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Variation in the uncoupling protein 2 and 3 genes and human performance

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Dhamrait SS, Williams AG, Day SH, Skipworth J, Payne JR, World M, Humphries SE, Montgomery HE. Variation in the uncoupling protein 2 and 3 genes and human performance. *J Appl Physiol* 112: 1122–1127, 2012. First published January 12, 2012; doi:10.1152/japplphysiol.00766.2011.—Uncoupling proteins 2 and 3 (UCP2 and UCP3) may negatively regulate mitochondrial ATP synthesis and, through this, influence human physical performance. However, human data relating to both these issues remain sparse. Examining the association of common variants in the *UCP3/2* locus with performance phenotypes offers one means of investigation. The efficiency of skeletal muscle contraction, delta efficiency (DE), was assessed by cycle ergometry in 85 young, healthy, sedentary adults both before and after a period of endurance training. Of these, 58 were successfully genotyped for the *UCP3*-55C>T (rs1800849) and 61 for the *UCP2*-866G>A (rs659366) variant. At baseline, UCP genotype was unrelated to any physical characteristic, including DE. However, the *UCP2*-866G>A variant was independently and strongly associated with the DE response to physical training, with *UCP2*-866A allele carriers exhibiting a greater increase in DE with training (absolute change in DE of $-0.2 \pm 3.6\%$ vs. $1.7 \pm 2.8\%$ vs. $2.3 \pm 3.7\%$ for GG vs. GA vs. AA, respectively; $P = 0.02$ for A allele carriers vs. GG homozygotes). In multivariate analysis, there was a significant interaction between *UCP2*-866G>A and *UCP3*-55C>T genotypes in determining changes in DE (adjusted $R^2 = 0.137$; P value for interaction $= 0.003$), which was independent of the effect of either single polymorphism or baseline characteristics. In conclusion, common genetic variation at the *UCP3/2* gene locus is associated with training-related improvements in DE, an index of skeletal muscle performance. Such effects may be mediated through differences in the coupling of mitochondrial energy transduction in human skeletal muscle, but further mechanistic studies are required to delineate this potential role.

uncoupling protein; endurance; genetic variation; single nucleotide polymorphism; gene-environment interaction

THE MOVEMENT OF PROTONS down their chemiosmotic gradient and across the inner mitochondrial membrane provides the energy with which ATP is generated by ATP synthase. However, a proton leak uncoupled to ATP synthesis is responsible for <50% of oxygen consumption in tissue such as resting skeletal muscle (37). This seems regulated by nuclear-encoded, mitochondrial-targeted uncoupling proteins (UCPs) of the mitochondrial anion-carrier superfamily, of which three mammalian forms are recognized (UCP1–3). UCP1 expression is limited to brown adipose tissue (BAT) (29). Expression of UCP2 mRNA is identified in tissues including liver, white adipose tissue (WAT), and cardiac and skeletal muscle (19, 22), and its protein in human heart (34) and liver (45). UCP3 mRNA expression is largely confined to skeletal muscle and BAT, with smaller amounts in WAT and in cardiac tissue (3, 32, 46).

The UCPs may regulate the mitochondrial proton leak, and with it mitochondrial reactive oxygen species (ROS) generation in vitro (16, 46) and in vivo (36, 44). In thymocytes, up to 50% of the basal proton leak is seemingly dependent on UCP2 expression, whose reduction is associated with elevation in ATP levels (29). A similar role for UCP3 (6, 8) is also implicated in skeletal muscle, with proton leak (*state 4* respiration) being reduced in skeletal muscle mitochondria isolated from the *UCP3*(−/−) knockout mouse in some (23, 47) but not all studies (6). In whole animal studies, fasting skeletal muscle ATP synthesis rate is twice as high in *UCP3* knockout mice than in controls, implying an increase in mitochondrial coupling when UCP3 expression is reduced (9).

Free fatty acids upregulate expression of skeletal muscle UCP2 and UCP3 (25, 39). Skeletal muscle UCP expression is also modulated by exercise training. Eight weeks of endurance training is associated with 54% and 41% decreases in UCP2 mRNA expression in rat heart and tibialis anterior (type IIa and IIb fast-twitch fibers) muscle, respectively, with no associated changes in soleus (slow twitch) muscle (2). Cortright et al. failed to identify such an effect, a disparity perhaps related to differences in feeding pattern between experiments (11). Nonetheless, in keeping, acute exercise in the mouse did reduce UCP2 expression. Meanwhile, skeletal muscle UCP3 expression is reduced in response to endurance training in both rodents and humans (2, 11, 41). UCP3 protein content is 46% lower in the skeletal muscle of endurance-trained cyclists than in healthy untrained men, although the same hierarchy of content [most abundant in type 2b fast glycolytic > type 2a fast oxidative-glycolytic >type 1 slow oxidative fibres (26)] is retained (38). Such changes are independent of endurance training-related neo-mitochondrial biogenesis (20): vastus lateralis mitochondrial volume increases by 47% with 6 wk of endurance training in healthy men, but relative UCP3 protein content and uncoupled mitochondrial respiration decrease by 53% and 18%, respectively (18).

A common, functional promoter *UCP2* gene variant (*UCP2*-866G>A, rs659366) lies at the junction between negative and positive cis-acting DNA segments in a region containing binding sites for hypoxia-, inflammation-, and pancreatic β-cell-specific-binding factors (17). This polymorphism appears associated with altered gene function: the rare (A) allele has been

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associated with lower gene transcription in somatic non-β-cells (30), more effective gene transcription in pancreatic β-cells with reduced markers of β-cell function (30), and measures of reduced glucose-stimulated insulin secretion (42). It has been associated with protection from obesity (17), but also with the presence of diabetes in obese subjects (30) and with increased plasma markers of oxidative stress and prospective cardiovascular risk in diabetic subjects (15). Meanwhile, a common promoter polymorphism has also been identified in the *UCP3* gene (*UCP3*-55C>T, rs1800849), the rare allele being associated with obesity in a recessive manner in several studies (7, 24, 35).

Delta efficiency (DE) is a measure of the efficiency of skeletal muscle contraction and represents the ratio of external work performed to the internal energy expended (21). It might thus be that the *UCP2*-866A allele would be associated with lower UCP2 activity in cardiac and skeletal muscle and thus increased mitochondrial coupling (increased "efficiency"). Similarly, the *UCP3*-55T allele may represent a thrifty genotype, with enhanced mitochondrial coupling and preservation of substrate supply. On this basis, we sought to test the hypothesis that common genetic variation at the *UCP2/UCP3* locus might be associated with exercise training-related changes in skeletal muscle DE.

MATERIALS AND METHODS

Subjects were drawn from two studies of training-related change in DE that have been previously reported (48, 49). Each study had appropriate ethics committee approval, with written, informed consent obtained from each participant. All subjects and staff were blind to genotype during experimentation and data analysis.

Study subjects. Males were consecutive healthy Caucasian male British army recruits selected for homozygosity for the *ACE* I/D variant who underwent 11 wk of target-orientated training, as previously reported (49). This comprised a mixture of upper body strength and lower limb strength/endurance exercise (49). Females were healthy Caucasian volunteers recruited from the student and staff populations of the Staffordshire University (48), who had not been involved in any structured training program during the previous 6 mo and who underwent an 8-wk endurance training program. This comprised three nonsupervised sessions per week at 70–80% of maximum heart rate (as derived from the test of maximal oxygen uptake), with 20-min sessions for *weeks 1–4* increased to 30-min sessions for *weeks 5–8*. Subjects were trained to regulate their exercise intensity using a Polar heart rate monitor (Polar Electro, Kempele, Finland), and regular contact was maintained throughout training to ensure compliance.

Subject phenotyping. Measures of height and body mass were recorded at baseline and again at the end of the training period. Resting blood samples were drawn from a superficial forearm vein before the training period for subsequent genetic analysis.

DE was assessed before and after training in all subjects. Briefly, subjects cycled on an electrically braked cycle ergometer (Lode Rehcor, Lode, Netherlands) at 60 rpm at external power outputs of 40, 60, and 80 W for 4 min per stage. Expired air was analyzed breath by breath using a Cardiokinetics measurement cart (Medical Graphics), and heart rate was monitored telemetrically (Polar Electro, Polar, Kempele, Finland). A conversion factor dependent on respiratory exchange ratio was applied to the oxygen uptake measured to give rate of energy expenditure (4). DE was calculated as (Δ work performed per minute)/(Δ energy expended per minute).

Genotyping. Genomic DNA was extracted from 5 ml of whole blood. Genotypes were determined with polymersase chain reaction (PCR) amplification of the target gene sequence using published primers and conditions (7, 17). For *UCP2*-866G>A (rs659366), the 360-bp PCR product was digested with the restriction endonuclease *Mlu1* to yield 290 + 70-bp fragments in G-allele carriers only. For *UCP3*-55C>T (rs1800849), the 194-bp product was digested with the enzyme *BsuR*1 to yield 110-, 64-, and 20-bp fragments for the C allele and 110- and 84-bp fragments for the T allele. Products were resolved on a 7.5% polyacrylamide gel and confirmed by two independent technicians blind to subject outcome, with discrepancies resolved by repeat genotyping (14).

Statistical analysis. DE and the change in DE with training were both normally distributed. Differences in baseline characteristics and DE were compared between genotype groups. For the whole sample, characteristics were compared between genotype groups (including those defined by the presence/absence of a specific allele) using one-way ANOVA, two-tailed unpaired *t*-tests, linear trend analysis, and one-way analysis of covariance with sex as a covariate. Within each sex, characteristics were compared between genotype groups and between allele groups using one-way ANOVA, two-tailed unpaired *t*-tests, and linear trend. DE responses to training were compared between genotype groups and allele groups using two-way ANOVA with repeated measures on one factor (time). All data were analyzed using SPSS (SPSS, IBM). Data are presented as means ± SD. Using a Bonferonni correction for multiplicity of testing (two gene loci), a P value of <0.025 was considered statistically significant for genetic association. A power calculation would suggest a sample size of 26 would yield 80% power ($\alpha = 0.05$, two tailed) to detect a 2% difference in DE after training between genotype groups in an additive model.

RESULTS

There were 85 subjects who completed training (28 women). There was no gender difference in baseline DE (baseline DE men 24.7 ± 2.6%, women 24.3 ± 2.7%; $P = 0.5$). Training resulted in a significant increase in DE overall (1.0 ± 3.5%; $P = 0.01$ compared with baseline). There was no gender difference in this increase in DE ($P = 0.9$), but the increase was only significant in the male sample (absolute change in DE men 1.0 ± 3.5%; $P = 0.04$ compared with baseline) and not in the smaller female sample (absolute change in DE women 0.9 ± 3.6%; $P = 0.2$ compared with baseline).

Data on those who had completed training and who were successfully genotyped for *UCP2*-866G>A (58/85; 68%) and *UCP3*-55C>T (61/85; 72%) are shown in Table 1. The low genotyping rate was due to degradation of DNA from the original DE study. There was no difference in baseline characteristics between those with and without genotype data.

Table 1. *Baseline characteristics of the 85 subjects who completed training, including genotype and rare allele frequencies for those subjects then genotyped for the UCP3−55C>T and UCP2−866G>A variants*

Trait	Mean (SD)
Age, yr	20.7 (4.4)
Mass, kg	70.4 (9.4)
Height, m	1.74 (0.08)
Delta efficiency, %	24.6 (2.6)
UCP3−55C>T (61 subjects)	
CC/CT/TT (*n*)	29/27/5
T allele frequency, 95% CI	0.303 (0.222–0.385)
UCP2−866G>A (58 subjects)	
GG / GA / AA (*n*)	21/22/15
A allele frequency, 95% CI	0.448 (0.358–0.539)

CI, confidence interval.

Table 2. *Training-related change in delta efficiency according to UCP2-866G>A and UCP3-55C>T genotypes*

UCP Genotype (*n*)		Delta Efficiency, %			
		Pre	Post	Absolute Change, %	Proportional Change, %
UCP2−866	GG (21)	24.6 ± 2.6	24.4 ± 2.8	−0.2 ± 3.6	0.2 ± 14.6
	GA (22)	24.3 ± 3.0	26.1 ± 3.1	1.7 ± 2.8	7.9 ± 12.6
	AA (15)	24.0 ± 2.2	26.2 ± 3.3	2.3 ± 3.7	10.1 ± 15.1
	A allele	24.2 ± 2.7	26.1 ± 3.1	2.0 ± 3.1	8.8 ± 13.5
P ANOVA		0.8	0.1	0.07	0.08
P linear trend		0.9	0.07	0.03	0.03
P GG vs. A allele		0.5	0.04	0.02	0.03
UCP3−55	CC (29)	24.2 ± 2.8	25.8 ± 2.9	1.5 ± 3.3	7.1 ± 13.4
	CT (27)	24.6 ± 2.7	25.3 ± 3.3	0.6 ± 3.5	3.3 ± 14.7
	TT (5)	26.1 ± 1.1	24.9 ± 1.2	−1.2 ± 1.8	−4.3 ± 6.6
P ANOVA		0.4	0.8	0.2	0.2
P linear trend		0.4	0.5	0.09	0.08

Data are expressed as means ± SD.

UCP2 and *UCP3* genotypes were consistent with predicted Hardy Weinberg frequencies, with the rare allele frequencies similar to those previously reported (7, 17). There was no evidence of linkage disequilibrium between the two genotypes (delta −0.14; $P = 0.73$).

There were no significant associations between either *UCP2* or *UCP3* genotype and any baseline measurements including BMI and DE (Table 2). However, *UCP2*-866A allele carriers had significantly higher gains in DE during training, with resultant higher DE after training (Table 2; absolute change in DE: −0.2 ± 3.6% vs. 1.7 ± 2.8% vs. 2.3 ± 3.7% for GG vs. GA vs. AA, respectively; $P = 0.03$ by linear trend; $P = 0.02$ for A allele carriers vs. GG homozygotes; Fig. 1). In univariate analysis, *UCP2*-866 genotype and the presence or absence of the *UCP2*-866A allele accounted for 8.4% and 7.4% (adjusted R^2, respectively) of the interindividual variability in the absolute change in DE associated with endurance training. This effect was independent of any baseline characteristic of age, gender, height, and mass, and remained significant in a multivariate model containing these characteristics. Although rare *UCP3*TT homozygotes had lower DE after training, this did not reach statistical significance (Table 2). Similar genotype effects for the two gene variants were seen in both men and women (Table 3), in keeping with a gender-independent effect. There was a significant interaction between *UCP2*-866G>A and *UCP3*-55C>T genotypes, and the associated change in DE with training (adjusted $R^2 = 0.137$, P for interaction = 0.003; Fig. 2). The interaction effect was independent of the effect of either single polymorphism and of any baseline characteristic (gender, height, and mass) such that, in a further multivariate model, the *UCP2*-866A allele and the interaction between *UCP2* and *UCP3* genotypes together accounted for 14.8% of the variation in training-related change in DE (adjusted R^2).

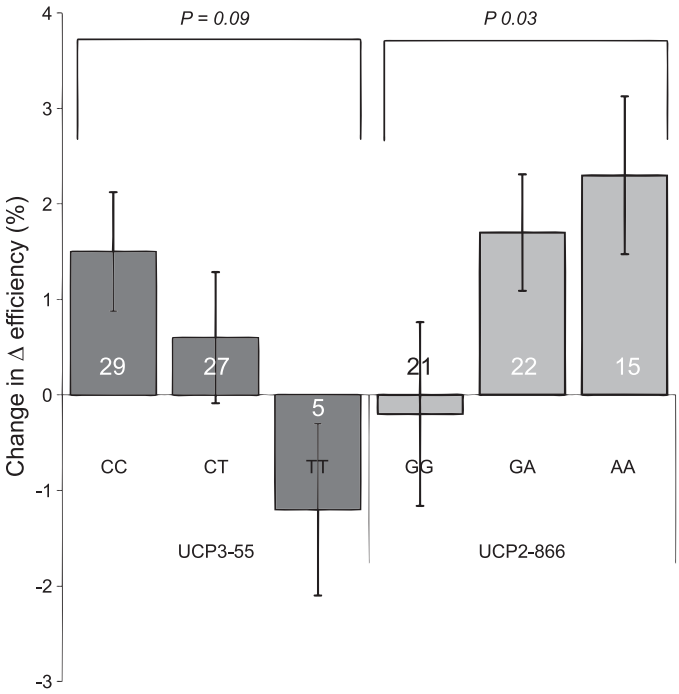

Fig. 1. Training-related changes in delta efficiency (means ± SE) according to *UCP3*-55C>T and *UCP2*-866G>A genotypes. Numbers within each genotype group are displayed at the base of each respective bar. *P* value relates to linear trend analysis.

DISCUSSION

We show, for the first time in humans, that variation in the UCP2 and UCP3 genes is associated with differences in the endurance training-related changes in DE. The *UCP2*-866A allele carriers had significant increases in DE (mean absolute increase of 2% DE) after training, whereas GG homozygotes had, on average, no change in DE after training. The small number of *UCP3*-55TT homozygotes tended to have lower DE after training. Statistical analysis suggested that these two SNPs might together account for up to 14.8% of interindividual variation in training-related change in DE. This suggestion of a role for UCPs in altering DE is supported by the findings of others in rodents: soleus muscle from mice, which overexpress *hUCP3*, produces greater heat energy during isometric exercise (i.e., is less efficient) than that from control animals (13).

Most of the observed association in this study was due to variation at the *UCP2*-866 locus, with *UCP2*-866A allele carriers benefiting from greater efficiency after formal supervised training. The *UCP2*-866G>A polymorphism has been shown to be functional in vivo and in vitro (30, 42). Promoter constructs of the −866A allele are associated with greater repression of transcription in somatic cells (30). Furthermore, the −866G>A variant appears to be strongly associated with functionality across the gene cluster (17), and the *UCP2*-866A

Table 3. *Training-related change in delta efficiency by gender according to UCP2-866G>A and UCP3-55C>T genotypes*

		Delta Efficiency, %					
		Pre		Post		Absolute Change, %	
UCP2−866G>A genotype (*n*)		Men	Women	Men	Women	Men	Women
UCP2−866	GG (men 10, women 11)	25.0 ± 2.9	24.3 ± 2.4	24.7 ± 1.8	24.2 ± 3.5	−0.3 ± 2.9	−0.1 ± 4.3
	GA (men 10, women 12)	23.7 ± 3.1	24.9 ± 3.0	25.4 ± 2.9	26.7 ± 3.2	1.7 ± 2.5	1.8 ± 3.1
	AA (men 10, women 5)	24.5 ± 1.7	22.9 ± 2.8	27.3 ± 3.5	24.0 ± 1.4	2.8 ± 3.9	1.1 ± 3.3
	A allele	24.1 ± 2.4	24.2 ± 3.0	26.3 ± 3.3	25.9 ± 3.0	2.2 ± 3.2	1.6 ± 3.1
P ANOVA		0.6	0.4	0.1	0.1	0.1	0.5
P linear trend		0.7	0.5	0.05	0.7	0.04	0.4
P GG vs. A allele		0.4	1.0	0.1	0.2	0.05	0.2
UCP3−55	CC (men 12, women 17)	24.5 ± 2.8	24.0 ± 2.9	25.9 ± 2.0	25.7 ± 3.5	1.4 ± 2.8	1.6 ± 3.7
	CT (men 18, women 9)	24.9 ± 2.8	24.2 ± 2.6	25.5 ± 3.4	24.7 ± 3.2	0.6 ± 3.6	0.5 ± 3.5
	TT (men 3, women 2)	25.5 ± 0.9	26.9 ± 1.0	25.6 ± 1.0	23.9 ± 0.4	0.0 ± 0.4	−3.0 ± 1.5
P ANOVA		0.8	0.4	0.9	0.7	0.7	0.2
P linear trend		0.5	0.3	0.8	0.4	0.4	0.1

Data are expressed as means ± SD.

genotype has been associated with markers of oxidative stress in diabetics and an increased risk of prospective coronary heart disease among healthy men (15).

Such effects may relate to UCP-dependent differences in mitochondrial proton leak/uncoupling, and thus in efficiency of ATP genesis relative to oxygen consumption. However, other mechanisms might be postulated. UCPs might remove fatty acid anions from the mitochondrial matrix to reduce mitochondrial lipotoxicity. This may occur so as to remove excess fatty acid anion oversupply, which would accumulate passively within the matrix according to membrane potential (40), or alternatively (as part of a fatty acid cycle) to allow removal of excess fatty acid during oversupply, which might otherwise lead to accumulation of intramitochondrial fatty acyl-CoAs and with consequent limitation of the CoA matrix pool available for fatty acid oxidation (27). Alternatively, both UCP2 and UCP3 might offer protection from mitochondrial ROS generation (16, 33) and may be activated by AMP kinase during fuel depletion (50) and alter insulin sensitivity in skeletal muscle (12).

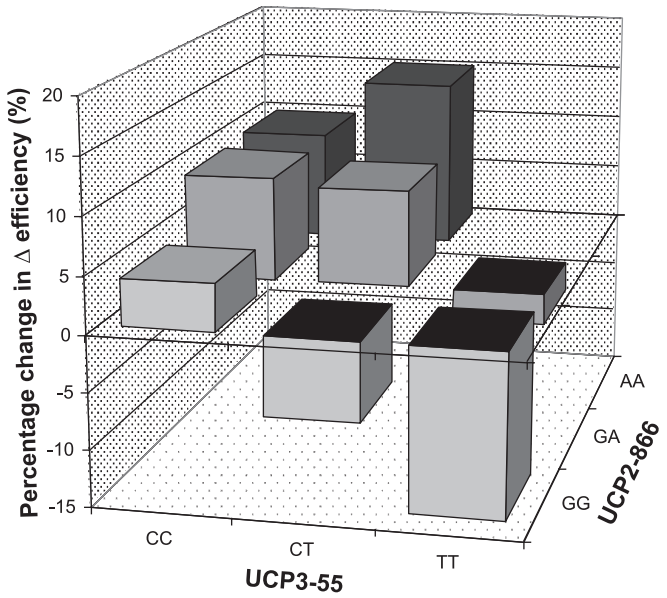

Fig. 2. Training-related changes in delta efficiency (means ± SE) by *UCP3* and *UCP2* haplotypes.

DE is a measure of efficiency of skeletal muscle contraction and represents the ratio of external work performed to the internal energy expended. As a phenotype, DE varies little between individuals, and reported changes with exercise, as here, are often numerically small. However, this does not mean that small changes in DE are without substantial biological impacts. Indeed, changes in economy of oxygen use may be associated with substantial differences in physical performance (10). As such, variation in DE (or in the genes that influence it) might be expected to influence endurance performance phenotypes or adiposity. No previous studies have explored the association of these genetic variants with DE measures. A study of the 89 fastest and 89 slowest Caucasian male South African Ironman triathletes revealed no clear difference in frequency of the *UCP3*-55C>T variant (28), although the body mass index of obese Caucasians may be negatively associated with physical activity levels in those of *UCP3*-55CC genotype (35). Meanwhile, linkage between a *UCP3* Y210Y(C>T) polymorphism and baseline body mass index and fat mass, and linkage with training-related changes in adiposity among whites have been suggested (31). However, in keeping with our suggestion of a role for *UCP* genotype in influencing metabolic efficiency, Buemann and colleagues found gross exercise efficiency at 40% maximal oxygen consumption to be higher among UCP2–55val/val homozygotes in a smaller study of 16 individuals genotyped for the *UCP2* exon 4 + 164C>T coding variant (5). Similarly, Astrup reported daily energy expenditure (adjusted for adiposity and spontaneous physical activity) to be lower among *UCP2–55* val/val homozygotes and spontaneous physical activity to be 20% higher (1).

Our study does have some weaknesses. First, our studies are individually not large and address only young Caucasian men and women. We would advocate that they should be extended to those of different age and ethnicity. Second, we did not control for alterations in diet over the training period. Although such an associated behavioral change cannot be discounted, we consider it perhaps unlikely to have occurred consistently (and in a manner dependent on UCP genotype) in such different training environments. Third, the training regimens applied to men and women, while both having a substantial component

relating to lower limb endurance, were different. However, the fact that a consistent genotype association was identified in both groups suggests a meaningful biological role for UCP genotype when interacting with an exercise training stimulus. Fourth, compliance with army training was uniform and complete, with all exercise being standardized, supervised, and target-orientated. For women, compliance with the nonsupervised training sessions was confirmed by means of regular contact. The physiological changes identified confirm that training had been undertaken by the cohort. Although it is possible that some reporting inaccuracy occurred, it seems unlikely that compliance failure would have occurred in a systematic way that was itself genotype-dependent. Finally, we are unable to ascertain conclusively which gene product (UCP2 or UCP3) is responsible for the observed association with DE, since the *UCP2* and *UCP3* genes are separated by only 7 kB in chromosomal region 11q13 (43). However, the lack of LD between the two genotypes studied might suggest independent effects of both UCP2 and UCP3 on performance. Much larger sample sizes, perhaps combined with deeper sequencing, would be required to address this issue. Furthermore, any causality would need to be determined from in vitro and in vivo human studies.

In summary, sequence variations at the *UCP3/2* gene locus are associated with differences in training-related gains in skeletal muscle DE. Further genetic studies are required to extend these results to larger study groups, including different ethnicities. Future work should investigate whether *UCP2* and/or *UCP3* are directly responsible for the observed changes in DE or whether they perform a permissive role, such as channeling fatty acids or reducing oxidative damage during high-intensity exercise.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: S.S.D., S.E.H., and H.E.M. conception and design of research; S.S.D., A.G.W., S.H.D., and J.R.P. performed experiments; S.S.D., S.E.H., and H.E.M. analyzed data; S.S.D., S.E.H., and H.E.M. interpreted results of experiments; S.S.D. prepared figures; S.S.D. and J.R.S. drafted the manuscript; S.S.D., A.G.W., S.H.D., M.W., S.E.H., and H.E.M. edited and revised the manuscript; S.S.D., A.G.W., S.H.D., J.R.S., J.R.P., M.W., S.E.H., and H.E.M. approved the final version of the manuscript.

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