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# Polymorphisms in Control Region of mtDNA Relates to Individual Differences in Endurance Capacity or Trainability

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**Abstract:** The purpose of this study was to investigate whether the polymorphisms in the control region of mitochondrial DNA (mtDNA) related to individual difference in the endurance capacity or trainability. Fifty-five sedentary males participated in this study and were submitted to an 8-week endurance training program. The $\dot{V}O_{2\,max}$ was determined before and after training. Total DNA was extracted from the blood, and the sequence of the mtDNA control region was determined. The polymorphism in the mtDNA control region was decided based on the "Cambridge sequence." In 29 of the 55 subjects, vastus lateralis muscle biopsy samples were taken at rest before and after the training program. MtDNA content and CS (citrate synthase) activity in skeletal muscle was measured as the phenotype of the polymorphisms in the mtDNA control region. The $\dot{V}O_{2\,max}$ increased to 48.2±6.3 ml/min/kg from 42.1±6.0 as a result of the 8-week training ($p<0.05$). The numbers of polymorphisms in determined 1,122 bp were 11.1±2.9 variable sites per person, and the total numbers of polymorphisms were 125 variable sites. The subjects were classified into two groups at each variable site, the Cambridge sequence (Cam) group and the non-Cambridge sequence (non-Cam) group. There were significant differences in pre-$\dot{V}O_{2\,max}$ between the two groups at each mtDNA nucleotide positions 16298, 16325, and 199, and in $\%\Delta\dot{V}O_{2\,max}$ at 16223 and 16362. Twenty-nine subjects who underwent the biopsy revealed significant differences in pre-CS activity at 194 and pre-mtDNA content at 514. Also, significant differences were found in the change rate of $\dot{V}O_{2\,max}$ and CS activity as a result of training between the two groups at 16519. In conclusion, it suggested that mtDNA polymorphisms in the control region might result in individual differences in endurance capacity or trainability. [Japanese Journal of Physiology, 52, 247–256, 2002]

*Key words*: mtDNA polymorphism, individual difference, $\dot{V}O_{2\,max}$, citrate synthase activity, mtDNA content.

It is well known that individual differences exist in endurance capacity or its trainability [1–3]. Several studies have shown that these individual differences are brought about by environmental effects such as life style and diet and by genetic predisposition [4–6]. The potential of several specific genes to cause individual differences in endurance capacity or trainability has been investigated [4, 7, 8].

The *angiotensin-converting enzyme* (*ACE*) gene, which has insertion (I)/deletion (D) polymorphism, is one of the most studied genes. Montgomery *et al.* [9] reported that elite high-altitude mountaineers had higher I allele frequency of the *ACE* gene than sedentary males did. Moreover, they reported that subjects with II homozygotes showed higher trainability during a 10-week endurance training program than individuals with ID heterozygotes or DD homozygotes did [9]. However, the results reported by Rankinen *et al.* [10]

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did not support their results in terms of the relation between *ACE* genotype and cardiorespiratory endurance. Although numerous studies have reported a relationship between these two items [9–14], it remains controversial. Furthermore, mitochondrial DNA (mtDNA) [15, 16], the *creatine kinase* gene [17–19] and the *Na*$^+$-*K*$^+$-*ATPase ∂2* gene [20] have been studied as genes that may dictate individual differences in endurance capacity or trainability. mtDNA is a double-stranded, circular molecule of 16,569 bp within the mitochondria [21] and has many more polymorphisms than seen in nuclear DNA [22]. mtDNA closely codes subunits in enzyme complexes of the oxidative phosphorylation system, and the noncoding region in mtDNA, only 1,122 bp in size, is called the control region [21]. Because mtDNA codes a mitochondrial respiratory chain for ATP generation in skeletal muscle, it is very likely that the polymorphisms in mtDNA relate to oxidative capacity in skeletal muscle or whole-body endurance capacity. Dionne *et al.* [15] reported that the polymorphism in mtDNA related to the individual differences in endurance capacity and trainability. However, in a follow-up study no relation was recognized between mtDNA polymorphism and endurance capacity [16]. We also studied about the relationship between mtDNA polymorphism and individual differences in endurance capacity or trainability. In a previous study, we focused on the coding region of mtDNA and examined the relation between endurance performance and mitochondrial capacity, which reflected the polymorphism in this region, excluding the influence of the individual nuclear DNA [23, 24]. However, we have not confirmed any link between individual differences in endurance capacity or trainability and the polymorphism in the mtDNA coding region. On the other hand, it has been reported that the control region has more polymorphisms than seen in the coding region of mtDNA [25]. The control region includes the binding site of transcription factor coded by nuclear DNA and is the origin of mtDNA replication and transcription. Therefore it is likely that the polymorphism in the control region of mtDNA brings about diversity in the sensitivity of binding between this region and the transcription factor increased by exercise stimulus. This may lead to an individual difference in oxidative capacity in skeletal muscle and thus account for individual differences in endurance capacity. No studies however, have until now targeted the link between polymorphism in the control region and the individual differences in endurance capacity and trainability.

In the present study, we examined whether polymorphism in this region relates to individual differences in endurance capacity or trainability. We also examined mtDNA and mitochondria content in skeletal muscle as phenotypes of the physiological factor influenced by polymorphisms in the control region.

## METHODS

**Subjects.** Fifty-five healthy sedentary males (age: 20.5±2.1 year; height: 172.6±6.3 cm; weight: 62.4±7.8 kg) volunteered to participate in the study. The subjects were informed of the possible risks inherent in the experimental procedures, and all gave their written informed consent. This study was approved by the office of the Department of Sports Sciences, University of Tokyo, and complied with its requirements for human experimentation.

**Experimental protocol.** The subjects exercised for 8 weeks at a frequency of 3.5 d a week. All subjects completely accomplished the training program. Supervised training was performed for 1 h at the 70% maximal oxygen uptake ($\dot{V}O_{2\,max}$) of each subject on a cycle ergometer. $\dot{V}O_{2\,max}$ was measured before and after training. To determine the sequence of mtDNA control region, a blood sample was taken from each subject before the training. In 29 of the 55 subjects, vastus lateralis muscle biopsy samples were taken at rest before and after the program.

**Measurement of $\dot{V}O_{2\,max}$.** $\dot{V}O_{2\,max}$ was determined on a cycle ergometer. The subjects sat quietly on a cycle ergometer for 3 min and warmed up at 0.5 kp for 4 min. They then pedaled at an increase rate of 0.5 kp/min until exhaustion. The expired air was analyzed breath-by-breath with an automatic expired gas analyzer (AE280, MINATO, Osaka, Japan). The criteria for $\dot{V}O_{2\,max}$ were respiratory exchange ratio $>$1.1, plateau in $O_2$ uptake, and a heart rate within 10 beats/min of the maximal heart rate predicted for age. All subjects achieved the $\dot{V}O_{2\,max}$ by at least two of these criteria.

**Determination of mtDNA control region sequence.** Total DNA was extracted from blood with a DNA extractor WB kit (Wako, Osaka, Japan). The control region in mtDNA was amplified by polymerase chain reaction (PCR) amplification, and the divided three parts overlapped one another. The primers and reaction conditions used for PCR amplification are shown in Table 1. PCR was performed in a PCR thermal cycler by means of a 50 μl reaction mixture containing 1× EX *Taq* buffer, dNTP Mix, EX *Taq* polymerase (all purchased from Takara, Tokyo, Japan), 10 pmol/μl of each forward and reverse primer, and 10 ng/μl total DNA. The PCR products were used in direct sequence (Espec Oligo Service Corp., Tsukuba). The polymorphism in the mtDNA

**Table 1. Primer sequence and reaction condition for PCR method.**

| | Forward primer | Reverse primer | Reaction condition | | | | Amplification site |
|---|---|---|---|---|---|---|---|
| | | | Denature | Annealing | Extension | Cycle | |
| PCR 1 | ACCATTAGCACCCAAAGCTAAGA | AGTTCACTTTAGCTACCCCCAAGT | 94°C 45 s | 65°C 45 s | 72°C 60 s | 30 | 15979–16489 |
| PCR 2 | CACCATCCTCCGTGAAATCAA | CTGTGGCCAGAAGCGGG | | | | | 16401–329 |
| PCR 3 | TGCCTCATCCTATTATTTATCGCA | CGTTTTACTCACTGGAACGGG | | | | | 142–726 |

control region was decided by means of the "Cambridge sequence" [26].

**Muscle biopsy.** Muscle samples were obtained from the vastus lateralis muscle by the percutaneous needle biopsy technique. Each sample was immediately frozen in liquid nitrogen and kept at −80°C until subjected to an analysis of mtDNA content and citrate synthase (CS) activity.

**Quantification of mtDNA.** The quantity of mtDNA in skeletal muscle was measured by Southern blotting. Total DNA was extracted after the digestion of muscle homogenates with proteinase K, followed by an extraction of protein with phenol–chloroform and precipitation with ethanol. Extracted DNA concentrations were determined spectrophotometrically. A sample of 2 μg of total DNA was digested with *Xho I* and loaded on 1% agarose gel. The restriction DNA separated by agarose gel electrophoresis was transferred to a nitrocellulose membrane and hybridized with [α-$^{32}$P]dCTP-labeled mtDNA. The membrane was washed and exposed to imaging plates (Fuji Film, Tokyo). The quantitation of the mtDNA content was normalized by the use of [α-$^{32}$P]dCTP-labeled 18S rRNA as an internal control.

**Citrate synthase activities.** As a marker for the amount of mitochondria in skeletal muscle, the maximum catalytic capacity of the mitochondrial matrix enzyme CS activity in skeletal muscle was measured. A spectrophotometric method as described by Srere [27] was used to determine the CS activity.

**Statistics.** All data were expressed as mean± SD. The paired *t*-test was used for comparison before and after training. The Mann-Whitney's *U*-test was used for a comparison of physiological variables before training between the Cambridge sequence (Cam) group and the non-Cambridge sequence (non-Cam) group. The analysis of covariance was used for a comparison of the change in physiological variables between two groups. The independent variables were prevalues and types of mtDNA sequence, and the dependent variable was the change rate of physiological variables. The probability values of $<0.05$ were considered significant.

## RESULTS

The $\dot{V}_{O_2\,max}$ increased to 48.2±6.3 ml/min/kg, from 42.1±6.0, as a result of 8 weeks of endurance training, averaging +15.0±9.8%. The effect of training caused large individual differences on the $\dot{V}_{O_2\,max}$, ranging from 0.1 to 54.7% (Table 2). The change rate of $\dot{V}_{O_2\,max}$ was correlated to the prevalue ($r=-0.451$, $p<0.01$). In the 55 subjects, the rate of polymorphism

per person was 1.0±0.3% over 1,122 bp. That is, 11.1±2.9 variable sites within the control region differed from the "Cambridge sequence," and the total occurrence of polymorphism in the 55 subjects was 125 variable sites. To compare the various phenotypes between the Cam group and the non-Cam group, we selected from these 125 variable sites the 34 where more than 2 subjects and fewer than 54 had the non-Cam (Table 3). The $\dot{V}O_{2\,max}$ before training (pre-$\dot{V}O_{2\,max}$) and the change rate of $\dot{V}O_{2\,max}$ resulting from training (%$\Delta\dot{V}O_{2\,max}$) between the Cam group and the non-Cam group were compared. The results showed that the non-Cam group (50.8±5.1 ml/min/kg, $n$=4) at the mtDNA nucleotide position 16298 had a significant higher pre-$\dot{V}O_{2\,max}$ than the Cam group did (41.4±5.6 ml/min/kg, $n$=51) ($p$=0.008) (Fig. 1A). The non-Cam group (36.9±3.9 ml/min/kg, $n$=5) at 16325 had a significantly lower pre-$\dot{V}O_{2\,max}$ than the Cam group did (42.6±6.0 ml/min/kg, $n$=50) ($p$=0.030) (Fig. 1B). But at 199, the non-Cam group (46.5±6.0 ml/min/kg, $n$=8) had a significantly higher pre-$\dot{V}O_{2\,max}$ than the Cam group (41.4±5.8 ml/min/kg, $n$=47) ($p$=0.040) (Fig. 1C). Moreover, the non-Cam

**Table 2. Individual differences in pre-$\dot{V}O_{2\,max}$ and the change rate of $\dot{V}O_{2\,max}$.**

| | Average | Range | |
|---|---|---|---|
| | | Min | Max |
| Pre-$\dot{V}O_{2\,max}$ (ml/min/kg) | 42.1±6.0* | 26.6 | 58.0 |
| %$\Delta\dot{V}O_{2\,max}$ (%) | 15.0±9.8* | 0.1 | 54.7 |

* $X$±SD.

**Table 3. Mitochondrial DNA polymorphism in the control region.**

| Variable site | Cambridge sequence | Non-Cambridge sequence | Subject's number with non-Cambridge | Investigated subject's number | Rate of non-Cambridge |
|---|---|---|---|---|---|
| 16129 | G | A | 9 | 55 | 16.4 |
| 16136 | T | C | 4 | 55 | 7.3 |
| 16172 | T | C | 3 | 55 | 5.5 |
| 16182 | A | C | 6 | 51 | 11.8 |
| 16183 | A | C | 14 | 55 | 25.5 |
| 16187 | C | T | 3 | 55 | 5.5 |
| 16189 | T | C | 16 | 55 | 29.1 |
| 16209 | T | C | 3 | 55 | 5.5 |
| 16217 | T | C | 7 | 55 | 12.7 |
| 16223 | C | T | 42 | 55 | 76.4 |
| 16245 | C | T | 4 | 55 | 7.3 |
| 16261 | C | T | 3 | 55 | 5.5 |
| 16278 | C | T | 4 | 55 | 7.3 |
| 16290 | C | T | 3 | 55 | 5.5 |
| 16298 | T | C | 4 | 55 | 7.3 |
| 16311 | T | C | 5 | 55 | 9.1 |
| 16319 | G | A | 6 | 55 | 10.9 |
| 16325 | T | C | 5 | 55 | 9.1 |
| 16362 | T | C | 33 | 55 | 60.0 |
| 16519 | T | C | 25 | 55 | 45.5 |
| 146 | T | C | 4 | 55 | 7.3 |
| 150 | C | T | 12 | 55 | 21.8 |
| 152 | T | C | 8 | 55 | 14.5 |
| 191 | A1 | A2 | 3 | 55 | 5.5 |
| 194 | C | T | 6 | 55 | 10.9 |
| 199 | T | C | 8 | 55 | 14.5 |
| 207 | G | A | 7 | 55 | 12.7 |
| 235 | A | G | 3 | 55 | 5.5 |
| 249 | A | delete | 3 | 55 | 5.5 |
| 303–309 | C7 | C8 | 24 | 54 | 44.4 |
| | | C9 | 6 | 54 | 11.1 |
| 320 | C | T | 3 | 50 | 6.0 |
| 489 | T | C | 38 | 53 | 71.7 |
| 499 | G | A | 3 | 54 | 5.6 |
| 514–523 | 5CA | 4CA | 12 | 54 | 22.2 |

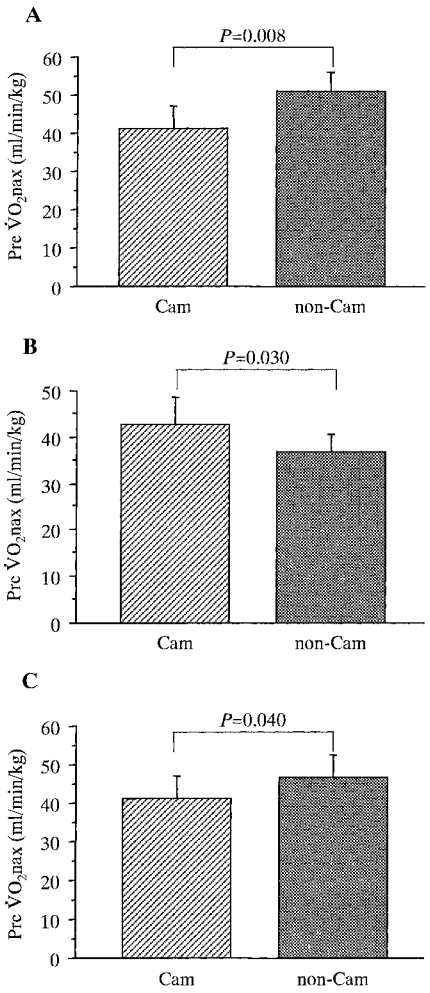


**Fig. 1. The relation between the polymorphisms in the control region of mtDNA and $\dot{V}O_{2\,max}$ before endurance training. A** shows a comparison of the pre-$\dot{V}O_{2\,max}$ between the Cam group ($n$=51) and the non-Cam group ($n$=4) at 16298. **B** shows a comparison of the pre-$\dot{V}O_{2\,max}$ between the Cam group ($n$=50) and the non-Cam group ($n$=5) at 16325. **C** shows a comparison of the pre-$\dot{V}O_{2\,max}$ between the Cam group ($n$=47) and the non-Cam group ($n$=8) at 199. These data were obtained from 55 subjects. The values show average±SD.

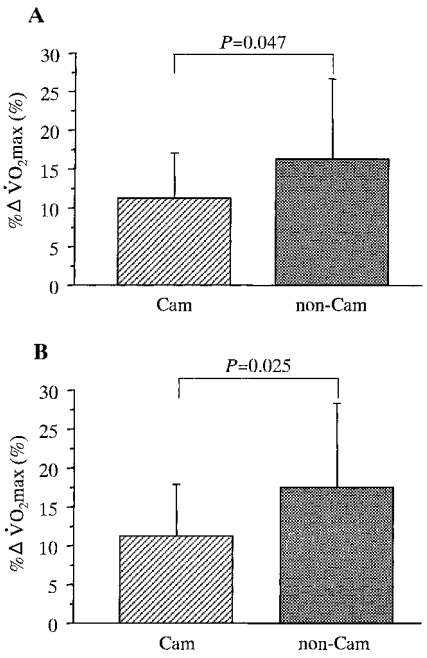


**Fig. 2. The relation between the polymorphisms in the control region of mtDNA and the change rate of $\dot{V}O_{2\,max}$ for endurance training. A** shows a comparison of the change rate of $\dot{V}O_{2\,max}$ for training between the Cam group ($n$=13) and the non-Cam group ($n$=42) at 16223. **B** shows a comparison of the change rate of $\dot{V}O_{2\,max}$ for training between the Cam group ($n$=22) and the non-Cam group ($n$=33) at 16362. These data were obtained from 55 subjects. The values show average±SD.

group (16.2±10.5%, $n$=42) at 16223 had a greater %Δ$\dot{V}O_{2\,max}$ than the Cam group (11.2±5.9%, $n$=13) ($p$=0.047) (Fig. 2A). And the non-Cam group (17.5±10.8%, $n$=33) at 16362 had a greater %Δ$\dot{V}O_{2\,max}$ than the Cam group (11.2±6.7%, $n$=22) ($p$=0.025) (Fig. 2B). The polymorphisms shown the significant difference between two groups in pre-$\dot{V}O_{2\,max}$, nucleotide positions 16298, 16325, and 199, were not haplotype. Furthermore, the polymorphisms shown the significant difference in %Δ$\dot{V}O_{2\,max}$ were not haplotype.

The relationship between $\dot{V}O_{2\,max}$ and mtDNA content or CS activity in skeletal muscle was investigated in the 29 subjects who underwent muscle biopsy. The $\dot{V}O_{2\,max}$ increased to 46.6±5.2 ml/min/kg, from 40.7±4.8 ($p$<0.01) (Fig. 3A). CS activity in the skeletal muscle increased to 17.4±4.5 nmol/min/mg tissue, from 12.1±3.6 ($p$<0.01) (Fig. 3B), and the mtDNA content in skeletal muscle significantly increased to 44.0±15.5, from 35.6±16.8 ($p$<0.05) (Fig. 3C). The change rates of $\dot{V}O_{2\,max}$ ($r$=−0.375, $p$<0.05), CS activity ($r$=−0.549, $p$<0.01), and mtDNA content ($r$=−0.673, $p$<0.01) were significantly correlated to each prevalue. No significant correlation between CS activity or mtDNA content in skeletal muscle and pre-$\dot{V}O_{2\,max}$ was observed (Fig. 4A, C). Similarly, there were no correlations in these changes caused by endurance training or %Δ $\dot{V}O_{2\,max}$ (Fig. 4B, D). In the 29 subjects, the polymorphisms in the control region possessed by more than 2 subjects and fewer than 28 were found in 10 variable sites. In these sites, $\dot{V}O_{2\,max}$, mtDNA content, and CS activity in skeletal muscle were compared between the Cam group and the non-Cam group. In the values before training, there were significant differences in the $\dot{V}O_{2\,max}$ between the two groups at 16325 (41.5±4.5 ml/min/kg, $n$=25, vs. 35.7±3.3 ml/min/kg, $n$=4, $p$=0.011) (Fig. 5A). And significant differences were found in the CS activity at 194 (12.5±3.6 nmol/min/mg tissue, $n$=26, vs. 8.8±1.1 nmol/min/mg tissue, $n$=3, $p$=0.032) (Fig. 5B) and

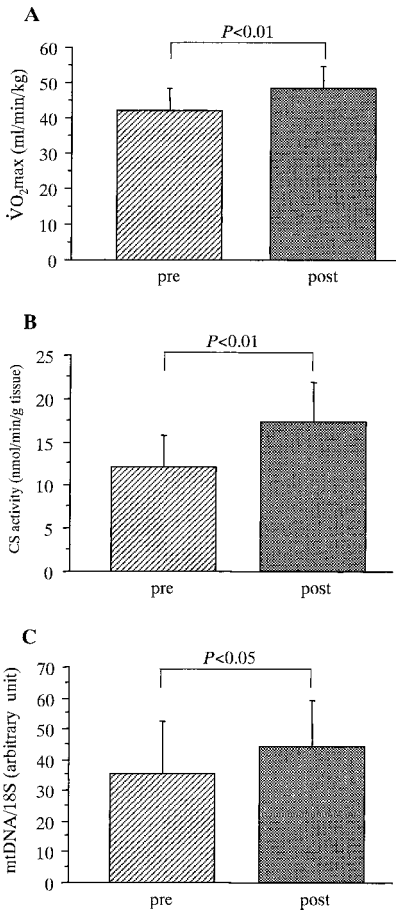


**Fig. 3. The $\dot{V}O_{2\,max}$, CS activity, and mtDNA/18S before and after endurance training. A**: $\dot{V}O_{2\,max}$; **B**: CS activity in skeletal muscle; **C**: mtDNA/18S in skeletal muscle. These data were obtained from 29 subjects. The values show average±SD.

in the mtDNA content at 514 (40.2±17.3, $n$=19, vs. 25.7±12.5, $n$=9, $p$=0.032) (Fig. 5C). The results also showed significant differences at the mtDNA nucleotide position 16519. A significant difference was noted in the $\%\Delta\dot{V}O_{2\,max}$ between the two groups at 16519 (18.0±6.1%, $n$=11, and 12.9±7.4%, $n$=18) ($p$=0.027) (Fig. 6A). The rate of change of CS activity caused by endurance training in the Cam group and the non-Cam group at mtDNA nucleotide position 16519 was 73.0±51.0% ($n$=11) and 37.6±47.8% ($n$=18), respectively ($p$=0.025) (Fig. 6B). However, significant difference occurred in the rate of change of mtDNA content in skeletal muscle between the two groups.

## DISCUSSION

The purpose of this study was to investigate whether polymorphisms in the mtDNA control region are linked to individual differences in endurance capacity or trainability. In 55 subjects, the sequence of the mtDNA control region was determined and the endurance capacity estimated before and after an 8-week endurance training program. Also, 29 of the subjects underwent a biopsy of the vastus lateralis muscle before and after the training period, and the mtDNA content and CS activity in skeletal muscle were measured. The relations among the polymorphisms of mtDNA and the phenotypes in skeletal muscle and endurance capacity or trainability were also investigated.

In our 55 subjects, pre-$\dot{V}O_{2\,max}$ significantly differed

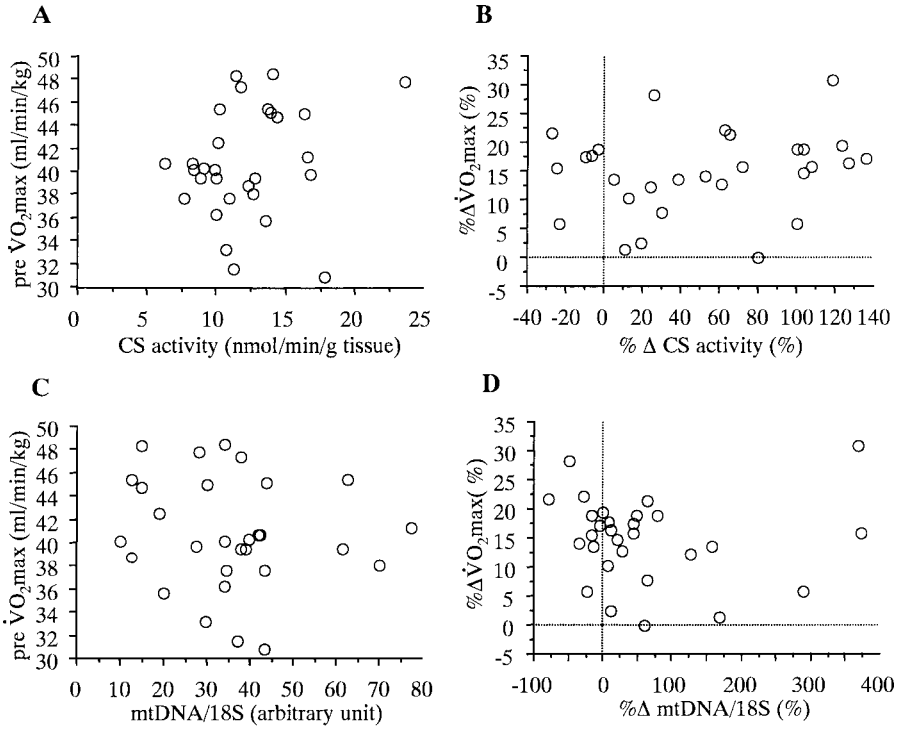


**Fig. 4. The relations between the pre-$\dot{V}O_{2\,max}$ and the pre-CS activity or mtDNA/18S in skeletal muscle and the relations between these change rates as a result of endurance training. A**, **C**: The relations between pre-CS activity ($r$=0.219, $p$=0.257) or mtDNA content ($r$=−0.142, $p$=0.4646) in skeletal muscle and pre-$\dot{V}O_{2\,max}$. **B**, **D**: The relation between the change rate of CS activity ($r$=0.198, $p$=0.301) or mtDNA/18S ($r$=−0.040, $p$=0.839) in skeletal muscle and the change rate of $\dot{V}O_{2\,max}$ as a result of endurance training. These data were obtained from 29 subjects.

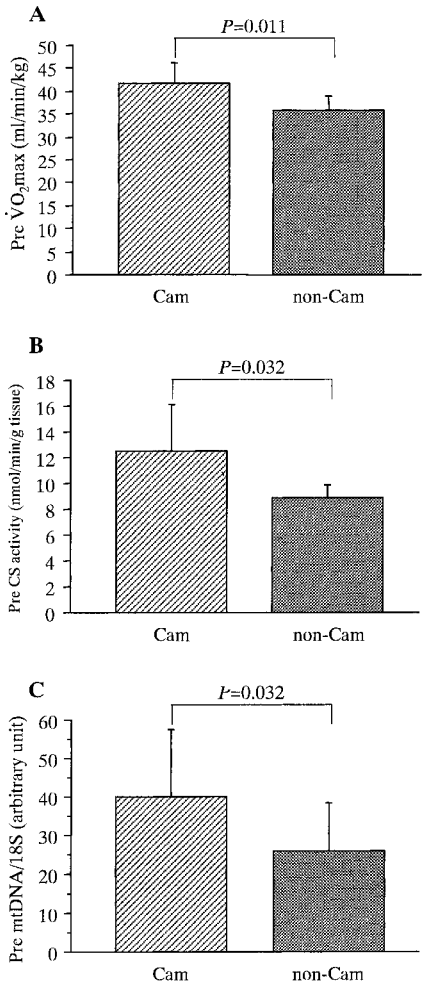


**Fig. 5. The relation between the polymorphisms in the control region of mtDNA, and $\dot{V}O_{2\,max}$, CS activity, and mtDNA/18S before endurance training. A** shows a comparison of the pre-$\dot{V}O_{2\,max}$ between the Cam group ($n$=25) and the non-Cam group ($n$=4) at 16325. **B** shows a comparison of the pre-CS activity between the Cam group ($n$=26) and the non-Cam group ($n$=3) at 194. **C** shows a comparison of the pre-mtDNA/18S between the Cam group ($n$=19) and the non-Cam group ($n$=9) at 514. These data were obtained from 29 subjects. The values show average±SD.

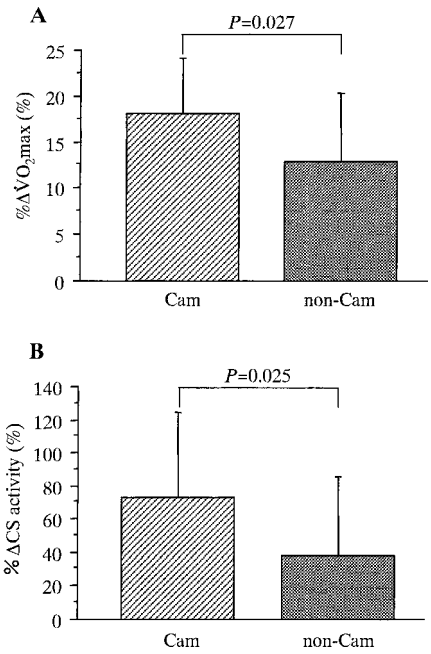


**Fig. 6. The relations between the polymorphisms in the control region of mtDNA and the change rates of $\dot{V}O_{2\,max}$ and CS activity as a result of endurance training.** These data show the relations between the polymorphism at 16519 of mtDNA (Cam group, $n$=11; non-Cam group, $n$=18) and the change rate of $\dot{V}O_{2\,max}$ (**A**) or CS activity (**B**) as a result of endurance training. These data were obtained from 29 subjects. The values show average±SD.

between the Cam group and the non-Cam group at nucleotide positions 16298, 16325, and 199. The non-Cam group at 16298 or 199 had higher pre-$\dot{V}O_{2\,max}$ than the Cam group did. The non-Cam group at 16325 had lower pre-$\dot{V}O_{2\,max}$ than the Cam group. Nucleotide positions 16298 and 16325 are between the Mt 5 sequence [28] and the Mt 3 sequence [29] in the control region, and 199 is within the H-strand origin [30]. Moreover, the non-Cam group at nucleotide position 16223 or 16362 showed a greater increase of $\dot{V}O_{2\,max}$ as a result of endurance training than the Cam group did. Nucleotide positions 16223 and 16362 are also between the Mt 5 sequence [28] and the Mt 3 sequence [29]. The Mt 5 sequence and the Mt 3 sequence exist in the 5′-flanking region of numerous mitochondrial genes coding for nuclear DNA [31–33]; the transcription of many genes for the mitochondrial respiratory system is coordinated by the binding of the Mt binding factor (MtBF) [32] to Mt elements. The present study does not reveal how the diversity in sequence within or close to the site relative to transcription or replication of mtDNA leads to the physiological difference, but it is possible that the polymorphisms in this region affect the transcription and replication of mtDNA. Moreover, since the different polymorphisms correspond to the individual differences in pre-$\dot{V}O_{2\,max}$ or %Δ$\dot{V}O_{2\,max}$, it is suggested that the polymorphisms of mtDNA contributing to individual differences in endurance capacity or trainability are different.

Twenty-nine subjects were examined by muscle biopsy. No significant correlation was found between endurance capacity and mtDNA content or CS activity in skeletal muscle. When the $\dot{V}O_{2\,max}$ and CS activity and mtDNA content in skeletal muscle before the training were compared between the two groups at each position, there were significant differences in the $\dot{V}O_{2\,max}$, CS activity, and mtDNA content at 16325, 194, and 514, respectively. Because this relation was

independent, it was unclear how a polymorphism at 16325 related to the $\dot{V}O_{2\,max}$ before training, and why the relation between the polymorphism at 194 or 514 and the phenotype in skeletal muscle had gone into the whole body phenotype. In regard to trainability, subjects with the Cam type at 16519 had greater $\%\Delta\dot{V}O_{2\,max}$. Furthermore, a similar difference in CS activity in skeletal muscle change resulting from training was recognized between the Cam group and the non-Cam group at 16519. Since CS activity in skeletal muscle reflects the amount of mitochondria present in skeletal muscle [34], it was suggested that the difference in the sequence at this position induced the difference in mitochondrial biogenesis by changing the binding activity of mtDNA transcriptional factor (mtTFA). Moreover, it is possible that the difference in this mitochondrial biogenesis brings about a difference in the oxidative capacity of skeletal muscle, resulting in a difference in the increase of $\dot{V}O_{2\,max}$ resulting from endurance training. In contrast, no difference existed between the two groups with respect to the change of mtDNA content in skeletal muscle as a result of endurance training, therefore it is very likely that the difference in mitochondrial biogenesis by training was induced by differences in transcription, but not by a replication of mtDNA. However, since mitochondrial biogenesis in skeletal muscle involves the expression of mitochondrial genes and transcriptional factor coded by nuclear DNA [35, 36], we need to further investigate these effects. Also, in the 29 subjects, polymorphism at 16519 showed varying changes in $\dot{V}O_{2\,max}$ and CS activity, whereas in all 55 subjects, including the 29, no significant difference in the change of $\dot{V}O_{2\,max}$ was found. Further investigations are needed to explain this apparent contradiction.

Dionne *et al.* [15] previously reported on the link between endurance capacity and polymorphism in mtDNA, and we tried to compare our results with theirs. In their study, 46 sedentary males engaged in a 12–20 week endurance training program. The mtDNA polymorphism of these subjects was then determined from digested patterns of mtDNA by the use of 22 kinds of restriction enzymes. Their results showed that subjects with the non-Cam at a nucleotide position of 13365 ($p<0.05$), 13470 ($p<0.05$), 15925 ($p<0.05$), or 4740 ($p<0.07$) had smaller $\dot{V}O_{2\,max}$ before training than the Cam subjects. Moreover, it was suggested that subjects with the non-Cam at nucleotide position 12406, 13365, 13470, or 15925 had lower trainability, whereas those with the non-Cam at 16133 had greater trainability ($p<0.05$). Of the above nucleotide positions, only 16133 is present within the control region. This polymorphism was not found in our subjects. The polymorphism in the control region detected by Dionne *et al.* [15] was at nucleotide positions 16133, 16274, 16350, and 16390, but the polymorphism in the present study was detected only at 16390, not at the others. It is impossible to decide whether the polymorphism at 16390 contributes to an individual difference in trainability because only two of our subjects showed polymorphism at 16390. Racial differences have been suggested as one reason for the observed differences between studies. MtDNA has been used as a population marker in studies of evolutionary biology, since unique mtDNA sequences are characteristic of different races and populations [37]. Moreover, the individual differences in endurance capacity or its trainability result from many environmental factors and genetic factors. For this reason, inconsistency arises in the results of several research projects about the relation between these differences and genetic factors. Thus we must undertake comprehensive study because the results are influenced by the subject's number. In general, many samples are necessary when we study about the genetic markers for some phenotypes. Further, a family study may be needed to raise reliability more.

In conclusion, we suggest that several polymorphisms might relate to individual differences in endurance capacity and trainability. More research will be needed to elucidate these relations and the polymorphisms of mtDNA.

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