we report that muscle disuse is associated with alterations of uncoupling protein 2 and 3 expression in skeletal muscle. Long-term exercise training leads to alterations of expression of these genes. Thus, physical activity appears to be a powerful regulator of uncoupling protein 2 and 3 expression in human skeletal muscle.

Subjects and methods

Tetraplegic subjects. The study protocol was reviewed and approved by the institutional ethics committee and informed consent was received from all subjects before they participated. We studied seven men with complete chronic lesion of the cervical spinal cord. The tetraplegic study participants received a thorough clinical examination, including routine blood and urine chemistry analysis and x-rays of the chest, spinal column and extremities. Condom drainage was used for bladder emptying in all subjects. Five subjects were treated against muscle spasms with baclofen (20–25 mg × 2–4) and one subject received additional treatment with diazepam (5 mg × 2). The control group consisted of nine healthy men. None of the study participants were tobacco users or were taking any other medication. The subjects were instructed to abstain from any form of strenuous physical activity for a period of 48 h before the experiment. All participants were asked about their exercise habits. None of the control subjects were elite athletes.

Muscle biopsy procedure and training protocol. Subjects reported to the laboratory following an overnight fast. All investigations were done before noon. Muscle biopsy specimens were obtained as described previously [21] under local anaesthesia (mepivacain chloride 5 mg/ml; Carbocain, Södertälje, Sweden) from the vastus lateralis portion of the quadriceps femoris muscle and immediately placed in liquid nitrogen.

A subgroup of tetraplegic patients ($n = 4$) participated in an 8-week physical training course of ESLC. Training consisted of seven exercise sessions a week (one session a day for 3 days and two sessions a day for 2 days). Training bouts were carried out as described previously [20] on a computer-controlled functional electrical stimulation exercise ergometer (ERGYS-I-Clinical Rehabilitation System, Therapeutic Alliances, Fairborn, Ohio, USA) [22, 23]. A doctor and a physiotherapist supervised all ESLC sessions. Subjects did not undergo ESLC bouts for 48 h before muscle biopsy.

Extraction of RNA and quantification of mRNA. Muscle biopsy specimens (25–35 mg) were removed from liquid nitrogen and homogenised using a polytron mixer in 1 ml guanidium thiocyantate-phenol solution (Sigma Tri-Reagent, Sigma, St. Louis, Mo., USA). Extraction of total RNA and cDNA synthesis was carried out as described previously [24]. We quantified UCP2 and UCP3 mRNA by reverse transcription followed by competitive PCR, using a synthetic multispecific standard with target sequences for UCP2, UCP3 and β–microglobin, as described in detail previously [24]. The UCP3 primer pair recognises sequences shared by both the long ($UCP3_L$) and short ($UCP3_S$) form of UCP3 transcripts [11].

Statistics. Data are presented as means ± SEM. Student's unpaired t test was used to analyse differences in expression of UCP2 or UCP3 between able-bodied and tetraplegic subjects. Individual values ($n = 4$) of UCP2 or UCP3 expression before and after ESLC training are illustrated in scatter diagrams.

Results

Clinical and anthropometric characteristics of the tetraplegic and able-bodied study participants are presented in Table 1. In response to a questionnaire, the able-bodied subjects reported moderate levels of exercise (2–3 times a week). Ages were similar in the two groups. The BMI of able-bodied subjects was within the normal range. As expected, BMI of the tetraplegic patients, although within the normal range, was reduced compared with able-bodied control subjects as a result of muscle wastage ($p < 0.01$). The full blood chemistry of a similar cohort has been described previously [20].

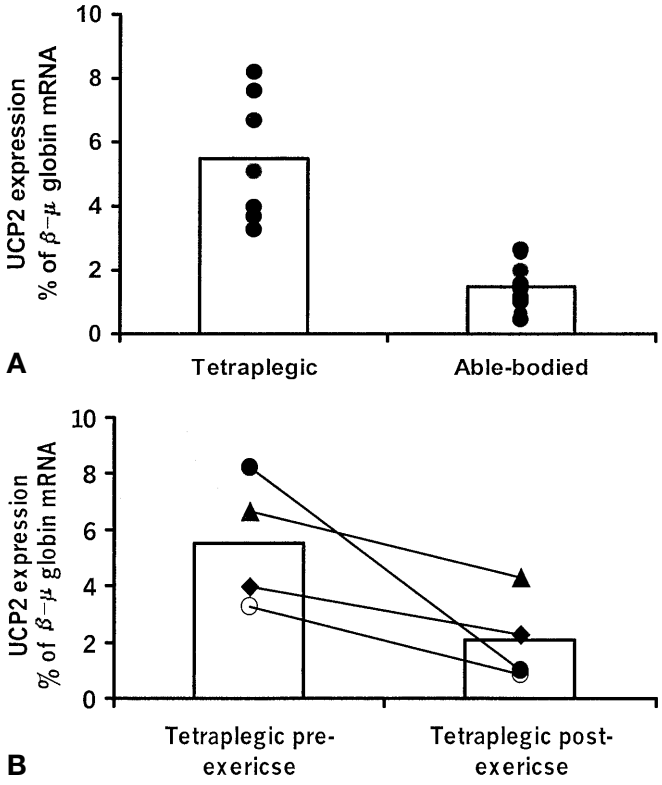

Fig. 1. **A** Expression of UCP2 mRNA in skeletal muscle from tetraplegic or able-bodied subjects. Individual results are reported as a percentage of β-microglobin mRNA. The superimposed bars show mean (5.49 ± 0.76 and 1.47 ± 0.26, for tetraplegic vs able-bodied subjects, means ± SEM, respectively) $p < 0.01$. **B** Expression of UCP2 mRNA in skeletal muscle from tetraplegic subjects before and after 8 weeks ESLC exercise training. Individual results are reported as a percentage of β-microglobin mRNA. The superimposed bar shows mean (5.52 ± 1.16 and 2.10 ± 0.79, for pre- vs post- exercise, means ± SEM, respectively)

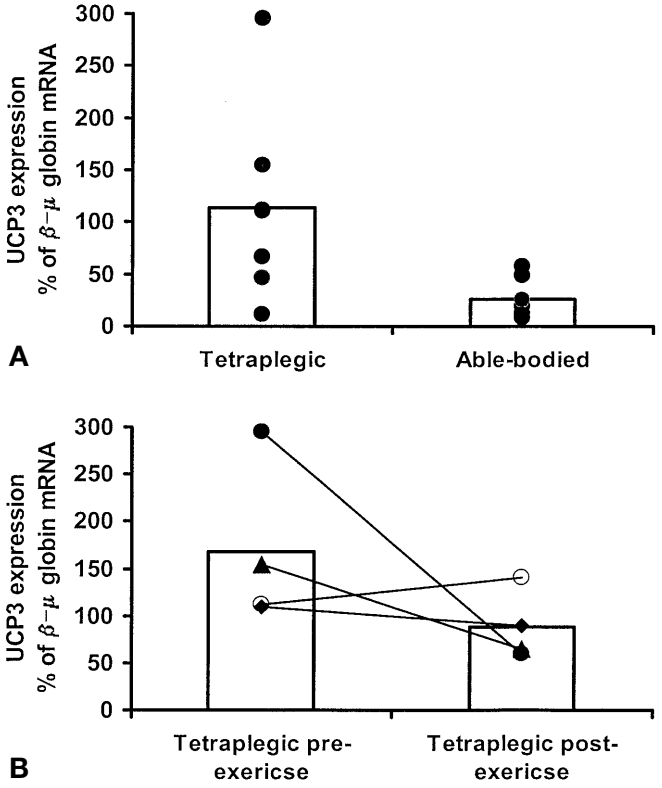

Fig. 2. **A** Expression of UCP3 mRNA in skeletal muscle from tetraplegic or able-bodied subjects. Individual results are reported as a percentage of β-microglobin mRNA. The superimposed bars show means (113 ± 35 and 26 ± 6, for tetraplegic vs able-bodied subjects, means ± SEM, respectively) $p < 0.05$. **B** Expression of UCP3 mRNA in skeletal muscle from tetraplegic subjects before and after 8 weeks ESLC exercise training. Individual results are reported as a percentage of β-microglobin mRNA. The superimposed bar shows means (167 ± 43 and 89 ± 15, for pre- vs post- exercise, means ± SEM, respectively)

Table 1. Subject characteristics

	Tetraplegic subjects	Able-bodied subjects
n	7	9
Age (years)	35 ± 2	30 ± 3
BMI (kg/m^2)	20.3 ± 1.2[a]	24.0 ± 0.4
Time since injury (years)	16.7 ± 4.8	–

Data are presented as means ± SE, [a] $p < 0.01$ significantly different compared with able-bodied subjects

Expression of UCP2 mRNA was assessed in skeletal muscle from tetraplegic or able-bodied people. Uncoupling protein 2 expression was 3.7-fold higher in skeletal muscle from tetraplegic than from able-bodied subjects ($p < 0.01$) (Fig. 1A). A subgroup ($n = 4$) of tetraplegic subjects undertook 8 weeks of ESLC training. Exercise training led to a pronounced reduction (62%)in UCP2 mRNA expression in skeletal muscle (Fig. 1B), achieving a similar expression to that in the able-bodied subjects (UCP2 mRNA 2.1 ± 0.8 vs 1.5 ± 0.3% of β-microglobin expression in exercised tetraplegic vs able-bodied subjects, respectively). Similar to the results for UCP2 expression, UCP3 gene expression was increased 4.1-fold in skeletal muscle from tetraplegic compared with able-bodied subjects ($p < 0.05$) (Fig. 2A). Exercise training led to a reduction in UCP3 gene expression in three of the tetraplegic subjects (Fig. 2B), although in contrast to UCP2, post-training UCP3 expression was still increased compared with able-bodied subjects (UCP3 mRNA 89 ± 15 vs 26 ± 6% of β-microglobin expression in exercised tetraplegic vs able-bodied subjects, respectively).

Discussion

Here we show that mRNA expression of UCP2 and UCP3 is considerably increased in skeletal muscle from tetraplegic subjects. Furthermore, long-term (8 weeks) intensive exercise training in a sub-group

of tetraplegic subjects led to a profound reduction in UCP2 expression, comparable with that in age-matched able-bodied subjects. Likewise training resulted in reduced UCP3 expression in three of the four tetraplegic subjects. In contrast to our results for UCP2, UCP3 expression remained increased compared with that in able-bodied subjects even after ESLC training.

Uncoupling protein 2 and UCP3 map to loci associated with hyperinsulinaemia and obesity [7]. Changes in the regulation of UCP2/3 have been associated with obesity and Type II (non-insulin dependent) diabetes mellitus, consequently, intense interest has focused on the regulation of these genes in skeletal muscle. We have recently reported UCP3 expression is reduced in skeletal muscle from lean, well controlled Type II diabetic subjects [24]. Reduced expression of UCP2 has been reported in adipose tissue and skeletal muscle of obese human beings [25, 26]. Furthermore, mutations in UCP3 have been reported in two obese and Type II diabetic probands [27]. Thus, alterations of uncoupling protein expression may play a part in metabolic disorders such as obesity and insulin resistance.

The increased UCP2 and UCP3 expression noted in skeletal muscle from tetraplegic subjects in this study is similar to the response noted in denervated rat skeletal muscle [19]. Interestingly, denervation of mouse muscle has been reported to lead to both increased [19] and reduced [28] UCP2 mRNA expression, while reducing UCP3 expression [19, 28]. Thus there appears to be notable species variation in the regulation of uncoupling protein gene expression in skeletal muscle. There is also conflicting data regarding the effect of exercise on UCP2 and UCP3 expression in rodent muscle. Exercise training has been reported to lead to down regulation [15] or no change [19] of UCP2 and UCP3 expression in rat skeletal muscle. In our study, intensive exercise training reduced the raised UCP2 expression returning it to normal in skeletal muscle from tetraplegic subjects. Thus in human beings, extreme muscle inactivity as observed with tetraplegia, appears to up-regulate skeletal muscle gene expression of UCP2 and UPC3. Furthermore, muscle activity through long-term exercise training decreases UCP2 expression in tetraplegic subjects. Whether exercise training of different intensity or different duration than that used in this study would modulate expression of UCP3 more profoundly is not known.

Subjects with chronic tetraplegia are characterised by low muscle mass and increased body fat, although their BMI is within normal limits [29, 30]. Training in chronic tetraplegic subjects has been shown to influence both insulin sensitivity and gross body composition [20, 30]. During short-term ESLC training, blood glucose and non-esterified fatty acid concentrations are decreased, due to a combined effect of increased whole body glucose uptake and decreased lipolysis [31]. Thus disturbances in glucose and fat metabolism in chronic tetraplegia [20, 29–32] coincide with higher UCP2 and UCP3 expression.

Increased facilitated thermogenesis in chronic tetraplegic subjects appears to be due to lower whole-body specific heat, reduced uptake of nutrients and severed pathways for central thermoregulatory mechanisms compared with able-bodied people [33]. Thus, it can be speculated that the altered UCP2 and UCP3 expression plays a part in the aberrant thermoregulation noted in chronic tetraplegic patients. Recent data, however, challenges the physiological importance of UCP2 and UCP3 as thermogenic mediators. For example, UCP1 ablated mice are unable to tolerate cold, despite pronounced up regulation of UCP2 gene expression in adipose cells [34]. Furthermore, food restriction, a situation which leads to the suppression of thermogenesis, leads to increased expression of both UCP2 and UCP3 in humans [16] and UCP3 in rats [12]. These results, together with those showing that UCP3 expression could be regulated by direct lipid infusion [17] or by dietary fat content [35, 36], are consistent with a role for UCP3 in regulating muscle lipid utilisation. Moreover, individuals heterozygous for a UCP3 splice polymorphism have reduced basal fat oxidation and an increased respiratory quotient, consistent with a role for UCP3 in metabolic fuel partitioning [27]. Interestingly, ESLC exercise training in tetraplegic subjects leads to greater reductions of blood glucose and non-esterified fatty acid concentrations compared with similar levels of exercise in able-bodied subjects [31].

In this study we have shown that skeletal muscle from tetraplegic subjects is characterised by increased expression of both UCP2 and UCP3 mRNA. Thus, extreme inactivity or cervical cord lesion leads to a higher expression of UCP2 and UCP3 in human skeletal muscle. Furthermore, intensive exercise training leads to a reduction of UCP2 expression in skeletal muscle from tetraplegic subjects to levels comparable with the expression in able-bodied people. Thus UCP2 expression appears to be regulated by exercise in human muscle, at least in a situation where expression is pathologically increased.

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