Plasma antibodies to heat shock protein 60 and heat shock protein 70 are associated with increased risk of electrocardiograph abnormalities in automobile workers exposed to noise

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Abstract In the living and working environment, stressful factors, such as noise, can cause health problems including cardiovascular diseases and noise-induced hearing loss. Some heat shock proteins (Hsps) play an important role in protecting cardiac cells against ischemic injury, and antibodies against these Hsps are associated with the development and prognosis of atherogenesis, coronary heart disease, and hypertension. Whether the presence of such antibodies is associated with abnormal electrocardiography (ECG) in stressed autoworkers exposed to chronic noise is presently unknown. Therefore, we investigated the association between the levels of plasma anti-Hsp60 and anti-Hsp70 with electrocardiograph abnormality in 396 autoworkers exposed to different noise levels by using Western blot, ECG, and multivariate logistic regression analysis. The results showed that the increase in levels of anti-Hsp70 was associated with a higher risk of ECG abnormalities characteristic of chronic myocardial ischemia ($P < 0.05$), conductive abnormality ($P < 0.01$), or heart displacement ($P < 0.05$); in contrast, elevated anti-Hsp60 was related to ECG abnormalities characteristic of sinus arrhythmia, chronic myocardial ischemia, and ectopic rhythm ($P < 0.01$ for all). Overall, high levels of both anti-Hsp70 and anti-Hsp60 were associated with significantly increased risk of ECG abnormalities (odds ratio [OR] = 1.73 and 95% confidence interval [CI] = 1.04–2.86 for anti-Hsp70 and OR = 1.36 and 95% CI = 1.07–1.72 for anti-Hsp60) with and without adjustment for cumulative noise exposure (OR = 1.96 and 95% CI = 1.20–3.21 for anti-Hsp70 and OR = 3.93 and 95% CI = 1.72–8.92 for anti-Hsp60). These findings suggest that the production of both anti-Hsp70 and anti-Hsp60 may be independent risk factors for the development and progression of abnormal ECG and therefore possibly cardiovascular diseases in autoworkers exposed to occupational noise.

INTRODUCTION

Stress response in humans can be induced by various environmental factors, including noise, high temperature, and viral infection (Benjamin and McMillan 1998; Falkowska-

Podstawka and Wernicki 2003). Occupational noise exposure constitutes a health problem because it causes disorders such as noise-induced hearing loss (NIHL) and sleep disturbance in humans (Folmer et al 2002; Borchgrevink 2003; Marcellini et al 2003; Bray et al 2004). Permanent hearing loss can be caused by chronic exposure equal to an average sound pressure level (SPL) of 85 dB (A) for 8 consecutive hours in a single day (Morata et al 1993), which is also sufficient to cause cardiovascular diseases (Babish 1998; Melamed et al 1999; Lusk 2002).

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Received 6 December 2004; Revised 31 January 2005; Accepted 8 February 2005.

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In response to stress, heat shock proteins (Hsps) fulfill a variety of physiological functions, including assisting in intracellular assembly, disassembly, stabilization, and internal transport of intracellular proteins to maintain normal structure and function of the cell. In addition to their functions as molecular chaperones, Hsps can protect organisms against certain diseases (Xu et al 1993; Rassow et al 1995; Hartl 1996; Xu and Wick 1996; Benjamin and McMillan 1998; Snoeckx et al 2001; Pockley 2002; Jin et al 2004; Mandal et al 2004; Sreedhar and Csermely 2004; Xiao et al 2004). For instance, humoral immune responses to Hsps were found in patients with some autoimmune diseases, although the antibody titers against Hsps were variable among the patients (Zugel and Kaufmann 1999a). Antibodies against Hsps have also been reported in individuals with hypertension and atherosclerosis (Benjamin and McMillan 1998; Wu et al 2001; Pockley et al 2003). In healthy individuals, anti-Hsps were occasionally detectable as well (Zugel and Kaufmann 1999b), which is not surprising in that Hsps are ubiquitous in living organisms (Schett et al 1995; Shingai et al 1995; Wu et al 1998, 2001; Pockley 2002; Xu 2002; Xiao et al 2004). However, it is unknown whether the presence of anti-Hsp antibodies plays any role in the development and progression of these diseases. Antibodies might serve as carriers for Hsps that deliver immunogenic peptides to antigen presenting cells for subsequent presentation to T cells or activate cells of the innate immune system. (Feige and van Eden 1996; Multhoff et al 1998; Multhoff 2002; Asea 2003). Therefore, the levels of anti-Hsps might be of clinical significance in the diagnosis and pathogenesis of certain atherosclerotic vascular diseases (Xu et al 1993; Kaufmann and Schoel 1994; Schett et al 1995; Hoppichler et al 1996; Pockley et al 2000; Pockley 2002; Xu 2002; Bason et al 2003).

Electrocardiography (ECG) is one of the clinical characteristics used for diagnosis of cardiovascular diseases, and specific changes of ECG patterns have emerged as markers for different stages of these diseases (Nakayasu et al 2004; Okin et al 2004). Moreover, enhanced levels of anti-Hsps become detectable in individuals with some cardiovascular diseases, such as atherosclerosis, coronary heart disease, and borderline hypertension (Rauch et al 1995; Pockley et al 2000; Mandal et al 2004). Over the past years, several studies have suggested that Hsps and anti-Hsps are likely related to cardiovascular events (Veres et al 2002; Pockley 2003).

In a recent study, we reported that the presence of anti-Hsp60 and anti-Hsp70 was linked to NIHIL in Chinese automobile workers (Yang et al 2004). However, it remained unknown whether occupational noise exposure might cause abnormal ECG and whether anti-Hsps levels might be markers for ECG abnormalities in stressed workers. These may be important for early detection and

prevention of cardiovascular diseases in the workers. Therefore, the aims of this study were to (1) explore a possible link between exposure level of occupational noise in the workplaces, the appearances of plasma anti-Hsp60 or anti-Hsp70, and abnormal ECG and (2) establish the association between enhanced anti-Hsp60 and anti-Hsp70 levels and abnormal ECG, which may serve as a biomarker for detection of early stages of cardiovascular diseases among exposed workers.

MATERIALS AND METHODS

Study subjects

A total of 396 autoworkers (269 men and 127 women) at the Dongfeng Motor Co (Shiyan, Hubei, People's Republic of China), who had been employed for at least 1 year, were included in this study. These subjects had a history of exposure only to occupational noise for at least 1 year but without exposure to other harmful factors, such as high temperature (greater than 32°C) or known toxicants (eg, organic solvent or polycyclic aromatic hydrocarbons) in workplaces (see Yang et al 2004). They had no history of fever or common infections such as influenza, diarrhea, pneumonia, or hepatitis within 1 month before medical examination because these infections may cause stress response due to inflammation. Furthermore, these subjects did not use any hearing protectors, such as earmuffs or disposable earplugs. The mean age of all subjects was 34.1 (SD = 7.0) years. The average employment time was 11.3 (SD = 7.4) years. The subjects were divided into control and exposed groups according to the level of occupational noise exposure, ie, the control group included 181 subjects who had been exposed to an occupational noise level of less than 85 dB (A) and the exposed group included 215 subjects who had been exposed to levels equal or greater than 85 dB (A). After having obtained informed consent, standardized occupational questionnaires were administered to collect occupational history from the subjects at the time of medical examination and to obtain information on employment history, tobacco smoking, and alcohol intake as well as medical and personal history. This study was approved by the Ethics and Human Subject Committee of Tongji Medical College.

Environmental noise monitoring

Noise exposure level at the selected workplaces was assessed with a sound pressure audiometer (BK-2231, Brüel and Kjaer Company, Naerum, Denmark) at 10 AM, 3 PM, and 5 PM for 3 consecutive days, twice per year, according to the Chinese national criterion for noise in workplace (Liu and Cai 1995). To evaluate the actual noise exposure level of the worker, cumulative noise exposure

(CNE) was calculated, based on the database of a 20-year noise exposure, according to monitoring data on A SPL and employment time calculated as follows: $\text{Exp}_c = \text{Leq} + 16.61 \times \log_{10}(T/T_0)$, dB (A) (Talbot et al 1999), where Exp_c is the CNE level; Leq , the time-weighted average exposed SPL A; T , the total adjusted time worked (in years); and T_0 , year 1.

Electrocardiography

Examinations of cardiac function of all workers were performed by experienced industrial physicians, and the ECG (ECG-6501, Nihon Kohden Corp, Tokyo, Japan) was recorded at the Institute of Occupational Medicine, Dongfeng Co, in a room that was away from the noisy workplace. Thereafter, according to the diagnosis standards of ECG (Jiang et al 2001), the subjects were classified into groups with normal or abnormal ECG. The normal group had no detectable abnormal changes of ECG, whereas the abnormal group had ECGs characteristic of sinus arrhythmia, left ventricular hypertrophy, chronic myocardial ischemia, conductive abnormality, ectopic rhythm, and others (Jiang and Song 2001). The diagnosis of the abnormal ECGs excludes other possible causes, such as tiredness or depression (or both), which may have contributed to ECG abnormalities in apparently healthy individuals.

Blood glucose and serum lipid and lipoprotein

To evaluate other risk factors of cardiovascular diseases, blood glucose levels were determined using glucose oxidation enzyme analysis (Chen 1997), and serum total cholesterol, serum triglycerides, and low-density lipoprotein cholesterol were determined using the methods described by Suen (1997). All these methods were the standard methods used in hospitals as recommended by the Chinese Medical Association (Chen 1997; Yan 2003), which is part of regular clinical evaluation of cardiovascular diseases.

Anti-Hsp60 and anti-Hsp70 levels

To detect the titers of anti-Hsp60 and anti-Hsp70, venous blood was collected, and the plasma of each sample was separated. Both recombinant human Hsp60 and Hsp70 were obtained with the expression of human complementary DNA coding and inducible Hsp70 in NaCl-induced *Escherichia coli* GJ1168 cells using pET30 as the expression vector (Tanguay et al 1993). The titers of anti-Hsp60 and anti-Hsp70 were determined by Western immunoblotting following the method described previously (Wu et al 1998; Yang et al 2004). In brief, the recombinant Hsp60 or Hsp70 was separated on polyacrylamide gel

electrophoresis, transferred to nitrocellulose membranes, and small membrane pieces (2×2 mm) containing Hsp60 and Hsp70 were incubated with plasma (diluted to 1:10) and the presence of anti-Hsp60 or anti-Hsp70 revealed using 3,3-diaminobenzidine tetrahydrochloride. The brown band present on the nitrocellulose strip was regarded as positive and no color band as negative (Wu et al 1998, 2001). The positive samples at a dilution of 1:10 were then diluted to 1:20, 1:40, and 1:80 to obtain final titer levels of the anti-Hsp60 and anti-Hsp70.

Statistic analysis

The clinical and laboratory measurements were built into a computerized database. Further statistic analysis of the data was carried out using the statistic analysis software (SAS 8.1) package. The Student's *t*-test and chi-square test were used for comparisons between various continuous and categorical variables, respectively. Cochran-Mantel-Haenszel Statistics test was used to assess the correlations between these variables. Crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were estimated in logistic regression models to measure the strength of association, ie, the probability of the occurrence of abnormal ECG given the exposure to noise or presence of the anti-Hsps. Statistical trends were tested in either general linear regression models or in logistic regression models. Both univariate and multivariate logistic regression models were fitted to estimate the associations. Statistic differences were based on the significance level of $P < 0.05$.

RESULTS

Selected characteristics of the subjects with and without exposure to noise

In this study, the noise exposure at a total of 55 workplaces was measured, of which 20 were considered non-hazardous occupational exposure spots (where the subjects in the control group worked) and 35 occupational noise exposure spots (where the subjects in the exposure group worked). The noise levels ranged between 65.0 and 74.7 dB (A) for the control spots and between 85.50 and 99.30 dB (A) for the exposure spots. As shown in Table 1, there was no difference in the distributions of sex, smoking, and tea drinking, nor any difference in height, weight, body mass index, history of cardiovascular disease, and the level of plasma anti-Hsp60 between the control group and the exposed group. However, compared with the control group, the exposed group was older ($P < 0.01$) and more likely to drink alcohol ($P < 0.05$), had a lower mean education level ($P < 0.01$) but a higher

Table 1 Characteristics and frequency of positive anti-Hsp70 and anti-Hsp60 in subjects by exposure status ($n = 396$)

	Control ^a	($n = 181$)	Exposed ^b	($n = 215$)	<i>P</i> value
Age ($n, \%$)					
<35 y	123	68.0	115	53.5	<0.01
≥35 y	58	32.0	100	46.5	
Sex ($n, \%$)					
Male	121	66.3	148	68.8	>0.05
Female	60	33.2	67	31.2	
Smoking ($n, \%$)					
Yes	65	5.9	91	42.3	>0.05
No	116	64.1	124	57.7	
Alcohol drinking ($n, \%$)					
Yes	30	16.6	53	24.7	<0.05
No	151	83.4	162	75.4	
Tea drinking ($n, \%$)					
Yes	94	51.9	118	54.9	>0.05
No	87	48.1	97	45.1	
Height (cm, mean $\pm$ SD)	167.12 $\pm$ 7.3		165.94 $\pm$ 7.7		>0.05
Weight (kg)	65.04 $\pm$ 12.7		64.24 $\pm$ 11.7		>0.05
Body mass index (kg/m ² , mean $\pm$ SD) ^c	23.15 $\pm$ 3.4		23.22 $\pm$ 3.2		>0.05
Education level (mean $\pm$ SD) ^d	4.27 $\pm$ 0.6		3.76 $\pm$ 0.7		<0.01
LAeq (dB (A)·y) (mean $\pm$ SD)	77.17 $\pm$ 3.6		89.61 $\pm$ 6.6		<0.01
CNE (dB (A)·y) (mean $\pm$ SD)	84.51 $\pm$ 3.9		98.71 $\pm$ 5.6		<0.01
History of CVD ($n, \%$)					
Yes	27	14.9	29	13.5	>0.05
No	154	85.1	186	86.5	
Abnormal ECG ($n, \%$)					
Yes	33	18.2	73	34.1	<0.01
No	148	81.8	142	66.1	
Anti-Hsp60 ($n, \%$) ^e					
1:10	9	5.0	17	7.9	>0.05
1:20	9	5.0	16	7.4	>0.05
1:40	9	5.0	15	7.0	>0.05
1:80	7	3.9	10	4.7	>0.05
Anti-Hsp70 ($n, \%$) ^e					
1:10	37	2.0	69	32.1	<0.01
1:20	11	6.1	31	14.4	<0.01
1:40	9	5.0	24	11.2	<0.05
1:80	4	2.2	11	5.1	>0.05

CNE, cumulative noise exposure; CVD, cardiovascular disease; ECG, electrocardiography.

^a Control group: subjects without occupational noise exposure.

^b Exposure group: subjects with occupational noise exposure.

^c Body mass index: ≥ 25 (1), < 25 (0).

^d Educational level: illiteracy (1), elementary school (2), junior high school (3), senior high school (4), junior college and higher (5).

^e Positive rates at different dilutions, which was calculated as: (no. of subjects positive for the test/all subjects tested) $\times$ 100%.

mean LeqA level ($P < 0.01$) and CNE level ($P < 0.01$) and higher detectable level of anti-Hsp70 ($P < 0.05$ for titers up to 1:40), as we previously reported (Yang et al 2004). More importantly, a greater percentage of the exposed group (34.1%) had abnormal ECG than the control group (18.2%) ($P < 0.01$) (Table 1).

CNE and ECG abnormality

Next, subjects were divided into normal ($n = 290$) and abnormal ECG groups ($n = 106$) on the basis of the outcomes of their ECG examinations. General and medical

characteristics of the subjects by ECG status are summarized in Table 2. The 2 groups did not differ in most of the characteristics including frequencies of cigarette smoking and alcohol and tea drinking, levels of blood glucose and total cholesterol and glycerol trioleate, proportion of hypertension, and family history of cardiovascular diseases, although the values of these characteristics were higher in the abnormal ECG group than in the normal ECG group. However, the abnormal ECG group had been exposed to a significantly higher level of CNE (94.9 ± 8.4 dB (A)·year) than the normal ECG group (91.2 ± 8.5 dB (A)·year) ($P < 0.01$) (Table 2).

Table 2 General and medical characteristics and CNE of subjects by ECG status

	Normal ECG (<i>n</i> = 290)	Abnormal ECG (<i>n</i> = 106)	<i>P</i> value ^a
General characteristics			
Age (y, mean ± SD)	34.12 ± 6.6	34.13 ± 7.9	>0.05
Sex ratio (M/F)	196/94	73/33	>0.05
Smoking (%)	42.1	32.1	>0.05
Alcohol drinking (%)	20.0	23.6	>0.05
Tea drinking (%)	53.1	54.7	>0.05
CNE (mean ± SD)	91.23 ± 8.5	94.93 ± 8.4	<0.01
Medical characteristics			
Body mass index (mean ± SD)	23.21 ± 3.2	23.13 ± 3.4	>0.05
History of CVD (%)	14.8	12.3	>0.05
Abnormal rate of systolic pressure (%)	6.2	8.5	>0.05
Abnormal rate of diastolic pressure (%)	27.2	22.6	>0.05
Blood glucose (mmol/L, mean ± SD)	4.55 ± 0.9	4.66 ± 0.7	>0.05
Total cholesterol (mmol/L, mean ± SD)	5.37 ± 1.4	5.40 ± 1.0	>0.05
Glycine trioleate (mmol/L, mean ± SD)	1.32 ± 0.9	1.17 ± 0.7	>0.05

ECG, electrocardiography; CNE, cumulative noise exposure; CVD, cardiovascular disease.

^a Two-sided χ^2 or Student's *t*-test for comparison between the two groups.**Table 3** Frequency of anti-Hsp70 and anti-Hsp60 of subjects by electrocardiography abnormalities

Groups	No. of workers	Anti-Hsp60 (+) ^a		Anti-Hsp70 (+) ^b		<i>P</i> value ^c	
		<i>n</i>	%	<i>n</i>	%	Anti-Hsp60 Ref.	Anti-Hsp70 Ref.
Normal	290	12	4.1	66	22.8		
Abnormal	106	14	13.2	40	37.7	<0.01	<0.01
Sinus arrhythmia	44	7	15.9	15	34.1	<0.01	>0.05
Left ventricular hypertrophy	21	1	4.8	5	23.2	>0.05	>0.05
Chronic myocardial ischemia	9	3	33.3	5	55.6	<0.01	<0.05
Conductive abnormalities	10	1	10.0	6	60.0	>0.05	<0.01
Ectopic rhythm	7	2	28.6	2	28.6	<0.01	>0.05
Others ^d	15	0	0.0	7	46.7	>0.05	<0.05
Total	396	40	10.1	106	26.8	—	—

^a Anti-Hsp60 (+): positive level of anti-Hsp60.^b Anti-Hsp70 (+): positive level of anti-Hsp70.^c χ^2 tests for comparison with the reference (Ref.) group.^d Others include heart displacement, electrical axis deviation.

Anti-Hsp70 and anti-Hsp60 electrocardiograph abnormality type

Of all subjects, 26.8% (106 of 396) presented enhanced anti-Hsp70 and 10.1% (40 of 396) enhanced anti-Hsp60 (Table 3). The percentages of subjects with enhanced levels of anti-Hsp70 were significantly higher in the abnormal ECG group (37.7%, 40 of 106) than in the normal ECG one (22.8%, 66 of 290). Similar findings were observed for anti-Hsp60 (13.2%, 14 of 106 in the abnormal ECG group and 4.1%, 12 of 290 in the normal ECG group) ($P < 0.01$). Although the incidences of positive anti-Hsp70 and anti-Hsp60 in the abnormal ECG groups tended to be higher than in the normal ECG group, only the subgroups of chronic myocardial ischemia, conductive abnormality, and other conditions (such as heart displacement, electrical axis deviation) were found to have significantly higher rates of positive anti-Hsp70 than that of the normal ECG group. Moreover, the positive rates of anti-Hsp60 were significantly higher in subgroups of si-

nus arrhythmia, chronic myocardial ischemia, and ectopic rhythm than that of the normal ECG group ($P < 0.05$ or $P < 0.01$). Although both anti-Hsp60 and anti-Hsp70 were associated with abnormal ECG in both univariate analysis and multivariate analysis, only anti-Hsp70 was independent of CNE.

Risk factors for ECG abnormality

Several potential confounders for ECG abnormality are listed in Table 4. The results showed that positive anti-Hsp70, daily sleep time, and CNE were associated with risk of ECG abnormalities. After adjustment for each other by including these factors in the same multivariate logistic model with or without CNE, those subjects who had enhanced anti-Hsp70 had 1.73-fold (95% CI = 1.04–2.86) or 1.96-fold (95% CI = 1.20–3.21) increased risk compared with subjects negative for anti-Hsp70 (Table 4), suggesting that the association between the levels of en-

Table 4 Logistic regression analysis of risk factors, anti-Hsp70, and electrocardiograph abnormalities

Variables	Regression coefficient		Standard error		Wald value		P value		OR (95% CI)	
	w/ CNE ^a	w/o CNE ^b	w/ CNE	w/o CNE	w/ CNE	w/o CNE	w/ CNE	w/o CNE	w/ CNE	w/o CNE
Constant	-4.322	0.290	1.664	1.122	6.748	0.067	0.009	0.796	—	—
CNE (dB (A)-y)	0.054	—	0.014	—	14.181	—	0.000	—	1.055 (1.026–1.085)	—
Smoking (cigarettes/y)	-0.564	-0.529	0.258	0.251	4.797	4.449	0.029	0.035	0.569 (0.343–0.942)	0.589 (0.360–0.963)
Marriage status ^c	-0.750	-0.552	0.330	0.318	5.148	3.010	0.023	0.083	0.473 (0.247–0.903)	0.576 (0.308–1.074)
Sleep time (h/d)	0.171	0.159	0.070	0.069	5.901	5.333	0.015	0.021	1.187 (1.034–1.362)	1.173 (1.024–1.343)
HS ^d	-0.838	-0.806	0.416	0.411	4.053	3.855	0.044	0.050	0.433 (0.191–0.978)	0.446 (0.200–0.999)
Anti-Hsp70 ^e	0.545	0.673	0.258	0.252	4.475	7.149	0.034	0.008	1.725 (1.041–2.860)	1.959 (1.197–3.208)

CI, confidence interval; CNE, cumulative noise exposure; OR, odds ratio.

^a w/ CNE: adjustment for other factors with CNE.^b w/o CNE: adjustment for other factors without CNE.^c Marriage status: single (1), married (2), divorce (3), remarriage (4).^d HS: health situation within the past 1 y: having illness (0), without damage or illness (1).^e Hsp70 (+) = 1, Hsp70(-) = 0.**Table 5** Logistic regression analysis of risk factors, anti-Hsp60, and electrocardiograph abnormalities

Variables	Regression coefficient		Standard error		Wald value		P value		OR (95% CI)	
	w/ CNE ^a	w/o CNE ^b	w/ CNE	w/o CNE	w/ CNE	w/o CNE	w/ CNE	w/o CNE	w/ CNE	w/o CNE
Constant	-4.489	-0.903	1.675	0.994	7.184	0.825	0.007	0.364	—	—
CNE (dB (A)-y)	0.056	—	0.014	—	15.322	—	0.000	—	1.058 (1.028–1.088)	—
Smoking (cigarettes/y)	-0.554	-0.542	0.259	0.253	4.577	4.597	0.032	0.032	0.575 (0.346–0.955)	0.582 (0.315–0.955)
Marriage status ^c	-0.694	—	0.332	—	4.384	—	0.036	—	0.499 (0.265–0.957)	—
Sleep time (h/d)	0.178	0.174	0.070	0.068	6.534	6.201	0.011	0.013	1.195 (1.042–1.371)	1.185 (1.037–1.355)
HS ^d	-0.909	-0.941	0.413	0.406	4.840	5.379	0.028	0.020	0.390 (0.176–0.864)	0.390 (0.176–0.864)
Work strength ^e	—	0.187	—	0.102	—	3.354	—	0.067	—	1.205 (0.987–1.471)
Anti-Hsp60 ^f	0.305	1.369	0.119	0.422	6.558	10.519	0.010	0.001	1.357 (1.074–1.715)	3.932 (1.719–8.922)

CI, confidence interval; OR, odds ratio; CNE, cumulative noise exposure.

^a w/ CNE: adjustment for other factors with CNE.^b w/o CNE: adjustment for other factors without CNE.^c Marriage status: single (1), married (2), divorce (3), remarriage (4).^d HS: health situation within the past 1 y: having illness (0), without damage or illness (1).^e Work strength: weak (1), moderate (2), strong (3), very strong (4).^f Hsp60 (+) = 1, Hsp60 (-) = 0.

hanced anti-Hsp70 and ECG abnormality may be independent of CNE. Similarly, after adjustment for the same factors in the same manner as described above, subjects with enhanced anti-Hsp60 had 1.36-fold (95% CI = 1.07–1.72) or 3.93-fold (95% CI = 1.719–8.922) increased risk compared with subjects negative for anti-Hsp60 (Table 5), suggesting that the association between the levels of enhanced anti-Hsp60 and ECG abnormality may be independent but likely confounded by CNE.

DISCUSSION

The effects of noise exposure are both auditory and non-auditory. The auditory effects are physiological, including hearing loss and interference with communications. The nonauditory effects include a variety of chronic health problems, such as sleeping problems, fatigue, and cardiovascular, autonomic, gastric and nervous disorders. Although the nonauditory effect of noise on health is generally quite weak (Borchgrevink 2003), the health effects depend on either level or length of occupational noise exposure, and the effect of long-time noise exposure on an individual's health is of particular concern. In this study, we found that the levels of both enhanced levels of anti-Hsp70 and anti-Hsp60 appeared to be associated with ECG abnormalities in the noise-exposed workers, but the association of anti-Hsp70 with ECG abnormalities seemed to be independent of CNE.

Numerous previous studies reported that Hsps can be induced in all organisms by various physiological stresses (eg, hyperthermia, hemodynamics, mutant proteins, and oxidative injury) and environmental stresses (high temperature, noise, xenobiotics). Some of these factors may produce multiple changes that ultimately affect protein structure and functions in living cells (Benjamin and McMillan 1998; Sreedhar and Csermely 2004). Hsps can confer cytoprotection against a number of stresses (Hightower 1991). However, when Hsps are expressed at elevated levels, they may become a target of immune responses (Xu et al 1999). In this study, no evidence for infectious factors associated with elevated level of anti-Hsp70 was found, and the elevated levels of anti-Hsp70 in workers exposed to high occupational noise appeared to be induced by abnormal stress from noise in the workplace (Yang et al 2004). One possible explanation is that occupational noise can trigger the synthesis of these Hsps (Lim et al 1993; Welch 1993). It is possible that as a result of the degradation of induced Hsps, peptides may be released from damaged cells and become targets of humoral and T cell-mediated immune response, ie, directed toward such Hsp-derived peptides. Findings from several studies support this hypothesis of an immune response to Hsps in chronic diseases, in which there was evidence for immunologic recognition of cross-reaction and auto-

immunity (Pockley 2002). Our previous studies indicated that the health of workers with long-time occupational noise exposure at a lower level was also affected (Wu et al 2001; Yang et al 2004). Therefore, the level of anti-Hsp70 may be a biomarker of abnormally experienced stresses in the workers exposed to occupational noise at lower levels but for a long period of time.

ECG abnormality is either a clinical characteristic of cardiovascular disease or a sign of early stage of cardiac function abnormality because the electrocardiogram has been widely applied as a basic cardiologic test for the patients with suspected or known heart disease and as a screening test for those with other conditions, such as coronary artery disease, cardiomyopathies, and left ventricular hypertrophy (Chen 1998; Zhang 1998; Sgarbossa et al 2001). Our findings showed that the presence of anti-Hsp70 and anti-Hsp60 was associated with ECG abnormalities; specifically, chronic myocardial ischemia and conductive abnormality were more likely to be associated with anti-Hsp70, whereas sinus arrhythmia, chronic myocardial ischemia, and ectopic rhythm were more likely associated with anti-Hsp60, which suggests that there might be an etiological link between the presence of either anti-Hsp70 or anti-Hsp60 and ECG abnormalities.

The clinical use of anti-Hsp70 antibody measurements in cardiovascular and cerebral diseases remains controversial. Some investigations (Portig et al 1997) have not found an association of anti-Hsp70 with cardiovascular diseases, whereas others have reported that their presence was correlated with an improved clinical outcome (Vogt et al 2004). However, others showed that Hsp60, Hsp70, anti-Hsp60, anti-Hsp65, and anti-Hsp70 were detectable in healthy individuals aged from 20 to 96 years and also showed a progressive decline in Hsp60 and Hsp70 levels and a trend of increase in Hsp70 antibody levels with age (Rea et al 2001). A significant correlation between anti-Hsp70 antibody and different vascular diseases has been reported in vascular patients, such as lower limb claudicants, lower-limb critical ischemia, and abdominal aortic aneurysms (Chan et al 1999), suggesting that Hsp70 and anti-Hsp70 might be involved in the pathogenesis and propagation of atherosclerosis. Other reports suggested that humoral immunity to Hsp70 was common in patients (in the first 48 hours after stroke onset) with ischemic stroke that was mainly caused by the development of vascular lesions and that elevated levels of anti-Hsp70 antibody could be triggering factors for stroke (Gromadzka et al 2001). Recently, Jin et al (2004) found that anti-Hsp70 antibody in patients (in the first 24 hours after stroke onset) with cerebral infarction was significantly higher than in controls, and this antibody decreased, even disappeared, during the recovery for 30 days.

Environmental stresses induce syntheses of Hsps,

which work as molecular chaperones, facilitating the synthesis, folding, assembly, and intracellular transport of many functional proteins (Hightower 1991; Morimoto et al 1994; Hartl 1996). Hsp70's cytoprotection was extensively observed in the hearts and brains of animals. Thus, Hsp70 protects heart from myocardial ischemic injury in heat shocked, transgenic animals and humans (Currie et al 1993; Marber et al 1995; Radford et al 1996; Plumier et al 1997; Rajdev et al 2000; Jin et al 2004). Interestingly, the presence of an antibody against Hsp70 contributes to autoimmunity through cross-reactivity between Hsps and tissue-specific proteins containing a similar epitope motif because of the high degree of amino acid sequence homology among Hsps of different species. Our findings suggest that antibodies against Hsp70 and Hsp60 might be involved in the pathogenesis or prognosis of cardiovascular diseases (or both). However, this hypothesis needs to be further confirmed in the follow-up study of the workers who had ECG abnormality and a history of exposure to CNE.

In summary, this study is the first to demonstrate associations between antibodies against human Hsp70 and Hsp60 and ECG abnormalities in autoworkers with an occupational noise exposure history. Thus, elevated levels of anti-Hsp70 and anti-Hsp60 were associated with ECG abnormality in workers exposed to high noise for a long time in their workplace, which may have an effect on their cardiac health; high noise levels may lead to increased plasma anti-Hsp70 and anti-Hsp60, which were associated with increased risk of ECG abnormality, particularly for anti-Hsp70. Therefore, the presence of enhanced anti-Hsp70 and Hsp60 may be a potential marker for susceptibility to cardiovascular disease associated with exposure to occupational noise. These findings are consistent with the notion that autoimmunity plays a role in the damage effect induced by occupational noise. However, the underlying molecular mechanisms for the association between noise, enhanced levels of Hsp70 and Hsp60, and ECG abnormalities among the exposed workers remain to be further investigated in larger studies.

ACKNOWLEDGMENTS

We are grateful to all volunteers who participated in the study and the medical personnel of Dongfeng Motor Co (Shiyan, Hubei) for generous collaboration in recruiting the subjects and collecting biological samples. This work was supported by research funds from the National Key Basic Research and Development Program (2002CB512905), the National Natural Science Foundation of China (NNSFC: 30200227 and 30430590). T.W. and R.M.T. also acknowledge financial support from the NNSFC of China and the Canadian Institute of Health Research of Canada for a research exchange program.

REFERENCES

- Asea A. 2003. Chaperokine-induced signal transduction pathways. *Exerc Immunol Rev* 9: 25–33.
- Babisch W. 1998. Epidemiological studies of the cardiovascular effects of occupational noise—a critical appraisal. *Noise Health* 1: 24–39.
- Bason C, Corrocher R, Lunardi C, et al. 2003. Interaction of antibodies against cytomegalovirus with heat-shock protein 60 in pathogenesis of atherosclerosis. *Lancet* 362: 1971–1977.
- Benjamin IJ, McMillan DR. 1998. Stress (heat shock) proteins: molecular chaperones in cardiovascular biology and disease. *Circ Res* 83: 117–132.
- Borchgrevink HM. 2003. Does health promotion work in relation to noise? *Noise Health* 5: 25–30.
- Bray A, Szymanski M, Mills R. 2004. Noise induced hearing loss in dance music disc jockeys and an examination of sound levels in nightclubs. *J Laryngol Otol* 118: 123–128.
- Chan YC, Shukla N, Abdus-Samee M, Berwanger CS, Standford J, Singh M, Mansfield AO, Stansby G. 1999. Anti-heat-shock protein 70 kDa antibodies in vascular patients. *Eur J Vasc Endovasc Surg* 18: 381–385.
- Chen HZ. 1998. Atherosclerosis and coronary heart disease. In: *Medicine*, ed Chen HZ. People Health Publishing House, Beijing, 246–274.
- Chen XM. 1997. Measurements of glucose and its metabolic products. In: *Clinic Laboratory Methodology*, ed Yang CS, Song NG. Tianjing Science and Technology Publishing House, Tianjing, China, 208–209.
- Currie RW, Tanguay RM, Kingma JG. 1993. Heat-response and limitation of tissue necrosis during occlusion/reperfusion in rabbit hearts. *Circulation* 87: 963–971.
- Falkowska-Podstawka M, Wernicki A. 2003. Heat shock proteins in health and disease. *Pol J Vet Sci* 6: 61–70.
- Feige U, van Eden W. 1996. Infection, autoimmunity and autoimmune disease. *EXS* 77: 359–373.
- Folmer RL, Griest SE, Martin WH. 2002. Hearing conservation education programs for children: a review. *J Sch Health* 72: 51–57.
- GBZ (National Criteria of China) 1. 2002. Hygienic standard for the Design of Industrial Enterprises. 5.
- Gromadzka G, Zielinska J, Ryglewicz D, Fiszur U, Czlonkowska A. 2001. Elevated levels of anti-heat shock protein antibodies in patients with cerebral ischemia. *Cerebrovasc Dis* 12: 235–239.
- Hartl FU. 1996. Molecular chaperones in cellular protein folding. *Nature* 381: 571–579.
- Hightower LE. 1991. Heat shock, stress proteins, chaperones and proteotoxicity. *Cell* 66: 191–197.
- Hoppichler F, Lechleitner M, Traweger C, Schett G, Dzien A, Sturm W, Xu Q. 1996. Changes of serum antibodies to heat shock protein 65 in coronary heart disease and acute myocardial infarction. *Atherosclerosis* 126: 333–338.
- Jiang ZZ, Suen RL, Wang YS. 2001. Diagnosis standard of electrocardiography. In: *Newly Brief Electrocardiograph*, ed Jiang ZZ, Song YC. Science and Technology Publishing House, Beijing, 431–441.
- Jin X, Xiao C, Tanguay R, et al. 2004. Correlation of lymphocyte heat shock protein 70 levels with neurologic deficits in elderly patients with cerebral infarction. *Am J Med* 117: 406–411.
- Kaufmann SH, Schoel B. 1994. Heat shock protein as antigens in immunity against infection and self-immunity. In: *The Biology of Heat Shock Protein and Molecular Chaperones*, ed Morimoto RI, Tissières A, Georgopoulos C. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 495–531.
- Lim HH, Jenkins OH, Myers MW, Miller JM, Altschuler RA. 1993.

- Detection of Hsp 72 synthesis after acoustic overstimulation in rat cochlea. *Hear Res* 69: 146–150.
- Liu WK, Cai RT. 1995. Noise. In: *Physical Factors and Occupational Medicine*, ed Liu WK, Cai RT. Science Publishing House, Beijing, 31–98.
- Lusk SL. 2002. Preventing noise-induced hearing loss. *Nurs Clin North Am* 37: 257–262.
- Mandal K, Jahangiri M, Xu Q. 2004. Autoimmunity to heat shock proteins in atherosclerosis. *Autoimmun Rev* 3: 31–37.
- Marber MS, Mestrl R, Chi S, Sayen MR, Yellon DM, Dillmann WH. 1995. Overexpression of the rat inducible 70-kD heat stress protein in a transgenic mouse increases the resistance of the heart to ischemic injury. *J Clin Invest* 96: 1446–1456.
- Marcellini L, Rosati MV, Ciarrocca M, Ursini A, Tomao E, Tomei F. 2003. Agricultural work: noise exposure and cardiovascular effects. *G Ital Med Lav Ergon* 25(Suppl): 229–230.
- Melamed S, Kristal-Boneh E, Froom P. 1999. Industrial noise exposure and risk factors for cardiovascular disease: findings from the CORDIS study. *Noise Health* 1: 49–56.
- Morata TC, Dunn DE, Kretschmer LW, Lemasters GK, Keith RW. 1993. Effects of occupational exposures to organic solvents and noise on hearing. *Scand J Work Environ Health* 19: 245–254.
- Morimoto RI, Tissières A, Georgopoulos C, eds. 1994. Progress and perspectives in the biology of heat shock proteins and molecular chaperones. In: *The Biology of Heat Shock Proteins and Molecular Chaperones*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1–30.
- Multhoff G. 2002. Activation of natural killer cells by heat shock protein 70. *Int J Hyperthermia* 18: 576–585.
- Multhoff G, Botzler C, Issels R. 1998. The role of heat shock proteins in the stimulation of an immune response. *Biol Chem* 379: 295–300.
- Nakayasu K, Nakaya Y, Oki Y, Nomura M, Ito S. 2004. Long-term follow-up in Japanese public office workers of the influence of blood pressure on ECG changes. *Circ J* 68: 563–567.
- Okin PM, Devereux RB, Nieminen MS, et al. 2004. Electrocardiography strain pattern and prediction of cardiovascular morbidity and mortality in hypertensive patients. *Hypertension* 44: 48–54.
- Plumier C, Krueger AM, Currie RW, Kontoyiannis D, Kollias G, Pargoulatos GN. 1997. Transgenic mice expressing the human inducible Hsp70 have hippocampal neurons resistant to ischemic injury. *Cell Stress Chaperones* 2: 162–167.
- Pockley AG. 2002. Heat shock proteins, inflammation, and cardiovascular disease. *Circulation* 105: 1012–1017.
- Pockley AG. 2003. Heat shock proteins as regulators of the immune response. *Lancet* 362: 469–476.
- Pockley AG, Georgiades A, Thulin T, de Faire U, Frostegard J. 2003. Serum heat shock protein 70 levels predict the development of atherosclerosis in subjects with established hypertension. *Hypertension* 42: 235–238.
- Pockley AG, Wu R, Lemne C, Kiessling R, de Faire U, Frostegard J. 2000. Circulating heat shock protein 60 is associated with early cardiovascular disease. *Hypertension* 36: 303–307.
- Portig I, Pankuweit S, Maisch B. 1997. Antibodies against stress proteins in sera with dilated cardiopathy. *J Mol Cell Cardiol* 29: 2245–2251.
- Radford NB, Fina M, Benjamin JJ, et al. 1996. Cardioprotective effects of 70-kDa heat shock protein in transgenic mice. *Proc Natl Acad Sci U S A* 93: 2339–2342.
- Rajdev S, Hara K, Kokubo Y, Mestrl R, Dillmann W, Weinstein PR, Sharp FR. 2000. Mice overexpressing rat heat shock protein 70 are protected against cerebral infarction. *Ann Neurol* 47: 782–791.
- Rassow J, Voos W, Pfanner N. 1995. Partner proteins determine multiple functions of Hsp70. *Trends Cell Biol* 5: 207–212.
- Rauch SD, San Martin JE, Moscicki RA, Bloch KJ. 1995. Serum antibodies against heat shock protein 70 in Meniere's disease. *Am J Otol* 16: 648–652.
- Rea IM, McNerlan S, Pockley AG. 2001. Serum heat shock protein and anti-heat shock protein antibody levels in aging. *Exp Gerontol* 36: 341–352.
- Schett G, Xu Q, Amberger A, Van Der Zee R, Recheis H, Willeit J, Wick G. 1995. Autoantibodies against heat shock protein 60 mediate endothelial cytotoxicity. *J Clin Invest* 96: 2568–2577.
- Sgarbossa EB, Birnbaum Y, Parrillo JE. 2001. Electrocardiographic diagnosis of acute myocardial infarction: current concepts for the clinician. *Am Heart J* 141: 507–517.
- Shingai R, Maeda T, Onishi S, Yamamoto Y. 1995. Autoantibody against 70 kD heat shock protein in patients with autoimmune liver disease. *J Hepatol* 23: 382–390.
- Snoeckx LH, Cornelussen RN, Van Nieuwenhoven FA, Reneman RS, Van Der Vusse GJ. 2001. Heat shock protein and cardiovascular pathophysiology. *Physio Rev* 81: 1461–1497.
- Sreedhar AS, Csermely P. 2004. Heat shock proteins in the regulation of apoptosis: new strategies in tumor therapy: a comprehensive review. *Pharmacol Ther* 101: 227–257.
- Suen YF. 1997. Measurements of serum lipid and serum lipoprotein. In: *Clinic Laboratory Methodology*, ed Yang CS, Song NG. Tianjing Science and Technology Publishing House, Tianjing, China, 218–220.
- Talbott EO, Gibson LB, Burks A, Engberg R, MeHugh KP. 1999. Evidence for a dose-response relationship between occupational noise and blood pressure. *Arch Environ Health* 54: 71–78.
- Tanguay RM, Wu Y, Khandjian EW. 1993. Tissue-specific expression of heat shock stress proteins of the mouse in the absence of stress. *Dev Genet* 14: 112–118.
- Veres A, Fust G, Smija M, et al. 2002. Relationship of anti-60 kDa heat shock protein and anti-cholesterol antibodies to cardiovascular events. *Circulation* 106: 2775–2780.
- Vogt S, Portig I, Kusch B, Pankuweit S, Sirat AS, Troitzsch D, Maisch B, Moosdorf R. 2004. Detection of Anti-hsp70 Immunoglobulin G antibodies indicates better outcome in coronary artery bypass grafting patients suffering from severe preoperative angina. *Ann Thorac Surg* 78: 883–889.
- Welch WJ. 1993. How cells respond to stress. *Sci Am* 268: 56–64.
- Wu T, Ma J, Chen S, et al. 2001. Association of plasma antibodies against the inducible Hsp70 with hypertension and harsh working condition. *Cell Stress Chaperones* 6: 394–401.
- Wu T, Yuan Y, Wu Y, He H, Zhang G, Tanguay RM. 1998. Presence of antibodies to heat stress protein in workers exposed to benzene and in patients with benzene poisoning. *Cell Stress Chaperones* 3: 161–167.
- Xiao C, Chen S, Yuan M, et al. 2004. Expression of the 60 kDa and 71 kDa heat shock proteins and presence of antibodies against the 71 kDa heat shock protein in pediatric patients with immune thrombocytopenic purpura. *BMC Blood Disord* 4: 1.
- Xu Q. 2002. Role of heat shock proteins in atherosclerosis. *Arterioscler Thromb Vasc Biol* 22: 1547–1559.
- Xu Q, Kiechl S, Mayr M, Metzler B, Egger G, Oberhollenzer F, Willeit J, Wick G. 1999. Association of serum antibodies to heat shock protein 65 with carotid atherosclerosis. Clinical significance determined in a follow-up study. *Circulation* 100: 1169–1174.
- Xu Q, Wick G. 1996. The role of heat shock proteins in protection and pathophysiology of the arterial wall. *Mol Med Today* 2: 372–379.
- Xu Q, Willeit J, Marosi M, et al. 1993. Association of serum antibodies to heat-shock protein 65 with carotid atherosclerosis. *Lancet* 341: 255–259.
- Yan SK. 2003. Suggestions for clinic methods of serum lipid and lipoprotein [in Chinese]. *Chin J Lab Med* 26: 182–184.
- Yang M, Zheng J, Yang Q, et al. 2004. Frequency-specific association

- of antibodies against heat shock protein 60 and 70 with noise-induced hearing loss in Chinese workers. *Cell Stress Chaperones* 9: 207–213.
- Zhang XM. 1998. Cardia arrhythmia. In: *Medicine*, ed Chen HZ. People Hygiene Publishing House, Beijing, 148–193.
- Zugel U, Kaufmann SH. 1999a. Immune response against heat shock proteins in infectious diseases. *Immunobiology* 201: 22–35.
- Zugel U, Kaufmann SH. 1999b. Role of heat shock protein in protection from and pathogenesis of infectious diseases. *Clin Micro Rev* 12: 19–39.