Eur J Appl Physiol (2000) 82: 407–412

ORIGINAL ARTICLE

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

Accepted: 15 April 2000

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

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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 QIAmp 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 $\mu mol \cdot l^{-1}$ of each primer, 200 $\mu mol \cdot l^{-1}$ of each dNTP, 1 U of Taq polymerase, 20 $mmol \cdot l^{-1}$ TRIS-Cl, 1.5 $mmol \cdot l^{-1}$ $MgCl_2$, 50 $mmol \cdot l^{-1}$ 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 α-error and a value of $P < 0.005$ was considered significantly different.

Table 1 Sequences of primers

Primers	Sequence	Position
HmtF2	CAG GAC ATC CCG ATG GTG CA	2999–3018
HmtR2	GGA GGC CTA GGT TGA GGT TGA	3613–3593
JF1	GGC AGA GCC C	3238–3247
	TAC CCA TGG CCA ACC TCC TA	3308–3327
JR1	GCC ATG GGT A	3317–3308
	GGG CTC TGC CAT CTT AAC AA	3247–3228

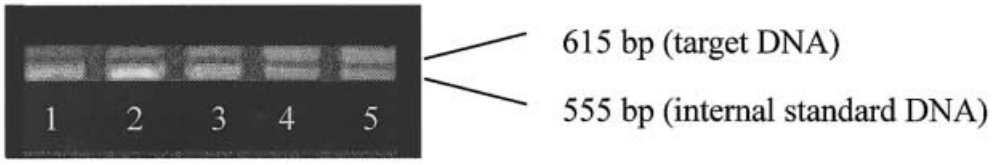

Fig. 1 Electrophoresis by polymerase chain reaction product. Known amounts of the serially diluted internal standard *DNA* were added to total cellular *DNA* extracted from peripheral blood leucocytes and amplified with primers hmtF1 and hmtR1. As indicated, two products were generated, the *upper one* derived from mtDNA (615 bp) and the *lower one* from internal standard *DNA* (555 bp). *Lanes 1–5* are coamplifications of varying amounts of internal standard *DNA*

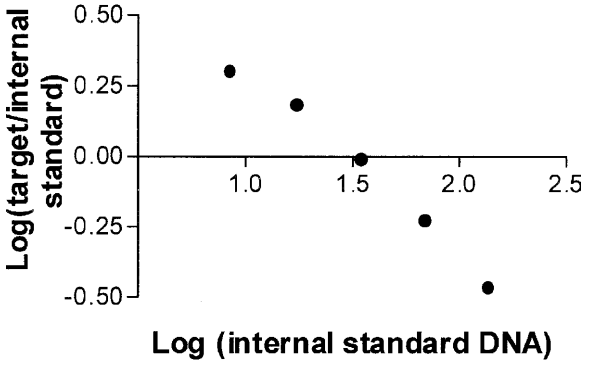

Fig. 2 Quantification of mitochondrial DNA (*mtDNA*) in peripheral blood leucocytes by competitive polymerase chain reaction. The ratio of each target:internal standard product was plotted against log (internal standard) to yield the equivalence point between internal standard and target DNA

Results

Baseline characteristics

Waist to hip ratio, systolic and diastolic blood pressures, TC, TG and LDL-C were significantly higher in the older than in the young age group (Table 2).

Changes in physiologic parameters before and after the exercise programme

In the young age group, systolic blood pressure, concentrations in the blood of fasting plasma glucose, fasting plasma insulin, TG and LDL-C significantly decreased and HDL-C increased following the exercise programme (all $P < 0.05$, Table 3). In the older age group, blood concentrations of fasting plasma glucose, TC and LDL-C decreased significantly following the exercise programme (all $P < 0.05$, Table 4).

Changes in mtDNA content and $\dot{V}O_{2\,max}$ before and after the exercise programme

In both group, the mtDNA content and $\dot{V}O_{2\,max}$ significantly increased after exercise training (Table 5). The

Table 2 Baseline clinical and biochemical characteristics of the subjects. *BMI* Body mass index, *W:H* waist to hip ratio, BP_s, BP_d systolic and diastolic blood pressures, respectively, *FPG* fasting plasma glucose concentration, *FPI* fasting plasma insulin concentration, *TC* total cholesterol concentration, *TG* tryglyceride concentration, *HDL-C, LDL-C* high and low density lipoprotein-cholesterol concentrations, respectively

	Young age group $n = 16$		Older age group $n = 14$		*P* value
	Mean	SD	Mean	SD	
Age (years)	24.8	6.2	66.7	5.8	–
BMI ($kg \cdot m^{-2}$)	23.1	2.8	25.1	4.9	NS
W:H	0.77	0.05	0.92	0.02	<0.005
BP_s (mmHg)	120.2	15.4	140.4	19.6	<0.005
BP_d (mmHg)	64.9	9.1	79.9	9.1	<0.005
FPG ($mmol \cdot l^{-1}$)	5.4	0.4	5.9	1.4	NS
FPI ($\mu IU \cdot ml^{-1}$)	11.1	6.3	10.4	4.8	NS
TC ($mmol \cdot l^{-1}$)	4.5	0.6	5.6	0.8	<0.005
TG ($mmol \cdot l^{-1}$)	2.1	0.6	3.6	2.2	<0.005
HDL-C ($mmol \cdot l^{-1}$)	1.7	0.3	1.4	0.2	NS
LDL-C ($mmol \cdot l^{-1}$)	2.4	0.5	3.5	0.7	<0.005

Table 3 Changes in physiological parameters in the young age group. Definitions as for Table 2

	Pre-training		Post-training		*P* value
	Mean	SD	Mean	SD	
BMI ($kg \cdot m^{-2}$)	23.1	2.8	23.0	2.4	NS
BP_s (mmHg)	120.2	19.6	109.5	17.5	<0.05
BP_d (mmHg)	64.9	9.1	65.7	7.4	NS
FPG ($mmol \cdot l^{-1}$)	5.4	0.4	4.6	0.6	<0.05
FPI ($\mu IU \cdot ml^{-1}$)	11.1	6.3	6.4	2.2	<0.05
TC ($mmol \cdot l^{-1}$)	4.5	0.6	4.6	0.7	NS
TG ($mmol \cdot l^{-1}$)	2.1	0.6	1.4	0.5	<0.05
HDL-C ($mmol \cdot l^{-1}$)	1.7	0.3	2.1	0.4	<0.05
LDL-C ($mmol \cdot l^{-1}$)	2.4	0.5	2.1	0.5	<0.05

Table 4 Changes in physiological parameters in the older age group. Definitions as for Table 2

	Pre-training		Post-training		*P* value
	Mean	SD	Mean	SD	
BMI ($kg \cdot m^{-2}$)	25.1	4.9	25.3	4.4	NS
BP_s (mmHg)	140.4	19.6	134.5	17.5	NS
BP_d (mmHg)	79.9	9.1	78.1	10.4	NS
FPG ($mmol \cdot l^{-1}$)	5.9	1.4	5.5	2.5	<0.05
FPI ($\mu IU \cdot ml^{-1}$)	10.4	4.8	9.1	3.0	NS
TC ($mmol \cdot l^{-1}$)	5.6	0.8	5.3	0.8	<0.05
TG ($mmol \cdot l^{-1}$)	3.6	2.2	3.5	1.3	NS
HDL-C ($mmol \cdot l^{-1}$)	1.4	0.2	1.4	0.2	NS
LDL-C ($mmol \cdot l^{-1}$)	3.5	0.7	3.2	0.8	<0.05

Table 5 Changes in mitochondrial DNA (mtDNA) content (in amol · 5 ng^{-1} genomic DNA) and maximal oxygen uptake ($\dot{V}O_{2\,max}$ in ml · kg^{-1} · min^{-1}) pre- and post-training

Subjects		Pre-training		Post-training		*P* value
		Mean	SD	Mean	SD	
Young	mtDNA	27.1	17.9	52.7	44.6	< 0.05
	$\dot{V}O_{2\,max}$	33.1	3.4	35.2	3.4	< 0.05
Older	mtDNA	15.3	10.2	32.1	30.0	< 0.05
	$\dot{V}O_{2\,max}$	24.3	5.3	30.3	7.3	< 0.05

basal mtDNA content in the young age group was significantly greater than that in the older age group.

Correlation between the mtDNA content and $\dot{V}O_{2\,max}$

There was a significant correlation between basal mtDNA content and $\dot{V}O_{2\,max}$ in the young age group ($r = 0.538$, $P < 0.05$) but not in the older age group (Fig. 3). The change of the mtDNA content was positively correlated with the change of $\dot{V}O_{2\,max}$ in both age groups (Fig. 4).

Discussion

In this study, we have shown that the mtDNA content of peripheral blood and $\dot{V}O_{2\,max}$ increased following a 10 week exercise programme in both young and older age groups. We have also found that the change of $\dot{V}O_{2\,max}$ was positively correlated with the change of the mtDNA content in healthy women.

Exercise increases mitochondrial enzyme activities (Williams et al. 1986). Trained subjects show a 40%–50% increase in the content of mitochondrial oxidative enzymes in their muscle with moderate training, in a similar exercise programme to that of our study (30–60 min daily at 70%–80% of $\dot{V}O_{2\,max}$, 3–5 times weekly) (Henriksson 1992). It was also reported that an approximately 25% increase in muscle $\dot{V}O_{2\,max}$ was observed in rats trained for 8–12 weeks by treadmill running [5.62 (SD 0.31) to 7.06 (SD 0.64) μmol · min^{-1} · g^{-1}]. In this study, there was also observed an increase in mitochondrial enzyme activity following exercise (approximately 70% for cytochrome oxidase and approximately 55% for nicotinamide adenine dinucleotide, reduced, cytochrome-c reductase; Robin-

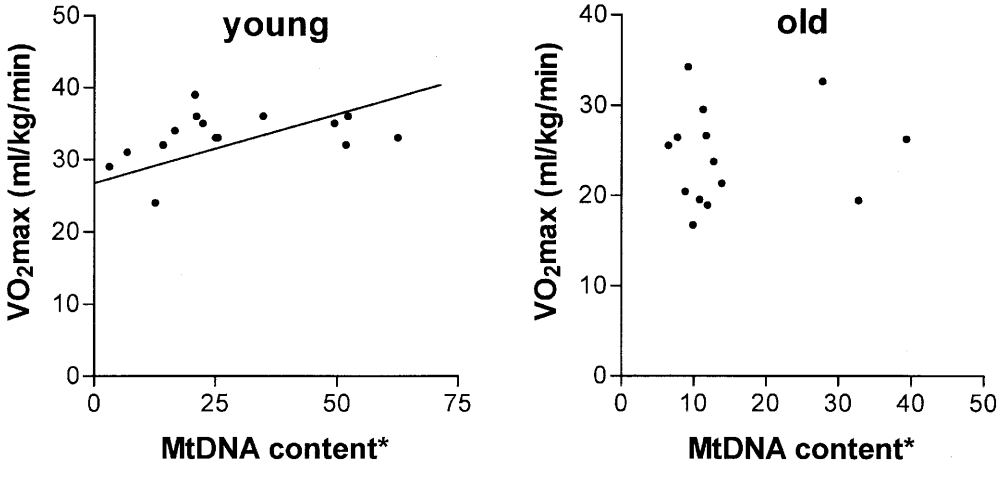

Fig. 3 Correlation between basal mitochondrial DNA (*mtDNA*) content and maximal oxygen uptake ($\dot{V}O_{2\,max}$) in both groups; there is a significant correlation in the young age group ($r = 0.538$, $P < 0.05$), but not in the older. *amol · 5 ng^{-1} genomic DNA

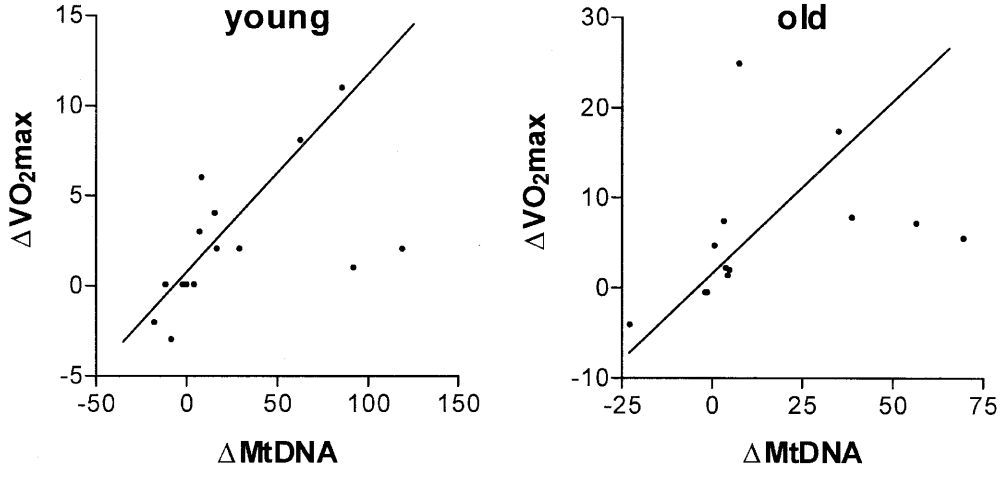

Fig. 4 Correlation between the change in mitochondrial DNA content (Δ*mtDNA*) and the change in maximal oxygen uptake ($\Delta\dot{V}O_{2\,max}$) in both groups; there are significant positive correlations in both groups (in young age group; $r = 0.739$, $P < 0.01$, in older age group; $r = 0.707$, $P < 0.01$)

son et al. 1994). Our results are in agreement with a recent report, which showed that mtDNA content in the muscle of normal subjects is correlated with $\dot{V}O_{2\,max}$ (Wang et al. 1999). They also showed that mtDNA content was correlated with mitochondrial enzyme activity. Although we did not measure mitochondrial enzyme activities, changes in the mtDNA content would reflect the changes in mitochondrial function following exercise.

There was a significant correlation between the pre-training mtDNA content and $\dot{V}O_{2\,max}$ in the young but not in the older age group (Fig. 3). The reason for poor correlation between pre-training mtDNA content and $\dot{V}O_{2\,max}$ in the older age group is not clear at present. It may be explained by the differences in quality and quantity of the mtDNA between the two age groups. It has been well reported that the mtDNA contents in various tissues of older subjects are lower than those in younger subjects (Renis et al. 1989; Corral-Debrinski et al. 1992). In addition, mtDNA deletion and mutation increase with increasing age (Katayama et al. 1991). It has been observed that mtDNA mutations were in the range of 5%–50% in older individuals but they were not present in the young individuals (Michikawa et al. 1999). In our older women, pre-training mtDNA content was already decreased and may not have been a good indicator of $\dot{V}O_{2\,max}$. It is, however, of interest that increases in mtDNA content in the older women were comparable to, even better than, those in the young women in response to the exercise programme.

The level of pre-training fitness of the young age group in our study appears to be lower when compared to other studies which were conducted in western countries (Clausen et al. 1996). However, it was close to the average $\dot{V}O_{2\,max}$ which was reported in the young Korean population [33.5 (SEM 1.0) $ml \cdot kg^{-1} \cdot min^{-1}$] (Yim 1999). The difference may be explained by ethnic or cultural factors. It is noteworthy that $\dot{V}O_{2\,max}$ had increased following the exercise programme in both age groups regardless of baseline mtDNA content. Interestingly, the effect of exercise on $\dot{V}O_{2\,max}$ was greater in older than in the young women. The young subjects might have been fitter at the beginning of the study; thus, the effects of exercise in the young age group would have been less.

We measured mtDNA content in peripheral blood because it was easy to obtain. Although measurement of the mtDNA content of metabolically active tissues would be more reliable, it is not practical in all subjects. However, we have recently found that the mtDNA content of peripheral blood is well correlated with those of muscle and liver in rats (unpublished data). Thus it could be that a change in mtDNA content of peripheral blood reflects those of metabolically active tissues such as liver and muscle.

In summary, a 10 week-moderate intensity, regular exercise increased the mtDNA content of peripheral blood and $\dot{V}O_{2\,max}$. It also decreased insulin resistance parameters in both the young and the older age groups. There was a significant correlation between the change in mtDNA content and the change in $\dot{V}O_{2\,max}$ in healthy women.

Considering that the mtDNA content of peripheral blood was shown to be lower in patients with type 2 diabetes, as well as when they were in the pre-diabetic stage (Lee et al. 1998), exercise could be helpful in increasing the mtDNA content in these patients. It will be interesting to investigate the effect of exercise on mtDNA in diabetic patients in the future.

Acknowledgements The authors thank Bong Sun Kang, Jeong Mi Kim and Soo Jin Jeong for their technical assistance. The work was supported by a grant from the Korean Ministry of Health and Welfare.

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