ORIGINAL ARTICLE

A six year follow up study of the subclinical effects of carbon disulphide exposure on the cardiovascular system

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Aims: A six year prospective cohort study was conducted to clarify whether the current carbon disulphide (CS_2) exposure level is low enough to prevent subclinical health impairment and/or to ameliorate health effects due to previous high exposure. This paper describes the effects on the cardiovascular systems.
Methods: The study subjects were 432 male workers exposed to CS_2 and 402 non-exposed workers in Japan, all of whom were examined in 1992–93. A total of 251 CS_2 exposed, 140 formerly exposed, and 359 non-exposed workers participated in the follow up survey (follow up rate 89.9%) in 1998–99. Mean duration of exposure was 19.3 years at the end the study. Mean CS_2 and 2-thiothiazolidine-4-carboxylic acid (TTCA) concentrations were 5.0 ppm and 1.6 mg/g creatinine. Health items examined were serum biochemical indices including lipids and coagulation-fibrinolysis factors, blood pressure, aortic stiffness, ophthalmography, and electrocardiography at rest and after Master's double 2 step test. Potential confounding factors were adjusted for.
Results: Incidence of ischaemic findings, defined as Minnesota codes I, IV_{1-3}, V_{1-3} (at rest and after the load), or receiving treatment for ischaemia, was significantly higher in the exposed workers, especially for the spinning/refining workers (adjusted OR 2.1; 95% CI 1.1 to 4.0) or the highest quartile of six year mean TTCA (adjusted OR 3.9; 95% CI 1.8 to 8.7), although the observed increase in risk was diminished when rigorous ECG criteria were applied. Incidence of retinal microaneurysm was increased with marginal significance. Among cardiovascular risk factors we examined, only blood pressure values were significantly increased in the exposed workers.
Conclusions: Increased risk of ischaemic electrocardiogram findings among Japanese viscose rayon workers was observed. Although its clinical significance is to be discussed, the current Japanese occupational exposure limit for CS_2, 10 ppm, would be high to prevent subclinical cardiovascular effects in this study population.

A large body of evidence has documented the adverse effects of occupational exposure to carbon disulphide (CS_2, CAS no. 75-15-0) on multiple organs. The cardiovascular and nervous systems are considered to be the principal targets affected by CS_2 exposure.[1 2] Currently, the Japan Society for Occupational Health[3] and the American Conference of Governmental Industrial Hygienists[4] recommend a time weighted average (TWA) of 10 ppm as the allowable occupational exposure limit for CS_2, whereas the Deutsche Forschungsgemeinschaft[5] of Germany reduced its MAK value to 5 ppm as the TWA concentration. It remains to be clarified whether the current CS_2 exposure level is low enough to prevent the occurrence of CS_2 related health impairments and/or to ameliorate health effects due to previous high exposure.

Various researchers have investigated and reported CS_2 effects on the cardiovascular system using mortality data[6–9] and subclinical indicators of cardiovascular functions or their risk factors.[10–13] Some studies showed a positive association between CS_2 exposure and outcome indices measured, while others did not. This discrepancy may be explained by differences in CS_2 exposure (intensity, duration) as well as differences in ethnic background, but a big issue to be solved is that most of the epidemiological studies in recent years were cross-sectional ones,[14] in which temporal association could not be guaranteed, and therefore it is difficult to conclude whether a causal relation exists at a relatively low concentration of CS_2.

We initiated a prospective cohort study with all Japanese rayon factories using sensitive indicators of subclinical health changes and detailed exposure assessment in 1992.[15 16] Study subjects' health was examined comprehensively, including cardiovascular, cerebrovascular, ophthalmological, neurological, neurobehavioural, and endocrinological aspects. In this paper, the results of CS_2 effects on the cardiovascular system, including ophthalmography after six years follow up will be presented.

SUBJECTS AND METHODS

Study base, follow up, and exposure assessment

The study design, results of follow up, and exposure assessment for six years were described previously.[15–17] Briefly, the baseline survey was done in 1992–93 at 11 rayon manufacturing factories in Japan. The study subjects were 432 male workers exposed to CS_2 and 402 male non-exposed workers from the same factories who had not been exposed to hazardous chemicals. In 1998 and 1999, the follow up survey was conducted and 750 subjects participated. The follow up rates were 89.9% in total, and 90.5% for the exposed and 89.3% for the non-exposed groups, respectively. None of the subjects had any medical history of cardiovascular diseases, including ischaemic heart diseases and hypertension with medical treatment at baseline, which we determined by checking companies' medical records and

Abbreviations: ECG, electrocardiography; LDL, low density lipoprotein; HDL, high density lipoprotein; Lp(a), lipoprotein a; OR, odds ratio; PWV, pulse wave velocity; TTCA, 2-thiothiazolidine-4-carboxylic acid; TWA, time weighted average

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Main messages

- This is a six year prospective cohort study of all Japanese viscose rayon factories, with detailed exposure assessment for CS_2 and sensitive indicators of subclinical cardiovascular effects.
- There is an association between exposure to CS_2 and increase in incidence of ischaemic findings defined as Minnesota codes with overall exposure levels for six years of 5 ppm (CS_2) and 1.6 mg/g creatinine (TTCA).
- However, the observed increase in risk for ischaemic changes is diminished when rigorous ECG criteria are applied.
- There is some evidence that not only exposure prior to the study but also exposure during the observation period has some significant impact on ischaemic findings, although its degree of contribution is unknown.
- A marginally significant increase is observed in incidence of retinal microaneurysm, but there are no exposure related changes in various risk factors of arteriosclerosis except for blood pressure.

Policy implications

- Re-evaluation of the current allowable occupational exposure limit for CS_2 may be required, while the clinical significance of the risk indicators should be discussed since the observed effects in this population are subclinical and their sizes are small.

through a self administered questionnaire. Among the workers exposed to CS_2, 251 continued to be exposed to CS_2 until the end of the study (CS_2 workers), but 140 workers were transferred to non-CS_2 jobs (ex-CS_2 workers), largely because three of the factories discontinued the production of rayon fibres during the study period between 1994 and 1995 due to changes in business circumstances. After the subjects' job records were summarised, some of the ex-CS_2 and the non-exposed workers were reclassified as CS_2 workers because they were still being exposed to CS_2 when they worked near the rayon production area; some non-exposed workers were excluded from the analysis because they had a history of CS_2 exposure for 1–8 years prior to the observation period. In this paper, the total number of subjects analysed statistically was 744 (CS_2 exposed workers, 259; ex-CS_2 workers, 133; and non-exposed workers, 352). Details of the follow up are described elsewhere.[17]

As all health examinations were not carried out on the same day, some subjects missed a few items depending on their work schedule. Table 1 presents a description of the demographic characteristics of the subjects at the time of the baseline survey and a summary of exposure assessment during the observation period. The frequencies of tobacco smokers and alcohol drinkers were slightly higher in the CS_2 workers, but no statistically significant differences were noted among the three groups. During the observation period, 1.6% (CS_2), 7.9% (ex-CS_2), and 7.1% (non-exposed) of the smokers had quit smoking; the frequency of smokers at the time of the follow up survey was as follows: CS_2 workers, 69.0%; ex-CS_2 workers, 61.7%; and non-exposed workers, 55.6%. For exposure assessment, we measured urinary 2-thiothiazolidine-4-carboxylic acid (TTCA) adjusted with creatinine level as the internal exposure index from 1992, and eight hour time weighted average CS_2 concentration in the breathing zone as the external exposure index from 1993 for each worker.

Risk factors for cardiovascular diseases

Blood biochemical indices examined were total cholesterol, HDL cholesterol, LDL cholesterol, apolipoprotein A-I, apolipoprotein B, lipoprotein a (Lp(a)) (follow up survey only), triglycerides, fibrinogen, tissue plasminogen activator, D-dimer, plasminogen activator inhibitor-1, and thrombin-antithrombin complex III. The subjects were asked to fast at least 12 hours before blood collection at the time of the follow up survey, while only non-fasting blood collection was performed at the time of the baseline survey. The samples were sent to a nationwide clinical laboratory within 24 hours and analysed with standard methods. Determination of LDL cholesterol and Lp(a) was done only at the follow up survey. The laboratory ran internal and external quality control programs to minimise measurement variation.

Subclinical indicator for vascular effect

All of the examinations and diagnoses described in the following sections were done under blind conditions in terms of subjects' exposure status. Blood pressure was measured by a doctor with a sphygmomanometer in an air conditioned room after a 15 minute rest. Aortic stiffness was evaluated by measuring carotid-femoral pulse wave velocity (PWV) (PWV-200, Fukuda Electric). Ultrasound measurement of the stiffness of the carotid artery was also done to obtain blood flow rate, maximal velocity of the blood, and stiffness parameter (QMF-2000XA, Hayashi-Denki Co). For the ophthalmography, a qualified doctor evaluated colour photo prints of ocular funds of both eyes for microaneurysm or retinal bleeding. Due to poor quality of an ophthalmograph, 33 subjects in the CS_2 exposed group, 54 in the ex-CS_2 group, and 82 in the non-exposed group were excluded from the statistical analysis for the follow up survey.

The exclusion occurred without relation to their exposure status or their ophthalmological findings because both examination and evaluation processes were performed under blind conditions. Since some of those had an ophthalmograph with good quality in the baseline survey, we compared the baseline prevalence of ophthalmological findings among them to explore the impact of such exclusion on the study results. The numbers of the subjects analysed here were 31 for the CS_2 exposed group, 42 for the ex-CS_2 group, and 45 for the non-exposed group.

Electrocardiography (ECG) and related symptoms

A 12-lead ECG at rest and after Master's double 2 step test were performed at both baseline and follow up surveys by an industrial physician or by our research team in the case when no industrial physician was available. ECG findings were evaluated by two cardiologists and coded according to Minnesota code 1982[18] under blind conditions. Codes I, IV_{1-3}, V_{1-3} at rest and after the load (in the original coding rule, these correspond to XI_{1-3} and XII_{1-3}) were considered as ischaemic signs, codes VI and VII were considered as conduction disturbance, and code VIII was considered as arrhythmia. If any subjects had medical treatment during the observation period, they were also considered to have positive findings. To take the clinical significance into account, we employed rigorous criteria for ischaemia, defined as any ECG changes (abnormal Q wave, QS pattern with complete bundle branch block, horizontal ST depression $\geqslant$2 mm, ST increase with reciprocal ST depression, and negative T wave

Table 1 Baseline characteristics of the study subjects and results of exposure assessment

	Non-exposed workers	Ex-CS_2 workers	CS_2 workers
Number	352	133	259
Age (y)‡	34.6	35.9	34.7
<35	137 (38.9%)	43 (32.3%)	108 (41.7%)
35–44	162 (46.0%)	80 (60.2%)	126 (48.6%)
⩾45	53 (15.1%)	10 (7.5%)	25 (9.7%)
Smoking			
Never	91 (25.9%)	27 (20.3%)	54 (20.8%)
Former	43 (12.2%)	13 (9.8%)	22 (8.5%)
Current (<30 cigarettes/day)	152 (43.2%)	72 (54.1%)	139 (53.7%)
Current (⩾30 cigarettes/day)	66 (18.8%)	21 (15.8%)	44 (17.0%)
Alcohol drinking			
Never or occasional	46 (13.1%)	23 (17.3%)	39 (15.1%)
Habitual (<45 g ethanol/day)	244 (69.3%)	90 (67.7%)	169 (65.3%)
Habitual (⩾45 g ethanol/day)	62 (17.6%)	20 (15.0%)	51 (19.7%)
Body mass index (kg/m^2)*	22.6 (2.8)	22.7 (2.7)	22.2 (2.5)
Education (% ⩾high school)	71.3%	62.4%	63.7%
Shift work (% shift workers)	88.6%	79.7%	87.6%
Coronary prone behaviour pattern (%)	20.2%	18.9%	18.9%
CS_2 exposure duration prior to the study (yr)‡	–	10.9 (2.3)	10.9 (2.3)
CS_2 concentration over the study period (ppm)‡	–	2.9 (1.8)†	5.0 (1.8)
TTCA over the study period (mg/g creatinine) ‡	–	1.3 (1.5)†	1.6 (1.9)

*Arithmetic mean (SD); ‡geometric mean (GSD).
†Average exposure duration during the study period was 2.0 years.

with ⩾50% of R amplitude or upright QRS) or as receiving medical treatment for ischaemic heart disease during the observation period. The London School of Hygiene Cardiovascular Questionnaire for ischaemia and myocardial infarction (Rose's questionnaire) was also done.[18] The coronary prone behaviour pattern was surveyed and determined through a self administered, brief Japanese type A behaviour pattern questionnaire.[19]

Statistical analysis

Exposure status was categorised into three groups (CS_2 workers, ex-CS2 workers, and non-exposed workers). To elucidate exposure-response relations, job title at baseline (that is, spinning/refining workers and other CS_2 workers), was first used as a surrogate marker of exposure. Eighteen CS_2 workers had been transferred to different CS_2 jobs during the study period, and allocated to a new job category in the case that job transfer occurred more than one year before the follow up survey. Exclusion of such workers did not change the results by separate analysis. Then, with the index of internal exposure, the subjects were classified into four roughly equal groups according to quartiles determined by six year TTCA levels.

In the analysis, both exposure to CS_2 during the observation period and previous exposure prior to the study were accounted for, although it seemed difficult to precisely separate out these two factors in the biological sense. First, we tested the differences between the exposed, ex-CS_2, and the non-exposed groups at baseline and at follow up, by which the effects of CS_2 exposure for their entire exposure period were examined. We also evaluated the changes during the six year observation period. For continuous data, intra-individual change over the observation period, defined as subtracting a value at the baseline survey from a value at the follow up survey, was compared for all variables except for LDL cholesterol and Lp(a). Moreover, we compared the health outcomes at follow up by including baseline values as covariates, in which baseline differences would be controlled for.[20] For binary data, incidences over the six year observation period were compared.

The normality of data distribution was examined, and an appropriate transformation was performed in order to obtain a normal distribution before the analysis for continuous data. Mean values of the variables were calculated and compared by analysis of variance followed by Dunnett's *t* test (non-exposed as a referent group). For incidence and prevalence data, proportions were compared by the χ^2 test or Fisher's exact method without any adjustment for the multiplicity of comparisons. To control for possible confounders simultaneously, multiple linear regression models were developed for the continuous outcome variables and multiple logistic regression models were developed for the binary outcome variables. Potential confounding factors included in the models were age (<35, 35–44, ⩾45 years), body mass index (BMI, kg/m^2), education level (junior high school, senior high school, or higher), smoking status (never, past, current (<30, ⩾30 cigarettes/day)), alcohol intake (never or occasional, habitual (<45, ⩾45 g ethanol/day)), shift work (yes or no), and coronary prone behaviour pattern (yes or no) at baseline. Systolic blood pressure and HDL cholesterol at baseline were added for ophthalmography, arterial stiffness, and ECG.

The effectiveness of all constructed models in describing outcome variables was checked by residual and collinearity analysis. Inclusion/exclusion of systolic blood pressure as a covariate into the full model did not change an estimate of a regression coefficient significantly. For blood biochemical indices, inclusion or exclusion of the subjects who used lipid lowering agents did not change the results (lipid lowering agent users were excluded from the analysis of the blood indices in table 2).

Sixteen of 133 (12.0%) ex-CS_2 workers and 15 of 352 (4.3%) non-exposed workers were exposed to solvents other than CS_2 during the study period, but none of those solvents showed any health risks with regard to the cardiovascular systems. Thus, we did not exclude such workers from the analysis. Some of the data were missing because of absence on the day of a research team visit or incompleteness of the questionnaire; both of these situations occurred without any relation to exposure status or health status. All p values were two sided. A test for trend of calculated OR was conducted by including exposure as a continuous variable in the regression model for the exposed group only. All statistical analysis was performed using the SAS package (Cary, NC).

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Table 2 Effects of exposure to carbon disulphide on the risk factors of cardiovascular disease in the follow up survey

	Non-exposed workers	Ex-CS_2 workers	CS_2 exposed workers	p value (ANOVA)
Lipid metabolism	(n = 347)	(n = 127)	(n = 253)	
Total cholesterol (mg/dl)‡	195 (1.2)	193 (1.2)	198 (1.2)	0.32
HDL cholesterol (mg/dl)‡	54 (1.3)	54 (1.3)	58 (1.3)*	<0.01**
LDL cholesterol (mg/dl)‡	119 (1.3)	117 (1.3)	118 (1.3)	0.93
Apo-AI (mg/dl)‡	138 (1.2)	139 (1.2)	145 (1.2)*	<0.01**
Apo-B (mg/dl)‡	97 (1.3)	96 (1.3)	97 (1.3)	0.87
Lp(a) (mg/dl)‡	13 (2.6)	11 (2.4)	11 (2.5)	0.31
Triglycerides (mg/dl)‡,§	108 (1.7)	104 (1.8)	102 (1.8)	0.50
Coagulation-fibrinolysis system	(n = 347)	(n = 127)	(n = 253)	
Fibrinogen (mg/dl)‡	241 (1.2)	244 (1.2)	240 (1.3)	0.69
Tissue plasminogen activator (ng/dl)‡	8 (1.7)	7 (1.7)	8 (1.6)	0.74
D-dimer (ng/dl)‡	50 (1.4)	52 (1.6)	50 (1.5)	0.65
Plasminogen activator inhibitor-1 (ng/dl)‡	39 (2.3)	40 (2.5)	42 (2.2)	0.52
Thrombin-antithrombin complex III (ng/dl)‡	2.2 (3.5)	1.8 (3.2)	2.4 (3.1)	0.16
Blood pressure (mm Hg)	(n = 336)	(n = 130)	(n = 250)	
Systolic blood pressure‡	119 (1.1)	118 (1.1)	121 (1.1)	0.05**
Diastolic blood pressure‡	71 (1.2)	71 (1.1)	73 (1.2)	0.23
Arterial stiffness				
	(n = 332)	(n = 129)	(n = 237)	
Aorta (pulse wave velocity, m/s)†	6.8 (0.9)	7.0 (0.9)	6.6 (0.9)	0.17
	(n = 325)	(n = 123)	(n = 240)	
Carotid artery (maximal blood flow, cm/s)‡	64.7 (1.3)	61.4 (1.2)	64.2 (1.2)	0.08

†Arithmetic mean (SD); ‡geometric mean (GSD).
§Subjects with non-fasting samples were excluded though inclusion of those showed similar results (n = 233 for CS_2 group, 124 for ex-CS_2 group, and 321 for non-exposed group, respectively).
**p<0.05 by ANOVA followed by Dunnett's *t* test; *p<0.05 compared to the non-exposed group.

RESULTS

Effects on risk factors or subclinical indicators for cardiovascular diseases

Table 2 shows the results of the measurement of blood biochemical indices in the follow up survey. For lipid metabolism, all items except HDL cholesterol and apolipoprotein A-I were comparable among the three groups. Prevalences of lipid lowering agent users at the time of the follow up survey were 4/259 (1.5%) among the CS_2 workers, 5/133 (3.8%) among the ex-CS_2 workers, and 2/352 (0.6%) among the non-exposed workers. When the multivariate analysis was applied, favourable increase of HDL cholesterol and apolipoprotein A-I remained significant, although we did not measure physical activity. For the coagulation-fibrinolysis system, there were no differences among the three groups at the time of follow up. Similar results were obtained for the baseline survey.

For blood pressure, 8/259 (3.1%) of the CS_2 workers, 2/133 (1.5%) of the ex-CS_2 workers, and 6/352 (1.7%) of the non-exposed workers were under treatment for hypertension and were taking antihypertensive agents at the time of the follow up survey ($p = 0.43$ by χ^2 test). Systolic blood pressure was small but significantly higher in the CS_2 exposed group compared to the non-exposed group after controlling for possible confounders in the follow up survey (antihypertensive agent users were excluded from the analysis of blood pressure in table 2). With the multiple regression analysis, a regression coefficient for the CS_2 group was 0.0103 (blood pressure value in mm Hg as a dependent valuable was logarithmically transformed to obtain normality; 95% CI 0.0029 to 0.0177), which indicates that systolic blood pressure of the CS_2 workers was 2.4% higher compared to the non-exposed workers. Similar results were obtained for diastolic blood pressure. Its regression coefficient was 0.0097 (95% CI 0.0007 to 0.0187), which is equivalent to a 2.3% increase for the exposed workers. In the baseline survey, geometric mean values of systolic/diastolic blood pressure were 121/70 mm Hg for the CS_2, 120/69 mm Hg for the ex-CS_2, and 119/69 mm Hg for the non-exposed, respectively ($p = 0.40$ by ANOVA). With multiple regression analysis, the difference between the CS_2 exposed and the non-exposed workers at baseline was small but statistically significant, while changes in systolic or diastolic pressures over six years were not statistically significant, when examined either by both intra-individual changes over six years or by comparison of blood pressure values at follow up with inclusion of a baseline value as a covariate. CS_2 workers were divided into two groups according to their job type or four groups according to six year TTCA level to examine whether exposure dependent relations exist. We found that blood pressure levels were increased in some of the exposed groups, but exposure dependency was unclear. There were no differences for any of the indices of arterial stiffness in either the baseline survey or the follow up survey.

Effects on ophthalmography and ECG

Table 3 shows the prevalence at baseline and at follow up, and incidence during the six year observation period for retinal microaneurysm and ischaemic findings among the CS_2 exposed, ex-CS_2, and non-exposed groups. The prevalence of microaneurysm of the retinal artery was about two times higher in the exposed group compared to the non-exposed group at follow up as well as at baseline. The odds ratio (OR) of having a retinal microaneurysm among the unaffected subjects at baseline, that is, the incidence, was also marginally significant after controlling for various possible confounders. When the subjects were stratified by age, the incidence of the exposed groups was higher than that of the non-exposed group through all age strata, although no statistical significance was detected between the exposed and the non-exposed groups (table 4). Among those who were excluded from the statistical analysis for the follow up

Table 3 Arteriosclerotic effects on the retinal artery and the coronary artery

	Non-exposed workers	Ex-CS_2 workers	CS_2 exposed workers	p value
Retinal microaneurysm				
Prevalence (baseline)	16/277 (5.8%)	15/108 (11.1%)	28/240 (11.7%)	0.04**
	1.0	2.1 (0.9–4.9)	2.2 (1.1–4.3)*	
Prevalence (follow up)	17/270 (6.3%)	7/79 (8.9%)	31/226 (13.7%)	0.02**
	1.0	1.7 (0.6–4.3)	2.7 (1.4–5.4)*	
Incidence over 6 years	11/219 (5.0%)	3/60 (5.0%)	17/184 (9.2%)	0.20
	1.0	1.4 (0.3–4.9)	2.3 (1.0–5.4)	
Coronary artery				
Ischaemic signs (defined as Minnesota codes I, IV_{1-3}, V_{1-3} or receiving treatment for ischaemia)				
Prevalence (baseline)	15/344 (4.4%)	1/130 (0.8%)	3/258 (1.2%)	0.02**
	–	–	–	
Prevalence (follow up)	27/343 (7.9%)	7/130 (5.4%)	32/257 (12.5%)	0.04**
	1.0	0.7 (0.3–1.7)	1.7 (0.9–3.1)	
Incidence over 6 years	21/329 (6.4%)	6/129 (4.7%)	30/254 (11.8%)	0.02**
	1.0	0.7 (0.3–1.9)	2.0 (1.1–3.6)*	
Ischaemia (defined as rigorous ECG findings such as ST depression ⩾2 mm or receiving treatment)				
Prevalence (baseline)	5/344 (1.5%)	0/130 (0%)	2/258 (0.8%)	0.37
	–	–	–	
Prevalence (follow up)	13/343 (3.8%)	4/130 (3.1%)	9/257 (3.5%)	0.93
	1.0	1.1 (0.3–3.8)	1.0 (0.4–2.7)	
Incidence over 6 years	12/338 (3.6%)	4/130 (3.1%)	9/255 (3.5%)	0.97
	1.0	1.1 (0.3–4.1)	1.1 (0.4–3.0)	

Results expressed as prevalence or incidence, with adjusted odds ratios underneath.
**$p<0.05$ by χ^2 test or Fisher's exact method; *$p<0.05$ compared to the non-exposed group by multiple logistic regression analysis, in which age, body mass index, education level, smoking status, alcohol intake, shift work, coronary prone behaviour pattern, systolic blood pressure, and HDL cholesterol at baseline and exposure variable were included as covariates.
Denominators are different because some of the subjects missed an ECG due to their work schedule or some were excluded from the analysis due to quality of an ophthalmograph (see text for details).

survey, the baseline prevalences of ophthalmological findings were 3/31 (9.7%) for the CS_2 exposed group, 5/42 (11.9%) for the ex-CS_2 group, and 4/45 (8.9%) for the non-exposed group, respectively.

With regard to the arteriosclerotic effect of carbon disulphide on the coronary artery, the results vary depending on the criteria of ECG. When ischaemic signs were defined as Minnesota codes I, IV_{1-3}, V_{1-3} (at rest and after the load) or receiving treatment for ischaemia, its incidence over the observation period was significantly higher in the CS_2 exposed group (11.8%) compared to the non-exposed group (6.4%) (table 3). In contrast, when more rigorous criteria were employed, both incidence and prevalence were comparable among the three groups. When the subjects were stratified by age, the incidence of the exposed groups was higher than that of the non-exposed group through all age strata, although there was no clear age related increase in incidence (table 4). A non-significant increase in the incidence of the exposed workers was observed in the ⩾45 years of age stratum (24.0%), while statistically significant increase was observed in the <35 years of age stratum (12.4%). The latter finding was coincident with a higher mean TTCA and CS_2 concentration in this age stratum. Two subjects for the exposed group and another two for the ex-CS_2 group were receiving medical treatment for ischaemic heart disease at the time of the follow up survey.

The prevalences of having symptoms for angina or possible myocardial infarction (Rose's questionnaire) were 16/259

Table 4 Age at baseline, exposure level, and incidence of retinal microaneurysm and ischaemic findings†

	Exposure index‡			Incidence	
	Exposure duration prior to the study (y)	TTCA (mg/g creatinine)	CS_2 (ppm)	Non-exposed workers	CS_2 exposed workers
Retinal microaneurysm					
Entire group	10.7 (2.3)	1.5 (1.9)	4.8 (1.8)	11/219 (5.0%)	17/184 (9.2%)
Age at baseline					
<35	6.1 (2.1)	1.9 (2.0)	6.0 (1.7)	5/78 (6.4%)	7/79 (8.9%)
35–44	16.0 (1.8)	1.3 (1.9)	4.0 (1.8)	3/99 (3.0%)	8/87 (9.2%)
⩾45	19.4 (1.6)	1.4 (1.9)	4.1 (1.9)	3/42 (7.1%)	2/18 (11.1%)
Ischaemic findings†					
Entire group	10.8 (2.3)	1.6 (1.9)	5.0 (1.8)	21/329 (6.4%)	30/254 (11.8%)*
Age at baseline					
<35	5.7 (2.1)	1.9 (1.9)	6.0 (1.7)	5/131 (3.8%)	13/105 (12.4%)*
35–44	16.5 (1.7)	1.4 (1.9)	4.3 (1.8)	10/150 (6.7%)	11/124 (8.9%)
⩾45	20.1 (1.5)	1.5 (1.9)	4.7 (2.0)	6/48 (12.5%)	6/25 (24.0%)

†Defined as Minnesota codes I, IV_{1-3}, V_{1-3} or receiving treatment for ischaemia.
‡Geometric mean (GSD).
*$p<0.05$ compared to the non-exposed workers by χ^2 test or Fisher's exact method.

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(6.2%) of the CS_2 workers, 12/132 (9.1%) of the ex-CS_2 workers, and 34/347 (9.8%) of the non-exposed workers at the time of the follow up survey (p = 0.27 by χ^2 test). In addition, the prevalence and incidence of ECG findings for conduction disturbance and arrhythmia were also comparable; for example, the prevalences for conduction disturbance and arrhythmia at the time of the follow up survey were 7/257 (2.7%) and 2/257 (0.8%) for the CS_2 workers, 2/130 (1.5%) and 3/130 (2.3%) for the ex-CS_2 workers, and 10/343 (2.9%) and 9/343 (2.6%) for the non-exposed workers, respectively.

To explore the exposure-response relation, we classified the exposed subjects according to their job title at baseline or to their internal exposure level over the study period, and their incidences were compared (table 5). Ischaemic findings were defined as Minnesota codes I, IV_{1-3}, V_{1-3} at rest or after the load or receiving treatment for ischaemia in this analysis. The overall OR as well as the OR for the highest quartile of six year TTCA were significantly increased for both ischaemic signs and retinal microaneurysm after controlling for age, although no significant regression coefficient was obtained by the test for trend. These relations remained significant when we accounted for various possible confounders simultaneously. The subjects in the high group had higher CS_2 exposure during the study period, but their exposure duration was shorter with younger age.

DISCUSSION

Effects of occupational exposure to CS_2 on the cardiovascular systems have been reported by various authors from different countries.[9 11 13 21–26] Recently, Sulsky *et al* reported the results of comprehensive review of the epidemiological studies for the possibility of cardiovascular effects of CS_2 and pointed out that mixed, inconsistent results were found for the association between CS_2 exposure and coronary heart disease or its indicators.[14] Our study offers the opportunity to evaluate the statistical association between CS_2 exposure and subclinical changes in various indices of the cardiovascular system because this is a cohort study with a high follow up rate (approx. 90%) and because various risk factors of the cardiovascular diseases were evaluated and controlled for. We found a statistical association between CS_2 exposure and subclinical ischaemic findings, defined by the Minnesota code, with a risk increase that was two times higher after controlling for major confounders among the Japanese rayon manufacturing workers. During the observation period, we undertook a detailed exposure assessment for all study subjects twice a year; their overall exposure levels to CS_2 for six years were 5.0 ppm (CS_2 personal air sampling) and 1.6 mg/g creatinine (urinary TTCA as internal exposure index). When the CS_2 exposed workers were divided into two or four groups according to their job titles or level of internal exposure, significant increases in the incidence were found for the spinning/refining workers (6.1 ppm for CS_2 and 2.0 mg/g creatinine for TTCA) or the highest quartile of the exposure (8.7 ppm and 3.6 mg/g creatinine), although there was no linear increasing trend of the risk. It should be noted that the observed increase in risk of ischaemic findings was diminished when rigorous ECG criteria were applied.

The biological mechanisms by which CS_2 induces toxic effects on the cardiovascular system have been proposed and investigated in experimental animals[1 27–31] as well as in humans.[11 32–34] In this study, we examined various risk indicators of arteriosclerosis such as serum lipid or the coagulation-fibrinolysis system, but there were no exposure related changes among these except blood pressure. This is consistent with the study in Taiwan in which increased ECG abnormality was observed while serum lipids levels were nearly identical to the reference group.[24] Kotseva also reported the results of a cross sectional study in Belgium in which the prevalence of ischaemic ECG findings was significantly higher among the exposed workers whose cumulative exposure index was 150 mg/m^3-year or more without any exposure related changes in blood pressure or

Table 5 Incidence of retinal microaneurysm and ischaemic findings† with relation to job title or six year internal exposure level

	Exposure index§			Incidence		Odds ratio‡	
	Exposure duration prior to the study (y)	TTCA (mg/g creatinine)	CS_2 (ppm)	Non-exposed workers	CS_2 exposed workers	Age adjusted	Age + other possible confounders adjusted
Retinal microaneurysm							
Entire group	10.7 (2.3)	1.5 (1.9)	4.8 (1.8)	11/219 (5.0%)	17/184 (9.2%)	2.0 (0.9–4.4)	2.2 (1.0–5.1)
By job title at baseline							
Other than spinning/refining	9.9 (2.2)	0.9 (1.8)	2.8 (1.8)		5/51 (9.8%)	2.2 (0.7–6.6)	3.2 (0.9–10.3)
Spinning or refining	11.0 (2.3)	1.9 (1.8)	5.9 (1.6)		12/133 (9.0%)	1.9 (0.8–4.6)	1.9 (0.8–4.8)
By internal exposure level for 1992–98							
Low	11.1 (2.2)	0.6 (1.4)	2.4 (1.7)		3/45 (6.7%)	1.4 (0.4–5.3)	1.9 (0.5–7.4)
Mid–low	12.1 (2.0)	1.3 (1.2)	4.4 (1.6)		5/46 (10.9%)	2.5 (0.8–7.5)	2.8 (0.9–8.9)
Mid–high	12.1 (2.5)	1.9 (1.1)	5.9 (1.3)		2/46 (4.3%)	0.9 (0.2–4.2)	0.5 (0.1–4.3)
High	8.3 (2.3)	3.5 (1.3)	8.3 (1.3)		7/46 (15.2%)	3.7 (1.2–10.6)*	3.8 (1.2–11.4)*
Ischaemic findings							
Entire group	10.8 (2.3)	1.6 (1.9)	5.0 (1.8)	21/329 (6.4%)	30/254 (11.8%)	2.1 (1.2–3.8)*	2.1 (1.1–4.0)*
By job title at baseline							
Other than spinning/refining	10.6 (2.2)	0.9 (1.8)	3.0 (1.8)		8/74 (10.8%)	2.0 (0.8–4.8)	2.1 (0.9–5.4)
Spinning or refining	10.9 (2.3)	2.0 (1.8)	6.1 (1.7)		22/180 (12.2%)	2.1 (1.1–4.0)*	2.1 (1.1–4.1)*
By internal exposure level for 1992–98							
Low	10.9 (2.2)	0.6 (1.5)	2.4 (1.7)		8/63 (12.7%)	2.2 (0.9–5.4)	2.3 (0.9–5.6)
Mid–low	12.4 (2.0)	1.3 (1.2)	4.6 (1.5)		4/63 (6.3%)	1.1 (0.4–3.4)	1.0 (0.3–3.1)
Mid–high	11.9 (2.3)	2.1 (1.1)	6.4 (1.4)		6/65 (9.2%)	1.5 (0.6–3.9)	1.6 (0.6–4.4)
High	8.6 (2.5)	3.6 (1.3)	8.7 (1.3)		12/63 (19.0%)	3.9 (1.8–8.7)*	4.2 (1.8–9.7)*

†Defined as Minnesota codes I, IV_{1-3}, V_{1-3} or receiving treatment for ischaemia.
‡OR for the non-exposed group was 1.0 as the referent.
¶Adjusted for age, BMI, education level, smoking status, alcohol drinking, shift work, coronary prone behaviour pattern, systolic blood pressure, and HDL cholesterol at baseline.
§Geometric mean (SD).
*$p<0.05$ compared to the non-exposed group by multiple logistic regression analysis, in which age, body mass index, education level, smoking status, alcohol intake, shift work, coronary prone behaviour pattern, systolic blood pressure, and HDL cholesterol at baseline and exposure variable were included as covariates.

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blood lipids.[34] Drexler *et al* found no relation between CS_2 exposure and various coronary risk factors including serum lipids, fibrinolytic activity, and electrolytes among German workers (median CS_2 exposure level and exposure duration were 4.0 ppm and 5.5 years, respectively) by a cross sectional design, and no effect of CS_2 exposure on ECG findings was detected.[12 35]

The only significant change we observed was a small increase in systolic and diastolic blood pressure in the exposed workers both at baseline and follow up surveys. However, if we combine the CS_2 exposed workers with the ex-CS_2 workers (ex-CS_2 workers were being exposed to CS_2 at the time of the baseline survey), the differences at baseline between the combined CS_2 exposed and ex-CS_2 group and the non-exposed group were no more statistically significant, although blood pressure values were still slightly higher for the combined group. This indicates that blood pressure levels at baseline of the ex-CS_2 workers from the closed-down factories were to some extent lower than those of the CS_2 workers from the remaining factories. As this discontinuation of exposure among ex-CS_2 workers was mostly due to changes in business policies of some of the participating companies, such selection was not health related, and could be by chance. Although the impact of CS_2 exposure over the six year observation period on blood pressure may not be substantial because six year intra-individual changes in blood pressure levels or comparison of blood pressure levels at follow up with inclusion of a baseline value as a covariate did not show significant differences, increase of blood pressure due to CS_2 exposure before the study period could relate to the increased risk of ischaemic findings in some degree, even if the difference was small. The biological plausibility of CS_2 toxicity on the cardiovascular system should be investigated further.

The major limitation of our study is a lack of information on the exposure that occurred before the study period. Generally, exposure concentrations to hazardous chemicals have tended to be improved by the introduction of effective industrial hygienic control measures in occupational settings. Such a downward trend of exposure was observed in this population even during the study period, as shown in our previous paper.[17] The most important question is whether the increased risk we observed was caused by the past exposure or by the recent exposure. One possible explanation for the observed association is that a higher exposure concentration of CS_2 before the observation period had strong effects on the cardiovascular systems of the exposed workers, and its progression by the normal aging process without relation to low level CS_2 exposure during the study period manifested itself in the increased risk of ischaemic findings. Unfortunately, we cannot know whether this is the case, but we performed additional analyses in order to assess the contribution of exposure over the observation period.

First, the exposed subjects were restricted to those whose exposure duration at baseline was less than five years and whose age at baseline was 20–44 ($n = 62$). The same number of subjects from the referent group was randomly selected from the non-exposed group with the frequencies of five year age category matched. Although the number of subjects analysed was not enough to reach statistical significance, incidences of ischaemic findings defined by Minnesota codes were 14.5% for the CS_2 exposed subjects and 4.8% for the non-exposed subjects ($p = 0.13$ by Fisher's exact method). Geometric means of CS_2 and TTCA during the observation period were 5.6 ppm and 1.7 mg/g creatinine, respectively. This may reflect the result in table 4 showing that the significant increase in ischaemic incidence in the exposed workers was coincident with a higher mean TTCA and CS_2 concentration in the $\leqslant 35$ years of age stratum where mean exposure duration was 5.7 years. Alternatively, we can compare the CS_2 exposed workers with the ex-CS_2 workers. Since mean age and exposure duration at the time when the study began were comparable between them, an observed difference could be a sign of CS_2 effects for the past six years. As already shown in table 3, the incidence of ischaemic signs was higher in the CS_2 workers than that in the ex-CS_2 workers (11.8% *v* 4.7%). When we directly compared the incidence of exposed workers with that in the ex-CS_2 workers who had been transferred to non-CS_2 jobs at least three years before the study ended, 110 ex-CS_2 subjects were identified. The incidence for the ex-CS_2 group was 4.5%, and OR adjusted for age, systolic blood pressure, HDL cholesterol, and other possible confounders was 2.7 ($p = 0.052$). These results indicate that not only the past exposure but also recent exposure had some significant impact on increased risk of ischaemic findings in this population, although its degree of contribution is unknown.

For ophthalmological effects, we obtained similar results as in our baseline report.[15] One important result here is that incidence over the observation period for the exposed workers was increased with marginal significance compared to the non-exposed workers, while that for the ex-CS_2 group was comparable with the non-exposed group. Although incidence and severity of the effects of CS_2 on the retinal artery have been reduced over time,[15] development of retinal microaneurysm could relate not only to the aging process but also to exposure to CS_2 under relatively low concentrations below 10 ppm. There are two studies evaluating the effect of exposure cessation on the ophthalmological findings. Sugimoto *et al* followed up 214 Japanese viscose rayon workers for five years.[36] They indicated that progression of retinopathy occurred in both the still exposed workers and those who had been transferred to jobs where there was no exposure, but none of the transferred workers showed progression of retinopathy if their exposure duration was 10 years or less. De Rouck *et al* reported the results of a four year follow up study of 29 CS_2 exposed workers in Belgium.[37] During the observation period, most of them were removed from CS_2 exposure. Prevalence and severity of retinal microaneurysm and small haemorrhage increased among workers in the relatively high CS_2 level area (at least 50 mg/m^3). Among workers at in areas of lower CS_2 (<10 mg/m^3), prevalence was unchanged while the number of microaneurysms increased. Since both studies did not have a non-exposed group as referents, it is difficult to separate out the effect of aging from the effect of exposure to CS_2. Since we did not show significant deterioration of the retinal micro-aneurysm among the ex-CS_2 workers whose mean exposure duration was 15.6 years at the end of exposure, past exposure would not relate to the progression of retinal microaneurysm in our population if exposure stopped. It should be noted that the significant increase in ophthalmological findings we observed here might be distorted to some extent because exclusion rates were sizable and different across the groups, even though such exclusion occurred randomly.

Our study results must be interpreted with caution besides the past exposure. First, an accurate estimation of real exposure is not simple, and it was not simple during the observation period. As we stated in the previous paper, when the exposure level was represented by maximum CS_2 and TTCA levels during a six year period for each subject (TTCA max , CS_2 max), the geometric means for those were 9.4 ppm and 4.6 mg/g creatinine, respectively.[17] Thus, the study subjects could have been exposed to CS_2 at levels near to or higher than the Japanese occupational exposure limit level (10 ppm) under certain working conditions, even though the average exposure level was maintained below the current occupational exposure limit during the observation period.

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Naturally, we cannot exclude the possibility that unmeasured confounding factors may have distorted the results or that the statistical adjustment of modifying or confounding factors may be insufficient. Moreover, since the observed effects of CS_2 in this population were subclinical and their sizes were small, the clinical significance of CS_2 exposure should be discussed as part of the risk management process.

In conclusion, using a longitudinal design with various subclinical indicators and detailed exposure assessment, we observed an increased risk of ischaemic ECG findings among Japanese viscose rayon workers. Exposure to CS_2 during the past six years was supposed to have some significant impact on the findings. Although our results may not be applicable to newly engaged workers and the clinical significance is to be discussed, the current Japanese occupational exposure limit, 10 ppm, would be high to prevent subclinical cardiovascular effects in this study population.

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