



PAPER

The association between the val/ala-55 polymorphism of the uncoupling protein 2 gene and exercise efficiency

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BACKGROUND: Energy expenditure may partly be determined by genetic variations in uncoupling proteins. We have previously found an increased physical activity but a similar 24-h energy expenditure (EE) in subjects with the val/val-55 UCP2 genotype compared to those with the ala/ala genotype which indicates that the val-55 allele is statistically associated with a higher metabolic efficiency.

DESIGN: EE during bicycling was determined by indirect calorimetry at three different loads (30, 40 and 60% of VO_{2max} in eight subjects with the val/val-55 genotype (35 ± 6 y, weight = 76.8 ± 13.6 kg, $VO_{2max} = 2.79 \pm 0.71$ l/min) and eight subjects with the ala/ala-55 genotype (37 ± 3 y, weight = 78.3 ± 16.5 kg, $VO_{2max} = 2.66 \pm 0.41$ l/min).

RESULTS: Incremental exercise efficiency across the three different work levels was higher in the val/val (25.3%, c.i. 24.2–26.4%) than in the ala/ala (23.6%, c.i. 22.5–24.7%) genotype $P < 0.05$. Gross exercise efficiency at 40% VO_{2max} was higher in the val/val ($15.3 \pm 0.6\%$) than in the ala/ala ($13.5 \pm 0.4\%$) group.

CONCLUSION: As the val/ala-55 polymorphism is located in a domain of the protein without any known function, the different exercise efficiency between the two genotypes most likely reflects a linkage disequilibrium with a functionally significant polymorphism in UCP2 or in the neighbouring UCP3 gene. The study suggests that variations in the UCP genes may affect not only basal metabolic rate but also influence energy costs of exercise.

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Introduction

Proton leak across the inner mitochondrial membrane allows for a heat dissipation which is uncoupled from the production of chemical energy in the form of ATP. The finding that permeability to protons is about five times greater in mitochondria from rat hepatocytes compared to lizard hepatocytes¹ suggests that mitochondrial uncoupling may play an important role in heat production in homeothermic animals. Proton leak has been estimated to be responsible for 52 and 26% of the energy expenditure in resting rat skeletal

muscles and liver, respectively.² Although fatty acid composition of the mitochondrial membrane probably plays an important role in its proton conductivity properties¹ uncoupling proteins constitute a particularly futile proton transport mechanism. Thermogenin, now referred to as uncoupling protein (UCP1), was the first of a group of three comparable proteins which have been characterized so far. UCP1 was demonstrated to be responsible for the high thermogenic capacity of the brown adipose tissue.³ More recently, a protein with a 59% amino acid similarity to UCP1 has been identified.^{4,5} In contrast to UCP1 which is exclusively expressed in brown adipose tissue⁶ this protein, now being termed UCP2, is expressed in a variety of different tissue including white adipose tissue and skeletal muscle.⁴ In experiments with yeast cells transfected with the UCP2 gene, an uncoupling capability of UCP2 similar to that of UCP1 has been observed as a reduction of inner mitochondrial

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membrane potential.⁴ Shortly after the detection of UCP2, a gene for a third protein (UCP3) was discovered with a 57% and 71% amino acid similarity with UCP1 and UCP2, respectively.⁷ Based on clone analyses the chromosomal distance between the UCP2 and UCP3 genes has been determined to be only 7 kilobases.⁸ UCP3 is predominantly expressed in skeletal muscles and brown adipose tissue⁷ and has also been shown to possess a reducing capability on the mitochondrial membrane potential in yeast.⁹ A thermogenic role of UCP3 is supported by the findings that transgenic mice overexpressing human UCP3 in skeletal muscles are hyperphagic but remain leaner than the wild-type.¹⁰

In large adult mammals like humans with little brown adipose tissue, UCP2 and UCP3 may play a greater role in thermogenesis than UCP1 as they are expressed in major organ systems. In fact, a positive correlation has been found in Pima Indians between sleeping metabolic rate adjusted for variations in body composition and UCP3 gene expression in the vastus lateralis muscle.¹¹ Furthermore, other investigations in humans have shown associations or linkages between energy expenditure (EE) and variations in the actual UCP2 or UCP3 genes or neighboring markers^{12,13} whereas other investigators reported no such relationships.¹⁴ Recently, we reported that the ala/val 55 polymorphism in the UCP2 gene was associated with differences in spontaneous physical activity (SPA) in healthy young caucasian Danish subjects of both gender during measurements in a metabolic chamber.¹⁵ By comparing 21 subjects having the ala/ala genotype to 10 subjects with the val/val genotype, the latter had a 20% higher 24-h SPA. Despite the higher SPA, 24-h EE adjusted for fat free mass was similar in the two groups and this yielded a lower 24-h EE in the val/val subjects if SPA was statistically accounted for.

As there are implications that uncoupling proteins may also play a role in determining exercise efficiency¹⁶ we found it relevant to investigate if genetic variability in UCPs may affect energy expenditure during low to moderate exercise. The study reported in the present paper therefore tests bicycling efficiency in groups of subjects which are homozygous for the ala and val alleles respectively using a protocol with different work loads.

Methods

Four men and four women with the val/val-55 genotype for the UCP2 gene (age 36.4 ± 2.1 y, weight 76.8 ± 4.8 kg, body mass index (BMI) 23.7 ± 0.9 kg/m², maximal oxygen consumption (VO_{2max}) 2.79 ± 0.25 l/min) were recruited from a group of 60 individuals who had been characterized for this polymorphism in a previous investigation.¹⁵ Because the ala/ala-55 genotype was more frequent amongst the 60 subjects previously tested (35 vs 17%) it was possible from this group to select subjects with ala/ala-55 who matched the val/val-55 group with regard to age, weight and VO_{2max} : (age 36.6 ± 1.1 y, weight 78.3 ± 5.8 , BMI 26.2 ± 1.5 kg/m², VO_{2max} 2.66 ± 0.15 l/min). The subjects were medically

examined at the previous investigation¹⁵ and appeared healthy without any endocrine disorders and had no regular intake of medicine.

Each subject was tested on an automatically braked bicycle (Ergoline 900, Jaeger) at three different work levels which were aimed to correspond to 20, 35 and 55% of the subjects individually determined VO_{2max} . Each work level was performed for 20 min and the three levels were imposed in sequence in a continuous test increasing from the lowest to the highest level. A pedalling rate of 70/min was maintained at all work levels. Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were measured by sampling expiratory air through a face mask (Oxycon Champion, Mijnhardt, The Netherlands) and EE was calculated from deWeirs equation.¹⁷ Only the last 10 min measurements of each work level were used to determine exercise efficiency. If oxygen consumption was unstable during this period we successfully selected another stable 10 min period within the 20 min periods, except for the highest level in one subject. Data from this case were discarded in the statistical analyses.

VO_{2max} was determined by a progressive bicycling test where the same equipment was used as in the efficiency tests. After 10 min 50 Watts the work load was increased by 20 Watts/min for men and 15 Watts/min for women until voluntary exhaustion while the pedalling rate was maintained at 70/min. As oxygen uptake was continuously measured during the VO_{2max} test we also used this test to determine the three different work loads for the efficiency test which should correspond to 20, 35 and 55% of VO_{2max} . Work loads one or two steps before reaching the aimed oxygen consumption were selected to account for the time lag in the subjects' oxygen uptake due to the rapidly increasing exercise intensity. Nevertheless, this decision proved to be insufficient as the actual relative intensities during the efficiency test were 30, 41 and 60% of VO_{2max} on average. However, the highest work load still appeared to be below the anaerobic threshold which occurred between 70 and 80% VO_2 for these subjects based on the observation of ventilatory thresholds during the VO_{2max} test.

To reduce the variation on substrate partitioning during the exercise test due to variation in the subjects habitual diet, we delivered a fixed diet which was prepared by a metabolic kitchen to be consumed at home during the two days prior to the efficiency tests. The diets energy content was individually determined according to an estimated daily energy requirement based on the subjects fat free mass (FFM). FFM was assessed by bioelectrical impedance (ANIMETER, H.T.S. Engineering, Odense, Denmark) using an algorithm given by Heitmann.¹⁸ Daily energy provided (kJ) was determined as $158.3 * FFM$ (kg) + 2071 since we used an equation for basal metabolic rate (BMR) developed from previous indirect calorimetry measurements performed in our department¹⁹ and assumed a physical activity level (PAL) factor of 1.45 which corresponds to a sedentary life style.²⁰ The diet had an energy distribution of 50, 35 and 15 energy-% carbohydrate,

fat and protein, respectively. The efficiency tests were performed in the morning after a 12-h fasting period. The subjects were instructed to refrain from any sports or other excessive physical activities throughout the last two days before the efficiency tests.

The subjects were characterized for the val/ala-55 UCP2 polymorphism by the restriction fragment length polymorphism (RFLP) technique. Genomic DNA was purified from leukocytes and a DNA sequence containing the actual polymorphism was amplified by polymerase chain reaction (PCR) using the primers 5'-catttggecgctgcaggccgg-3' and 5'-gggccagtgcgcgctgcag-3'. The PCR product was digested with *BbvI* as previously described.²¹

Calculations and statistics

Gross efficiency for each work level was calculated as total external work output divided by EE determined from respiratory data. We also determined incremental work efficiency in each subject by the regression slope for EE against work output by entering the three different observations in linear regression analyses.

Anthropometric measures, VO_{2max} , gross efficiency, respiratory exchange ratio (RER) and relative exercise intensity were compared at each exercise level between the two groups by one way ANOVA using the SIGMASTAT program (Jandel Scientific, Erkrath, Germany). Split plot analyses were applied to test group differences in RER and gross efficiency when including all three work levels by the use of SPSS version 10.0 (SPSS Inc, Chicago, IL). Covariance analyses were performed with STATGRAPHICS software, version 4.2 (Graphic Software Systems Inc, Rockville, MD). The incremental work efficiency in the two groups was compared by ANOVA weighed with the standard deviations of the estimates of the regression slopes. The GLM procedure in the SAS 6.12 statistical software package (SAS Institute Inc, Cary, NC) was used for this analysis.

Results

The subjects exercised at similar relative intensities in the two groups at all three work levels (Table 1). Gross efficiency was higher in the val/val compared to the ala/ala subjects at

the intermediate level whereas the difference did not reach statistical significance at the lowest and highest levels (Table 1). Incremental efficiency was 25.4%, c.i. 24.3–26.5% in the val/val group which was higher than the 23.6%, c.i. 22.5–24.7% calculated for the ala/ala subjects ($P=0.03$). There was no significant difference between the two groups in RER at any work level, however, a split plot analysis across the three levels disclosed a trend of a higher RER in the val/val genotype (Table 1). RER did not correlate with relative exercise intensity within each of the three levels. Gross efficiency did not correlate significantly with RER at any work level ($r^2 < 0.19$, $P > 0.10$) and the group difference of gross efficiency at the intermediate level remained significant after the inclusion of RER as a covariate in the analysis.

Discussion

The present finding of an association between a polymorphism in the UCP2 gene and exercise efficiency when assessed both as gross and incremental efficiency suggests that UCP2 itself or other proteins encoded for in neighboring genes may be involved in EE during exercise. Observations of negative correlations between exercise efficiency and the level of UCP2 and UCP3 gene expression in vastus lateralis muscle support this hypothesis.¹⁶ The same polymorphism did not associate with sleeping EE in our former study¹⁵ where 60 individuals were measured in a respiration chamber and this was in line with a Swedish study performed in a closely related population where no relationship between the ala/val-55 polymorphism and BMR was found.¹⁴ However, the val/val genotype had a lower 24-h EE after adjustment for differences in FFM, FM and spontaneous physical activity in our former study which points in the same direction as the increased exercise efficiency of the val/val subjects found in the present study with subjects selected from the former study. It may therefore be hypothesized that a certain level of physical activity is required before the actual relationship becomes physiologically significant. Nevertheless, an association of the ala/val-55 polymorphism with sleeping EE has previously been reported in non-diabetic Pima Indians. Hence, the dependence of the results on study populations indicates that the positive findings may reflect a linkage disequilibrium between the ala/val-55

Table 1 Exercise data for the three different work levels

Level	Relative intensity % VO_{2max}			Respiratory exchange ratio (RER)			Gross efficiency %		
	1	2	3	1	2	3	1	2	3
val/val	29.6 (1.9)	40.8 (1.6)	59.5 (1.5)	0.881 (0.010)	0.898 (0.005)	0.926 (0.004)	10.8 (0.5)	15.3 (0.6)	18.1 (0.5)
ala/ala	30.3 (1.1)	40.5 (0.9)	59.7* (0.7)	0.862 (0.007)	0.883 (0.006)	0.915* (0.005)	9.7 (0.3)	13.5 (0.4)	17.0* (0.5)
<i>P</i>	0.78	0.88	0.92	0.16	0.09	0.14	0.11	0.02	0.13
<i>P*</i>		0.81			0.07			0.06	

Data are mean and s.e.m. (brackets). * $n=7$ as the one subject who did not reach a stable VO_2 was excluded.
*P** the three different levels analyzed pooled in split plot analyses.

polymorphism and other polymorphisms having a functional significance rather than the polymorphism, *per se*, playing an active role. Furthermore, if analogy to the UCPI protein is made the position of codon 55 of the UCP2 gene can be deduced to correspond to a domain which has no known function and the valine for alanine would probably not result in any change in the tertiary structure of the protein.²¹

Relationships between RMR and various microsatellite markers next to the UCP2 gene demonstrated by linkage analyses performed in 640 French Canadians¹² further supports the theory that genetic variation in the chromosomal vicinity of the UCP2 gene plays an important role in the determination of EE. However, due to the short distance between the UCP2 and UCP3 genes, these genetic markers may also represent variation in UCP3. In favour of a more important role for UCP3 in thermoregulation than UCP2 is the observation in rats that the UCP3 gene expression, in contrast to the UCP2 gene expression, is sensitive to thyroid hormone.⁹ Furthermore, UCP2 lacks both of the two histidine residues which have been found to be crucial for the H⁺ translocation function of UCP1 in one²² but not in another²³ study. UCP1 sustains some of its uncoupling activity with one of these histidine residues preserved.²² UCP3 only lacks one of them²² and an uncoupling role for UCP3 is indicated by the finding that skeletal muscle mitochondria of UCP3 gene knockout mice are more coupled (increased state 3/state 4 respiration ratio).²⁴ Finally, UCP3, but not UCP2, gene expression in skeletal muscles has been reported to be positively correlated with sleeping energy expenditure in Pima Indians.¹¹

The transmembrane proton transport by uncoupling proteins seems to be dependent on the presence of free fatty acids (FFA) but their actual role is still unclear.²⁵ However, if fatty acids take an active part in the proton translocation, the fatty acid distribution between the intermembrane space and matrix and, in turn, fat oxidation may be affected by the level of UCP activity. In favour of a regulatory role of UCP2 and UCP3 in fat utilization are the observations that gene expression of UCP2 and UCP3 in the skeletal muscles is upregulated by energy restriction^{26,27} and that UCP3 gene expression both in humans²⁸ and rodents²⁹ has been shown to increase by the elevation of circulatory FFA, *per se*, and by aerobic training.³⁰ In support of an important role of UCP3 in fatty acid oxidative capability is the observation in African Americans that individuals who are homozygous for an exon 6 splice donor stop mutation which causes the loss of the last transmembrane motif of the protein have a substantial higher resting respiratory quotient (RQ).³¹ In our former respiration chamber study we found a higher RQ in the val/val compared to the ala/ala carriers¹⁵ and in the present study the val/val genotype was associated with a borderline significantly higher RER during exercise. The higher RER did not explain the higher exercise efficiency in this group of participants because it remained higher after RER was included as a covariate when gross efficiency was examined.

Both a low 24-h EE³² and a high 24-h RQ³³ measured under standardized conditions have been suggested to predispose for weight gain. An impaired UCP function could therefore be speculated to cause overweight. In fact, a negative correlation between skeletal muscle UCP3 gene expression and BMI has been found in Pima Indians.¹¹ Moreover, an insertion/deletion polymorphism in an untranslated part of exon 8 of the UCP2 gene has been observed to be related to BMI in these Indians.¹³ Furthermore, linkage analyses on a French Canadian population have revealed associations between body fat percent and microsatellite markers encompassing the UCP2 gene. In contrast, similar BMI was seen in val/val-55 and ala/ala-55 UCP2 genotypes in 60 randomly recruited Danish subjects¹⁵ and genotypes of this polymorphism have also been found to be equally distributed among obese and non-obese Danish men²¹ which also was the case for another identified polymorphism of the UCP2 gene, an insertion variant of its 3' untranslated region.³⁴ The apparent absent relationship between adiposity and the val/ala-55 polymorphism despite that, in the same population, it seems to relate to energy expenditure and fat oxidation may be explained by its possible associations with counteracting factors. One possibility could be that the increased exercise efficiency is compensated for by more physical activity. Findings from our previous study of a higher 24-h spontaneous physical activity score in the val/val-55 subjects may imply this.¹⁵ Locomotions might be directly facilitated by a greater exercise efficiency or the val/ala-55 UCP2 polymorphism may be contemporarily associated with energy expenditure and an unknown factor which influences spontaneous physical activity. Lack of obesity in UCP3 knockout mice despite the demonstration of an increased mitochondrial coupling²⁴ may also suggest that other thermogenic mechanisms may compensate for a reduced uncoupling activity.

Conclusion

The present investigation has detected an association between exercise efficiency and a previously characterized common polymorphism in the UCP2 gene causing the substitution of valine for alanine. The result is in concordance with a previous finding of a relationship between the same polymorphism and adjusted 24-h EE. Due to the short distance between the UCP2 and UCP3 genes we cannot exclude the possibility that these results are due to a linkage disequilibrium with a significant variation in the UCP3 gene. The characterizations of genetic variations with influence on exercise efficiency may have implications for the understanding of biological factors with interests for both obesity and sports.

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