Frequency Dependence of the Suppressive Effects of Vibration on Atherosclerosis in the Rabbit

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Summary: Whole-body vibration suppresses the development of atherosclerosis in the rabbit (Oki and Matoba, 1987). The present study was designed to clarify whether the effect of vibration on atherosclerosis depends on the frequency of vibration. Longitudinal vibrations at a frequency of 30 or 60 Hz was applied to 12 New Zealand white rabbits for 12 weeks. The gradual decrease in body weight and blood hematocrit in the vibration groups with time were parallel to the changes in the controls. The rate of increase in serum lipid concentrations induced by a cholesterol-rich diet was significantly suppressed in the vibration groups, as compared to the controls. This may be due to the vibration and not the diet. The aortic wall was thinner at 60 Hz than at 30 Hz, whereas the ratios of trace metals (Ca/Mg and Zn/Cu) in the aortic tissues were smaller at 30 Hz. The area of plaque formation in the intima was smaller at 60 Hz than at 30 Hz ($p < 0.05$). Thus, the suppressive effect of vibration on the development of atherosclerosis in the aorta may be greater at a frequency of 60 Hz than at 30 Hz. Vibration may play an important role in lipid metabolism.

Key words: whole-body vibration — atherosclerosis — metal concentrations — vibration frequency — plaque formation

Introduction

The effects of whole-body vibration on the organs and tissues of humans and animals may depend on the frequency of the vibration (Ljung and Sivertsson, 1975; Hudlicka and Wright, 1978; Matoba and Chiba, 1989). In previous experiments, whole-body vibration suppressed the development of atherosclerosis in rabbits on a cholesterol-rich diet and also reduced the rate of increase in serum lipid concentrations (Oki and Matoba, 1987). The present study was designed to clarify the relationship between the vibration frequency and the suppressive effect of vi-

bration on the development of atherosclerosis in the rabbits.

Materials and Methods

Seventeen male New Zealand white rabbits, weighing an average of 2.3 kg, were arranged in order of body weight and divided into 3 groups: group A consisted of 6 rabbits exposed to vibrations of 30 Hz. Group B consisted of 6 rabbits exposed to vibrations of 60 Hz, and the others were controls (Group C). All the rabbits were fed 100g of 1% cholesterol in their diet per day for 12 weeks. The com-

position of metals per 100 g of food was 1,640 mg of calcium (Ca), 240 mg of magnesium (Mg), 4.06 mg of zinc (Zn) and 1.13 mg of copper (Cu). Each rabbit in groups A and B was placed in a cage that was mounted on the platform of a vibrator (Akashi Seisakusho Co.; ASE-385). Longitudinal vibrations with an acceleration level of 10 m/s^2 at each frequency were administered, every other day for 30 minutes, over a period of 12 weeks. The noise generated by the vibrator was approximately 68 dB inside and 58 dB outside the cage. To avoid the bias of noise, the control rabbits were placed in a cage close to the operating vibrator.

The following parameters were observed. Body weight was measured every week. Hematocrit, serum total cholesterol (TC), serum triglycerides (TG), and serum Ca, Mg, Zn and Cu were measured every 4 weeks. Blood samples were obtained from auricular veins, 12 hours after fasting; and the sera were stored at -85°C . Serum metals were measured by ashing with nitric acid and perchloric acid. The ash was dissolved by adding 1N HCl and 0.5% lanthanum chloride by weight. The metal concentrations were determined with an atomic absorption/flame emission spectrophotometer (Shimazu Co.; AA 640-12). After 12 weeks of experiments, all the rabbits were intravenously anesthetized with sodium pentobarbital (Nembutal®: 35-45 mg/kg), and the aortas were rapidly removed and frozen with liquid nitrogen. For light microscopic examination, a part of each aorta was fixed in 10% formalin and embedded in paraffin for staining with Hematoxylin-Eosin and Elastica-Van Gieson methods. Histologically, the ratios of intima (I) to I plus media (M), $I/I+M$, were calculated at the maximal and minimal wall thickness sites from about 30 measurements at each site. The area of plaque formation in the intima was analyzed with an image analysing system (Cosmozone 98; Nikon). The metal

concentrations in the aortic tissues were measured by the same methods that were used for the serum samples.

Results

1. Body weight, hematocrit, serum total cholesterol and triglycerides.

Table 1 shows the changes in body weight, hematocrit, serum total cholesterol (TC) and serum triglycerides (TG) induced by vibration. The body weights in all groups increased approximately 15% after 12 weeks. No significant differences among the 3 groups were observed in the rate of increase in body weight after each week. Significant food refusals were not encountered during the experimental period. The hematocrit for each group averaged approximately 42% at the beginning of the experiment. The values decreased gradually with time to 29.2%, 28.8% and 27.3% for groups A, B and C, respectively, by the 8th week. No significant differences were noted between the 3 groups. Thereafter, the hematocrit increased slightly to approximately 31% in each group by the 12th week.

The mean value for TC in the controls increased markedly from 42.7 mg/dl to 1274, which is about 30 times the basal level, following the 1% cholesterol diet. The rates of increase of TC in groups A and B were much less. The mean values of TC in groups A and B increased from 31.8 to 652 mg/dl and from 23.4 to 560 mg/dl, respectively. There was a significant difference between the values before and after the experimental period in each group. The TG in the controls doubled by the end of the experiments, whereas the TG in groups A and B were reduced by the vibration.

2. Histological findings.

An abundant plaque formation on the intimal surface was observed in the as-

ending aorta in all groups. In the descending aorta, however, the degrees of plaque formation were much different for each group. The areas of plaque formation in ascending and descending thoracic aortas in groups A, B and C were $20.05 \pm$

2.09 (mean $\pm$ SE) %, $13.38 \pm 8.28\%$ and $30.86 \pm 11.32\%$, respectively. A significant difference was noted between the areas in group A and group B ($p < 0.05$). Histologically, the thickness of the intima (I) and media (M) in the thoracic aorta was

TABLE 1
Body weight, hematocrit, serum total cholesterol and triglycerides in each group before and after the experiments

	Group A	Group B	Group C
Body weight (kg):			
Before	2.29 ± 0.04	2.36 ± 0.07	2.31 ± 0.06
12 wks after	2.63 ± 0.05	2.69 ± 0.04	2.76 ± 0.07
Hematocrit (%):			
Before	41.9 ± 0.7	41.6 ± 0.7	41.6 ± 0.8
12 wks after	32.8 ± 0.9	31.0 ± 0.9	28.7 ± 1.6
Total cholesterol (mg/dl):			
Before	31.8 ± 6.0	23.4 ± 6.4	42.7 ± 13.4
12 wks after	651.8 ± 97.4	559.6 ± 83.2	1274.0 ± 208.7
Triglyceride (mg/dl):			
Before	92.2 ± 8.1	90.1 ± 14.4	104.2 ± 20.3
12 wks after	79.1 ± 15.9	61.7 ± 11.4	209.8 ± 71.6

mean $\pm$ standard error; * $p < 0.05$, † $p < 0.01$

TABLE 2
The ratio of intima to intima plus media at the maximal and minimal sites of aortic wall thickness, with or without plaque formation

	Ratio of intima/intima + media	
	Maximal site	Minimal site
Plaque (+)		
Group A (30 Hz)	0.111 ± 0.049	0.022 ± 0.004
Group B (60 Hz)	0.074 ± 0.060	0.012 ± 0.002
Group C (controls)	0.167 ± 0.039	0.014 ± 0.002
Plaque (—)		
Group A (30 Hz)	0.383 ± 0.041	0.132 ± 0.067
Group B (60 Hz)	0.364 ± 0.044	0.018 ± 0.003
Group C (controls)	0.488 ± 0.044	0.097 ± 0.048

mean $\pm$ SE

TABLE 3
Metal concentrations in serum and aortic tissues

Group	n	Calcium	Magnesium	Zinc	Copper
Serum (mg/dl)					
A: 30Hz	6	13.5±0.2	2.42±0.04	0.199±0.009	0.107±0.016
B: 60Hz	6	13.7±0.3	2.50±0.04		0.134±0.012
C: controls	5	13.4±0.2	2.16±0.09		0.146±0.017
Arotic tissues (μg/g dry defatted weight)					
A: 30Hz	6	1107±256	200±10	82.7±3.0	3.10±0.21
B: 60Hz	6	1406±285	220±18	91.5±3.2	3.38±0.82
C: controls	5	1414±505	197±19	89.8±2.5	2.24±0.21

mean±standard error; *p<0.05, †p<0.01

Fig. 1. Ratios of calcium/magnesium and zinc/copper in aortic tissues

Each column represents a mean±standard error.

measured at the maximal and minimal sites of wall thickness. The ratio of I to I+M in each with or without plaque tended to be less in group B than in group A (Table 2).

3. Metal concentrations.

Table 3 compares the metal concentrations in the sera and aortic tissues from the 3 groups at the end of the experimental period. No significant differences were observed in the serum metal concentrations of the groups at the start of the experiments. By the 12th week, the Mg concentrations in groups A and B were

significantly higher than in the controls. The zinc (Zn) and copper (Cu) concentrations did not change, significantly, in any of the groups.

The Ca and Zn concentrations in aortic tissues from group A tended to be lower than in the other groups. The Cu concentration in group A was significantly higher than in the controls ($p<0.05$). Figure 1 shows the ratios of Ca to Mg concentrations (Ca/Mg) and of Zn to Cu concentrations (Zn/Cu). The ratios of Ca/Mg and Zn/Cu in the vibration groups tended to be lower than in the controls. In the vibration groups, the value for group A tended to be lower than that for group B. The value for Zn/Cu in group A was significantly different from the controls ($p<0.05$).

Discussion

The increases in serum total cholesterol and triglycerides induced by a cholesterol-rich diet were suppressed by vibration. However, the body weight and hematocrit were not significantly changed by vibration. These findings indicate that suppression of the rate of increase in serum total cholesterol and triglycerides is not dependent on the cholesterol-rich food,

but on the vibration. Vibration may play an important role in lipid metabolism.

Trace elements in aortic tissues could be directly or indirectly involved in the atherosclerotic process (Mertz, 1982; Aalbers and Houtman, 1985). Calcium concentrations are positively correlated to atherosclerosis, and magnesium concentrations are negatively correlated. The ratio of Ca to Mg should be proportional to the amount of plaque formation in the intima of the aorta. A high ratio of Zn to Cu, due to a deficiency of Cu, is positively related to the degree of atherosclerosis (Klevay, 1975); however Cu concentrations are not changed in the atherosclerotic aorta (Senapati et al. 1985). In the present study, a significantly higher ratio of Zn to Cu, due to a deficiency of Cu, was obtained in the controls as compared to the vibration groups. Accordingly, the ratios of Zn to Cu and Ca to Mg may be involved in the development of atherosclerosis.

The mechanism by which vibration suppresses the development of atherosclerosis is not clear. The rate of suppression in the development of atherosclerosis may depend on the frequency of vibration. The ratios of Ca/Mg and Zn/Cu in the aortic tissues were smaller at 30 Hz than at 60 Hz, which were smaller than the controls. The area of plaque formation and the ratio of the intima to the intima plus media in the aortic walls were smaller at 60 Hz vibrations than at 30 Hz. The vibration frequency influences blood flows which might result in the suppression of plaque formation. Vibrations at frequencies of 1-400 Hz caused prompt reductions in the active forces of vascular smooth muscle in isolated preparations of rat portal vein and rabbit thoracic aorta (Ljung and Sivertsson, 1975). At all frequencies from 30 to 150 Hz, contractions of isolated dog saphenous arteries were reduced significantly to approximately 60% of the controls (Lindbald et al. 1986). Skeletal muscle blood flow

in the rabbit was augmented by vibration due to a considerable dilation of the resistance vessels (Hudlicka and Wright, 1978). Acute exposure to vibration frequencies of 30 to 40 Hz produced vasodilation in the skin (Joyce et al. 1969). The myocardial blood flow was increased by vibrations at 120 Hz and decreased at 50 Hz (Matoba and Chiba, 1989). A hypothesis has been proposed that vibration exerts a direct action on the contractile process by causing an increased rate of detachment of actin-myosin cross-linkages (Ljung and Sivertsson, 1975). The changes of blood flow may be related to the suppression of plaque formation. In conclusion, whole-body vibration suppresses the development of atherosclerosis induced by a cholesterol-rich diet and also inhibits the increase of serum lipid concentrations. The degree of these changes may be dependent on the frequency of the vibration.

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