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Effect of exercise on the mitochondrial DNA content of peripheral blood in healthy women

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Abstract Exercise decreases insulin resistance and increases maximal exercise capacity as estimated from maximal oxygen uptake (\( \dot{V}O_2 \max \)). Recent reports have demonstrated that the mitochondrial DNA (mtDNA) content of blood is correlated with \( \dot{V}O_2 \max \) in healthy subjects (mean age 31 years) and is inversely correlated with insulin resistance parameters. The aim of this study was to determine the effect of regular exercise on the mtDNA content in the peripheral blood of 16 healthy young women of mean age 24.8 (SD 6.2) years and 14 healthy older women of mean age 66.7 (SD 5.8) years. The exercise programme lasted for 10 weeks and consisted of three sessions a week, each of 1 h and aiming to attain 60%–80% of \( \dot{V}O_2 \max \). The mtDNA content of peripheral blood was measured by competitive polymerase chain reaction. The \( \dot{V}O_2 \max \) had significantly increased following the exercise programme (from 33.1 (SD 3.4) to 35.2 (SD 3.4) ml · kg \(^{-1} \) · min \(^{-1} \) in the young and from 24.3 (SD 5.3) to 30.3 (SD 7.3) ml · kg \(^{-1} \) · min \(^{-1} \) in the older women, both \( P < 0.05 \)). Exercise decreased systolic blood pressure, and concentrations of triglyceride, low density lipoprotein-cholesterol (LDL-C), glucose and insulin in the blood of the young and of total cholesterol, LDL-C and glucose in that of the older women. High density lipoprotein-cholesterol (HDL-C) in the young women was increased by exercise. The mtDNA content significantly increased following the exercise programme in both groups (from 27.1 (SD 17.9) to 52.7 (SD 44.6) amol · 5 ng \(^{-1} \) genomic DNA in the young and from 15.3 (SD 10.2) to 32.1 (SD 30.0) amol · 5 ng \(^{-1} \) genomic DNA in the older women, both \( P < 0.05 \)). There was a significant positive correlation between the change in mtDNA content and the change in \( \dot{V}O_2 \max \) (\( r = 0.74 \) in the young and \( r = 0.71 \) in the older women, both \( P < 0.01 \)). In conclusion, 10 weeks of moderate intensity, regular exercise increased the mtDNA content in peripheral blood and decreased insulin resistance parameters. This data suggests that increase in the mtDNA content may be associated with increased insulin sensitivity.

Key words Mitochondrial DNA · Maximal exercise capacity · Exercise · Competitive polymerase chain reaction · Insulin resistance

Introduction

The mitochondrion is an intracellular organelle in which occur oxidative phosphorylation and chemical reactions such as the citric acid cycle. Contrary to other organelles, the mitochondrion has its own DNA consisting of 16,569 base pairs. Mitochondrial DNA (mtDNA) codes 13 genes, which are essential for oxidative phosphorylation, and also codes ribosomal RNA and transfer RNA which are involved in the expression of these genes. It has been shown that originally, mitochondria were independent cells, which had aerobic respiration, but later became symbiotic with anaerobic cells, retaining their own DNA (Gray et al. 1999). The mtDNA has a shorter half-life and higher mutational rate than nuclear DNA, because it is easily exposed to free radicals,
by-products of oxidative phosphorylation and damaged by them. It has also been reported that mtDNA content is decreased by oxidative damage following drug treatment (Arnaudo et al. 1991).

Point mutations of mtDNA have been reported to be associated with diabetes (Reardon et al. 1992). In our prospective population-based study, the subjects having lower mtDNA content in the peripheral blood had a higher chance of contracting diabetes within 2 years. We have also found that mtDNA content of the peripheral blood was correlated with clinical parameters of insulin resistance such as blood pressure and waist to hip ratio (Lee et al. 1998). This may suggest that decrease in mtDNA content of peripheral blood could reflect the state of the insulin resistance syndrome. Taken together, decreases or abnormalities in mtDNA caused by environmental factors could contribute to the development of diabetes.

Exercise has been found to increase insulin sensitivity in people both with and without diabetes (Landt et al. 1985; Rodnick et al. 1987; Devlin 1992; Brown et al. 1997; Dengel et al. 1998; Walker et al. 1999). The effects of physical training on increasing the rate of glucose metabolism have been explained by multiple factors such as increased muscle mass, augmented muscle blood flow, increased capillary area, enhanced mitochondrial oxidative enzyme capacity and activation of the glucose transport system (Koivisto et al. 1986).

Since the mtDNA content is correlated with insulin resistance parameters and exercise decreases insulin resistance, we hypothesized that exercise might increase the mtDNA content of blood in normal subjects. To examine the correlation between the mtDNA content and whole body oxygen consumption and also to show whether exercise increases the mtDNA content of blood, we investigated the changes of the mtDNA content of peripheral blood and maximal exercise capacity as estimated from maximal oxygen uptake ($\dot{V}O_2$ max) before and after an exercise programme.

### Methods

#### Subjects

We recruited 16 healthy young women, aged 18–30 years (young age group) and 14 healthy elderly women, aged 55–70 years (old age group) from a centre for aerobic exercise. The subjects showed no disabilities with regard to cardiopulmonary function. No subject had any history of diabetes, hypertension or smoking. Baseline electrocardiogram, pulmonary function, and blood pressure tests were made and medical histories taken to check on heart and pulmonary function.

#### Protocol

**Exercise programme**

All the subjects took part in an exercise programme that consisted of three sessions a week for 10 weeks. Each session lasted for 1 h during which it was aimed to reach 60%–80% of the $\dot{V}O_2$ max. The average percentage of attendance for all subjects was above 90%.

### Measurements

Body mass index, waist to hip ratio, systolic and diastolic blood pressure, the mtDNA content of peripheral blood, $\dot{V}O_2$ max, concentrations of fasting plasma glucose, fasting plasma insulin, total cholesterol (TC), triglyceride (TG), low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C) were measured using conventional methods before and after the 10 week exercise programme in both groups.

### Quantification of mtDNA

The DNA content was measured as described previously (Lee et al. 1998; Park et al. 1999). Blood samples were centrifuged and the buffy coat layer was separated and stored at −70 °C till measurement. Total DNA was extracted using a QIAamp tissue kit (QIAGEN, Chatworth, Calif., USA). The DNA concentration of each sample was measured using a spectrophotometer (Beckman, Fullerton, Calif., USA). The internal standard was designed to use the same primer set as the target gene but to yield a different sized polymerase chain reaction (PCR) product (555 compared to 615 bp). It was prepared by PCR using the specially designed primers shown in Table 1. Two independent PCR amplifications using sets of hmtF2 and JR1, and hmtR2 and F1, produced 259 and 316 bp, respectively. Secondary PCR amplification using the above products and primers hmtF2 and hmtR2 produced a 555 bp fragment containing sequences from mtDNA positions 2,999–3,247 and 3,308–3,613, with deletion of the intervening 60 bp (from position 3,248–3,307). The known amounts of the serially diluted internal standard were added to 5 ng of total cellular DNA and subjected to PCR using a set of primers. The final volume of the PCR reaction was 20 μl, containing 0.4 μmol · l$^{-1}$ of each primer, 200 μmol · l$^{-1}$ of each dNTP, 1 U of Taq polymerase, 20 mmol · l$^{-1}$ TRIS-CI, 1.5 mmol · l$^{-1}$ MgCl$_2$, 50 mmol · l$^{-1}$ KCl, 0.05% Tween 20, and 0.0001% gelatin. Reactions took place under the following conditions: one cycle of 5 min at 94 °C, and 30 cycles of 30 s at 94 °C, 40 s at 57 °C and 40 s at 72 °C and a final extension of 7 min at 72 °C. The PCR product was analysed on an agarose gel by electrophoresis. Gels were stained with ethidium bromide and photographed under UV light (Fig. 1). The intensities of the target DNA band (615 bp) and competitor band (555 bp) were quantified using NIH Image (image software available from the National Institutes of Health, USA). The ratio of each target mtDNA product; internal standard product was plotted against log (internal standard) to yield the equivalence point between internal standard and target mtDNA (Fig. 2). The $r$ values of the standard curves were between 0.95 and 1.00. Inter-assay variance of mtDNA measurement was 12.2%.

### Statistics

The characteristics of the subjects were analysed by the Mann-Whitney test. The Spearman correlation coefficient was used for analysis of simple correlation. We used the Wilcoxon-signed rank test to analyse changes of parameters before and after exercise. Values at $P < 0.05$ were considered statistically significant except in the analysis of comparison of basal characteristics where Bonferroni’s correction was used to reduce $\alpha$-error and a value of $P < 0.005$ was considered significantly different.

### Table 1  Sequences of primers

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence</th>
<th>Position</th>
</tr>
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<tbody>
<tr>
<td>HmtF2</td>
<td>CAG GAC ATC CCG ATG GTG CA</td>
<td>2999–3018</td>
</tr>
<tr>
<td>HmtR2</td>
<td>GGA GGC CTA CCA GTG TGA GTG TGA</td>
<td>3613–3593</td>
</tr>
<tr>
<td>F1</td>
<td>GCC AGA GCC C</td>
<td>3238–3247</td>
</tr>
<tr>
<td>JR1</td>
<td>GCC ATG GTG A</td>
<td>3217–3308</td>
</tr>
<tr>
<td>JRF</td>
<td>GGG CTC TGC CAT CTT AAC AA</td>
<td>3247–3228</td>
</tr>
</tbody>
</table>